

# Herbivory Increases Fruit Set in *Silene latifolia*: A Consequence of Induced Pollinator-Attracting Floral Volatiles?

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**Abstract** Although the effect of herbivory on plant reproduction has been investigated in some detail, little is known about how herbivores affect floral signalling. Here, we investigated the effect of foliar herbivory by the African Cotton Leafworm (*Spodoptera littoralis*) on floral signalling and fruit set in the White Champion (*Silene latifolia*). We found no effects of herbivory on floral traits involved in visual signalling (flower number, corolla diameter, calyx length, petal length) or in amount of nectar produced. However, *Spodoptera*-infested plants emitted higher amounts of the two floral volatiles, (Z)-3-hexenyl acetate and  $\beta$ -ocimene, than control plants. Open pollinated, infested plants also were found to produce more fruits than control plants, but only with nocturnal pollinators. Experimental addition of the two induced floral volatiles to non-infested *Silene* flowers also led to the production of more fruits with nocturnal pollination. This suggests that higher fruit production in herbivore-infested plants was

caused by increased nocturnal pollinator attraction, mediated by the induced floral emission of these two volatiles. Our results show that the effects of herbivory on plant reproductive success are not necessarily detrimental, as plants can compensate herbivory with increased investment in pollinator attraction.

**Keywords** Pollination · Herbivory · Flower scent · Volatiles

## Introduction

Reproduction often imposes the main resource demand on a plant (Reekie and Bazzaz 2005). According to Lloyd's (1980) serial adjustment hypothesis, the relative resource allocation is continuously modulated and interconnected as the allocation of resources to one function comes at the expense of another. For instance, an elevated investment in the production of fruits due to high pollination success may subsequently affect reproductive traits such as flower shape (Brys et al. 2011) or longevity (Abdala-Roberts et al. 2007). Similarly, fruit or seed predation may increase the overall flower production as a result of reallocation of the reproductive resources (Freeman et al. 2003). Physiological trade-offs in resource allocation are shaped by natural selection on plant reproduction (Agrawal 2011a; Reekie and Bazzaz 2005).

Nevertheless, the reproductive investments (at each stage) also are interlinked with selection on survival, for example, imposed by leaf or root herbivory (Reichman and Smith 1991). Thus, as defense and reproduction both directly rely on the available resources, it can be expected that any investment in defense will have a direct effect on investments in reproduction. For instance, herbivore-induced compounds can compete biosynthetically with the production of pollinator-attracting volatiles (Agrawal 2011b). Accordingly,

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a trade-off between defense and reproduction often has been found in studies that have jointly investigated herbivory and reproductive performance (e.g., Belsky 1986; Bergelson and Crawley 1992; Bergelson et al. 1996; Freeman et al. 2003; Lohman et al. 1996; Marquis 1992; Strauss et al. 1996, 2002). Although herbivory usually triggers defense at the cost of reproduction (Strauss et al. 2002), in a few other cases herbivory has been shown to increase reproductive output (Lucas-Barbosa et al. 2013; Poveda et al. 2003). In addition to direct trade-offs, however, herbivory also may indirectly impact on plant reproduction, namely through ecological trade-offs (Strauss et al. 2002). This is the case when plant defense interacts with the attraction of mutualists such as pollinators, for example, through altered volatile signalling. Herbivory has indeed been shown to decrease floral attractiveness by triggering the induced emission of defense compounds that were associated with changes in pollinators visitation (Kessler and Halitschke 2009; Kessler et al. 2011; Poveda et al. 2003, 2005). Examples in which herbivory increases floral attractiveness through the induction of pollinator-attracting volatiles are not yet known.

In our study, we estimated the direct and indirect effects of a leaf herbivore on the pollination success of *Silene latifolia*. We used the generalist herbivore, *Spodoptera littoralis*, that has recently established itself in large areas of the Mediterranean region. We investigated the potential alteration of chemical and morphological plant traits and the related pollination fitness. We asked the following specific questions: 1) Does foliar herbivory alter the plants' floral display? 2) Does foliar herbivory alter floral volatile emission? 3) What are the consequences of herbivory for plant pollination (fruit and seed set)?

## Methods and Materials

**Study System and Source of Biological Material** The White Campion, *Silene latifolia*, is a short-lived perennial species that occurs naturally as a weed in disturbed and agricultural habitats. This species is pollinated mainly by nocturnal insects (especially moths), but diurnal pollination also occurs (Young 2002). Among moths, *Hadena bicruris* is a common nursery pollinator in *S. latifolia*. In nursery pollination mutualism, the pollinator lays eggs into female flowers when pollinating, and the moth caterpillars feed on developing seeds as their breeding site (Dufaÿ and Anstett 2003). Since *S. latifolia* is a dioecious species, successful fruit set is strictly dependent on pollinator visitation.

During summer 2011, seeds of *S. latifolia* were collected from a natural population (30 randomly selected female plants) located near the University Campus of Monte Sant'Angelo (Naples, Southern Italy). Fruits were collected randomly, and seeds were pooled together and vernalized at

4 °C. At the beginning of each experimental season (2012 and 2013), a part of the collected seeds was sown in pots with sterilized soil (to avoid interfering effects from potential root herbivores) from the same locality of the source population; germinated plants were grown outside for 3 months, and when they started flowering, were used for experimental treatments.

