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RELATIONSHIPS

## Herbivory affects male and female reproductive success differently in dioecious *Silene latifolia*

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### Abstract

In dioecious plants, reproductive efforts of male and female plants can be differentially affected by herbivory due to sex-specific allocation and re-modulation of resources. Here, we investigate the effects of foliar herbivory by the polyphagous species *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) on male and female reproductive traits of *Silene latifolia* (Poir.) (Caryophyllaceae). We set up experimental populations of male and female control and *Spodoptera*-infested plants and estimated flower morphology and phenology, and plant reproductive success. Herbivory did not affect floral traits involved in visual signalling to pollinators (flower size). Female reproductive success did not differ between *Spodoptera*-infested and control plants in terms of seed set and seed germination. On the other hand, *Spodoptera*-infested male plants produced fewer flowers than male control plants and maintained fewer flowers during flowering peaks. By experimental reduction in male flower number, we showed that male flower number is directly correlated with male reproductive success (pollen export to female flowers). This strongly suggests that male reproductive success is reduced after herbivory, whereas female reproductive success remains unaffected. The fact that females are more tolerant against herbivory is in accordance with sexual selection theory, which predicts that females, in contrast to males, are not selected for maximum investment in reproductive traits such as floral signalling, and should thus be more flexible in the allocation of resources.

### Introduction

Herbivory is common in all plants and countless adaptations have been evolved by plant species to reduce its detrimental effects (Ehrlich & Raven, 1964; Benson et al., 1975). Through the reduction in photosynthetic parts and the elicitation of a defence response, herbivory imposes a strong reduction in the resources that the plant can allocate to vegetative and reproductive organs (Obeso, 2002). Therefore, plants facing herbivory need to re-modulate the allocation of resources to maximise their reproductive success (Bazzaz et al., 1987; Herms & Mattson, 1992). This re-modulation involves a complex trade-off between

survival and reproductive traits, as the increase in investment in one trait may come at the expense of another (Herms & Mattson, 1992; Obeso, 2002).

The different responses of plants to herbivory and the impact of herbivory on resource allocation in plants have been described in detail (Boege & Marquis, 2005; Núñez-Farfán et al., 2007). The strategies of plants to cope with herbivory typically involve a reduction in flower number or size (Elmqvist & Gardfjell, 1988; Quesada et al., 1995; Mutikainen & Delph, 1996), an alteration of nectar contents (Adler et al., 2006), or a change in emission of volatile compounds (Theis et al., 2009; Dicke & Baldwin, 2010; Lucas-Barbosa et al., 2011; Schiestl et al., 2014). Assuming that female function is more costly than male function (Lloyd, 1979; Freeman et al., 1981; Charlesworth & Morgan, 1991), it could be expected that foliar herbivory would predominantly result in a decrease in female

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reproductive success. However, the effects of herbivory on reproductive investment on hermaphroditic and monoecious plants are difficult to interpret given that both male and female functions are present in the same flower or on the same plant (Quesada et al., 1995; Lehtilä & Strauss, 1999; Paige et al., 2001; Schiestl et al., 2014).

