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PLANT ANIMAL INTERACTIONS

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Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set

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Abstract Separate and combined effects of root and leaf herbivores on plant growth, flower visitation and seed set were tested in a factorial experiment using potted mustard, *Sinapis arvensis*, at an old fallow field. A 50% leaf removal by cabbageworms (*Pieris rapae*) when the seedlings had their first four leaves reduced plant height and shoot mass, and delayed the onset of flowering. Root herbivory by two wireworms (*Agriotes* sp.) over the whole experiment changed flower visitation; the number of flower visitors per plant was higher in plants with root herbivores than in plants without root herbivores. Combined leaf and root herbivory affected flowering period, number of fruits per plant and number of seeds per fruit. Plants attacked by leaf and root herbivores had a shorter flowering period and produced fewer fruits per plant than plants with root herbivores only. Although the experimental plants faced major herbivore-induced growth changes, plant reproduction (seed set and weight per plant) was similar in all treatments, documenting their ability to effectively compensate for leaf and root herbivory.

Keywords Cabbageworms · Compensatory plant growth · Herbivory · *Sinapis arvensis* · Wireworms

Introduction

Traditionally, below-ground and above-ground communities have been investigated separately even though both systems show high interdependence (Wardle 1999; Masters et al. 2001; Van der Putten et al. 2001; Brown and Gange 2002; Scheu and Setälä 2002). The interactions between above- and below-ground organisms have only recently become a major field of study. Many of these studies focus on the effects of decomposers on plant growth (Scheu and Parkinson 1994; Bonkowski et al. 2000), on above-ground plant–herbivore interactions (Scheu et al. 1999; Bonkowski et al. 2001) and on the effects of below-ground herbivores on plants, above-ground herbivores and their natural enemies (Brown et al. 1987; Brown and Gange 1989; Gange and Brown 1989; Moran and Whitham 1990; Masters and Brown 1992; Masters et al. 1993, 2001; Nötzold et al. 1998). Although the effects of below-ground organisms on plant growth have been investigated, their effect on plant reproduction is hardly studied (but see Brown et al 1987; Maron 1998; Nötzold et al 1998; Masters et al. 2001). Further, the relative importance and the combined effects of below-ground and above-ground herbivores on plant performance, fitness and on flower visitation are not known.

Plant fitness is affected by foliar herbivory through changes in plant traits or by altered pollinator visitation patterns of damaged plants (Lehtila and Strauss 1997, 1999; Strauss 1997; Mothershead and Marquis 2000). Seed production may be substantially reduced by herbivory, even when herbivores do not feed directly on reproductive tissue. This may be due to reduced resources available for flower, pollen or seed production or because pollination rates are lowered in damaged plants. Changes in floral characters may reduce attractiveness to pollinators. Foliar herbivory may change the amount or chemistry of nectar (Hambäck 2001), the number or morphology of flowers (Karban and Strauss 1993; Strauss et al 1996; Lehtila and Strauss 1997, 1999), the flowering phenology (Hambäck 2001) or the height of plants (Strauss 1997; Mothershed and Marquis 2000; Hambäck 2001). Root herbivores may affect the flowering period, the number of flowers and the size of the flowerheads (Brown et al 1987; Nötzold et al 1998; Masters et al 2001). Accordingly, leaf and root herbivores (separately

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and in combination) may be expected to reduce the number and quality of flowers and fruits, and thereby, overall plant fitness. The objective of this study was to analyze the effect of leaf herbivores (caterpillars, *Pieris rapae*) and root herbivores (wireworms, *Agriotes* sp.) on plant growth, flower visitation and seed set, using an annual, cross-pollinated plant (*Sinapis arvensis*).

Materials and methods

Experimental set-up

The experiment was carried out in a greenhouse and an adjacent fallow field in Göttingen (Lower Saxony, Germany) in 2001. The experiment was set up in a two-factorial design. The effects of root herbivores (R) and leaf herbivores (L) on plant growth and reproductive parameters of mustard (*S. arvensis*) were investigated. The plants were subjected to four treatments (Control, R, L, RL). Six replicates of each treatment were established. We used 10-l pots that were filled with soil taken from a 10-year-old fallow field located on a limestone plateau east of Göttingen. The soil was defaunated by heating to 75–80°C for 2 h. Two wireworms (larvae of the click beetle *Agriotes* sp.) were added to each pot as the root herbivore treatment, which is equivalent to a density of ca. 30 individuals/m^2. Wireworms are patchily distributed and may reach densities of up to several hundred per square meter. The density chosen corresponds to that of patches frequently found in the field (K. Poveda, personal observation). Third-instar larvae of *P. rapae* (cabbageworms) were reared from eggs of butterflies collected around Göttingen and used as leaf herbivores.

