

Plant size affects mutualistic and antagonistic interactions and reproductive success across 21 Brassicaceae species

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Abstract. Plant size has been hypothesized to be a major driver of biotic interactions. However, it is little understood how plant size affects plant mutualists vs. antagonists and the plant's resulting reproductive success. We established a common garden experiment covering an interspecific plant size gradient (from 10 to 130 cm height) across 21 annual Brassicaceae species, thereby standardizing features of habitat and surrounding landscape. We assessed flower-visiting pollinators and florivores (pollen beetle adults and larvae) and the resulting effects of all these flower-visiting insects on plant reproductive success. Besides flower characteristics (size, abundance, color), plant size had a generally positive effect on abundance and species richness of pollinators as well as on abundance of pollen beetle adults and larvae. Pollen beetles reduced seed number as well as thousand-seed weight, whereas pollinators increased seed number only. Overall, increasing plant size led to less thousand-seed weight but had no effect on seed number, indicating counterbalancing effects of herbivory and pollination. In conclusion, seed number of large plant species should benefit from locations with many pollinators and few herbivores and small plant species' seed number from locations with few pollinators and many herbivores.

Key words: bee (Apoidea); Germany (city of Göttingen in Lower Saxony); herbivory; *Meligethes aeneus*; multitrophic interaction; pollen beetle; pollination.

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INTRODUCTION

Body size is a well-known and major predictor of patterns and processes in ecology, with predator and prey body masses determining food-web and population dynamics (Brose 2010). This is true not only for animals, but also for plants, where intraspecific as well as interspecific height differs greatly and can be a major predictor of richness of associated organisms and niches filled (Feeny 1976, Lawton 1983, Schlinkert et al. 2015a). Large plants are highly conspicuous and may be highly attractive for organisms as they offer larger microhabitat area as well as greater quantity and variety

of resources, enhancing number and size of populations of particular herbivorous species, thereby offering a greater range of biotic interaction partners (Feeny 1976, Lawton 1983). Positive impacts of variations in plant size on the abundance and diversity of associated insects are mainly known from intraspecific field studies (Donnelly et al. 1998, Haysom and Coulson 1998, Gómez 2003, but see Tscharntke and Greiler 1995), which often suffer from unstandardized features of local habitat and surrounding landscape. However, different (and even opposing) biotic interactions and resulting differences in plant reproductive success have not yet been studied comprehensively

under standardized conditions and across a broad range of closely related plant species.

Plant reproductive success is known to be strongly affected by flower-visiting insects. Insect pollination normally leads to an increase in number and quality of seeds and fruits (e.g., Bommarco et al. 2012) and is of great importance for the reproductive success of more than 87% of the species-level diversity of flowering plants (Ollerton et al. 2011), including 75% of the major crops (Klein et al. 2007, Garibaldi et al. 2013). A decline in insect-pollinated plant species often goes along with a decline in pollinator diversity (Biesmeijer et al. 2006, Gabriel and Tschamntke 2007). On the other hand, antagonists such as pollen beetles (e.g., *Meligethes aeneus* Fab.) may reduce the reproductive success of plants. Larvae of pollen beetles feed on pollen, while adults feed on different flower components; in both cases, feeding damage can result in stalks without fruit or weakened fruits and therefore in reduced seed numbers (Williams 2010).

If large plants attract more insects having an impact on seed number or quality than small plants, then differences in plant size may result in differences in the plant's reproductive success. Large plants may benefit from mutualistic interactions and suffer from antagonistic interactions—but what is actually more important for the plant's fitness and reproductive success? Are mutualists and antagonists equally attracted by large plants? Is it after all an advantage or a disadvantage for a plant to be large, or do beneficial and detrimental effects on plant reproductive success outweigh each other? In short, do relative plant size differences among co-occurring plant species modulate their relative reproductive performance in that they affect flower-visiting insects?

While many studies showed effects of flower parameters such as size, cover, amount, or color on flower-visiting insects (Giamoustaris and Mithen 1996, Hegland and Totland 2005, Scheid et al. 2011), studies testing the effects of plant size (i.e., the presentation height of flowers) on mutualistic and antagonistic flower-visiting insects are scarce. Thereby these studies focus either on pollinators (Klinkhamer et al. 1989, Donnelly et al. 1998, Gómez 2003) or on feeding damage by flower herbivores (Williams and Free 1979, Sletvold and Grindeland 2008, Schlinkert

et al. 2015b). Moreover, no study tested the relative importance of mutualistic vs. antagonistic flower-visiting insects in relation to plant size (i.e., height of plants) involving the final outcome in terms of the plant reproductive success (plant size and reproductive success only: O'Connell and Johnston 1998, Dickson and Petit 2006; plant size, pollinators, and reproductive success, but not florivores: Gómez 2003, Ehrlén et al. 2012; plant size, feeding damage to flowers, and reproductive success, but not flower herbivores and pollinators: Williams and Free 1979, Sletvold and Grindeland 2008, Schlinkert et al. 2015b, all studies but the last named focused on intraspecific plant size gradients). Besides, plant size moderated effects on mutualists and antagonists might further influence the ecological niches of co-occurring plant species, their interspecific competition, the plants' overall fitness, and consequently long-term evolutionary processes (Herrera and Pellmyr 2002).