Unlike hermaphroditic species, dioecious species have a completely separate allocation to male and female functions. Nevertheless, the difference in sex-specific responses to herbivory in dioecious plants has been only scantily investigated (Ågren et al., 1999; Cornelissen & Stiling, 2005; Avila-Sakar & Romanow, 2012). Resource allocation is dramatically different in male and female plants, reflecting the fundamental biological roles of the two sexes (Barrett & Hough, 2013). That is, whereas the male plant's primary function is to export as much pollen as possible, the female plant functions are more complex, because they encompass both the attraction of pollinators and the production of progeny. The different functions of the sexes are clearly reflected in different resource allocation strategies: male plants are typically selected to allocate most of their resources into growth, flower production, and pollinator attraction, but less into chemical defences (Lloyd & Webb, 1977; Ågren et al., 1999; Avila-Sakar & Romanow, 2012). Female plants, on the other hand, need a more sophisticated modulation of their resources because reproduction, growth, and defence all impact on their reproductive success (Boecklen et al., 1994; Delph, 1999; Obeso, 2002; Cepeda-Cornejo & Dirzo, 2010; Maldonado-López et al., 2014). This difference in sex-specific resource allocation strategies has been even advocated as one possible explanation for the evolution of dioecy in flowering plants (Ashman, 2002; Avila-Sakar & Romanow, 2012) and has led to the formulation of the male-biased herbivory hypothesis, which states that female plants should be better defended against herbivores than male plants (Ågren et al., 1999; Cornelissen & Stiling, 2005). However, aside from any potential sex-bias in herbivore resistance, male, and female plants can also differ in patterns of resource allocation to reproduction after herbivore attack, as female plants can modulate a greater fraction of their resources (allocated in the several steps of their reproductive performance) than their male counterparts (Lloyd, 1980; Lovett Doust & Lovett Doust, 1983). To shed new light on this hypothesis, we investigated whether leaf herbivory by the polyphagous African cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), differently affects male and female reproductive success in the dioecious plant white campion, *Silene latifolia* (Poir.) (Caryophyllaceae). In detail, we tested whether herbivory altered *S. latifolia* floral display (flower size and number) and

whether this alteration had an effect on the reproductive performance (seed set, siring seeds) of the two sexes.

## Materials and methods

### Study species

*Silene latifolia* is a dioecious, short-lived perennial found in open disturbed habitats such as field margins and roadsides (Goulson & Jerrim, 1997). Its distribution area includes Central Europe and the Mediterranean Basin (Baker, 1948). *Silene latifolia* is sexually dimorphic for floral, leaf, and resource allocation traits (Delph et al., 2002). Male plants produce more flowers, but these are shorter-lived (Carroll & Delph, 1996; Meagher & Delph, 2001) and emit a larger amount of pollinator-attracting VOCs than female flowers (Waelti et al., 2009).

All plants employed in the experiments were grown from seeds collected in a natural population near the Campus of Monte Sant'Angelo (Naples, southern Italy). Seeds were placed in pots with sterilised soil (collected from the natural population site) for germination. Plants at the beginning of the flowering period were used in the experiments.

*Spodoptera littoralis* is a generalist and polyphagous moth, native to Egypt where it is considered a major pest of a wide variety of host plants (Amin & Gergis, 2006). Its distribution area surrounds the Mediterranean basin and largely overlaps with that of *S. latifolia*. *Spodoptera littoralis* lines of the individuals used in our experiments, originated from a wild population in Alexandria (Egypt) and were reared in captivity at the Swedish University of Agricultural Science, Alnarp, Sweden, and refreshed with new wild-collected individuals approximately every 6 months. Eggs obtained from this rearing were placed in a climate chamber at 22 °C, 60% r.h., and L18:D6 photoperiod in the Department of Biology of the University of Naples Federico II. After hatching, first instars were reared on artificial diet (Elzinga et al., 2003) and, when at second instar (after 3–4 days), were fed with young leaves of *S. latifolia*.

### Plant treatments

We performed a series of experiments to test effects of *S. littoralis* folivory on phenotypic traits and reproductive success of male and female plants of *S. latifolia*. At the beginning of the experimental season, *S. latifolia* seeds, collected from local wild plants, were sown in pots. Germinated plants were grown for 4 months and, when they started flowering, were used for experimental treatments.

In all experiments, we set up male and female control and *Spodoptera*-infested plants. Infested plants were subjected to folivory by two-second instar *S. littoralis* caterpill-

lars. At this larval stage (0.8–1.0 cm long), caterpillars were considered suitable for experiments and placed in a clip cage to prevent movement along the plant. A clip cage was made of a Petri dish perforated and assembled on the plant without injuring it, to contain the leaf and two caterpillars. Every 2 days, the feeding caterpillars were replaced with new second-instar caterpillars to keep larval stage constant. Every 3 days, as soon as at least half the surface of the leaf was consumed, the clip cages with caterpillars were moved to a different leaf. For the *Spodoptera*-infested plants, herbivory continued during the entire experimental period. Control plants were grown under the same conditions as *Spodoptera*-infested plants, with empty clip cages attached around a leaf. Control and *Spodoptera*-infested plants were placed in an open experimental area, at a distance of 10 m between groups.