Seeds of mustard from a regional wild population (provided by the Botanical Garden, Göttingen) were sown on 9 July 2001 in the greenhouse. Young seedlings (with two true leaves) were transplanted into small pots. On 21 July 2001 they were transplanted into the experimental pots, to which the wireworms had been added. Six days after transplantation, two cabbageworms were put on the seedlings, which had their first four true leaves. We let the larvae consume 30–50% of the leaves, which took approximately 2 days. This level of leaf damage is well within the range recorded in the field (Strauss et al. 1996). Any other leaves subsequently produced were left undamaged. On July 30 all the pots were set in random order in the field.

Sampling and statistical analyses

Data on plant height, shoot mass, beginning and end of the flowering period were collected for each plant. When fruits turned yellow and the plants began to die (mid October 2001) fruits and shoots were collected and oven dried for 3 days at 60°C. The number of fruits (pods) of each plant was counted. Twenty fruits were randomly selected, and seeds of these fruits counted and weighed. These data were used to calculate the number of seeds and seed weight per plant. Total number of flowers produced by each plant was estimated by counting the pedicels left on the inflorescences.

Insects visiting the flowers were observed during the flowering period, 1 August to 20 September 2001. Each plant was observed for a total of 45 min, divided into three observation periods of 15 min. These observation periods were distributed randomly on 3 different days and at different hours each day, between 10 a.m. and 4 p.m. For each plant the number of open flowers was counted. The number of flower visitors was calculated per open flower and per 15-min interval.

Data were analyzed by two-way analysis of variance (ANOVA). Differences between means were inspected using Tukey's honesty significant difference test. We correlated the number of fruits per plant with the number of seeds per fruit. Statistical analyses were performed using Statistica 5 (StatSoft 1995).

Results

Leaf herbivory changed plant height, beginning and length of the flowering period (Table 1). Shoot mass was reduced by leaf herbivores with marginal significance (6.5 g±0.58 and 8.14 g±0.63, mean±SE for plants with and without leaf herbivory, respectively; Table 1). Plants attacked by leaf herbivores were smaller (Fig. 1a) and began to flower later (Fig. 1b) than plants without leaf herbivores. The flowering period depended on the combination of root and leaf herbivory treatments (Table 1). Plants attacked by both herbivores had a shorter flowering period than plants attacked by root herbivores only (Fig. 1c). Although the total number of flowers produced per plant was similar in all treatments, there was an interaction between the effects of root and leaf herbivores on the number of fruits per plant (Table 1). Plants with only root herbivores produced more fruits than plants with both herbivores (Fig. 2a). Plants with many fruits had fewer seeds per fruit than plants with few fruits (r=–0.53, n=24, P=0.007). Accordingly, the number of seeds per fruit depended on both root and leaf herbivory (Table 1). Plants with both herbivores had a similar number of seeds per fruit as control plants. In contrast, plants with either root or leaf herbivores had fewer seeds per fruit than plants with both herbivores, and plants with root herbivores had fewer seeds per fruit than control plants (Fig. 2b).

There was no significant effect of root herbivores, leaf herbivores or both on the number of flowers (range of arithmetic means: 231–275, n=6) or on the total number (1,395–1,579) and mass of seeds per plant (2.01–2.43 g; Table 1), i.e., overall plant reproduction was not affected despite the changes in growth parameters.

Table 1 ANOVA table of F -values on the effect of root herbivores (R) and leaf herbivores (L) on plant height, shoot biomass, onset of flowering, period of flowering, total number of flowers, number of fruits per plant, number of seeds per fruit, number of seeds per plant, seed weight per plant and number of flower visitors per flower

Treatment	Plant height	Shoot mass	Onset of flowering	Period of flowering	Total flowers	Fruits per plant	Seeds per fruit	Seeds per plant	Seed weight	Visitors per flower
L	4.6*	3.3$^{(*)}$	27.4***	8.9**	<0.1	0.6	1.9	0.6	1.7	1
R	0.5	<0.1	2.8	0.6	<0.1	<0.1	0.2	<0.1	0.1	4.5*
R×L	4.2$^{(*)}$	0.7	0.4	7.0*	1.6	5.1*	21.1***	<0.1	<0.1	<0.1