To test for the effect of herbivory, we used the generalist Egyptian Cotton Leafworm, *Spodoptera littoralis*. This species, native to Northern Africa where it is known as a major pest of numerous crops (El-Sayed et al. 2009), is actually also established in many South-European countries with migratory populations that invade the northern part of Europe (CABI/EPP0 1997). *Spodoptera littoralis* current distribution largely overlap with *S. latifolia* around the Mediterranean basin so that it is likely the two species have been already in contact. In our study, larvae used for infesting plants were obtained from the Swedish University of Agricultural Science (Alnarp, Sweden). These larvae originated from Egypt and had been reared in captivity for several generations. As previous feeding experience can influence host plant preferences, larvae from hatched eggs were first reared on artificial diet (following a recipe for noctuid species described in Elzinga et al. 2002) for 5 days and then fed exclusively with *S. latifolia* leaves until used for experimental purposes (after 3–4 days, typically when at second instar). In that way, larvae experienced the test plant species before the beginning of the experiments and immediately started consuming leaves when in the clip cages.

**Plant Treatment** Plants subjected to the leaf herbivory treatment were infested with 2 sec instar *S. littoralis* caterpillars. Feeding was restrained to leaves by containing caterpillars in clip cages made from petri dishes; caterpillar development and feeding was monitored regularly (approximately every second day). All caterpillars were replaced (approx. every 5–6 days) by new second instars to keep larval stage constant and limit differential effects caused by different developmental stages. As caterpillars consumed on average half a *Silene* leaf in 3 days, clip cages with the caterpillars were moved to a different leaf when required. For experimental treatments, *Silene* plants were subject to herbivory for the entire experimental period (4 weeks). Control plants were equipped merely with empty clip cages.

**VOCs Collection** In spring 2012, floral volatiles organic compounds (VOCs) were collected in a non-destructive way. They were sampled from individual flower headspace from 31 females and 32 male plants from the same germinated seeds that were used for pollination experiments. Each flower was sampled only once, in the first evening of opening. Flowers from each plant were sampled before infestation (pre-infestation;  $N=31$  females/32 males), 2 days after infestation (early post-infestation;  $N=27$  females/32 males) and 1 week after

infestation (late post-infestation;  $N=17$  females/26 males) from herbivore-treated and control plants. Flowers were enclosed in Nalophan® oven bags. Air was pulled out of the bag at a rate of 100 ml/min over a glass tube filled with 20 mg Tenax TA (60/80 mesh, Supelco Bellefonte, PA, USA). Scent was collected on the same day for the same treatment for 30 min between 9.00 and 12.00 pm.

For the analysis of floral VOCs, gas chromatography with mass selective detection (GC-MSD) was used. Samples were injected into a GC (Agilent 6890 N, Agilent Technologies, Santa Clara, CA, USA) using a Gerstel thermodesorption system (TDS3, Gerstel Mühlheim, Germany) with a cold injection system (KAS4; Gerstel Mühlheim, Germany). For thermodesorption, the TDS was heated from 30 to 240 °C at a rate of 60 °C/min, and held at the final temperature for 5 min. The KAS was set to -150 °C during trapping of eluting compounds from the TDS. For injection, the KAS was heated to 250 °C at a rate of 12 °C/s, and the final temperature was held for 3 min. The GC was equipped with a DB-5 column (0.32 mm ID, 0.25 µm film thickness, 30 m length), and helium was used as carrier gas at a flow rate of 2 ml/min. Compound determination and quantification was done using a mass selective detector (Agilent MSD 5975). Compounds were identified tentatively by comparison of spectra obtained from the natural samples with those from a reference collection (NIST library) followed by verification and quantification using synthetic standard compounds. The Agilent MSD ChemStation program was used for quantification. To quantify individual compounds, synthetic standards of all compounds were analyzed in three different amounts (1, 10, and 100 ng) on the GC-MSD system, and specific target ions were selected for each compound. The peak areas of target ions were used to calculate calibration curves and subsequent quantification implemented in the ChemStation software (Agilent). All amounts of VOCs were calculated in ng per flower per liter sampled air. Compounds that were found in less than 30 % of all plant individuals (*i.e.*, methyl benzoate, veratrol) were not included in the analysis.

**Floral Trait/Reward Measurements** In 2012, three plant traits important for visual attraction of pollinators (Young 2002) also were measured from the same plants used for scent collection using a digital calliper: corolla diameter, petal length, and calyx length. A total of 47 flowers (23 from control plants and 24 from *Spodoptera*-infested plants) was measured. We also estimated amount and concentration of nectar in *Spodoptera*-infested and control plants by using a hand-held sugar-refractometer (Eclipse, Bellingham & Stanley Ltd, Kent, UK).