To test whether herbivores would consume different amounts on male and female plants, we performed an additional experiment with 20 male and 20 female *S. latifolia* plants. From each plant, one leaf was placed on a millimeter paper and photographed. Leaf area was calculated with ImageJ 1.33 software (Wayne Rasband, National Institute of Health, Bethesda, MD, USA). Then a clip cage was assembled on the plant, to contain the measured leaf and two-second instar caterpillars. After 2 days, the clip cage was removed and leaf area was calculated as above. Amount of herbivore damage was estimated as the difference between the leaf area before and after the herbivore treatment for male and female *S. latifolia* plants.

#### Flower morphology, flower number, and phenology of plants

We measured four floral traits (corolla diameter, petal length, petal width, and calyx height) fundamental for visual attraction of diurnal and nocturnal pollinators (Young, 2002) from male and female *Spodoptera*-infested and control plants. Measurements were taken from 16 control (number of flowers = 41) and 16 *Spodoptera*-infested female plants (number of flowers = 44) and from 32 control (number of flowers = 44) and 16 *Spodoptera*-infested male plants (number of flowers = 47) in late afternoon or early morning when flowers were open.

To estimate the effect of herbivory on flower number and phenology of male and female *S. latifolia* plants, we set up an experiment with 29 control female plants and 29 *Spodoptera*-infested female plants and 40 control male plants and 39 *Spodoptera*-infested male plants placed in the same experimental area, at a distance of 10 m between groups. Plants were infested with herbivores before the onset of flowering and herbivory treatment continued during the entire experimental period. Both treatments were monitored daily and the number of flowers was recorded from 23 May to 1 July 2013.

#### Estimation of female reproductive success

At the end of the flowering period, we recorded the number of fruits from female plants from the phenology experiment and counted and weighed seeds. Furthermore, we estimated the germination rate of seeds of control and *Spodoptera*-infested plants. Seeds were washed in a solution of sodium hypochlorite 5% (wt/vol) diluted 1:5 (obtaining a solution of ca. 1%) and then rinsed thoroughly with distilled water. After washing, seeds were scattered on wet sterile filter paper in Petri dishes (50 seeds per Petri dish, 10 Petri dishes) for a total of 500 seeds per treatment (50 seeds from each plant). Petri dishes were placed in a climate chamber (20 °C day period, 10 °C overnight) and every day, we took note of the number of germinated seeds up to the end of the experimental period (20 days).

#### Estimation of male reproductive success

To assess whether the number of male flowers is a good predictor of male reproductive success (pollen export to female flowers) and how the reduction in this number affects male reproductive success, we performed a pollination limitation experiment by manipulating the number of male flowers. Plants for this experiment were preventively placed in a net house, to avoid loss of pollen for males and pollination for female plants. After 1 week, we set up two treatments in two different experimental areas of the university campus: the first treatment consisted of a plot with three male and 10 female flowers (with three male and five female plants) and the second of a plot with 10 male and 10 female flowers (with three male and five female plants). For both treatments, we carried out 10 replicate plots (by inverting the experimental areas of two treatments for each replicate). During the experiment, plants were exposed to natural pollinators for 72 h. During this time, we kept the number of flowers constant, by removing newly developing buds, to ensure the maintenance of the initial conditions. At the end of both treatments, fruits and seeds from female plants were counted and used as indirect proxy of male siring success.

#### Statistical analysis

Floral phenology of control and *Spodoptera*-infested plants in both sexes was obtained by calculating the mean number of flowers produced daily with confidence intervals (95% CI). A significant difference in phenology was considered when the CI bars of two treatments were not overlapping. For all other analyses, where two independent samples were compared, t-test was used with the level of significance set to 0.05. All analyses were carried out in IBM SPSS Statistics v.21 (IBM-SPSS, Armonk, NY, USA).

