

Benefits and costs to pollinating, seed-eating insects: the effect of flower size and fruit abortion on larval performance

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Abstract Plant–pollinator interactions are well-known examples of mutualism, but are not free of antagonism. Antagonistic interactions and defenses or counter-defenses are expected particularly in nursery pollination. In these systems, adult insects, while pollinating, lay their eggs in flowers, and juveniles consume the seeds from one or several fruits, thereby substantially reducing plant fitness. The outcome of such interactions will depend, for the plant, on the balance between pollination versus seed predation and for the larvae on the balance between the food and shelter provided versus the costs imposed by plant defenses, e.g., through abortion of infested fruits. Here, we examine the costs and benefits to the larvae in the nursery-pollination system *Silene latifolia/Hadena bicruris*. Using selection lines that varied in flower size (large- vs. small-flowered plants), we investigated the effects of variation in flower and fruit size and of a potential defense, fruit abortion, on larval performance. In this system, infested fruits are significantly more likely to be aborted than non-infested fruits; however, it is unclear whether fruit abortion is effective as a defense. Larger flowers gave rise to larger

fruits with more seeds, and larvae that were heavier at emergence. Fruit abortion was frequently observed (ca. 40% of the infested fruits). From aborted fruits, larvae emerged earlier and were substantially lighter than larvae emerging from non-aborted fruits. The lower mass at emergence of larvae from aborted fruits indicates that abortion is a resistance mechanism. Assuming that lower larval mass implies fewer resources invested in the frugivore, these results also suggest that abortion is likely to benefit the plant as a defense mechanism, by limiting both resources invested in attacked fruits, as well as the risk of secondary attack. This suggests that selective fruit abortion may contribute to the stability of mutualism also in this non-obligate system.

Keywords Flower number · Nursery pollination · Pollination mutualism · Pre-dispersal seed predation · *Silene alba*

Introduction

Plant–pollinator interactions are well-known examples of mutualism, but are not free of antagonism. Antagonistic interactions and defenses or counter-defenses are expected particularly in systems in which the adult pollinator lays its eggs in the flower and juveniles act as seed predators (nursery pollination; Dufaÿ and Anstett 2003; Kephart et al. 2006). For the plant, the outcome of this interaction will depend on the balance of pollination benefits versus seed predation costs. Similarly, fitness of the seed predating and pollinating insect will likely be affected by the quantity and quality of food provided by the developing fruit to its larvae, but also crucially by the plant's ability to control damage (Bronstein 1992; Holland et al. 2004b).

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Control mechanisms by the plant are indeed likely to have a large impact on fitness of both the plant and the insect if they affect growth and survival, and thus demographic rates of the seed predator.

Fruit abortion may represent such a control mechanism, as indicated by its occurrence in several nursery-pollination systems, where the plant aborts the attacked seeds or fruits. Beyond the effect of limiting the costs to a given plant (by limiting the investment of resources in infested fruits and by lowering the risk of attack of more fruits on the same plant), both selective abortion of infested fruits, and random abortion of fruits (i.e., abortion irrespective of whether flowers or fruits have eggs or not) can limit the population size of the pollinating and seed-predating insect (Holland and DeAngelis 2001, 2006; Westerbergh and Westerbergh 2001). In the well-studied case of the yucca/yucca moth interaction, different *Yucca* species employ different strategies to control damage: abortion of flowers with high loads of eggs or larvae (Addicott 1986; Pellmyr and Huth 1994; Richter and Weis 1995), reduction of seed number available to developing larvae by protecting some seeds from consumption (Ziv and Bronstein 1996), or larval starvation caused by a physical barrier that prevents larvae from reaching the fertilized seeds (Bao and Addicott 1998). In the senita cactus/senita moth interaction, reduction in damage is achieved by random fruit abortion, irrespective of egg/larval load (Holland et al. 2004a).

