How plant reproductive success is determined by the interplay of antagonists and mutualists

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Abstract.   Plant reproductive success is often the outcome of mutualistic and antagonistic plant–animal interactions, which can be moderated by landscape composition. Studies addressing single plant–animal interactions are common, but studies simultaneously considering multiple plant–animal interactions in a landscape context are still scarce. We selectively excluded flower-visiting insects on phytometer plants and quantified how mutualistic and antagonistic interactions shaped the reproductive success of a common annual plant, wild mustard (*Sinapis arvensis*). Floral herbivory by larvae of rape pollen beetles (*Meligethes* spp.) strongly reduced fruit production, but could be minimized by insecticide application. Total seed production (the product of fruit production and seeds per fruit) strongly increased with pollinator visitation. On average, pollinator access to plants enhanced seed numbers by 754%. Insecticide treatment almost redoubled this number. The landscape composition (proportion of semi-natural habitats in 1000 m radius) surrounding phytometer plants did not affect plant–animal interactions, presumably due to the high dispersal ability of both the pollen beetles and the major pollinators (syrphid flies, bumblebees). In conclusion, pest control increased reproductive success only in the case of sufficient pollination.

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INTRODUCTION

The reproductive success of plants is often the outcome of mutualistic and antagonistic plant–animal interactions. Animal-mediated pollination plays a key role for the sexual reproduction of 88% of global angiosperm plant species and 70% of the major global crop species (Klein et al. 2007, Ollerton et al. 2011). At the same time, plant reproduction is often impaired by negative plant–animal interactions (Zhang et al. 2007). Among these, pest damage can severely reduce plant reproductive output such as the number of produced fruits or seeds.

Many studies have emphasized the importance of landscape composition for pollination services (Steffan-Dewenter et al. 2001, Ricketts et al. 2008, Holzschuh et al. 2012). Besides honeybees (*Apis mellifera*), wild bees (Hymenoptera: Apoidea) are major pollinators in agricultural systems (Garibaldi et al. 2013). Wild bees depend on high-quality nesting sites and continuous food supply, which are often subject to the availability of semi-natural habitats (Steffan-Dewenter and Tscharntke 1999, Öckinger and Smith 2006). Agricultural expansion and intensification at the expense of (semi-)natural habitats can cause reductions in wild bee abundance and species richness, putting the reproductive success of animal-pollinated plants situated in simplified agricultural landscapes at risk (Steffan-Dewenter et al. 2001, Tscharntke et al. 2005, Holzschuh et al. 2012).

Most arthropod pests undergo multiple life stages during their reproductive cycle, with different impact on plant reproduction. For example, while adults of rape pollen beetles (*Meligethes* spp.) feed on pollen of open Brassicaceae flowers, their larvae develop within flower buds, often of the same plant species. Larval development of pollen beetles can restrict flower and pod development (Lamb 1989, Schlinkert et al. 2015*a, b*). Changes in landscape composition can alter the spatial distribution of pollen beetles and degree of local plant infestation (Gladbach et al. 2011). Thereby, the response of pests to landscape composition may differ from those of pollinators. For example, loss of semi-natural habitats may not only reduce pollinator diversity but also negatively impair natural enemies of crop pests (e.g., parasitoids or predatory arthropods; Thies and Tscharntke 1999). Therefore, reduced parasitism in structurally simplified agricultural landscapes can result in higher pollen beetle abundances and crop damage (Thies and Tscharntke 1999). However, how landscape context moderates the effects of larvae and adults of pollen beetles on plant reproductive success is largely unresolved.

When investigating plant–animal interactions in a landscape context, many studies focus either on pollination or pest damage. Studies that experimentally address both mutualistic and antagonistic plant–animal interactions are still scarce (Herrera 2000, Herrera et al. 2002, Shackelford et al. 2013). This is despite changes in landscape composition might lead to unforeseen consequences for plant reproductive success including interactions between pollination and pest damage (Zhang et al. 2007, Shackelford et al. 2013). For instance, herbivory has been shown to modify plant–pollinator networks (Hoffmeister et al. 2016). Traditionally, many studies have focussed on herbivory of non-reproductive plant tissues (Strauss 1997, Lucas-Barbosa 2016). In contrast, the consumption of flowers prior to pollination has received less attention (McCall and Irwin 2006). Yet, floral herbivory (florivory) destroying floral organs in an early stage can offset later positive effects of mutualistic flower visitors and may thus strongly affect eventual plant reproductive success (Rodríguez-Rodríguez et al. 2015).

Here we investigated how two important plant–animal interactions—pollination and floral herbivory—are mediated by landscape composition and interact to shape plant reproductive success. We conducted our study using a landscape-scale design and experiments on a pollinator-dependent, nectar-offering plant, wild mustard (*Sinapis arvensis*, L.), whose flowers are frequently attacked by rape pollen beetles (*Meligethes* spp.). We separated mutualistic from antagonistic interactions using four experimental exclusion treatments: (1) pollinator exclusion, (2) rape pollen beetle exclusion, (3) exclusions of both pollinators and rape pollen beetles, and (4) no exclusion of any species. To assess the effects of landscape composition, these treatments were replicated in 10 landscapes of varying composition: five landscapes with high proportions of semi-natural habitats and five landscapes with low proportions. We addressed the following hypotheses: (1) Pollinator abundance and richness on potted *S. arvensis* plants (hereafter referred to as "phytometer plants") are higher in landscapes with high proportion of semi-natural habitats. (2) The abundance of rape pollen beetles on phytometer plants is higher in landscapes with low proportion of semi-natural habitats. (3) Competition for flowers with adult rape pollen beetles leads to reduced pollinator visitation. (4) Reproductive success of wild mustard increases with pollinator abundance and richness and negatively relates to abundances of adult rape pollen beetles and the damaging effects of their larvae on flower buds.