**Pollination Success** In 2012, we performed a first pilot experiment on *Spodoptera*-infested and control female plants. To test for effects of herbivory on the attractiveness of plants

for pollinators, plants were exposed to natural pollinators at the Naples University Campus (where *S. latifolia* naturally occurs). In order to disentangle potential differences in responses of diurnal and nocturnal pollinators to herbivory we split the herbivory- and control plants into two treatment groups. One group (nocturnal pollination:  $N=11$  control plants;  $N=10$  *Spodoptera*-infested plants) was covered with a fine net every morning (ca. 8.00 am), and the net was removed every evening (ca. 7.00 p.m.); the second group (diurnal pollination:  $N=8$  control plants;  $N=10$  *Spodoptera*-infested plants) was covered every evening (ca. 7.00 pm), and the net was removed every morning (ca. 8.00 a.m.). At the end of the flowering period, all pollinated flowers that developed into fruits were recorded. When ripe, individual fruits were harvested and stored for subsequent analysis of their seed number and weight.

In the same spring, to test for the effects of herbivory on fruit production due to resource allocation, an additional set of *Spodoptera*-infested ( $N=10$ ) and control female plants ( $N=10$ ), were covered with a net during the whole flowering period. Five flowers of each plant were hand-pollinated with an excess of pollen collected from surrounding male plants and fruits were collected when ripe. Seeds from these fruits also were counted and weighed.

**Manipulation of Floral Scent** In spring 2013, we performed a more detailed study on the floral traits that had varied significantly with our treatments in 2012 to investigate causal links between pollinator behavior and these traits. In particular, to test for the effects of the induced floral VOCs on pollinator attraction, we experimentally manipulated the floral scent. A mixture of the two floral scent compounds that significantly increased after *S. littoralis* herbivory, (*Z*)-3-hexenyl acetate and  $\beta$ -ocimene, were used in this experiment. (*Z*)-3-Hexenyl acetate (Sigma Aldrich, purity  $\geq 92$  %) and  $\beta$ -ocimene (mixture of isomers, Sigma Aldrich purity  $\geq 90$  %) were diluted in dichloromethane (HPLC grade; see Online Resource 1 for concentrations). Rubber septa (GR-2, 5 mm Supelco, Bellefonte, PA, USA) were soaked in the mixture for 1 h, and afterwards were allowed to evaporate for 1 h. In a pre-experiment, scent emitting from these septa was collected and analyzed as described for flower scent; the amounts of compounds in the soaking-solution was adjusted so that emission rates matched the mean amounts of 2–3 *S. latifolia* flowers (Online Resource 1). Scent-emitting septa were fixed on a thread and hung onto non-infested *S. latifolia* plants ( $N=19$ ). We added five scented septa for each plant close to five open flowers. The same septa were progressively moved to the new open flower as soon as the old flower started wilting (approx. every 2 days). In this way, each plant always carried, among other flowers, five fresh flowers with added odor septa during the whole experimental period (*i.e.*, up to the end of the flowering time) with septa constantly emitting scent.

In the same experiment, we also set up a group of control plants equipped with septa soaked in dichloromethane only ( $N=11$ ), a group of *Spodoptera*-infested plants ( $N=18$ ), and a group of mechanically damaged plants ( $N=18$ ). Damage was produced by removing roughly half a leaf with a scissor every 3 days. This damage was comparable to the average amount of leaf tissue consumed by *S. littoralis* caterpillars during the same time period.

All treatment groups were placed in the same experimental area, at a distance of 10 m between each group. Based on results of the 2012 pollination experiment, all treatment groups were exposed only to nocturnal pollinators (*i.e.*, starting at 7.00 p.m. and ending at 8.00 a.m.). Plants were monitored regularly during the experimental period, and at the end of flowering period, all pollinated flowers that developed into fruits were recorded.

**Statistical Analyses** For two-group comparisons (floral traits, nectar) between herbivory and control plants, *MannWhitney U tests* were used. For four group comparisons between herbivory, control, scent-augmented, and mechanically damaged plants, *Kruskal-Wallis tests* with *Mann-Whitney U tests* for a *posteriori* multiple comparison, were used, with the significance level set to  $P=0.01$  (Bonferroni correction). For the floral VOCs analysis, the three stages of VOC sampling were analysed separately: stage 1 (before infestation), stage 2 (2 days after infestation) and stage 3 (1 week after infestation). Amounts of all compounds were  $\ln(1+x)$  transformed. General linear models (GLM) were evaluated for all compounds with amount of each compound as dependent variable, and sex of the plant and treatment as fixed factor. All analyses were performed using SPSS statistical package (SPSS Inc., Chicago, IL, USA).

## Results

In the floral scent collected in the spring 2012, 18 compounds were detected and analysed (Table 1 and 2). Two volatiles ( $\beta$ -ocimene and (*Z*)-3-hexenyl acetate) showed (marginally) increased emissions when compared to controls, but only in (early) post-infestation (Fig. 1). For  $\beta$ -ocimene, a significant effect of herbivory was found ( $P=0.014$ ; Online Resource 2), and for (*Z*)-3-hexenyl acetate, a marginally significant interaction between herbivore and sex was found ( $P=0.061$ ; Online Resource 2). Both compounds were emitted in higher amounts in herbivore-treated plants than control (Fig. 1). The two isomers of  $\beta$ -ocimene did not show clear, distinct peaks and were thus quantified together. For (*Z*)-3-hexenyl acetate, the interaction between herbivore and sex indicated that increased emission was sex-specific; indeed only female

flowers showed increased amounts of this VOC after herbivory (Fig. 1; Tables 1 and 2).