Unlike the yucca/yucca moth and senita cactus/senita moth associations (obligate mutualism; Fleming and Holland 1998), the interaction studied here between *Silene latifolia* and *Hadena bicruris* is not obligate and is therefore considered to be a basal form of nursery pollination (Dufaÿ and Anstett 2003; Bernasconi et al. 2009). However, there is evidence for potential specialization in this system, at least to some degree: the two species have similar geographic distributions, flowering and oviposition are synchronous (Biere and Honders 1996; Bopp and Gottsberger 2004; Wright and Meagher 2003), moths respond to specific scent compounds emitted by the flowers (Dotterl et al. 2006), and *H. bicruris* larvae grow better on *S. latifolia* than on other host species (Bopp and Gottsberger 2004). In the *S. latifolia/H. bicruris* interaction, experimental infestation of flowers with eggs of the seed predator leads to a significantly higher probability of fruit abortion compared to sham-manipulated fruits (Jolivet and Bernasconi 2006). It is therefore important to investigate whether, also in this less specialized system, fruit abortion in response to infestation is an effective control mechanism. This implies testing whether fruit abortion can reduce damage to the plant (i.e., serve as a defense mechanism by reducing costs of herbivory) and effectively lower larval performance in terms of growth or survival prospects (i.e., serve as a resistance mechanism by

reducing herbivore performance). In addition, both a plant's propensity to abort its fruits (Stephenson 1981), as well as larval performance on that plant, may depend on variation in flower size or in fruit size, and therefore variation in resource allocation per flower or per fruit should also be taken into account when studying the effect of fruit abortion on larval performance.

In this study, using artificial-selection lines that differ in flower size, we investigated the effects of: (1) fruit abortion, and (2) experimentally controlled variation in flower size (resulting in variation in fruit size) on larval performance in the *S. latifolia/H. bicruris* system (i.e., resistance). The use of these selection lines provided us with the variation needed to ensure the statistical power to assess the impact of flower or fruit size. Assuming that abortion serves as a defense against moth attack, we predicted that fruit abortion would reduce the damage to the plant by reducing the amount of resources invested in attacked fruits, resulting in a cost to larvae (i.e., lower larval performance) on aborted compared to non-aborted fruits. We further expected that large fruits would provide better resources for the developing larvae, or differ potentially in their attractiveness to ovipositing females, and that large-flowered plants might have a different propensity to abort their fruits than small-flowered plants. Consistent with the idea that abortion acts as an effective control mechanism in this non-obligate system, our results indicate that fruit abortion reduces the benefits to the larva (i.e., resistance). Assuming that lower larval mass at emergence implies a smaller investment of resources by the plant, this also suggests that fruit abortion thereby reduces the costs that the plant suffers by limiting investment in attacked fruits.