METHODS

Study region and study sites

The study was conducted in the agricultural surroundings of Göttingen, Lower Saxony, Germany (51°31′58″ N, 9°56′07″ E). We selected 10 study landscapes of different composition: five landscapes with high proportions of semi-natural habitats in a radius of 1000 m (7–15%; 8.2% $\pm$ 2.7%; mean $\pm$ standard deviation throughout) and five landscape with low proportions (0–2%; 0.4% $\pm$ 0.9%). Mapping was based on aerial images using Google Earth software and subsequent ground-truthing in the field. In the following, we refer to the composition of these landscape as "complex" and "simple," respectively. Semi-natural habitats were dominated by calcareous grassland, hedgerows, orchards, and fallows. The remaining habitats were mainly dominated by intensively

managed fields of wheat, maize, or oilseed rape (OSR). Oilseed rape in particular may influence flower visitors. Thus, we mapped the percentage of OSR cover in a 1000 m radius surrounding each experimental site. All study sites were selected in a way that minimized the proportion of rural settlements and forests in the 1000 m radius.

Study species

We used wild mustard (*Sinapis arvensis*, L.) as a phytometer plant species (Appendix S1: Fig. S1). This annual self-incompatible species of the Brassicaceae family is an archaeophyte common to temperate agricultural regions worldwide. Offering nectar in an open floral architecture, it is attractive to pollinators such as bees and hoverflies (Steffan-Dewenter and Tscharntke 1999). Similar to the closely related OSR (*Brassica napus*), *S. arvensis* is infested by rape pollen beetles (*Meligethes* ssp., Nitidulidae), with larvae developing within flower buds (Schlinkert et al. 2015a). Seeds develop within pods, with the number of seeds relying on pollination success (Steffan-Dewenter and Tscharntke 1999, Parsche et al. 2011). Podless stalks are a typical sign of damage by rape pollen beetles (Thies and Tscharntke 1999). Notably, as in other Brassicaceae, the number of seeds per pod

strongly depends on successful pollination; however, this is not generally the case for the number of produced pods per plant (Mesquida et al. 1988, Hudewenz et al. 2014).

Plants were sown on 28 April 2015 (seeds; Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany; soil, Fruhstorfer Einheitserde T25; HAWITA, Vechta, Germany) and grown in the greenhouse. From 12 May onward, all plants were transplanted into 3-L pots as soon as two true leaves had emerged and adapted to outside conditions. In order to prevent dehydration during hot weather conditions, we added a water storing granulate to the soil (BROADLEAF P4; BOSSE, Costa Mesa, California, USA). Potted plants were fertilized twice with a standard NPK fertilizer and watered as needed.

Experimental setup

We used an experimental setup based on exclusion treatments to study the individual and combined effects of pollination and floral herbivory on reproductive success of *S. arvensis* (Fig. 1). We separated the contributions from mutualistic and antagonistic plant–animal interactions by using four treatments: (1) pollinator exclusion, (2) rape pollen beetle exclusion, (3) full exclusion of both

Fig. 1. Experimental design. At each of the 10 study sites, 24–32 potted individuals (see *Methods*) of *Sinapis arvensis* were randomly placed on grassy field margins next to cereal fields and arranged in two shifted rows (a, b). For selective exclusion of pollinators, inflorescences were covered with gauze bags that were only permeable to rape pollen beetles (bag treatment; c). For exclusion of all visiting insects, inflorescences were covered with Osmolux bags (d). For excluding rape pollen beetles, inflorescences were sprayed using a standard, bee-neutral pesticide.

pollinators and rape pollen beetles, and (4) no exclusion of any flower visitors. With the exception of the full exclusion treatment, all treatments were applied to eight separate plant individuals on each study site, resulting in a total of 264 plant individuals used in the experiment.

To study the effects of floral herbivory by rape pollen beetles in the absence of pollination (pollinator exclusion), we used gauze bags (mesh width: 3 mm) that are permeable for rape pollen beetles but not for the pollinators of *S. arvensis* (H. Schlinkert, *unpublished data*, Fig. 1c). Owing to limited availability of these gauze bags, we used them only for two inflorescences per plant. Three additional inflorescences were covered with punched crisp bags that similarly excluded pollinators and allowed access of rape pollen beetles (no significant difference in exclusion effectiveness).

To measure the effects of pollinators in the absence of floral herbivores (rape pollen beetle exclusion), we used a neonicotinoid insecticide (Biscaya; Bayer CropScience, Monheim, Germany; 240 g/L Thiacloprid). The insecticide is classified under bee conservation category IV and is commonly used in the cultivation of vegetables and field crops such as OSR. We used a dose of 1 mL Biscaya mixed with 1 L water, as recommended by the manufacturer for the application in OSR. We sprayed inflorescences the first time prior to flowering and before placing plants in the field (4–5 June). Pesticide application was then repeated throughout the study period three more times on the initially sprayed inflorescences. By spraying the insecticide directly on the inflorescences, we restricted the effects of the experimental treatment to the flowers of plant individuals. Thus, the pesticide treatment only affected florivorous flower visitors, for example, pollen beetles, in contrast to affecting all herbivorous insects, as would be the case in a systemic application (e.g., using seed coating).

Previous studies have established that *Sinapis arvensis* is partially self-incompatible, and well suited for studying effects of changes in flower visitor communities with landscape composition on plant reproduction (Steffan-Dewenter and Tscharntke 1999, Gladbach et al. 2011, Parsche et al. 2011). To verify the plant species' dependency on pollination and to set up a baseline of plant reproductive success without any flower visitation by mutualistic or antagonistic visitors,

we also conducted a treatment involving the full exclusion of all flower visitors. To this end, we covered five inflorescences per plant individual with Osmolux bags (Pantek, Montesson, France, Fig. 1d). Osmolux bags are made of breathable plastic, which avoids rotting of inflorescences or fruits. Due to logistical constraints and the assumption that landscape context does not affect flower visitation of fully covered inflorescences (i.e., flower visitation was not possible), the full exclusion treatment was only implemented at three study sites.