The examined floral traits did not differ between *Spodoptera*-infested and control plants (corolla:  $U=245.5$ ,  $Z=-0.649$ ,  $P=0.516$ ; calyx:  $U=268.0$ ,  $Z=-0.170$ ,  $P=0.865$ ; petal:  $U=250.0$ ,  $Z=-0.553$ ,  $P=0.580$ ). There also was no difference in nectar amount ( $U=344.5$ ,  $Z=-1.006$ ,  $P=0.314$ ) or sugar concentration ( $U=192.5$ ,  $Z=-0.519$ ,  $P=0.604$ ) (Table 3).

In the 2012 pollination experiment, we found higher fruit production in *Spodoptera*-infested compared to control plants, but only with nocturnal pollination ( $U=27.5$ ,  $Z=-2.001$ ,  $P=0.045$ ), whereas there was no difference in fruit production among diurnally pollinated plants ( $U=30.0$ ,  $Z=-0.896$ ,  $P=0.370$ ; Fig. 2). *Spodoptera*-infested and control plants showed no significant differences in terms of number of seeds per fruit produced ( $U=39.0$ ,  $Z=-0.132$ ,  $P=0.895$ ) or of seed weight ( $U=33.0$ ,  $Z=-0.662$ ,  $P=0.508$ ). The hand pollination experiment also showed no differences between herbivory- and control plants in fruit set ( $U=40.5$ ,  $Z=-0.756$ ,  $P=0.481$ ), number of seeds per fruit ( $U=290.5$ ,  $Z=-0.389$ ,  $P=0.697$ ), or seed weight ( $U=297.0$ ,  $Z=-0.263$ ,  $P=0.793$ ). However, comparison of seed set between open pollinated and hand pollinated flowers showed significant differences ( $\chi^2=21.925$ ,  $df=3$ ,  $P<0.01$ ; Online Resource 3) indicating that open pollinated plants were pollen-limited.

In the 2013 scent manipulation experiment, when only nocturnal pollination was allowed for, we found different fruit production between the test groups ( $\chi^2=25.886$ ,  $df=3$ ,  $P<0.01$ ; Fig. 3). Plants with floral scent compounds added produced more fruits than herbivore-treated plants ( $U=85.0$ ,  $Z=-2.617$ ,  $P=0.008$ ), control plants ( $U=19.0$ ,  $Z=-3.689$ ,  $P<0.001$ ) and mechanically damaged plants ( $U=37.5$ ,  $Z=-4.071$ ,  $P<0.001$ ). Herbivore-treated plants produced more fruits than control plants ( $U=40.5$ ,  $Z=-2.639$ ,  $P=0.008$ ), and showed a trend towards higher fruit set than mechanically damaged plants ( $U=84.0$ ,  $Z=-2.489$ ,  $P=0.13$ ). Control and mechanically damaged plants were not different ( $U=84.5$ ,  $Z=-0.661$ ,  $P=0.521$ ). Number of flowers was not different among herbivore-treated, control, and mechanically damaged plant groups ( $\chi^2=4.353$ ,  $df=2$ ,  $P=0.113$ ).

## Discussion

Although herbivory often has been shown to compromise reproduction, it also may trigger increased attractiveness of flowers to pollinators (Poveda et al. 2003). Our study using the generalist herbivore, *Spodoptera littoralis*, feeding on *Silene latifolia* plants shows that herbivore feeding increased fruit production, at least in the short term (Figs. 2 and 3). This effect was limited to nocturnal pollination, and likely caused by increased attractiveness of flowers. We did not detect any

**Table 1** Floral volatiles (ng/h/flower; mean±s.e.m.) of control and *Spodoptera littoralis*-infested female *Silene latifolia* plants. The infestation stages refer to pre-infestation, early post-infestation (2 d after infestation with herbivores) and late post-infestation (1 week after infestation with herbivores)