Materials and methods

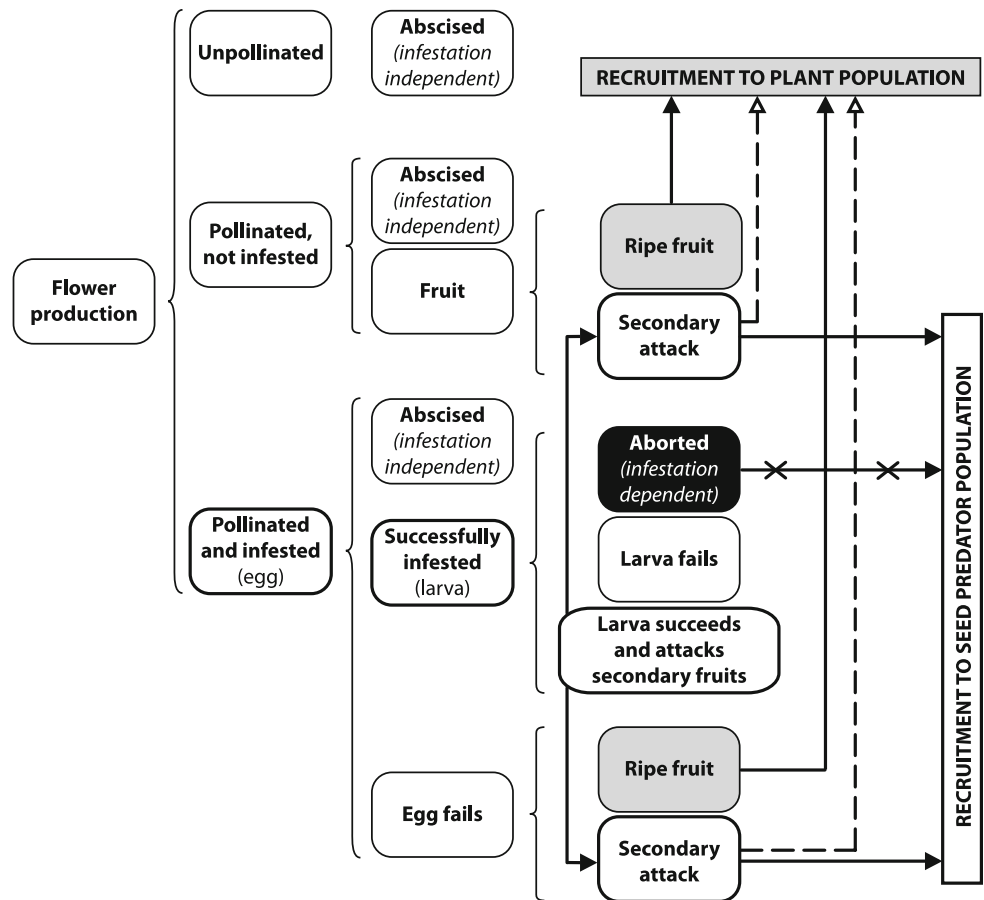
Study system

The white campion *Silene latifolia* (Poiret) [= *Silene alba* (Miller) Krauss, = *Melandrium album* (Miller) Garcke; Caryophyllaceae] is a short-lived perennial, dioecious plant native to Europe and found in disturbed habitats (Goulson and Jerrim 1997; Bernasconi et al. 2009). *S. latifolia* is dioecious and sexually dimorphic for several traits, including calyx width and floral display (Delph et al. 2002). Male plants carry more, but smaller and shorter-lived flowers than females (Carroll and Delph 1996; Meagher and Delph 2001; Young and Gravitz 2002). Fruits contain up to several hundreds of seeds (Jolivet and Bernasconi 2007). *S. latifolia* flowers from April to October; its white flowers open and start emitting scent at dusk (Jurgens et al. 1996). Nocturnal (moths) and diurnal (e.g., hoverflies) pollinators visit the plant

(Jurgens et al. 1996; Shykoff and Bucheli 1995; Van Putten et al. 2003; Young 2002). The moth *Hadena bicruris* Hufnagel (Lepidoptera: Noctuidae) is considered to be the main pollinator in the European native range (Brantjes 1976; Jurgens et al. 1996). This moth has a dual role as pollinator and seed predator (Fig. 1). Adult females of this moth lay usually a single egg inside or on a female flower (Brantjes 1976). After hatching, the first instar larva enters the fruit (primarily infested fruit). After consuming the developing seeds, the larva (usually fourth or fifth instar) leaves this primarily infested fruit through the enlarged entrance hole and starts feeding on other fruits on the same plant (secondarily infested fruits). On secondarily infested fruits larvae are often larger than the fruit, and parts of their body are thus exposed to parasitoids (Biere et al. 2002). Larval development from eclosion to pupation lasts around 3–4 weeks and larvae need several fruits (three to five) to complete development. The moth is present from May to October in most (over 90% in a recent survey; Wolfe 2002) European populations, with two or more overlapping generations per year (Elzinga et al. 2007), and high prevalence (i.e., often 50% or more of the fruits are attacked (Biere and Honders 1996; Elzinga et al. 2005; Wolfe 2002).