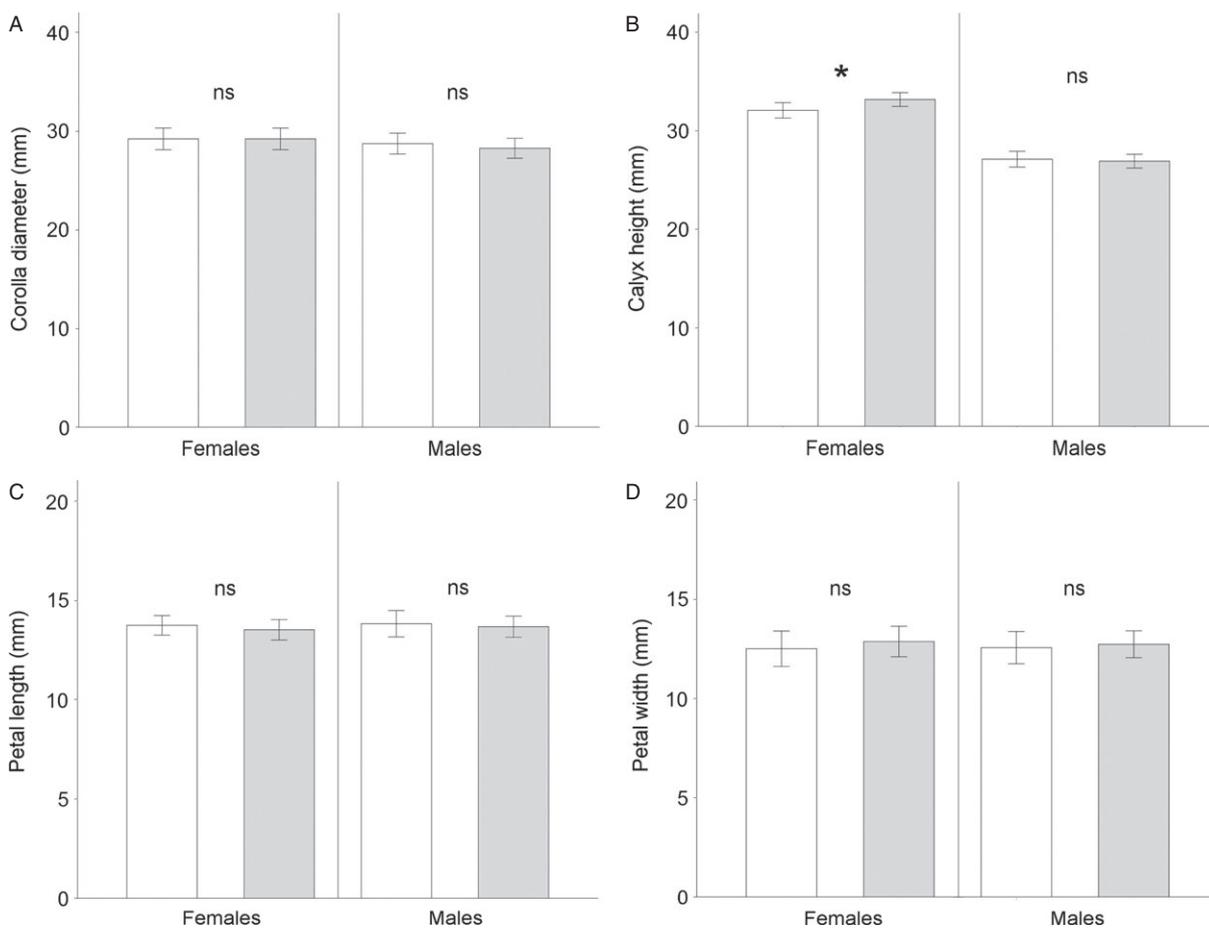
## Results

Herbivory did not affect floral traits, both in male plants (corolla diameter:  $t = 0.645$ ,  $P = 0.52$ ; petal length:  $t = 0.354$ ,  $P = 0.72$ ; petal width:  $t = -0.320$ ,  $P = 0.75$ ; calyx height:  $t = 0.381$ ,  $P = 0.70$ ; all d.f. = 87) and female plants (corolla diameter:  $t = 0.001$ ,  $P = 1.0$ ; petal length:  $t = 0.627$ ,  $P = 0.53$ ; petal width:  $t = -0.619$ ,  $P = 0.54$ ; all d.f. = 83), except marginally for calyx height in female plants ( $t = -2.100$ , d.f. = 83,  $P = 0.039$ ) (Figure 1A–D). The amount of leaf damage by herbivory was not significantly different between female and male plants (mean  $\pm$  SE damaged leaf area =  $1.86 \pm 0.27$  vs.  $1.97 \pm 0.18$  cm<sup>2</sup>;  $t = -0.772$ , d.f. = 27,  $P = 0.45$ ).

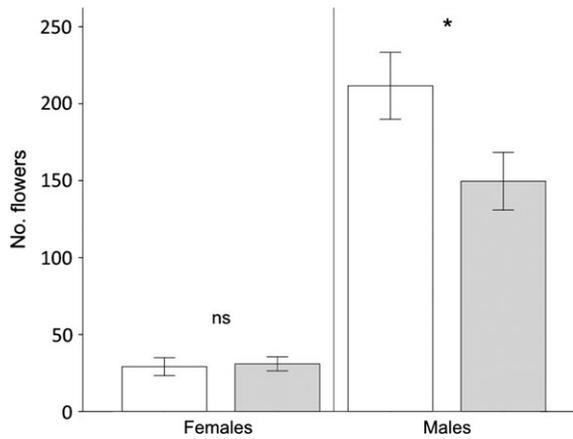
The number of flowers did not differ between control and *Spodoptera*-infested female plants ( $t = 0.492$ , d.f. = 77,  $P = 0.62$ ), whereas *Spodoptera*-infested male plants showed a significant decrease in flower number

compared to control male plants ( $t = -4.327$ , d.f. = 58,  $P < 0.001$ ) (Figure 2). Flower phenology showed four synchronic flowering peaks in both male and female plants, with significant differences between control and *Spodoptera*-infested male plants (Figure 3).