VOCs	Female plants		Infestation stages			
	Pre-infestation		Early post-infestation		Late post-infestation	
	Control (16)	<i>Spodoptera</i> -infested (15)	Control (12)	<i>Spodoptera</i> -infested (15)	Control (8)	<i>Spodoptera</i> -infested (9)
<b>FAD</b>						
Nonanal	690.5±55.0	688.9±46.5	734.7±94.4	764.2±50.3	931.6±86.6	866.6±66.8
Decanal	115.5±15.2	101.7±7.4	113.9±12.4	117.7±9.0	211.1±43.4	132.0±18.7
(Z)-3-Hexenyl acetate	548.6±169.2	539.5±140.2	<b>677.7±407.1</b>	<b>2080.6±1113.6</b>	650.5±303.5	542.9±356.7
<b>Aromatics</b>						
Benzaldehyde	395.5±73.6	219.2±44.7	230.2±54.6	254.9±69.0	327.2±181.7	129.1±24.8
Benzyl alcohol	0.3±0.10	0.1±0.03	0.1±0.02	0.2±0.03	0.1±0.03	0.2±0.03
Benzyl acetate	433.1±212.4	524.3±288.1	283.0±247.4	328.1±202.8	822.2±553.1	1.5±0.3
Phenylacetaldehyde	6571.7±1334.3	3710.1±859.6	4847.6±1454.7	4609.4±1344.2	3747.3±1474.1	2501.2±1016.5
Phenylethyl alcohol	755.1±281.4	434.9±192.8	727.7±312.0	869.1±399.7	257.8±161.4	193.8±122.9
Guaicol	0.9±0.3	0.5±0.2	1.1±0.7	0.9±0.4	0.5±0.2	0.8±0.4
Methyl salicylate	8.0±3.8	3.6±2.6	2.0±1.8	3.8±3.5	5.2±2.2	5.1±5.0
Phenyl propanol	260.4±120.6	2253.7±1181.4	182.5±156.7	2559.2±1492.0	2820.6±2601.8	193.7±135.5
Benzyl benzoate	148.0±56.4	96.2±37.1	73.6±35.3	232.2±138.1	118.8±56.0	17.0±13.6
<b>Terpenoids</b>						
α-Pinene	1.9±0.3	1.4±0.3	1.5±0.3	1.9±0.2	3.5±0.8	3.1±1.0
β-Ocimene	188.0±21.3	249.4±45.8	<b>188.5±33.5</b>	<b>361.2±62.9</b>	211.9±46.6	169.5±67.9
Limonene	170.8±27.8	154.9±15.0	197.1±45.0	248.3±55.6	220.4±28.1	241.5±37.00
Lilac aldehyde A	207.7±94.2	213.1±116.3	35.0±15.7	52.3±22.2	45.0±39.3	17.0±8.2
Lilac aldehyde B	139.0±58.3	119.5±57.3	36.3±14.5	45.8±20.8	31.2±25.2	8.6±6.5
Lilac aldehyde C	21.5±10.5	19.3±12.3	4.3±2.0	3.7±2.4	4.0±4.2	0.7±0.8

FAD are fatty acid derivatives. Volatiles with significant differences are given in bold. For statistical analysis, both sexes were analysed together using a GLM; significant effects of “treatment” (β-ocimene) or “treatment \* sex” ((Z)-3-hexenyl acetate) were found

direct effect of herbivory on reproductive fitness, as hand-pollinated, herbivore-infested plants did not produce fewer fruits and seeds than control plants. These findings show that *S. latifolia* can increase its fruit production upon herbivore attack, a strategy similar to overcompensation, through increased floral attractiveness for pollinators via induced floral volatiles.

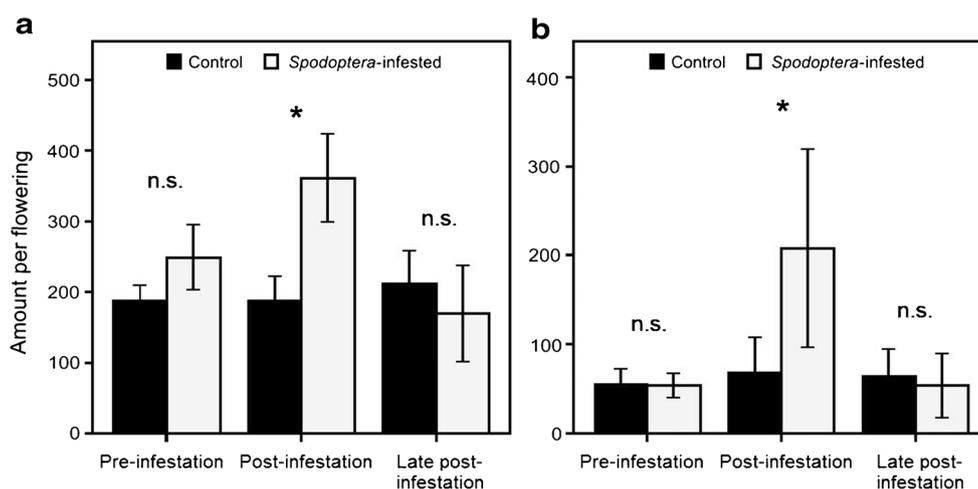
Generally, herbivory can have both direct and indirect effects on plant reproductive performance (Strauss et al. 2002). Direct effects on plant fitness are mediated by the removal of vegetative or reproductive tissues and by the reduction of available resources that can be allocated to fruits and seeds (Strauss et al. 2002; Zangerl and Berenbaum 2009). In our experiments, such direct effects were not detectable as hand-pollinated *Spodoptera*-infested *Silene* plants did not suffer reduced seed set compared to control plants (Online Resource 3). This indicates that the herbivore damage imposed in our study did not significantly compromise allocation of resources to either fruits or seeds.