Plants respond to experimental egg infestation by increased flower or fruit abortion, which suggests that abortion may be a plant response to reduce damage (Jolivet and Bernasconi 2006). Indeed flowers infested with an egg were significantly and substantially (sixfold probability) more likely to abscise than sham-manipulated flowers (i.e., in which we inserted the toothpick in the flower without placing an egg), the latter having a rate of abscission of 0–2.6% (Jolivet and Bernasconi 2006). Also, among marked fruits of naturally pollinated plants that were dropped there was a significant excess of primary infestation compared to fruits which were not prematurely dropped (J. A. Elzinga and G. Bernasconi, unpublished data). Because of this, we refer to *fruit abortion* if it is known that the flowers are infested (as after experimental infestation in our study) rather than fruit abscission (a term that we use for non-infested fruits, see Fig. 1, or for fruits of unknown infestation status). In this we do not follow Stephenson (1981), who uses abscission for damaged fruits and abortion as a response to resource limitation, because it is not straightforward to distinguish the roles of damage versus resource limitation in determining the premature shedding of developing fruits in plant species that frequently face seed predation and may therefore be selected to produce an

Fig. 1 Schematic diagram of flower fates (pollination, infestation, infestation-independent abscission, infestation-dependent abortion, secondary attack) and their contribution to plant and seed-predator populations, based on the example of the interaction between *Silene latifolia* and the associated pollinating and seed-predating insect *Hadena bicruris*. Although larvae in aborted fruits can emerge, it is likely that most of them will perish. *Secondarily* attacked fruits may also abort, if they are attacked early during development. *Dashed lines* Secondarily attacked fruits may occasionally ripen and disperse a few seeds. Under resource limitation, flower and fruit fates will feedback on flower production



excess of flowers. Abortion in this system acts at the level of the fruit rather than of seeds. Fruits contain only one larva because a single egg is deposited (Brantjes 1976) or, in the rare cases of multiple oviposition, because of larval competition and cannibalism. We use the term *larval emergence* to describe the stage when the larva exits the primary fruit, after which it will try to locate secondary fruits. Although under natural conditions many larvae in aborted fruits probably perish, some may still emerge, but their chances to survive will depend on their success in locating secondary fruits to complete their development.

Plant material and rearing conditions

To simultaneously investigate the roles of fruit abortion and of fruit size (and provisioning) on larval performance, and of fruit size on the propensity to abortion, we conducted our experiment using plants derived from seeds arising from two artificial-selection programs (Delph et al. 2004). Using two American source populations, two replicate “large-flowered” (LF) lines and two replicate “small-flowered” (SF) lines were created by selection on calyx width (Delph et al. 2004). Because of a flower size vs. flower number trade-off, LF plants produce fewer flowers compared to SF plants (Delph et al. 2004). LF and SF selection lines were crossed within line type and between replicates in the generation preceding our experiments to eliminate inbreeding effects. The advantages of using selection lines originating from a common genetic background are that it avoids confounding variables that cannot be excluded with natural variation in flower size, and it provides clear-cut phenotypic differences thereby increasing statistical power. However, the flower sizes in these lines are within the range observed for natural variation (Delph et al. 2002). We germinated 20 seeds per family in Petri dishes filled with glass pearls and water, then transplanted the seedlings to Jiffy peat pellets. After 30 days, we potted the plants individually (pots with 16 cm³ of 1:3 sand:soil mixture; Tref-De Baat BF4, GVZ-Bolltec, Zurich), and kept them in a pollinator-free greenhouse [16 h light at 22°C and 8 h dark at 18°C, 45% relative humidity, natural daylight was supplemented by lamps (EYE Clean-Ace, 6500 K, 400 W; Iwasaki Electronics, Japan)].