The present study focuses on mutualistic and antagonistic flower-visiting insects and their effect on plant reproductive success along a plant size gradient, covering 21 plant species of the family Brassicaceae in a common garden experiment. The interspecific approach of the study allows for a broad plant size gradient without manipulation by, for example, fertilization or cutting of plants. As differences in plant size may go along with differences in other species-specific characteristics, we chose closely related species without significant correlation between size and phylogeny and disentangled effects of important characteristics from those of plant size per se by their use as covariables. We tested the following hypotheses:

Increasing plant size enhances the abundance of flower-visiting pollinators, pollen beetle adults, and pollen beetle larvae (1.1–1.3), while flower characteristics (number, size, and color) have an additional impact on these insects (1a–c). Pollinator abundance has a positive effect on seed number and thousand-seed weight per plant individual (2.1), while abundance of pollen beetle adults and pollen beetle larvae has a negative effect (2.2–2.3).

Finally, we answered the questions whether there is a trade-off between beneficial and detrimental effects of mutualists and antagonists along the plant size gradient, leading to similar seed numbers and thousand-seed weights across

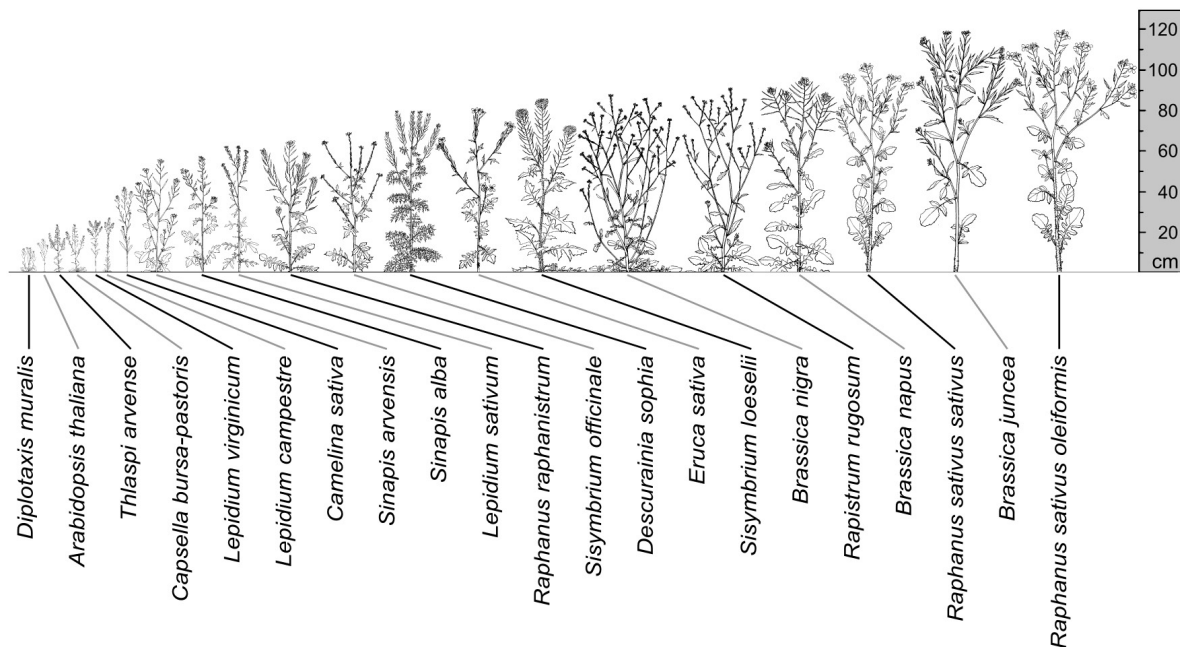


Fig. 1. Size (height) of the different plant species of this study is shown relative to one another. Drawings from Schlinkert (2014).

species—or whether plant size differences among co-occurring plant species modulate their relative reproductive success.

METHODS

Study site and sampling design

In 2010, we established a common garden experiment in the city of Göttingen (Lower Saxony, Germany; 51.5° N, 9.9° E). The study site was located in a grassland area dominated by grasses and herbs including numerous Brassicaceae species for a long time. For the experiment, we chose 25 widespread annual plant species of the Brassicaceae family covering a gradient in plant height. Four plant species could not be brought to full flowering between mid-June and mid-July 2010 and were excluded from the data set to avoid phenological differences in the local insect community of the study area. The remaining 21 plant species varied considerably in plant height: average height ranged from 12.7 cm \pm 2.4 cm (mean \pm SD throughout) (*Diplotaxis muralis* (L.) DC.) to 120.5 cm \pm 6.8 cm (*Raphanus sativus* L. *oleiformis*) (mean size of the different plant species shown relative to one another in Fig. 1). Plant

height of the different species, being highly correlated with the species' plant biomass ($P < 0.001$; Spearman's rho = 0.788), was not significantly related to the species' time of full flowering ($P = 0.365$; Spearman's rho = 0.208) or to phylogenetic relatedness of the species (Appendix S1). The 21 Brassicaceae species were similar in many characteristics such as the typical flower shape of the family, the presence of secondary plant substances (glucosinolates), and their pollination ecology in that insect pollinators can increase seed set of all species (Klotz et al. 2002). Plant species were either indigenous weeds (nine species), cultivated plants (eight species, also occurring in the wild), or neophytes (four species, established for long time in the wild).

We established a field of 100 square plots with a size of 1 m² each and a distance of 30 cm to each other (for a photograph of the experimental site, see Appendix S2). Four plots per plant species were arranged in monoculture in a completely randomized design. We irrigated and weeded regularly, once fertilized all plots equally (NPK fertilizer 15:6:12), and managed the plants to not exceed plot borders and to reach a plant cover of about 100% per plot until the time of full blossom

(for plant number per plot of the different species, see Appendix S3).