Indirect or ecological trade-offs between defense and pollination are those defense responses that negatively interact with the attractiveness of a plant to pollinators (Strauss et al. 1996). Such effects can be mediated by decreased flower- or inflorescence size (Barber et al. 2012; Lehtila and Strauss 1997; Poveda et al. 2005), amounts of rewards, secondary defense metabolites present in nectar (Adler et al. 2006), induced repellent volatile organic compounds (VOCs; Kessler and Halitschke 2009; Zangerl and Berenbaum 2009), or reduced attractive floral VOCs (Bruinsma et al. 2014; Pareja et al. 2012; Schiestl et al. 2014). In our study, however, no ecological trade-offs were detectable; we found no short-time effects of leaf herbivory on floral display (*i.e.*, flower number, corolla diameter, petal length, and calyx length), or reward in *Silene* flowers (Table 3). In a previous study on *S. dioica*, herbivory stimulated flowering in damaged plants, but after a severe defoliation (50 and 100 %) (Elmqvist and Gardfjell 1988). Here, the amount of defoliation was less than 10 %, possibly explaining the absence of effect on flower number. In

**Table 2** Floral volatiles (ng/h/flower; mean±s.e.m.) of control and *Spodoptera littoralis*-infested male *Silene latifolia* plants. The infestation stages refer to pre-infestation, early post-infestation (2 days after infestation with herbivores) and late post-infestation (1 week after

infestation with herbivores). FAD are fatty acid derivatives. For statistical analysis, both sexes were analysed together using a GLM; significant effects of “treatment” ( $\beta$ -ocimene) or “treatment \* sex” ((*Z*)-3-hexenyl acetate) were found

VOCs	Male plants		Infestation stages			
	Pre-infestation		Early post-infestation		Late post-infestation	
	Control (16)	<i>Spodoptera</i> -infested (16)	Control (16)	<i>Spodoptera</i> -infested (16)	Control (13)	<i>Spodoptera</i> -infested (13)
<b>FAD</b>						
Nonanal	676.4±46.7	706.1±44.7	858.6±69.9	802.1±48.6	862.0±115.6	776.6±76.9
Decanal	96.5±6.7	101.4±6.9	127.8±8.4	140.0±10.3	137.5±19.3	154.6±16.8
( <i>Z</i> )-3-Hexenyl acetate	547.8±177.3	676.4±188.1	751.2±199.2	485.0±130.7	620.0±289.5	733.9±319.3
<b>Aromatics</b>						
Benzaldehyde	224.5±42.2	256.3±62.4	190.0±28.1	338.3±75.9	180.5±34.2	206.4±54.6
Benzyl alcohol	0.2±0.10	0.3±0.10	0.1±0.04	0.2±0.10	0.3±0.10	0.1±0.02
Benzyl acetate	476.3±184.3	290.0±105.7	429.8±238.3	442.2±267.1	444.7±216.5	229.7±134.0
Phenylacetaldehyde	4695.7±1136.9	4798.0±1407.5	3660.1±898.2	6031.8±1540.5	4545.3±1350.1	4179.5±1273.2
Phenylethyl alcohol	620.5±163.0	472.8±183.6	506.4±231.3	775.2±324.8	519.1±205.1	338.1±160.8
Guaicol	7.3±5.6	6.9±5.5	6.7±4.3	10.9±8.2	1.4±0.8	7.2±5.8
Methyl salicylate	14.2±5.9	16.4±9.0	8.0±4.4	7.8±3.9	4.8±2.4	4.3±1.9
Phenyl propanol	1774.8±1378.7	1780.9±1231.4	466.7±236.4	1894.4±677.9	461.4±281.5	1537.8±1164.7
Benzyl benzoate	158.9±49.4	108.9±34.1	335.8±191.2	100.8±57.2	97.8±46.7	61.9±32.5
<b>Terpenoids</b>						
$\alpha$ -Pinene	2.2±0.4	2.2±0.4	2.0±0.3	1.7±0.2	3.4±0.5	2.5±0.5
$\beta$ -Ocimene	220.8±49.1	300.5±51.6	<b>341.3±72.4</b>	<b>415.7±69.9</b>	220.6±72.6	242.3±68.8
Limonene	326.1±148.4	157.7±17.3	217.6±36.5	138.4±11.8	198.9±26.5	247.6±79.9
Lilac aldehyde A	286.0±112.1	332.8±127.7	169.6±47.2	165.8±53.6	175.1±77.8	62.7±31.2
Lilac aldehyde B	113.9±34.7	188.6±65.6	102.2±28.4	102.8±32.5	108.3±47.7	37.5±17.5
Lilac aldehyde C	24.4±11.9	24.5±9.1	15.8±5.1	11.8±5.1	20.7±10.2	5.1±3.9



**Fig. 1** Changes in the emission of  $\beta$ -ocimene (a) and (*Z*)-3-hexenyl acetate (b) in control (black bars) and *Spodoptera littoralis*-infested (grey bars) female *Silene latifolia* plants. Flowering stage 1: before herbivory, 2: 2 days after infestation, 3: 1 week after infestation. In the early post-infestation stage, the two compounds were emitted in

significantly increased amounts in *Spodoptera*-infested plants. For (*Z*)-3-hexenyl acetate, this effect was only marginally significant and only present in females (marginally significant interaction between herbivory and sex, see Online Resource 2) (\* significant difference at  $P < 0.01$ )