Finally, to test the combined effects of pollination and floral herbivory on plant reproductive success (no exclusion), plants were studied without any treatment thus enabling access of both pollinators and rape pollen beetles to inflorescences.

Potted plants were arranged in two shifted rows with randomly arranged treatments and embedded into soil at ground level (Fig. 1; Appendix S1: Fig. S1). At full blossom of phytometer plants (26–28 June), we collected all inflorescences of half of all plant individuals (i.e., four individuals) for the treatments untreated, pollinator exclusion, and pesticide application in each study site. These inflorescences were used for later determination of flower bud damage by rape pollen beetle larvae (see section *Bud damage estimation*). All plant individuals from which inflorescences had been collected were directly removed from the study sites. The remaining plant individuals were used for pollinator surveys. In addition, following the end of the main flowering period and the finishing of pollinator surveys (1–9 July), they were transported back to the greenhouse area for pod ripening and later assessment of reproductive success.

Pollinator surveys

To estimate pollinator visitation rates, we recorded all flower visits by insects during 5-min observation per plant individual and observation round. Flower visitors were identified on the wing or assigned to morphospecies. Following each observation, we captured all visiting bee species with a sweep net during another period of 5 min. Pollinator richness was then defined as the combined information from pollinator observations (visiting morphospecies) and sweep netted bee species. Captured bees were killed with acetate, stored separately, and continuously numbered for later assignment to plant individuals. Plants with

bagged inflorescences were not included in pollinator observations and captures, as pollinators were excluded from their inflorescences. We conducted three observation rounds as soon as all plants across all sites had started flowering (first round, 17–21 June; second round, 21–25 June; third round, 26–29 June). Due to heavy rape pollen beetle infestation, not all plant individuals could be observed during the third observation round (Appendix S1: Fig. S2). Pollinator surveys were carried out on warm days with suitable weather conditions (no rain, no heavy wind) and between 09:00 hours and 17:00 hours, with different times of day across repeated observations. In addition to the likely mutualistic flower-visiting taxa, we also counted the number of adult rape pollen beetles per inflorescence on each plant individual. Furthermore, we quantified the flower abundance of each plant individual at the onset of each observation round. To this end, we recorded the number of inflorescences with more than one open flower and calculated the mean number of open flowers of 30 randomly chosen inflorescences. Flower abundance per individual was then estimated as the product of flowering inflorescences and the previously

calculated mean number of open flowers per inflorescence.

Bud damage estimation

To quantify floral herbivory by rape pollen beetle larvae, we dissected 20 randomly picked buds of each of the previously harvested plant individuals. Subsequently, we calculated the percent bud damage as the proportion of damaged buds (with or without larvae inside) per plant. Finally, we calculated the mean percent bud damage per treatment and site across the four plants used for dissection.

Reproductive success of phytometer plants

To quantify the effect of pollination and floral herbivory on plant reproductive success, ripe infructescences were harvested from the remaining four plants per treatment and site between 16 July and 9 August. From each plant, we randomly took five infructescences and counted the number of pods and podless stalks. Then, if present, we randomly took ten pods per infructescence and counted the number of seeds per pod. Hence, two different components of plant reproductive success were measured: (1) the mean number of pods across five infructescences per plant (hereafter called "fruit production") and (2) the mean number of seeds per pod across 50 pods per plant (hereafter called "seeds per fruit"). In addition, as a third component, we calculated (3) the total combined success as the product of fruit production and the number of seeds per fruit for each plant individual (hereafter called "total seed production").

Statistical analyses

To account for the repeated observations of plant individuals within the same study site, we used linear mixed-effects models (in the case of normally distributed response variables) and generalized linear mixed-effects models (in the case of Poisson-distributed response variables) to model our data. We included site, observation round, and plant individual identity as random factors. When necessary variables were $\log(x + 1)$ or sqrt(x)-transformed. All numerical explanatory variables were scaled to zero mean and unit variance to ease comparisons of effect sizes.

We first tested for the effects of landscape composition (complex vs. simple), exclusion treatment, and the interaction of the two factors on pollinator visitation rate and pollinator species richness of *S. arvensis*. These models also included the abundance of adult rape pollen beetles as predictor (to test for competition with pollinators), the surrounding OSR cover, and the flower abundance of *S. arvensis* individuals. Each response variable was analyzed in a separate model. The models only included data from *S. arvensis* plants of the treatments untreated and pesticide application. We did not include data on visitation rate or species richness of pollinators on *S. arvensis* plants that were assigned to the treatments pollinator exclusion and full exclusion, as for these plant individuals pollinator visitation had been experimentally prevented.

Variation in the abundance of adult rape pollen beetles was modeled using the same model structure as above (excluding rape pollen beetle abundance as explanatory variable); here data from individuals of the treatment pollinators excluded were also included in the analysis. In addition, we modeled the mean percentage of damaged flower buds (due to pollen beetle larvae having developed within) of each treatment

per study site as a function of landscape, treatment, and co-variates.

Finally, we quantified the combined and separate effects of mutualistic and antagonistic plant–animal interactions on plant reproductive success. We assumed pollinator visitation rates and richness to be zero for treatments with pollinator exclusion. In the same way, adult rape pollen beetle abundances and bud damage were regarded as zero for the total exclusion treatment; all assumptions were confirmed by our observations in the field. This enabled us to include reproductive success data from all treatments into analyses. We then set up three models, one for each of the three measures of reproductive success as response variable: fruit production, seeds per fruit, and total seed production. Explanatory variables were pollinator visitation rate, pollinator species richness, adult rape pollen beetle abundance, and bud damage by rape pollen beetle larvae, with mean values per plant individual across observation rounds for pollinator visitation rates, pollinator species richness, and rape pollen beetle abundance (because some plant individuals could only be observed twice; Appendix S1: Fig. S2).