Survey of flower-visiting insects: pollinators, pollen beetle adults, and pollen beetle larvae

Within a period of four weeks between mid-June and mid-July, we sampled flower-visiting hymenopteran, dipteran, and lepidopteran insects (assumed to be pollinators) on every plot during its specific period of full blossom, conducting three runs with varying daytime (morning, midday, afternoon) and excluding cold or wet days. Each run consisted of a 5-min observation period for assessing the abundance of pollinators without disturbance and a subsequent net 5-min. catching period (handling time not included) to identify pollinators to species level. Pollinators were pooled for each plot.

Number of pollen beetle adults was recorded once on five randomly chosen and individually marked plant individuals on every plot at its time of full blossom. Abundance of pollen beetle adults per plot was extrapolated by multiplying the mean number per plant individual of each plot with its number of plant individuals.

To assess the abundance of pollen beetle larvae per plot, we harvested all inflorescences from one-quarter of each of two randomly selected plots per plant species. The harvest of inflorescences took place at the time of full blossom of each plot, after flower-visiting insects and flower cover were sampled and the number of plant individuals per plot was recorded. Thereby we left the five individually marked plant individuals of each plot so that they could develop pods. Inflorescences were stored at -20°C until buds >2 mm and flowers were dissected to collect the pollen beetle larvae. Abundance per plot was extrapolated by multiplying the mean number of pollen beetle larvae per plant individual of each plot with its number of plant individuals.

Survey of plant size, flower parameters, seed number, and thousand-seed weight

Plant size (i.e., plant height from the ground to the top of the plant), petal length, and flower quantity per plant individual were recorded at the time of full blossom at five randomly selected plant individuals and flowers of each plot. Mean

values of plant size and petal length were calculated for each plot. Flower quantity per plot was extrapolated by multiplying the mean number of flowers per plant individual of the relevant plot with its number of plant individuals. Flower cover was estimated per plot by eye (and the first author only). Flower color was species-dependent and either yellow or white.

We assessed seed number as quantitative measurement of the plant reproductive success. Additionally, we calculated the thousand-seed weight (average single seed's weight \times 1000) as an indication of seed quality: It is known for different species that large size and high weight of seeds can positively contribute to seedling establishment in offering greater food reserve; hence, seedlings from heavy seed can be superordinate competitors (Hanumaiah and Andrews 1973, Houssard and Escarré 1991, Ellis 1992, but see Hatzig et al. 2015). Therefore, we counted the pods of the five individually marked plant individuals at the plants' individual time of full ripeness. A subset of 20 randomly selected ripe and still closed pods per plant individual was opened, seeds per pod were counted, they were oven-dried for 48 h at 60°C , and their dry weight was recorded. Thousand-seed weight per plant individual was calculated by multiplying the average single-seed weight of the plant individual with 1000. Seed number per plant individual was extrapolated by multiplying the seed number per pod with the number of pods per plant individual. Mean values of seed number and thousand-seed weight per plant individual were calculated for each plot.

To compare seed number and thousand-seed weight between different plant species, we accounted for the species-specific potential using the realized percentage of their potential instead of absolute values (relationships between absolute values of plant characteristics and plant size are shown in Appendix S4). The potential of a plant species was defined as the mean of 10 maximum values based on 40 randomly selected individuals per species. Thereby we referred to the maximum values of the used breeding lines under natural conditions at our experimental site, originating from plant individuals with access by pollinating insects and comparably low levels of herbivory ($63.6\% \pm 26.0\%$ of the mean proportional feeding

damage per plant species). In the following, we refer to the percentage in which one plant individual realized the species' maximum potential as seed number (percentage) and thousand-seed weight (percentage).

Statistics: effect of plant size on pollinators, pollen beetle adults, and pollen beetle larvae

We first tested for effects of plant size on flower-visiting insects, using linear mixed-effects models with plant species included as random effect to avoid pseudoreplication and to account for species-specific features, for example, intra-specific size variation (four plots per plant species). Response variables were abundance of pollinators as well as their species richness and abundance of pollen beetle adults and their larvae per plot. As covariates we included flower size, quantity, and color with their two-way interactions but omitted flower cover as this results from and is positively correlated with flower number (results of a multiple regression using means per plant species: $P = 0.009$, centered and standardized estimate \pm SE = 0.870 ± 0.299) and flower size ($P = 0.014$, centered and standardized estimate \pm SE = 0.816 ± 0.299).

Statistics: effects of pollinators, pollen beetle adults, and pollen beetle larvae on seed number and thousand-seed weight

In a second step, we tested the effect of flower-visiting insects on seed number (percentage of the species' maximum) and thousand-seed weight (percentage), again using linear mixed-effects models. Abundance of pollinators (strongly correlated with their species richness, results of a simple correlation using means per plant species: Pearson's correlation coefficient = 0.919 , $P \leq 0.001$), abundance of pollen beetle adults, and abundance of their larvae including two-way interactions were used as explanatory variables.

Statistics: overall effect of plant size on seed number and thousand-seed weight

Finally, we analyzed the net effect of plant size on seed number (percentage of the species' maximum) and thousand-seed weight (percentage). Again, we used the same modeling approach as described above (i.e., linear mixed-effects models).

Statistics: general procedure for all models

Testing for correlations among all explanatory variables of each model, we found only the significant relationships ($P < 0.05$) between plant size and log-transformed petal length (Pearson's correlation coefficient = 0.51) and between log-transformed petal length and log-transformed flower quantity (Pearson's correlation coefficient = -0.66), as well as between the log-transformed abundance of pollinators and pollen beetle adults (Pearson's correlation coefficient = 0.59) and between the log-transformed abundance of pollen beetle adults and pollen beetle larvae (Pearson's correlation coefficient = 0.60). Multicollinearity was not an issue as variance inflation factors were < 3 for all models in this study (Zuur et al. 2010). Response and explanatory variables were either log- or arcsine-square-root-transformed whenever necessary to account for homoscedasticity and normal error distribution, which was confirmed by examining diagnostic plots.