Hand pollination and crosses

For crosses, we used eight LF full-sib families and eight SF full-sib families, and as far as possible used three females per family (2 lines × 8 families × 3 females = 48 females). Because some plants did not flower, we were able to cross 44 females [SF, (7 families × 3 females) + (1 family × 2 females) = 23 plants; LF, 7 families × 3

females = 21 females]. For pollen donors, we used males from the same selection line as the female, but from a different family, and as far as possible males were used only once. We used 37 males to pollinate the 44 females (SF, one male used in four crosses; LF, four males used in two crosses; all other 32 males used only in one cross). We brushed three anthers on the stigmatic surface, which ensures full seed set (A. Burkhardt and G. Bernasconi, unpublished data). On each female, we pollinated three replicate flowers (second, third and fourth flower produced, for 132 flowers pollinated). The first flower was left unpollinated because flower production shuts down very quickly if first flowers are pollinated, which suggests resource limitation of flower and seed production (Meagher and Delph 2001).

Estimation of fruit quality in LF and SF selection lines

We used one control fruit per plant (resulting from the pollination of the second flower) to estimate variation between selection lines and among individual plants in fruit size and seed provisioning. These estimates were then entered as covariates in the analysis of larval performance (see below). When the control fruit was ripe, we measured maximum fruit length and fruit width with callipers (precision 0.1 mm), and estimated fruit volume assuming an ellipsoid (volume = $1/6 \times \pi \times \text{width}^2 \times \text{length}$). We measured the total seed mass per fruit (milligrams), and assessed number of seeds and individual seed size with a seed counter equipped with an optical sensor (Elmor C3; Elmor Angewandte Elektronik, Schwyz, Switzerland). For each fruit we calculated mean seed size (micrometers) and we quantified the %C and %N (CHNS-932 analyzer; Leico, St. Joseph, Mich.) in a dried (24 h at 80°C) and ground (Mixer Mill MM 300; Qiagen, Basel) subsample of the seeds (2.7–3.3 mg of ground powder, to the nearest microgram; Mettler MT5, Greifensee, Switzerland). The seed C/N ratio was calculated as %C divided by %N for each fruit. Total C and total N were calculated as the %C or %N, respectively, multiplied by the total seed mass per fruit (milligrams). The rationale of measuring the C/N content of seeds, along with the above variables, was to dissect how variation in flower size (calyx width), which was the target of artificial selection (see “Plant material and rearing conditions”), affected different components of fruit size and seed provisioning, and whether such variation in allocation may be associated with larval performance and the propensity to abort.

Variation in the number of fertilized ovules per fruit and or fruit abortion may additionally depend on pollination quality. Although we used ample amounts of pollen in hand pollinations (see above), pollen of *S. latifolia* also varies in germination rates (Jolivet and Bernasconi 2007; Teixeira

and Bernasconi 2008). We preliminarily verified that there was no significant difference in the in vitro pollen germination rate of the SF and LF males used for pollination (Table 1), so that selection line differences in seeds per fruit or in the propensity to abort cannot be ascribed to variation among pollen donors, but represent differences among SF and LF female plants.

Assessment of larval performance and fruit abortion

To measure larval performance and plant defenses, we infested the third and fourth flowers produced on each female plant (hereafter “first infested fruit” and “second infested fruit”, respectively). Experimental infestation allowed us to examine the effect of flower size and fruit provisioning on larval performance and on the propensity to fruit abortion, whereas a study examining larval performance after natural oviposition could confound the effects of flower size and fruit provisioning, given that moths may choose particular flowers (e.g., the most profitable ones) for oviposition (Milinski 1997). Immediately after pollination we introduced a fertilized egg of *H. bi-cruris* in the corolla tube and carefully placed it close to the ovary using a wet toothpick, and bagged the flower to cage the larva when it emerged. Since the toothpick is much smaller than the inner diameter of the corolla tube, eggs could be placed very easily in both SF and LF flowers. We used eggs collected in a natural population near the campus of Lausanne University, Switzerland (46N31, 6E38). The eggs were stored for 1–3 days on a moist filter paper at 4°C before use. We could thus ensure that the eggs used for experimental infestation were fertilized, since fertilized eggs turn brown. Typically eggs hatch 3–4 days

after having been laid (Elzinga et al. 2002). Assignment of eggs to plants was randomized.