All non-significant interaction terms ($P < 0.05$) were dropped from the final models.

Analyses were done within the R statistical environment version 3.2.3 (R Development Core Team 2015), with add-on packages lme4 ver. 1.1-11 (Bates et al. 2015) and multcomp ver. 1.4-4 (Hothorn et al. 2008).

Results

Altogether, 2623 pollinator visits were observed during the three observation rounds, which were mainly from syrphid flies (1288) and wild bees (868 visits; bumblebees, 692 visits; Appendix S1: Fig. S2). Among the sweep netted bees (133 individuals), 21 species were discriminated (Appendix S1: Table S1), with the genera *Bombus* (70) and *Lasioglossum* (47) being most abundant.

Landscape and treatment effects on pollinators and rape pollen beetles

Unexpectedly, landscape composition affected neither pollinator visitation rates nor pollinator richness (Fig. 2a, b, Table 1). Visitation rate but not pollinator richness was higher on pesticide-sprayed plants than on unsprayed plants (Fig. 2a, b,

Table 1). Abundance of rape pollen beetle was not related to pollinator visitation rate or richness (Table 1). Excluding pollinators from plants neither affected the abundance of adult pollen beetles nor the proportion of damaged buds by their larvae (Table 1). Pesticide application did not affect the abundance of adult beetles; however, it strongly reduced bud damage by beetle larvae (Fig. 2c, d, Table 1). The reduction in bud damage by pesticide application was stronger in simple as compared to structurally complex landscapes (significant interaction Table 1, Fig. 2d). Oil seed rape cover had only weak effects on pollinators and rape pollen beetles (Table 1). At the local scale, higher flower abundance of *Sinapis arvensis* plants attracted both higher numbers and richness of pollinators as well as adult rape pollen beetles (Table 1).

Mutualistic and antagonistic effects on reproductive success

All measures of plant reproductive success differed significantly among treatments (Fig. 3). Fruit production, that is, the mean number of developed pods across five infructescences per plant, was 11 ± 3 for untreated plant individuals (Fig. 3a). Taking into account podless stalks, $59\% \pm 14\%$ of all potential pods were thus realized. In comparison, excluding pollinators slightly reduced fruit production (10 ± 3; $42\% \pm 15\%$). Pesticide application increased fruit production (20 ± 5; $71\% \pm 11\%$), as did full exclosure of all invertebrates (32 ± 15; $95\% \pm 7\%$). Similarly, the number of seeds per fruit, that is, the mean number of produced seeds across 50 pods per plant, differed among treatments (Fig. 3b). Here exclosure of pollinators or all invertebrates resulted in the lowest seed production, whereas untreated and pesticide-sprayed plants produced considerably more seeds per pod. The overall reproductive success per plant individual, as measured by fruit production × seeds per fruit, was highest for plants for which pollinator access was possible and antagonists had been inhibited using pesticide application (Fig. 3c). Hence, on average pollinator access to plants enhanced the total seed production by 754% as compared to plants where pollinators were selectively excluded, a number that almost redoubled when in addition pesticide was applied (Fig. 3c).

Overall, pollinator access was critical for reproductive success of *S. arvensis* plants. Fruit
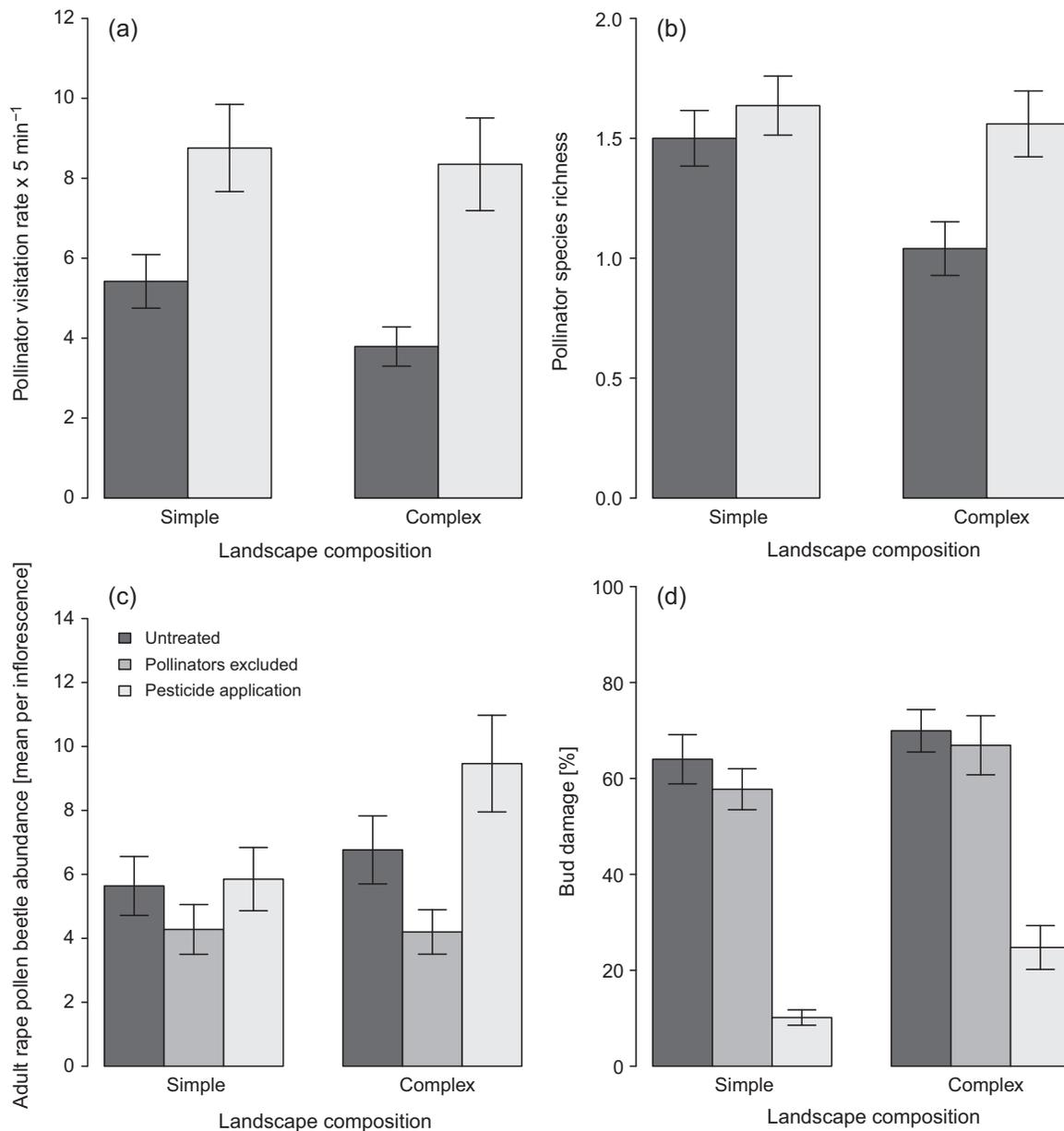
Fig. 2. Effects of landscape composition and treatment on (a) pollinator visitation rates, (b) pollinator species richness, (c) mean abundance of adult rape pollen beetles per inflorescence, and (d) the percentage of damaged flower buds on *Sinapis arvensis* phytometer plants. Shown are raw data. Bars indicate mean ± 1 SE.