When we considered female reproductive success, herbivory did not affect number of seeds per fruit ( $t = 1.101$ , d.f. = 18,  $P = 0.29$ ), weight of seeds ( $t = -1.596$ , d.f. = 18,  $P = 0.13$ ), nor germination rate of seeds ( $t = -1.184$ , d.f. = 17,  $P = 0.25$ ) (Table 1). Experimental reduction in flowers (from 10 to three flowers) in male plants led to a significant decrease in number of pollinated flowers (i.e., fruits) in female plants ( $t = -3.708$ , d.f. = 18,  $P = 0.002$ ) (Figure 4A), whereas the number of sired ovules (seeds per pollinated flower) did not show a significant difference ( $t = 0.827$ , d.f. = 73,  $P = 0.41$ ) between treatments with 10 or three male flowers (Figure 4B).



**Figure 1** Floral traits—mean ( $\pm$  SE) corolla diameter, calyx height, petal length, and petal width—in control (white bars) and *Spodoptera*-infested (grey bars) female and male *Silene latifolia* plants. Herbivory does not affect floral display in female nor in male plants, with the exception of a marginally significant difference in calyx height (indicated with \*; t-test:  $P = 0.039$ ; ns, not significant).



**Figure 2** Mean ( $\pm$  SE) total number of flowers produced in control (white bars) and *Spodoptera*-infested (grey bars) female and male *Silene latifolia* plants during the flowering period (t-test: \* $P < 0.05$ ; ns, not significant).