For each infested fruit (two fruits with larvae per plant, in eight cases only one larva developed) we recorded two components of larval performance: larval age at emergence (days since egg infestation), and larval mass at emergence (milligrams). We calculated larval mass gain as larval mass divided by larval age at emergence. We recorded fruit abortion, which can be recognized as a gradually extending abscission zone through the pedicel at the base of the fruit that ultimately causes the fruit to drop. When there was no sign of larval presence in the fruit 10 days after infestation (no frass extruding from the fruit), we crossed and infested an additional flower on the same plant, and so on, until we obtained two successful infestations per plant (up to a maximum of nine attempts). We counted the number of attempts necessary to achieve the first and the second successful infestation per plant (henceforth, “first infestation” and “second infestation”, respectively).

Statistical analyses

We ran generalized linear mixed models (GLMM) with family as a random factor (to account for repeated measures within full-sib families) on fruit-quality traits (measured in the control fruit), larval performance (measured in the first and second infested fruits) and on probability of abortion. Further, abortion and covariates (fruit quality, performance of first larva) were entered in more complete models where applicable and as described below. All initial models included the two-way interactions between fixed factors and the covariates. We applied a stepwise reduction procedure to GLMMs by deleting terms with $P > 0.06$, and we present

Table 1 Fruit and seed traits in small- (SF; $n = 23$ females) and large-flowered (LF; $n = 21$ females) selection lines of *Silene latifolia*

Plant trait	Selection line		Ratio of mean ^a	Test statistic	P
	LF	SF			
Number of seeds	335.90 ± 91.64	184.91 ± 54.58	1.82	$\chi^2 = 11.22$	<0.001
Fruit volume (mm ³)	869.69 ± 101.88	510.95 ± 124.18	1.70	$F = 33.06$	<0.001
Total seed mass (mg)	257.22 ± 62.95	122.10 ± 43.26	2.11	$F = 17.06$	<0.01
Total C (mg)	117.61 ± 28.91	55.97 ± 19.55	2.09	$F = 17.14$	<0.01
Total N (mg)	7.18 ± 1.87	3.43 ± 1.15	2.10	$F = 17.06$	<0.01
Mean seed size (µm)	1,822 ± 22	1,757 ± 32	1.04	$F = 5.15$	0.07
%C	45.69 ± 0.19	45.91 ± 0.31	1.00	$F = 2.56$	0.13
%N	2.80 ± 0.06	2.84 ± 0.11	0.99	$F = 0.52$	0.48
Seed C/N	16.35 ± 0.34	16.26 ± 0.57	1.01	$F = 0.28$	0.60
Pollen germination	19.24 ± 12.32	15.10 ± 9.39	1.27	$\chi^2 = 0.37$	0.54

Since these traits may additionally depend on the quality of the pollen used, we also show in vitro pollen germination of the pollen donor used in hand pollination (SF, $n = 20$ males; LF, $n = 17$ males). All analyses performed with generalized linear mixed models (GLMM) accounting for within-family repeated measures. Data are mean ± SD; SD was calculated on family means ($n = 15$ families)

^a Ratio of mean: LF/SF

the minimal adequate models and log-likelihood ratio tests (LLRT) in the results. For all models, where applicable, we transformed the variables to improve homogeneity of variances and normality of errors, or we corrected for overdispersion.

To investigate whether selection lines differed in fruit quality, we ran univariate GLMMs with selection line as a fixed effect, family as a random effect and normal errors for each of the response variables: log (fruit volume), total seed mass, total C and N content; proportion of C and N (all angularly transformed), C/N ratio in the seeds (inverse transformed), mean seed size. We assumed quasi-Poisson errors for number of seeds per fruit, and binomial errors for in vitro pollen germination. We ran a mixed-effects analysis of covariance to test for the effect of selection line and in vitro pollen germination (as a covariate) on the number of seeds per fruit.