**Table 3** Values (mean±SD) of *Silene latifolia* flower corolla, calyx and petals (cm), nectar amount (μl) and concentration (%), in control plants and *Spodoptera littoralis*-infested plants. The examined floral traits did not significantly differ between *Spodoptera*-infested and control plants (Mann–Whitney U tests, significance level set to  $P=0.01$ )

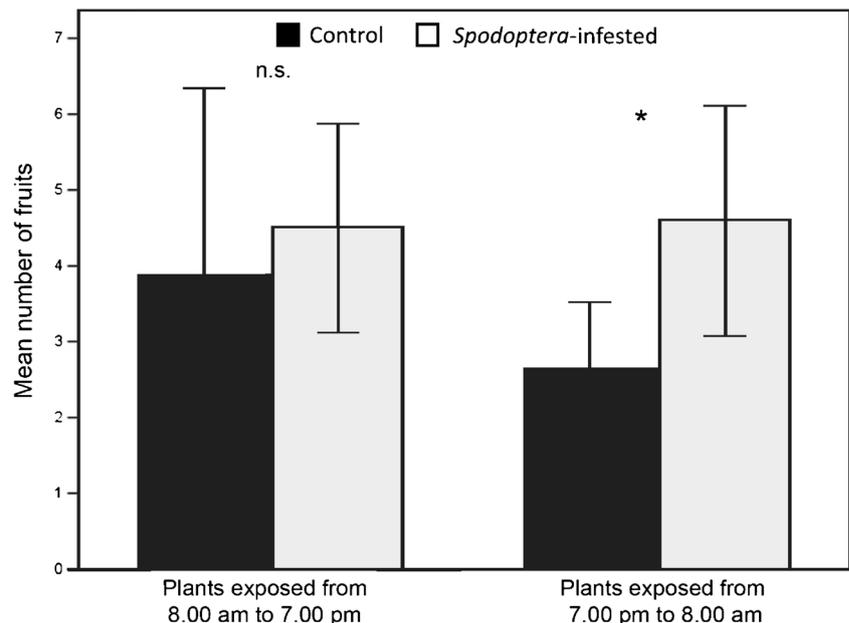
Floral trait	Control plants (23)	<i>Spodoptera</i> -infested plants (24)
Corolla width	2.4±0.3	2.4±0.2
Calyx length	2.7±0.3	2.7±0.2
Petals length	1.1±0.2	1.1±0.1
Nectar amount	16.1±20.1	9.5±9.3
Sugar concentration	22.0±19.3	26.5±22.5

accordance with earlier studies in *Brassica* (Lucas-Barbosa et al. 2013; Poveda et al. 2003), we found that herbivory increased fruit production (Figs. 2 and 3). This effect was apparent during both experimental seasons, and likely was caused by increased attractiveness of flowers to pollinators, as the effect could be successfully mimicked by adding synthetic floral scent compounds to non-infested flowers, and it was limited to nocturnal pollination. Therefore, overcompensation as an alternative mechanism causing higher fruit production is unlikely. In addition, *Spodoptera*-infested plants had the same seed set as control plants. Seed set (*i.e.*, the number of sired ovules) represents an indirect measurement of pollinator efficiency. Comparable pollination efficiency indirectly suggests that *Spodoptera*-infested plants do not attract a different *spectrum* of pollinators than control plants, but that herbivory leads to increased attraction of similar, effective pollinators. Increased pollinator attraction as compensation

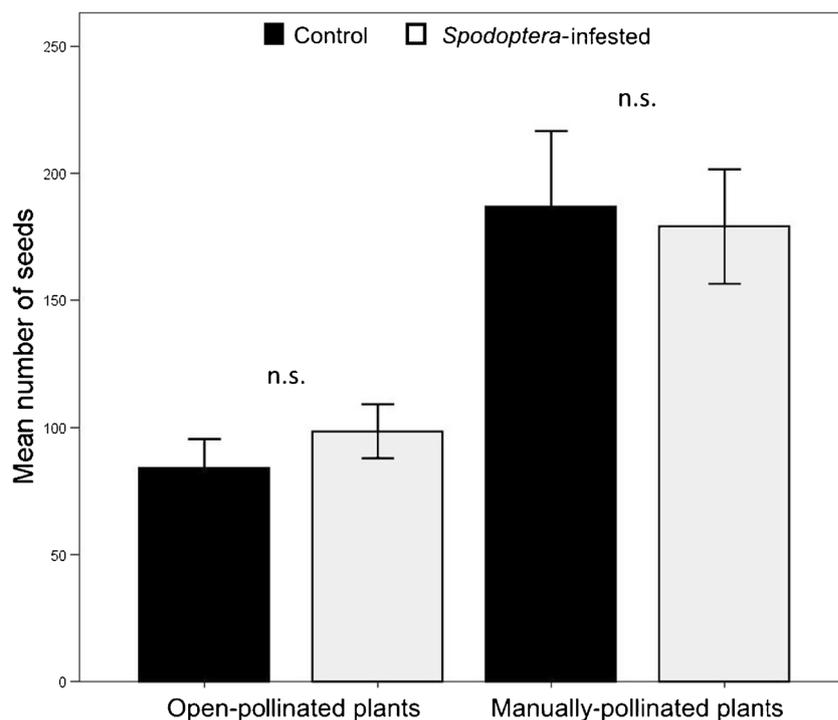
for herbivory can be expected to evolve with higher propensity in outcrossing species (Abel et al. 2009) such as the dioecious *S. latifolia*. More studies are needed, however, to better understand the effects of plant mating system and life history on herbivore-mediated phenotypic plasticity in floral traits (Campbell and Kessler 2013).