production was marginally positively related to pollinator visitation rates (Fig. 4a). Similarly, there was a strong positive effect on the number of seeds per fruit, also increasing the total seed production (fruit production × seeds per fruit; Table 1, Fig. 4b, c). Reproductive success was not related to pollinator richness. Bud damage by larvae of pollen beetles reduced fruit production but not the number of seeds per fruit (Table 2). Interestingly, adult rape pollen beetles did not negatively affect plant reproductive success. Instead, high beetle abundance was not related to fruit production (Fig. 4d) and was even positively related to the number of seeds per fruit

Table 1. Summary statistics of linear mixed-effects models testing the effects of landscape composition, exclusion treatment, adult rape pollen beetle abundance, percent oilseed rape (OSR) cover, and flower abundance of observed *Sinapis arvensis* individuals on visitation rate and species richness of pollinators, as well as abundances of adult rape pollen beetles and flower bud damage due to pollen beetle larvae.

	Response			
Source of variation	Estimate	SE	Z	P
Pollinator visitation rate				
(Intercept = landscape simple and treatment untreated)	1.436	0.264	5.431	<0.001
Landscape complex	−0.141	0.352	−0.400	0.689
Treatment pesticide application	0.235	0.092	2.559	0.011
Adult rape pollen beetle abundance	−0.019	0.033	−0.561	0.575
OSR cover	−0.319	0.176	−1.815	0.070
Flower abundance *S. arvensis*	0.554	0.036	15.186	<0.001
Pollinator species richness				
(Intercept = landscape simple and treatment untreated)	0.784	0.129	6.098	<0.001
Landscape complex	−0.067	0.182	−0.369	0.712
Treatment pesticide application	0.001	0.048	0.027	0.979
Adult rape pollen beetle abundance	0.040	0.026	1.507	0.132
OSR cover	−0.045	0.091	−0.496	0.620
Flower abundance *S. arvensis*	0.195	0.029	6.667	<0.001
Adult rape pollen beetle abundance				
(Intercept = landscape simple and treatment untreated)	1.348	0.618	2.183	0.029
Landscape complex	0.299	0.378	0.792	0.429
Treatment pollinator exclusion	0.261	0.244	1.066	0.286
Treatment pesticide application	−0.228	0.095	−2.410	0.016
Landscape complex × treatment pollinator exclusion	–	–	–	–
Landscape complex × treatment pesticide application	–	–	–	–
OSR cover	−0.035	0.189	−0.188	0.851
Flower abundance *S. arvensis*	0.276	0.059	4.705	<0.001
Bud damage (mean % per treatment and site)				
(Intercept = landscape simple and treatment untreated)	61.381	8.230	7.459	<0.001
Landscape complex	10.302	12.335	0.835	0.404
Treatment pollinator exclusion	−2.190	8.411	−0.260	0.795
Treatment pesticide application	−52.246	3.222	−16.216	<0.001
Landscape complex × treatment pollinator exclusion	7.175	9.503	0.755	0.450
Landscape complex × treatment pesticide application	8.851	3.912	2.262	0.024
OSR cover	6.699	6.077	1.102	0.270
Flower abundance *S. arvensis*	−1.870	2.047	−0.914	0.361

Note: Treatments included in models with pollinators as response include untreated and pesticide application; for models with rape pollen beetles or bud damage as response, untreated, pollinators excluded, and pesticide application were included.

(Fig. 4e), resulting in an overall positive effect on the total number of seeds produced (Table 2, Fig. 4f).

DISCUSSION

Our study shows that plant reproductive success is driven by mutualistic and antagonistic plant–animal interactions. Even though enhanced pollinator visitation has the potential to increase plant reproductive success, it cannot compensate for negative effects of herbivory of flower buds preceding flower development. Furthermore, in generalist plants such as our phytometer species *Sinapis arvensis*, the surrounding landscape composition has only weak effects on the outcome of these interactions, presumably because of the generalist-dominated interacting community.