$^{(*)}$ P <0.1; * P <0.05; ** P <0.01, *** P <0.001

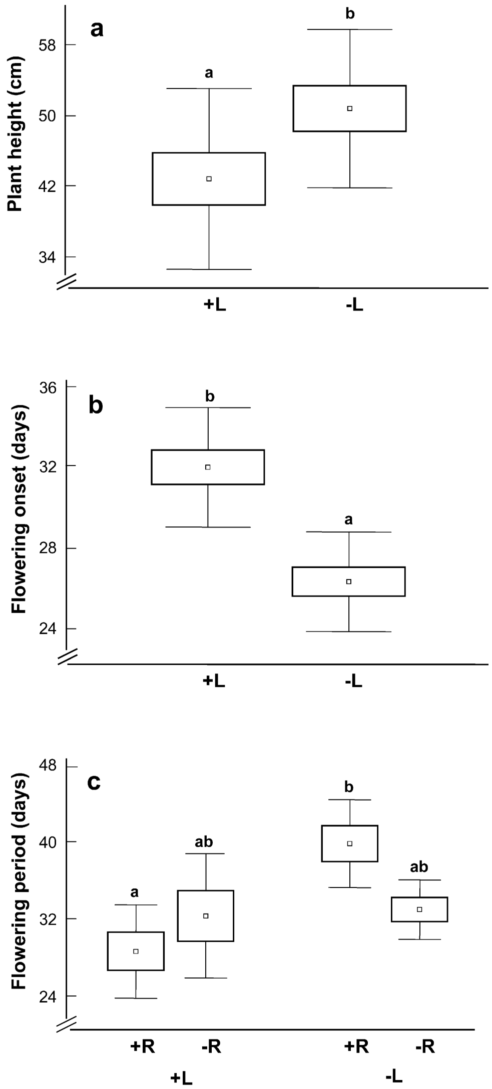

Fig. 1 Effect of root herbivores (*R*) and leaf herbivores (*L*) on plant height (**a**), flowering onset (days to the beginning of the flowering period) (**b**) and the length of the flowering period (**c**) of *Sinapis arvensis* (mean ± 1SE and 1SD). Treatments with different letters are significantly different (Tukey's test, $P<0.05$)

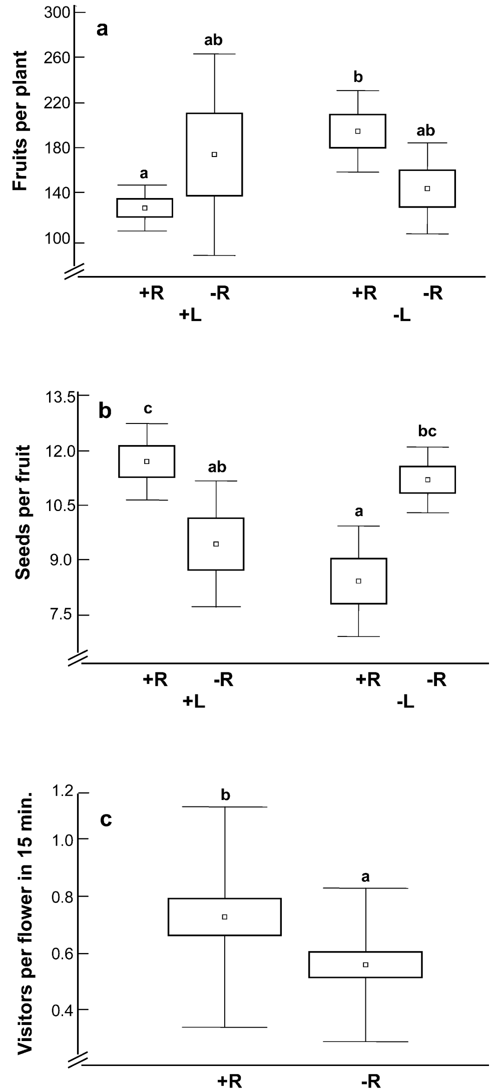

Fig. 2 Effect of root herbivores (*R*) and leaf herbivores (*L*) on the number of fruits per plant (**a**), the number of seeds per fruit (**b**) and the number of visitors per flower (**c**) of *Sinapis arvensis* (mean ± 1SE and 1SD). Treatments with different letters are significantly different (Tukey's test, $P<0.05$)

Altogether 859 flower visitors were observed. The most abundant visitor was the honeybee (*Apis mellifera*) with 486 visits, followed by hover flies (Syrphidae) with 265 visits (mainly of the species *Eristalis tenax*). The remaining visitors were predominantly bumblebees (*Bombus* spp.) and flies (Diptera). The number of visitors per flower was affected by root herbivores (Table 1) and was higher in plants with root herbivores than in plants without root herbivores (Fig. 2c).

Discussion

Although interest in interactions between below- and above-ground organisms has increased in recent years, no studies have considered the combined effects of root herbivores and leaf herbivores on flowering, flower visitation and seed set of plants (but see Masters et al. 2001). The results of this paper show that mustard plants responded strongly to root and leaf herbivores, but in the end, plant reproduction was not significantly affected by any of the treatments highlighting the flexibility and compensatory power of *S. arvensis* in response to herbivore damage.