In addition to documenting increased fruit set after herbivory, our study also shows that this effect is significantly enhanced (compared to the herbivory treatment) by the addition of two floral scent compounds,  $\beta$ -ocimene and (*Z*)-3-hexenyl acetate, to flowers of non-infested plants (Online Resource 1). These two compounds were emitted in increased amounts after herbivory (Tables 1 and 2; Fig. 1). (*Z*)-3-Hexenyl acetate is a well-known, inducible compound in leaves of many plants, and is thought to be important for plant indirect defense, *i.e.*, the attraction of parasitoids to herbivore-infested plants (Pichersky and Gershenzon 2002). Its role in pollinator attraction presently is unknown. In *Silene*, the induction of this compound in flowers may also serve indirect defense functions, possibly affecting caterpillars of the nursery pollinator *Hadena bicruris* feeding inside flowers or other herbivores via attraction of generalist predators. This might explain the female-specific induction of this compound, because females develop fruits and seeds, and thus should invest more in (indirect) defense.  $\beta$ -Ocimene, the other induced compound, is a well known pollinator-attracting volatile in flowers (Raguso 2008; Schiestl 2010). It also has been shown to be herbivore-inducible, and also may serve indirect-defense functions (Dicke et al. 1990; Kessler and Baldwin 2001). None of the two compounds has been reported as an attractive volatile for the nursery pollinator *Hadena bicruris* (Dötterl et al. 2006), but it is known that a range of nocturnal moths

**Fig. 2** Mean number of fruits produced in control (black bars) and *Spodoptera littoralis*-infested (grey bars) plants exposed alternatively to diurnal (from 8 am to 7 pm) or nocturnal (from 7 pm to 8 am) pollinators in 2012. Higher fruit production was found in herbivory-treated plants exposed to nocturnal pollinators when compared to control plants (\* significant difference at  $P<0.05$ )



**Fig. 3** Mean number of fruits produced by the different treatment groups in 2013. Plants were exposed only to nocturnal pollinators (from 7 pm to 8 am). Plants with added floral scent compounds produced significantly more fruits than herbivore-treated plants, mechanically damaged plants and control plants. Different letters indicate significant differences



visits and pollinates *S. latifolia* flowers in the field (Jürgens et al. 1996; Young 2002), which may be responsible for the increased visitation after herbivory.

In the 2013 scent manipulation experiment, plants with added synthetic floral scent had a significantly higher fruit set than *Spodoptera*-infested plants (Fig. 3). The most obvious explanation of this difference in the scent-added treatment seems to be that, in our experimental setting, the effect of septa emission is throughout the course of flowering time, while in infested-plants the *Spodoptera* damage causes only a spike in scent production despite its continued presence. Indeed, the increase in  $\beta$ -ocimene and (*Z*)-3-hexanyl acetate was detectable only shortly after herbivores were added to the plants (2 days after infestation). Later the effect disappeared (Fig. 1; Online Resource 2) suggesting an ecological cost for a prolonged emission of these attractive compounds.

Presently, we cannot conclude whether the responses of *S. latifolia* to *S. littoralis* infestation are specific to this herbivore, or generally induced by (leaf-feeding) herbivores. Although in S-Italy *Spodoptera* has only recently been established and may not be a natural herbivore of local plants, *S. latifolia* also is common in Northern Africa (Seybold 1990) where it may have interacted with this herbivore. In addition, *S. latifolia* is a pioneer plant that might frequently face novel herbivores, depending on the different environments it colonizes. The species has become successfully established in the US, where it is now considered an invasive plant (Wolfe et al. 2004). Therefore, selection for a co-evolved response to any specific herbivore is not expected to be very strong in *Silene*.

We, therefore, suggest that the responses documented here are within the general reaction norm of herbivore-mediated responses in this plant species.

Overall, our findings show that it should not necessarily be expected that there is a conflict between defense and reproduction, because plants can maintain or increase fitness upon herbivore attack. As this study lacks a documentation of the direct effects of the two compounds, (*Z*)-hexenyl acetate and  $\beta$ -ocimene, on pollinator behavior, further studies that directly address the pollinator attractiveness to these herbivore-induced volatiles are needed.

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