Pollinator and pollen beetle exclusion treatment

Pesticide spraying increased pollinator visitation rates on phytometer plants significantly. Recent work by Kessler et al. (2015) demonstrated that honeybees and bumblebees prefer
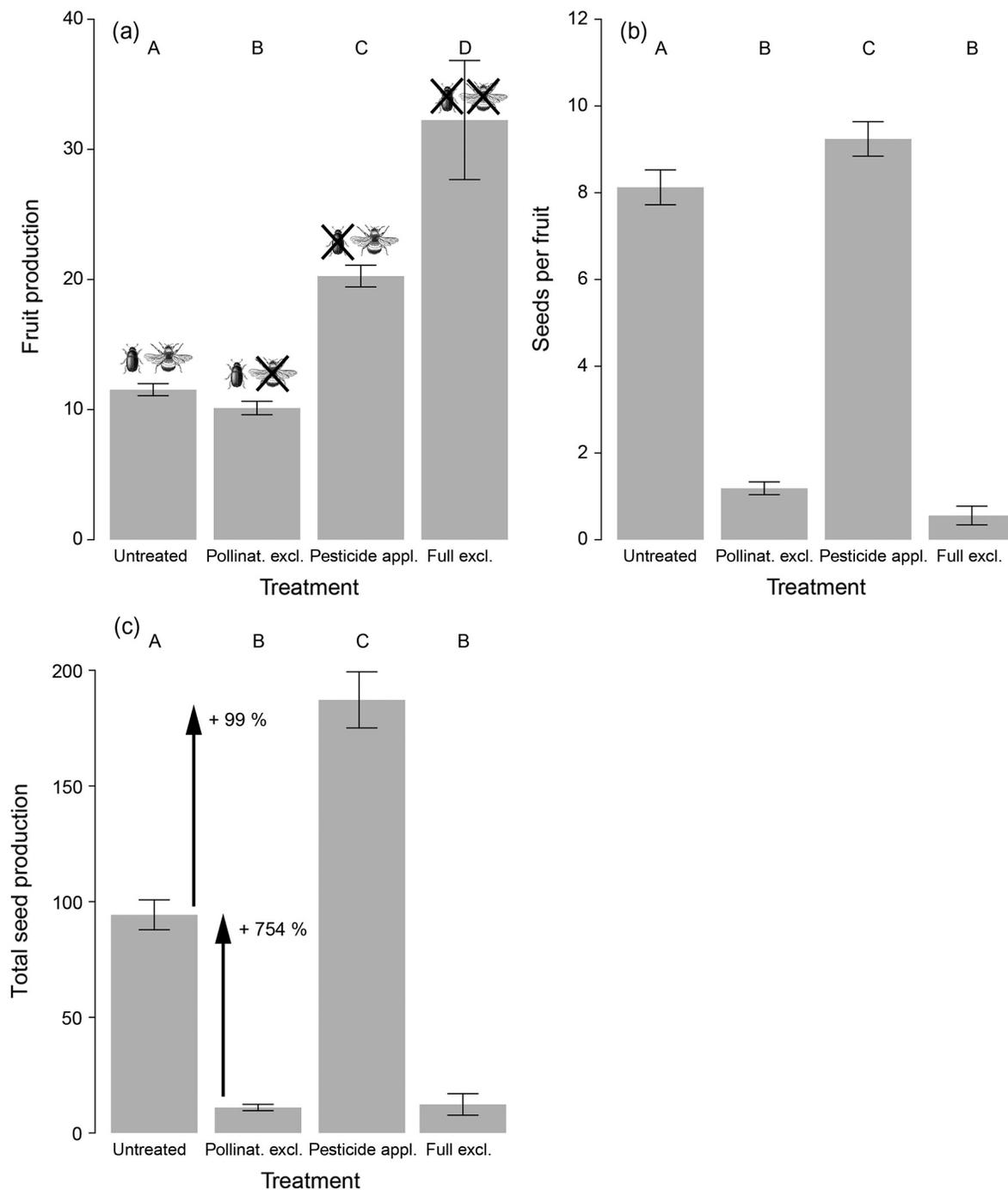
Fig. 3. Effects of treatment on (a) fruit production, (b) the number of seeds per fruit, and (c) the total seed production of *Sinapis arvensis.* Shown are raw data. Symbols indicate treatment effects on rape pollen beetles and pollinators. Allowing access of pollinators increases the total seed production (fruit production × seeds per fruit; Fig. 3c) by 754% (pollinators excluded–untreated); this number almost doubles when in addition rape pollen beetles are excluded (untreated–pesticide application). Letters indicate significant ($P < 0.05$) differences between treatments (Tukey contrasts from a linear mixed-effects model with study site as random factor).
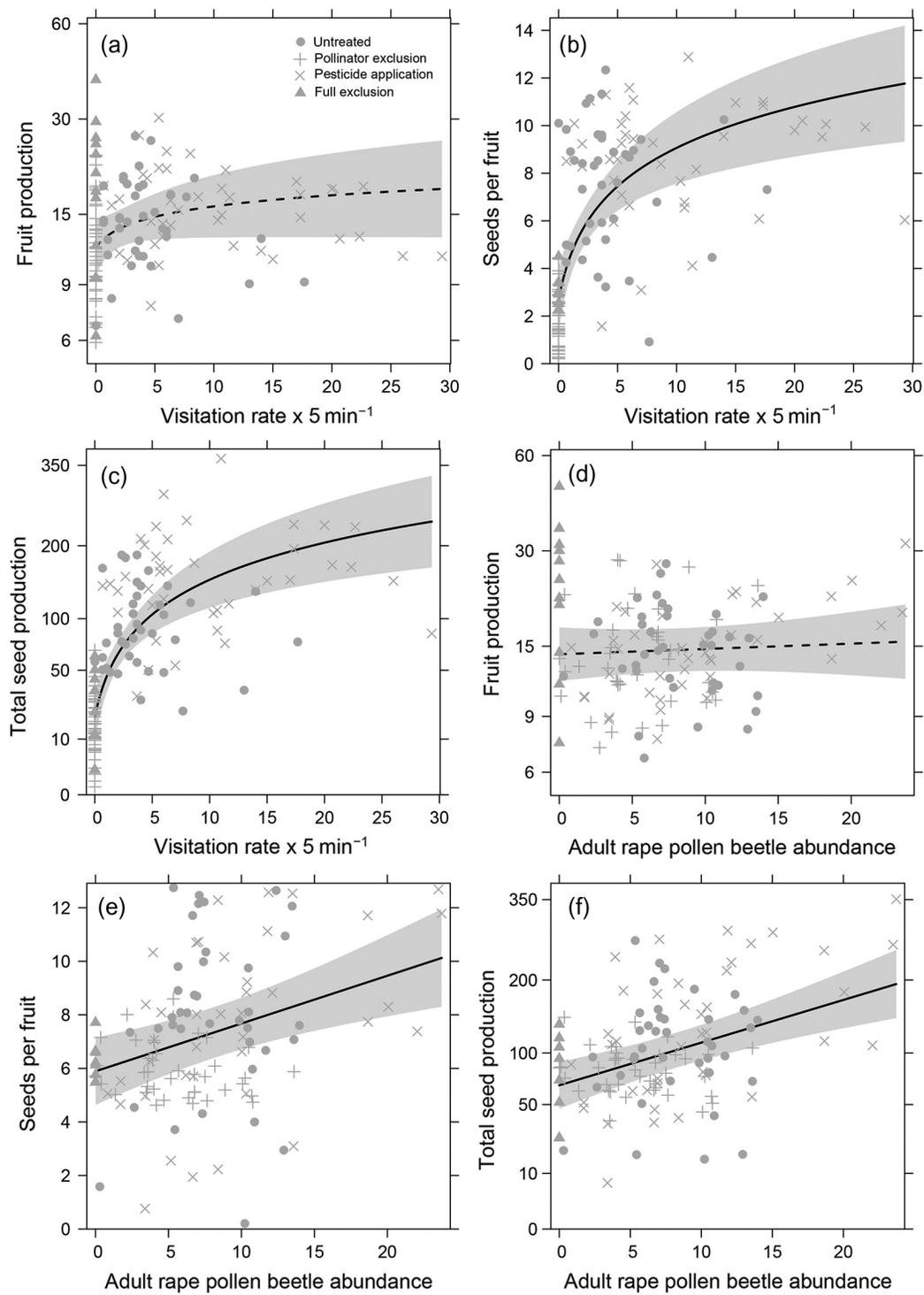
Fig. 4. Partial residual plots of the effects of pollinator visitation rate and the abundance of adult rape pollen beetles on the three measures of plant reproductive success: fruit production, seeds per fruit, and total seed production. Shown are fitted models (lines) and 95% confidence intervals (shaded areas). Solid lines indicate significant ($P < 0.05$) relationships. Symbols indicate the different exclusion treatments.