To compare larval performance (mass and age at emergence) between selection lines and between plants that aborted or did not abort fruits, we ran univariate GLMM (using selection line and fruit abortion as a fixed factor, with family as a random factor) with covariates. Based on the examination of bivariate Pearson's correlations (Table 2) among fruit traits, we selected the following independent covariates: number of seeds per fruit, seed C/N, and the proportion of germinated pollen grains as control for pollen quality. The first and second larva were treated separately in different models. In the GLMM for performance of the second larva we additionally entered the performance of the first larva (mass, and age of first larva serving as a covariate for mass and age, of second larva, respectively).

We ran univariate GLMMs with binomial errors to investigate the correlates of fruit abortion. For abortion of the first fruit, the initial model included selection line and family, plus the following covariates: number of seeds per fruit, seed C/N, proportion of germinated pollen grains, number of infestation attempts, and larval mass gain of the first larva, and all two-way interactions. For abortion of the second fruit, the initial model contained selection line and fruit abortion of the first fruit as fixed factors, family as random factor, and the following covariates: seed C/N, larval mass gain of the first larva and total number of attempts to obtain both larvae, all two-way interactions between selection line and covariates, the interaction between selection line and total number of attempts, and that between selection line and abortion of the first fruit. Number of seeds per fruit and proportion of germinated pollen grains were not included as covariates in the second initial model because they did not significantly explain the variance in abortion of the first infested fruit. We analyzed all data using the R software version 2.6.2 (R Development Core Team 2006). Unless specified, data are given as mean \pm SD.

Results

Differences between selection lines in fruit quality

LF plants produced fruits with approximately twice as many seeds, and double the fruit volume, total seed mass, total C and total N as SF plants (Table 1). These variables were generally positively correlated (Table 2). LF plants

Table 2 Correlations between fruit and seed traits in *S. latifolia*

	Fruit volume	Number of seeds	Total seed mass	Total C	Total N	Seed C/N	Mean seed size
Fruit volume	1	0.582 <i>0.023</i>	0.662 ^a <i>0.007</i>	0.663 ^a <i>0.007</i>	0.652 ^a <i>0.008</i>	0.213 <i>0.446</i>	0.544 <i>0.036</i>
Number of seeds		1	0.948 ^a <i><0.001</i>	0.949 ^a <i><0.001</i>	0.943 ^a <i><0.001</i>	0.189 <i>0.501</i>	0.106 <i>0.708</i>
Total seed mass			1	1 ^a <i><0.001</i>	0.999 ^a <i><0.001</i>	0.091 <i>0.746</i>	0.374 <i>0.17</i>
Total C				1	0.999 ^a <i><0.001</i>	0.088 <i>0.756</i>	0.372 <i>0.172</i>
Total N					1	0.04 <i>0.888</i>	0.382 <i>0.16</i>
Seed C/N						1	-0.265 <i>0.34</i>
Mean seed size							1

Pearson's correlation coefficients (r) are given. Two-tailed error probabilities for the null hypothesis that $r = 0$ are highlighted in *italics*. See "Materials and methods" for definitions of variables. All correlations are calculated using family mean, $n = 15$ families

^a Significant after Bonferroni correction (nominal $\alpha = 0.017$, i.e., $0.05/28$ tests)

tended to produce slightly (+4%) larger seeds than SF plants. Selection lines did not differ significantly in the provisioning of individual seeds, i.e., seeds of both lines contained the same amount of C and N, and in similar proportions (there was also no significant difference in C/N; Table 1). Thus LF plants produced larger fruits with more seeds, and differed from SF plants in food quantity rather than food quality.

Effects of selection line, fruit abortion and fruit quality on larval performance

In agreement with the finding that LF plants produce larger fruits, larvae were significantly larger at emergence on LF than SF plants (Fig. 2). For both the first and second larva, mass at emergence was significantly affected by selection line (Table 3).