We calculated corrected Akaike's information criterion for small sample sizes (AICc) for all subsets of the full model to identify the most parsimonious models which best explained the respective response variable. To account for nonindependency between some of the explanatory variables, we used parameter weights to identify variables and interactions between variables that consistently contributed to the information content of the models. Parameter weights were calculated by averaging models with the lowest AICc values in a Δ AICc range of 2, which represents similar goodness-of-fit (Burnham and Anderson 2002, Grueber et al. 2011). Explanatory variables and interactions with a parameter weight ≥ 0.6 were defined as important for the relevant response variable and are presented in the results. Estimates with SE were assessed from the summary table of the model with the lowest AICc value involving all explanatory variables with a parameter weight ≥ 0.6 (parameter weights, estimates, and SE of all tested variables of every calculated model are provided in Tables 1 and 2). We centered and standardized all continuous predictors prior to analyses to improve their interpretability (Schielzeth 2010).

All statistical analyses were conducted in the R environment (R Development Core Team 2015). Variance inflation factors were calculated using

Table 1. Effects of plant size and covariables on abundance per plot of flower-visiting insects.

| Flower-visiting insects | | pl. size | fl. size | fl. quant. | fl. col. | pl. size: fl. size | pl. size: fl. quant. | pl. size: fl. col. | fl. size: fl. quant. | fl. size: fl. col. | fl. quant.: fl. col. |
|-------------------------|------|--------------|--------------|--------------|--------------|--------------------|----------------------|--------------------|----------------------|--------------------|----------------------|
| abd. pol. | pw | 1.000 | 1.000 | 1.000 | 0.450 | 1.000 | 1.000 | 0.220 | 0.000 | 0.000 | 0.000 |
| | est. | 0.064 | 0.668 | 0.609 | – | –0.410 | –0.492 | – | – | – | – |
| | SE | 0.117 | 0.139 | 0.104 | – | 0.124 | 0.096 | – | – | – | – |
| abd. PB ad. | pw | 1.000 | 1.000 | 1.000 | 1.000 | 0.290 | 0.110 | 1.000 | 1.000 | 0.240 | 0.340 |
| | est. | 1.153 | 2.111 | 0.428 | 0.657 | – | – | 0.818 | –0.942 | – | – |
| | SE | 0.307 | 0.415 | 0.254 | 0.283 | – | – | 0.316 | 0.283 | – | – |
| abd. PB la. | pw | 0.810 | 1.000 | 0.000 | 1.000 | 0.450 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| | est. | 1.334 | 2.716 | – | 0.074 | – | – | – | – | 1.910 | – |
| | SE | 0.538 | 0.491 | – | 0.595 | – | – | – | – | 0.562 | – |

Notes: abd. pol. = abundance pollinators (log-transformed); abd. PB ad. = abundance pollen beetle adults (log-transformed); abd. PB la. = abundance pollen beetle larvae (log-transformed); pl. size = plant size (in cm); fl. size = flower size (petal length in mm, log-transformed); fl. quant. = flower quantity (log-transformed), fl. col. = flower color. Parameter weights (pw) refer to a delta 2 AICc range. Explanatory variables and interactions with a parameter weight ≥ 0.6 (bold) were defined as important for the relevant response variable and will be discussed. Estimates (est.) with SE were assessed from the summary table of the lme model with the lowest AICc involving all explanatory variables with a parameter weight ≥ 0.6 and are centered and standardized to improve their interpretability. For effects of plant size and covariables on species richness of pollinators, see Appendix S5.

the HH package (Heiberger 2015). For the calculation of mixed-effects models and their AICc values, we used the nlme package (Pinheiro et al. 2015) and the dredge function in the MuMIn package of R (Barton 2015). For the visualization (not for the calculation of mixed-effects models) of interactions of two continuous explanatory variables, we converted one of them into a categorical variable, using the medians of the upper and the lower half of the data. In case of opposing trends, we added a line predicted by the variable's overall median to show the medium trend.

RESULTS

Effect of plant size on pollinators, pollen beetle adults, and pollen beetle larvae

We recorded overall 3538 pollinators of 79 species, dominated by 2526 individuals of 26 bee species (arithmetic mean \pm SE: 25 ± 2 pollinator

individuals and 8 ± 0 pollinator species per plot), 7322 pollen beetle adults (613 ± 108 extrapolated individuals per plot) and 8182 pollen beetle larvae (1107 ± 323 extrapolated individuals per plot) (see Appendix S3 for all parameters per plant species).