## Discussion

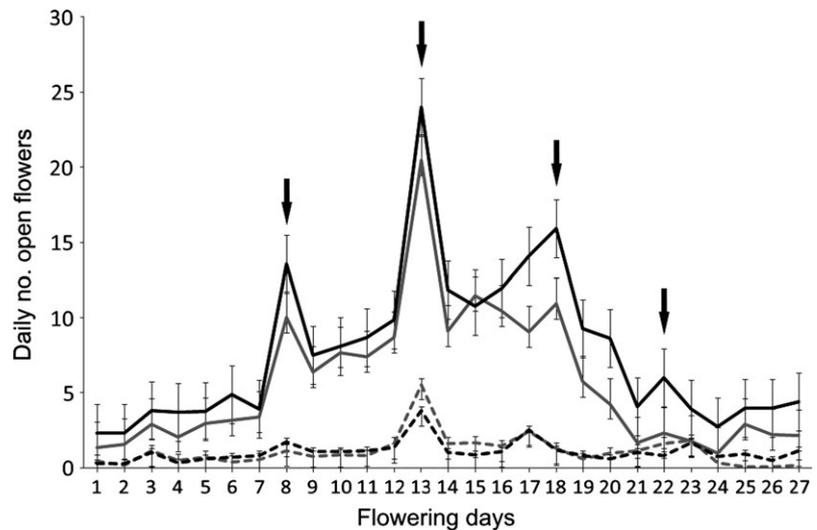
Herbivory has a direct effect on plant reproductive performance because the removed plant tissue and the resources allocated to defence reduce the potential amount of

resources allocated to reproduction (Bazzaz et al., 1987; Strauss et al., 2002; Zangerl & Berenbaum, 2009). However, investments in defence do not necessarily come at the expense of reproductive efforts (Bazzaz et al., 1987; Coley, 1988). In this study, we show that males and females of a dioecious plant respond differently to herbivory, suggesting sex-specific resource allocation strategies.

The production of the floral display is costly for a plant (Bond & Maze, 1999), so flower size and number of flowers can be affected by a reduction in allocable resources. In contrast with what has been reported for hermaphroditic plant species (Lehtilä & Strauss, 1999; Poveda et al., 2005; Barber et al., 2012), in our study, three of four investigated floral traits (viz., corolla diameter, petal length, and petal width) were not influenced by herbivory, neither in female nor in male plants. Only calyx height increased marginally in female infested plants, but this trait is not important for pollinator attraction (Klinkhamer & de Jong, 1990; Hirota et al., 2012).

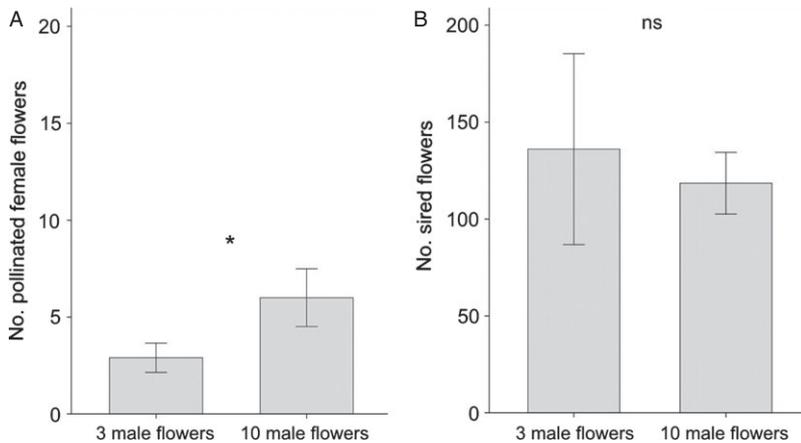
Although the timing of peak flowering does not change in herbivore-infested plants, we found a significant reduction in flower production only in *Spodoptera*-infested male plants. This effect was particularly pronounced after the main flowering peak. This response pattern is in agree-

**Figure 3** Phenology [mean ( $\pm$  95% confidence interval) daily number of open flowers] in control (black lines) and *Spodoptera*-infested (grey lines) female (broken lines) and male (solid lines) *Silene latifolia* plants recorded from 23 May to 1 July 2013. Arrows indicate main flowering peaks.