Table 2. Summary statistics of linear mixed-effects models examining the effects of pollinator visitation rate, pollinator species richness, adult rape pollen beetle abundance, and bud damage by pollen beetle larvae on three measures of reproductive success of *Sinapis arvensis*: fruit production (mean number of produced pods per plant individual), seeds per fruit (mean number of seeds per pod), and total seed production (fruit production × seeds per fruit).

Source of variation	Response			
	Estimate	SE	Z	P
Fruit production				
(Intercept)	2.606	0.069	37.92	<0.001
Pollinator visitation rate	0.133	0.076	1.748	0.081
Pollinator species richness	−0.046	0.074	−0.627	0.530
Adult rape pollen beetle abundance	0.019	0.035	0.541	0.588
Bud damage	−0.312	0.038	−8.312	<0.001
Seeds per fruit				
(Intercept)	5.650	0.388	14.551	<0.001
Pollinator visitation rate	2.846	0.545	5.224	<0.001
Pollinator species richness	0.512	0.526	0.974	0.330
Adult rape pollen beetle abundance	0.888	0.252	3.520	<0.001
Bud damage	0.375	0.266	1.414	0.157
Total seed production				
(Intercept)	8.039	0.399	20.155	<0.001
Pollinator visitation rate	3.479	0.584	5.957	<0.001
Pollinator species richness	0.260	0.564	0.461	0.645
Adult rape pollen beetle abundance	1.206	0.270	4.465	<0.001
Bud damage	−0.398	0.284	−1.400	0.162

sucrose solutions laced with neonicotinoids to solutions lacking the pesticides. In contrast, repellent effects of neonicotinoids have been shown for hoverflies (Easton and Goulson 2013). Higher pollinator visitation rates on pesticide-sprayed plants are therefore unlikely to have resulted solely from attraction of wild bees. Instead, they are more likely a result of reduced floral herbivory and hence greater attractiveness of plants to flower visitors: First, pesticide application reduced the density of adult rape pollen beetles on inflorescences. Second, the application strongly reduced bud damage by pollen beetle larvae (in simplified agricultural landscapes from 61% for untreated plants to 9% for sprayed plants, Table 1), allowing these buds to develop into flowers at a later stage. In addition, floral

herbivory can alter the emission of flower volatiles which act as cues for pollinators (Zangerl and Berenbaum 2009, Barber et al. 2012). Instead, flower-rich plants seemed to be very attractive to both pollinators and pollen beetles at the same time. This corresponds to recent studies emphasizing flower abundance and plant size driving the attractiveness of Brassicaceae to plant mutualists and antagonists (Schlinkert et al. 2015a, b).