Plant size had an overall positive effect on all flower-visiting insects (Fig. 2A–C, E; for parameter weights and estimates with SE of all tested variables of every model, see Table 1; for pollinator species richness, see Appendix S5; for model selection tables, see Appendix S6). Abundance of pollinators increased with increasing plant size, while this effect decreased and even reversed with increasing size and number of flowers (Fig. 2A, B). Likewise, species richness of pollinators increased with increasing plant size (for details and further effects, see Appendix S5; for model selection tables, see Appendix S6). The abundance of pollen beetle adults increased with

Table 2. Effects of abundance per plot of flower-visiting insects on plant reproductive success.

| Plant reproductive success | | abd. pol. | abd. PB ad. | abd. PB la. | abd. pol.: abd. PB ad. | abd. pol.: abd. PB la. | abd. PB ad.: abd. PB la. |
|----------------------------|------|--------------|---------------|--------------|------------------------|------------------------|--------------------------|
| Seed number (%) | pw | 0.660 | 0.780 | 0.670 | 0.100 | 0.150 | 0.670 |
| | est. | 0.017 | –0.033 | 0.011 | – | – | –0.030 |
| | SE | 0.010 | 0.013 | 0.011 | – | – | 0.013 |
| Thousand-seed weight (%) | pw | 0.460 | 0.680 | 0.370 | 0.090 | 0.240 | 0.240 |
| | est. | – | –4.834 | – | – | – | – |
| | SE | – | 2.917 | – | – | – | – |

Notes: Seed number (asin-sqrt-transformed) and thousand-seed weight in percentage of the species' maximum. For further information, see legend to Table 1.

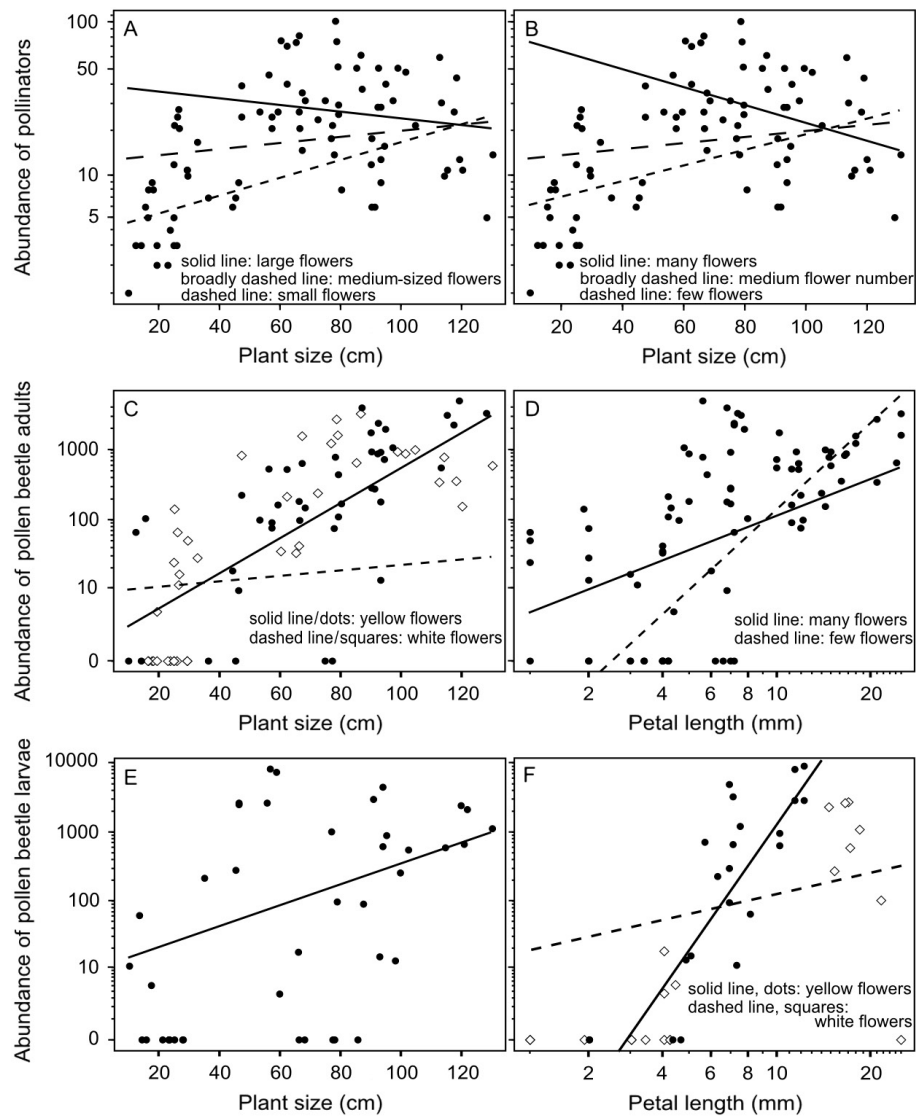


Fig. 2. Effects of plant size and important covariables on abundance per plot of (A, B) pollinators, (C, D) pollen beetle adults, and (E, F) pollen beetle larvae. Axes of variables were transformed corresponding to analyses (petal length, abundance of pollinators, abundance of pollen beetle adults, and abundance of their larvae: log transformation). Predictions derive from the model with the lowest AICc including all important explanatory variables (parameter weight ≥ 0.6). Parameter weights and estimates with SE of shown effects: (A) parameter weight = 1, estimate with SE = -0.410 ± 0.124 ; (B) parameter weight = 1, estimate with SE = -0.492 ± 0.096 ; (C) parameter weight = 1, estimate with SE = 0.818 ± 0.316 ; (D) parameter weight = 1, estimate with SE = -0.942 ± 0.283 ; (E) parameter weight = 0.810, estimate with SE = 1.334 ± 0.538 ; (F) parameter weight = 1, estimate with SE = 1.910 ± 0.562 . To visualize interactions of two continuous explanatory variables (A, B, D), we converted one of them into a categorical variable, using the medians of the upper and the lower half of the data. In case of opposing trends, we added a line predicted by the variable's overall median to show the medium trend (small flowers = 4 mm, medium flowers = 6.8 mm, large flowers = 11.7 mm; few flowers = 349.5, medium flower number = 734.8, many flowers = 4741.5).