Plant performance

Plants that were attacked by leaf herbivores at an early stage were smaller and their flowering period was delayed and shorter. The reduction in size and shoot mass found in our experiment may have been caused by a reduced photosynthetic area (Crawley 1983; Kinsmann and Platt 1984; Marquis 1984; Strauss 1991), since a 50% removal of the leaf area at an early stage has been documented to translate into a reduction in total leaf area by 25% over a plant's lifetime (Strauss et al. 1996). Flowering phenology depends on the resources plants allocate for reproduction, as documented for *Raphanus raphanistrum* (Strauss et al 1996). The delayed flowering onset in *S. arvensis* in response to leaf herbivory in part was caused by abortion of the first flowers (K. Poveda, personal observation) which presumably resulted from a reduction in resources allocated to inflorescences. Flower abortion is known to depend strongly on the resources available (Stephenson 1981; Stirling et al. 2002). In plants attacked by leaf herbivores the photosynthetic tissue was reduced strongly. Reduced allocation of resources to inflorescences in plants attacked by leaf herbivores was presumably also responsible for the reduced production of pollen and nectar; however, in the end, leaf herbivores did not affect the number and mass of seeds, i.e., plant reproduction.

Plants attacked by root herbivores had a longer flowering period and a higher number of fruits than plants attacked by both herbivores. However, at the density used in this experiment root herbivores did not significantly affect plant growth and plant reproduction. In contrast, root herbivores in most studies detrimentally affected plant growth (Powell and Myers 1988; Gange and Brown 1989; Nötzold et al. 1998) and plant reproductive parameters (Powell and Myers 1988; Brown and Gange 1990; Maron 1998; Masters et al 2001). However, parts of the root system when removed by feeding may be replaced rapidly (Simberloff et al. 1978; Andersen 1987; Riedell 1989). Also, plants may respond by increased lateral root proliferation to compensate for damage (Brown and Gange 1990). This may benefit the plant, since lateral roots enhance the uptake of nutrients and water. Ridsdill Smith (1977) reported foliage yield of ryegrass to be unaffected by feeding of scarabaeid larvae. Riedell (1989) even found the yield in corn to be increased at low corn rootworm (*Diabrotica* spp.) density. Since the number of wireworms used in our experiment was low (two per pot), mustard compensated root damage.

In plants with both root and leaf herbivores the flowering period was shortened and fewer fruits per plant were produced compared to plants with root herbivores only. Obviously, these plants suffered most because of the loss of photosynthetic tissue and roots, both important for nutrient acquisition. Plants are known to respond to root herbivory by mobilizing carbohydrate reserves and directing them to the attacked area which is termed re-growth potential (Davidson et al. 1970). Usually plant re-growth potential increases with plant size, so damage by root feeders should be less important in large plants (Davidson et al. 1970). In our experiment plants damaged by leaf herbivores were smaller, which likely reduced the re-growth potential of plants additionally attacked by root herbivores.

Plant reproduction

Plants strongly responded to the treatments, but in contrast to our expectations, the differences in plant height, flowering onset, flowering period, and number of fruits did not result in significant differences in the number of seeds produced. Interestingly, all plants had the same number of total flowers, but the number of fruits differed between treatments and plants with many fruits had fewer seeds per fruit than plants with many fruits. Obviously, the mustard plants compensated for reduced fruit numbers with an increase in seed number per fruit. Different compensatory mechanisms contributed to the ultimately similar plant fitness. Plants often produce more ovules within flowers than they turn into seeds (Stephenson 1981). This allows plant reproduction to adjust to changes in resources available. In our experiment plants attacked by both herbivores produced far fewer fruits than plants attacked by root herbivores only. Presumably, this was due to early flower and fruit abortion. It is well documented that defoliation results in a reduced number of fruits due to increased fruit abortion; however this may not be associated with a decrease in the number of seeds (reviewed by McNaughton 1982). In our study, mustard plants attacked by leaf herbivores aborted flowers at an early stage, leaving only the pedicels on the inflorescence (K. Poveda, personal observation). Since the number of pedicels was taken to estimate the number of flowers, this may have resulted in an overestimation of the number of flowers produced by a plant.

Herbivory significantly affected the number of visiting pollinators per flower but flower visitation did not affect reproduction; all plants produced a similar number of seeds. Plants attacked by root herbivores had more flower visitors than plants without root herbivores. Attack by leaf herbivores is known to affect flower morphology and, thereby flower visitation (Conner 1996; Lehtila and Strauss 1997; 1999; Mothershed and Marquis 2000). Surprisingly, attack by root herbivores resulted in an increased number of flower visitors possibly due to enhanced nectar production.

In conclusion, above- and below-ground herbivores affected plant height, shoot mass, flowering phenology, fruit set and even the number of flower visitors. Ultimately, however, none of these changes resulted in changes in the number and mass of seeds produced, i.e., in plant reproduction. This documents effective compensatory responses of *S. arvensis* to both leaf and root herbivory.

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