Mutualistic and antagonistic effects on reproductive success

Pollinator visitation played a major role for plant reproductive success. Selective pollinator exclusion and full exclusion of all arthropods resulted in very low numbers of seeds per pod and a significantly reduced overall reproductive output as compared to untreated plants (Fig. 3c) and those with pesticide-sprayed inflorescences. In contrast, pollinator species richness played no role for plant reproductive success. Overall, observed pollinator richness was low; hence, functional complementary may have played only a minor role (Blüthgen and Klein 2011). Particularly in plants with a generalized pollination syndrome such as *S. arvensis*, flower visitation by dominant species can better predict pollination than pollinator richness (Vázquez et al. 2005, Winfree et al. 2015). As expected, our study emphasizes the detrimental roles of herbivory during early flower development. Fruit production, that is, the number of pods produced, was highest for plants where oviposition of beetle eggs into flower buds had been prevented. Interestingly, fruit production (i.e., the number of pods per plant) was even higher for plants of the full exclusion treatment that also excluded pollinators (using flower bags, Fig. 3a). To understand this apparent discrepancy, it needs to be noted that *S. arvensis* can compensate for low pollination rates (as simulated in this treatment) by enhancing pod production. However, as the number of seeds per pod strongly depends on pollination, these pods remain largely empty; thus, excluding both herbivores and pollinators still results in very low seed production at pod and plant level (Fig. 3b, c). Hence, pollination remains pivotal to plant reproductive success in Brassicaceae (Mesquida et al. 1988, Hudewenz et al. 2014). The non-additive effects of herbivory and pollination imply that overall seed production is highest when negative

effects of florivory on early flower production are prohibited, so that later positive effects of pollinators can result in high seed production per pod. Obviously, these effects can also be reversed when herbivory follows pollination (e.g., mammal browsing of fruits produced after successful pollination, Herrera 2000). Nevertheless, in both cases, only in the absence of herbivores can pollination eventually enable successful plant reproduction.

A surprising finding of our study was that high numbers of adult rape pollen beetles enhanced plant reproductive success, in particular the number of seeds per fruit. A subsequent investigation revealed that this effect was mainly apparent on pesticide-sprayed plants (Appendix S1: Fig. S3). The neonicotinoid does not affect the pollen beetles directly on contact, but requires the ingestion of the sprayed plant tissue to take effect (Thieme et al. 2010). Prior to ingesting, adult rape pollen beetles moved around unimpededly foraging on pollen or searching for oviposition places. Hence, rape pollen beetles initially thought to counteract pollination service may have behaved as legitimate pollinators when their antagonistic potential was inhibited. This is also suggested by other studies showing that rape pollen beetles can behave as pollinators, even in the absence of pesticide application (Crowson 1988, Gómez 2005).

Landscape composition effects on pollinators and rape pollen beetles

Contrary to expectations, neither pollinator visitation rates nor pollinator species richness were higher on phytometer plants situated in landscapes with high amount of semi-natural habitat as compared to structurally simple landscapes. In general, semi-natural habitats represent extensively used, resource rich habitats for pollinators (Öckinger and Smith 2006). Two studies also using *S. arvensis* as phytometer plants in the same study region as ours showed that bee visitation rates declined with increasing distance to semi-natural habitats (Steffan-Dewenter and Tscharntke 1999, Parsche et al. 2011). However, effects of habitat loss may not become visible until less than 5% of semi-natural habitat remains (Winfree et al. 2009). In addition, the majority of the observed pollinators on *S. arvensis* were hoverflies, of which many species respond positively to arable land (Haenke et al. 2009,

Jauker et al. 2009). Second-most abundant were bumblebees that can be highly mobile (foraging distances up to 3000 m; Walther-Hellwig and Frankl 2000, Westphal et al. 2006), whereas solitary bees (foraging up to a few hundred meters; Gathmann and Tscharntke 2002) were rarely observed (Appendix S1: Fig. S2). The prevalence of generalist, mobile species may thus explain the equally high levels of pollinator abundance, richness, and pollination services in both studied landscape types.

Adult rape pollen beetles were influenced neither by landscape composition nor by the land-cover of OSR. Rape pollen beetles are very mobile (Thies et al. 2003, Gladbach et al. 2011). Hence, their responses may be related to landscape characteristics beyond the 1000-m scale (Rusch et al. 2013). Other studies found decreasing abundance of pollen beetles with increasing area of semi-natural habitats, probably due to enhanced pest control by parasitoids (Thies and Tscharntke 1999, Thies et al. 2003). However, natural (e.g., woodland) and semi-natural habitats (e.g., grassland) do not only support parasitoids but can also increase pollen beetle densities by providing habitat for hibernation (Rusch et al. 2013). Landscape management for pest control hence needs to be regionally adapted as well as to the pest and crop species in question (Veres et al. 2013). Our findings support that herbivory and pollination have consistent effects on plant fitness even with variation in landscape context. Hence, the two may be more related to correlated evolution of mutualism- and antagonistic-related plant traits than to concurrent changes in landscape composition (Herrera et al. 2002).

Conclusions

By altering the attractiveness of plants and destroying floral structures, florivores have strong detrimental effects on plant reproductive output. We find that these become particularly apparent at early stages of plant reproduction when larvae develop within flower buds, which offsets the positive effects of later-visiting mutualistic flower visitors. While pesticide application may enhance seed production, pollination remains the most-limiting factor for the number of seeds produced by our phytometer plant,

Sinapis arvensis. There is growing interest in the interplay of mutualistic and antagonistic plant–animal interactions in a landscape perspective (Shackelford et al. 2013). We find that landscape composition plays only a minor role for the reproductive output of the generalist plant *S. arvensis*. This may reflect the high mobility and generalist habitat use of the major pollinator species (syrphids, bumblebees) and pests (pollen beetles) in our study. In general, both local and landscape-scale factors usually affect species interactions. Hence, agricultural management at local and landscape scales needs to be adapted to foster positive ecosystem services and at the same time preventing unforeseen consequences of disservices.

Acknowledgments

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