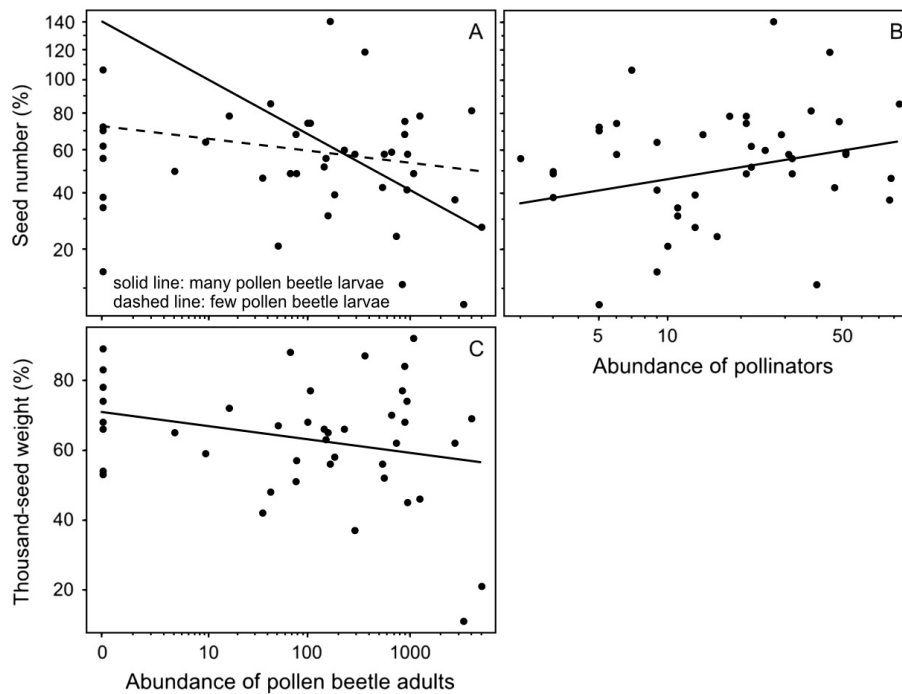


Fig. 3. Effects of abundance per plot of flower-visiting insects on (A, B) seed number (percentage of the species' maximum) and (C) thousand-seed weight (percentage). Axes of variables were transformed corresponding to analyses (seed number: asin-sqrt-transformation; abundance of pollen beetle adults and pollinators: log transformation). Predictions derive from the model with the lowest AICc including all important explanatory variables (parameter weight ≥ 0.6). Parameter weights and estimates with SE of shown effects: (A) parameter weight = 0.670, estimate with SE = -0.030 ± 0.013 ; (B) parameter weight = 0.660, estimate with SE = 0.017 ± 0.010 ; (C) parameter weight = 0.680, estimate with SE = -4.834 ± 2.917 . To visualize interactions of two continuous explanatory variables (A), we converted one of them into a categorical variable, using the medians of the upper and the lower half of the data (few pollen beetle larvae = 6; many pollen beetle larvae = 2207).

increasing plant size, while plots with yellow flowers showed the strongest increase (Fig. 2C). Further, pollen beetle abundance was positively related to petal length, especially on plots with few flowers (Fig. 2D). The abundance of pollen beetle larvae was positively related to plant size (Fig. 2E). Additionally, petal length had a positive influence on the abundance of pollen beetle larvae, mainly on plots with yellow flowers (Fig. 2F).

Effects of pollinators, pollen beetle adults, and pollen beetle larvae on seed number and thousand-seed weight

On average, the plant species realized $57.4\% \pm 2.8\%$ of their species' maximal seed number and a mean thousand-seed weight of $62.1\% \pm 1.8\%$ of the species' maximum (see Appendix S3 for all). Seed number (percentage

of the species' maximum) decreased with increasing abundance of pollen beetle adults, particularly on plots with high abundance of pollen beetle larvae, and increased with increasing pollinator abundance (Fig. 3A, B; for parameter weights and estimates with SE of all tested variables of every model, see Table 2; for model selection tables, see Appendix S6). Thousand-seed weight (percentage) was negatively related to adult pollen beetle abundance (Fig. 3C), while it was not affected by the abundance of pollinators or pollen beetle larvae.

Overall effect of plant size on seed number and thousand-seed weight

We did not detect an effect of plant size on seed number (percentage of the species' maximum, asin-sqrt-transformed: parameter weight = 0),

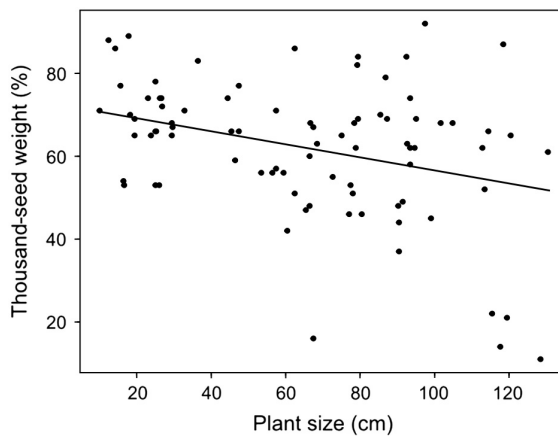


Fig. 4. Effect of plant size on thousand-seed weight (percentage of the species' maximum). Predictions derive from the model with the lowest AICc including all important explanatory variables (parameter weight ≥ 0.6). Parameter weights and estimates with SE of the shown effect: parameter weight = 1, estimate with SE = -5.291 ± 2.650 .

while thousand-seed weight (percentage) decreased with increasing plant size (parameter weight = 1, estimate = -5.291 , SE = 2.650; Fig. 4; for model selection tables, see Appendix S6).