**Table 1** Female reproductive success estimation in terms of mean number and weight of seeds per fruits and germination rate of seeds in *Silene latifolia* plants

	Control	<i>Spodoptera</i> -infested	t	d.f.	P
No. seeds per fruit	167.35 $\pm$ 22.56	137.68 $\pm$ 14.71	1.101	18	0.29
Weight of seeds per fruit (g)	0.10 $\pm$ 0.03	0.14 $\pm$ 0.01	-1.596	18	0.13
Germination rate (%)	81.80 $\pm$ 6.47	90.67 $\pm$ 3.21	-1.184	17	0.25



**Figure 4** Mean ( $\pm$  SE) number of (A) pollinated flowers and (B) sired seeds per pollinated flower in treatments with 3 or 10 male flowers (t-test: \* $P < 0.05$ ; ns, not significant).

ment with sexual selection theory, which suggests that male individuals are selected for maximum pollinator attractiveness, i.e., the production of as many flowers as possible and maximum scent production (Willson, 1979; Waelti et al., 2009). As a consequence, in male plants, the reduction in allocable resources after an herbivore attack directly comes at the expense of flower production, this being the male main reproductive investment (Gross & Soule, 1981). This herbivory effect is particularly enhanced in our study system as *S. latifolia* is the only dioecious plant studied so far for which the cost of reproduction is higher for male plants rather than for female plants (Delph, 1999). Generally, in other dioecious species, female plants show higher reproductive effort associated with less growth, higher photosynthetic rates, delayed, or less frequent reproduction, earlier death, and spatial segregation (Obeso, 2002). In *S. latifolia*, although female plants acquire less carbon and allocate more biomass to reproduction (Gross & Soule, 1981; Gehring & Monson, 1994; Laporte & Delph, 1996), male plants suffer most in the competition for limited resources (Delph, 1999; Delph et al., 2005). Male plants exhibit a higher cost of reproduction probably due to a smaller vegetative and total biomass, less tolerance of high density, and limiting nutrients (Lawrence, 1963; Lovett Doust et al., 1987; Gehring & Linhart, 1993; Delph & Meagher, 1995; Delph et al., 2002), and shorter life than female plants (Lovett Doust et al., 1987; Carroll & Mulcahy, 1993; Gehring & Linhart, 1993; Taylor, 1994). Furthermore, the cost of reproduction is affected by fecundity selection that promotes high flower production in male plants at the expense of their growth and maintenance (Delph et al., 2005; Delph & Herlihy, 2012).

Although male reproductive success is notoriously difficult to quantify (Klinkhamer et al., 1994), we showed that the number of male flowers affects the number of polli-

nated flowers (fruits) and thus the overall number of sired seeds. Therefore, as expected, a reduction in flower number leads to lower male reproductive success. Interestingly, we found that the number of male flowers did not correlate with the number of sired seeds per pollinated flower. This is indirect evidence that pollen siring ability is not dependent on male flower number (as it likely depends on pollinator efficiency), because the number of sired seeds per fruit remains unchanged between treatments.

In our study, *S. littoralis* caterpillars inflicted the same damage to male and female plants. However, whereas herbivory reduces male reproductive success, female reproductive success was not affected by herbivory. In a recent study, Cozzolino et al. (2015) showed that *Spodoptera*-infested *S. latifolia* female plants had increased fruit production, likely mediated by increased production of pollinator-attracting floral scent compounds. This supports the idea that, in contrast to male plants, the reproductive effort is not yet maximised in female plants (Lloyd, 1980) and can be enhanced upon herbivore attack. Our finding highlights that female plants are more resistant than male plants in maintaining the reproductive success in the face of leaf herbivory and indirectly supports the general hypothesis of higher tolerance of female plants towards herbivory (Ashman, 2002).

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