DISCUSSION

The results of this study supported our hypothesis that plant size is a major driver of biotic interactions: Plant size had a positive effect on mutualistic pollinators as well as on antagonistic pollen beetles. In line with our hypothesis, mutualistic and antagonistic interactions had contrasting effects on the plant reproductive success in terms of seed number (percentage of the species' maximum), yet thousand-seed weight (percentage) was negatively influenced by antagonists only. Regarding seed quantity, increasing benefits from mutualistic interactions and losses from antagonistic interactions seemed to be counter-balanced with increasing plant size; thus, no overall effect on seed numbers could be found.

Effect of plant size on pollinators, pollen beetle adults, and pollen beetle larvae

In support of our hypothesis, plant size was positively correlated with abundance of flower-visiting insects. Mutualistic pollinators as well

as antagonistic pollen beetles were generally more abundant on large than on small plants in accordance with studies testing the effect of differences in intraspecific plant size on pollinators (Geber 1985, Donnelly et al. 1998, Gómez 2003) and on feeding damage to flowers (Williams and Free 1979, Sletvold and Grindeland 2008). Not only abundance of pollen beetle adults, but also abundance of their larvae increased with increasing plant size in our study, although parasitism rates of pollen beetle larvae were higher on large compared to small plants (Appendix S7). Large plants may be visually and chemically more apparent to insects than small plants, as they are more exposed and emit volatiles via a large surface area, which may be used by insects for host location (Bruce et al. 2005). This high conspicuousness of large plants may be followed by higher finding rates of associated organisms (Feeny 1976, Lawton 1983) and may be particularly true for flower associated insects if plant size refers to inflorescence height as it was the case regarding the studied plant species (Fig. 1; see also Schlinkert et al. 2015b, showing a correlation between plant size and feeding damage to flowers and fruits, but not to leaves and stems). Additionally, large plants may be highly attractive to associated insects by offering large microhabitat area and high quantity and variety of resources (Feeny 1976, Lawton 1983). Attractiveness of large plants may be particularly high for flower-visiting insects, in that flowers of large plants are often more exposed to the sun than those of small plants that are often shaded by the larger plants (Klinkhamer et al. 1989).

The covariates petal length and flower quantity were of high importance for flower-visiting insects; in particular, petal length positively influenced the abundance and species richness of pollinators, the abundance of pollen beetle adults, and the abundance of their larvae. Long petals may be seen as signal of reward as flower size is often positively related to nectar and pollen production (Cohen and Shmida 1993) and, independently of reward, enhances the detectability of the flower, leading to reduced searching time for flower-visiting insects particularly in areas with low flower densities (Cohen and Shmida 1993, Hegland and Totland 2005). Pollen beetle adults are shown to be selective not only for feeding but for oviposition as well and lay

most eggs in large (2–3 mm) buds (Ekblom and Borg 1996). Flower quantity had a positive effect on the abundance and species richness of pollinators and the abundance of pollen beetle adults, presumably because of the large number of food opportunities with low movement costs and reduced competition among flower-visiting insects (Klinkhamer et al. 1989, Ohashi and Yahara 2002, Scheid et al. 2011). Particularly, the interaction between petal length and flower quantity affected abundance of pollen beetle adults as well as species richness of pollinators, implying that high flower cover, combining the advantages of large and numerous flowers, was an attractive signal for flower-visiting insects. The abundance of pollen beetle larvae was not affected by flower quantity. Oviposition of pollen beetles appeared to be more related to the quality (size) of flowers and buds. Abundances of pollen beetle adults and their larvae were higher on plots with yellow flowers in comparison with plots with white flowers. A preference for yellow colors by pollen beetles has been already shown (e.g., Giamoustaris and Mithen 1996). Contrarily, pollinators showed no color preference, although, for example, *Pieris* and *Eristalis* adults, also present in our study in moderate numbers ($3.2\% \pm 0.7\%$ of total pollinator abundance, which was dominated by bees), have been shown to prefer yellow to white flower morphs of wild radish *Raphanus raphanistrum* L. (Kay 1976).

The interactions between plant size and the covariates petal length, flower quantity, and flower color affected the strength of the plant size effect on flower-visiting insects. The generally positive effect of plant size was strengthened, neutralized, or even weakened (Fig. 2A–C; Appendix S5: Fig. S1A, C) by highly attractive traits such as large petals or numerous flowers: The attractiveness of plots with large or many flowers on pollinators was high, diminishing the influence of plant size in comparison with plots with small or few flowers. This emphasizes the great importance of petal length and flower number for pollinators (Hegland and Totland 2005). The signal effect of yellow flowers on pollen beetles was strengthened by increasing plant size (Fig. 2C), probably due to free visibility of exposed flowers, while white flowers were less attractive even if plants were tall.

Effects of pollinators and pollen beetles on seed number and thousand-seed weight

In support of our hypothesis, seed number (percentage of the species' maximum) was positively affected by pollinator abundance and negatively affected by pollen beetle abundance. The positive effect of pollinator abundance on the seed number (percentage) corroborates previous studies on the dependence of seed set in Brassicaceae on insect-mediated pollination (Steffan-Dewenter and Tschamntke 1999, Morandin and Winston 2005, Parsche et al. 2011). Pollinator species richness, which was highly correlated with pollinator abundance, may have further had a positive influence on seed set by complementary pollination (Klein et al. 2003, Hoehn et al. 2008). The negative effect of pollen beetle adults on seed number (percentage), which was reinforced by abundance of pollen beetle larvae, probably resulted from their feeding on flower components. Florivory by pollen beetles often leads to podless stalks and to decreased seed number (Williams 2010, Appendix S8). Although mainly adult pollen beetles cause damage, their pollen-feeding larvae can also weaken pods and cause podless stalks (Williams 2010, see Appendix S8).

Thousand-seed weight (percentage of the species' maximum) was negatively affected by pollen beetle adult abundance, while pollinators had no effect. Plants with a high amount of damaged flowers, for example, by feeding of pollen beetles, may create compensatorily new flowers (Williams and Free 1979), possibly at the expense of the remaining flowers' thousand-seed weight.

Overall effect of plant size on seed number and thousand-seed weight

Strikingly, the negative impacts of the antagonists on seed number (percentage of the species' maximum) were neutralized by the benefits derived from increased mutualistic interactions with pollinators, leading to similar seed numbers across the plant size gradient in the 21 Brassicaceae species under the specific conditions of our study. Besides the ability of some brassicaceous plants to compensate for pollen beetle damage (e.g., Williams and Free 1979), this finding points toward a balance between mutualistic and antagonistic biotic interactions that allows for the coexistence of a wide range of

differently sized Brassicaceae species. However, such a balance or trade-off between mutualists and antagonists may change with environmental conditions. Likewise, Geber (1985) did also not find a difference in seed set between flowers of large and small *Mertensia* plants, although large plants attracted more bumble bees. Consistent with our suggestion of an equilibrium between mutualistic and antagonistic biotic interactions, Geber (1985) stated that observed flower-visiting bumble bees comprised not only mutualists, but also nectar robbers, which may have a negative effect on seed set (Malooof and Inouye 2000). Other studies showed positive (Dickson and Petit 2006), negative (Ehrlén et al. 2012), or study site-dependent effects (O'Connell and Johnston 1998, Gómez 2003) of intraspecific plant size or flower height on plant reproductive success. The different results of these studies indicate that species identity or study site-specific effects, for instance, differences in the relative abundance of antagonists and mutualists, significantly influence the overall effect of plant height on the resulting reproductive fitness. Using an interspecific approach and standardized local conditions, we found that both mutualists and antagonists are generally attracted by large plants. Consequently, we hypothesize that large plants have an advantage over small plants in case of few herbivores and many pollinators and that small plants have an advantage over large plants in case of many herbivores and few pollinators (Fig. 5).

Plant size and related species-specific traits

Plant size differences can be related to species-specific traits and responses to herbivory and/or pollination, thereby confounding effects of plant size per se. Such potentially confounding factors can be overcome using intraspecific plant size gradients. However, intraspecific plant size gradients are the result of different treatments (such as fertilization and cutting) and thus do vary, for instance, in levels of plant nutrition or visual attractiveness of the modified plants. In contrast, studies using interspecific plant size gradients have potentially to deal with species-specific variation in defense and compensation strategies, composition of plant volatiles, and differences in plant and flower morphology (Lawton 1983, Herrera and Pellmyr 2002). We minimized

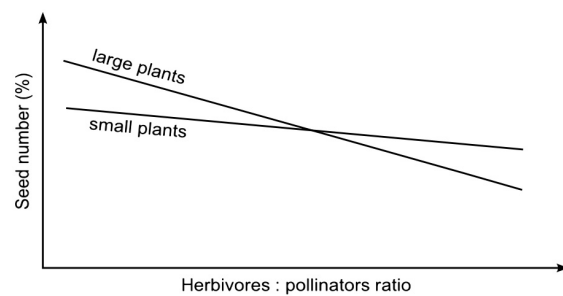


Fig. 5. The herbivore-to-pollinator ratio is hypothesized to shape the relative advantage of plant size. High pressure by herbivores should favor small plants, but high pollinator densities should favor large plants. The crossing point indicates the equilibrium of advantage (to the left of the crossing point) and disadvantage (to the right of the crossing point) for large plants we found at our study site regarding the seed number.

the influence of potentially confounding species-specific traits with our selection of related species with no correlation between plant size and phylogeny. We further disentangled plant size and flower morphology effects using flower traits as covariables in the analyses. Including the plant species as random effect in our models, we additionally accounted for random variation in species-specific traits that potentially confounded effects of plant size. Our results, based on a large interspecific plant size gradient and standardized local conditions, provide further evidence that mutualistic and antagonistic interactions and their effects on plant fitness are largely driven by plant size and thus might have implications for interspecific competition and long-term evolutionary processes (Herrera and Pellmyr 2002).

CONCLUSIONS

Plant size turned out to be a comprehensive driver of the interactions between plants and their flower visitors, while flower characteristics also played an important role. Regarding seed production, large plant species benefited from more mutualistic interactions by pollinators, but also suffered from more pollen beetles than small plant species. Hence, the counterbalancing effects of mutualistic and antagonistic flower-visiting

insects on seed production in our study led to outweighed advantages and disadvantages for large plant species compared to small ones. Whether being larger or smaller than adjacent plants is an advantage should depend on the ratio of pollinators to florivores in the habitat, given that the plant is sensitive to pollinators and florivores. This implies that landscape changes that affect the ratio of pollinators to herbivores (e.g., resulting from agricultural intensification) may affect reproduction of plants in dependence of their size. Moreover, these changes might also affect coevolutionary processes that shape the local populations of interacting plants, antagonists, and mutualists (Thompson 1998, Herrera and Pellmyr 2002). In general, our highly standardized experiment along a broad interspecific plant size gradient illustrates how variation in plant size drives the complex interaction networks between plants and their flower-visiting mutualists and antagonists.

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