Importantly, we found also clear evidence that fruit abortion significantly decreases larval performance. First, at emergence the larvae on aborted fruits had reached less

than half the mass of larvae growing on non-aborted fruits (Fig. 2; Table 3). For the second larva, both the abortion of the previous infested fruit on the same plant (abortion of the fruit with the first larva) and the abortion of the fruit on which the second larva itself was growing had a significant effect on larval mass at emergence. If the first fruit had been aborted, the larva growing on the second fruit was smaller. Further, the second larva was significantly heavier, the higher the seed C/N ratio. Finally, fruit abortion also significantly affected larval age at emergence. Fruit abortion was the sole significant explanatory variable for larval age at emergence for both the first and the second larva (Table 3). Larvae emerged significantly earlier (approximately 3 days; Fig. 2) from aborted compared to non-aborted fruits. The larval mass gain over time (milligrams per day) was significantly smaller in aborted fruits (first, 6.28 ± 1.70 ; second, 6.12 ± 2.58) than in non-aborted fruits (first, 12.19 ± 3.74 ; second, 9.47 ± 3.42), and in SF fruits (first, 7.37 ± 1.21 ; second, 5.00 ± 0.87) compared to LF fruits (first, 13.13 ± 3.20 ; second, 10.41 ± 2.78).

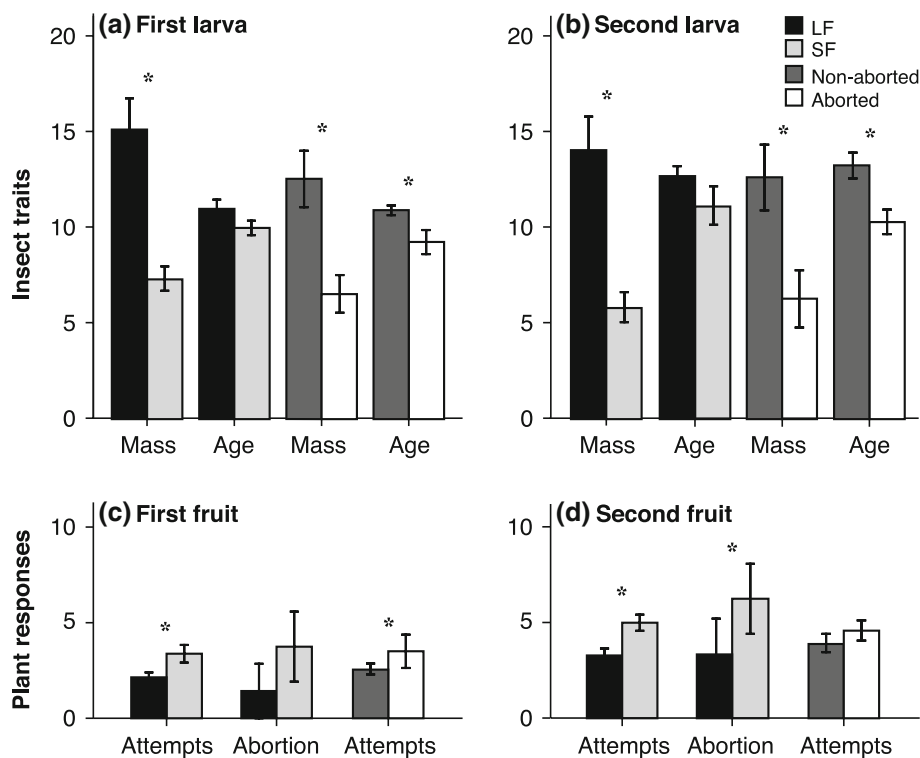


Fig. 2 Performance of *H. bicruris* larvae by selection line [small-flowered (SF); large-flowered (LF) *S. latifolia*] and by fruit abortion for **a** the first and **b** second larva per plant. Plant responses by selection line and fruit abortion for **c** the first and **d** the second infested fruit. Two fruits (first and second fruit) on each plant were infested (first and second larva). Performance was measured as: larval mass (mg) and larval age (days) at emergence from the primary fruit;

plant responses as proportion of infested fruits that were aborted ($\times 10$ to improve readability of the graph) and as the number of attempted infestations needed to obtain a successfully infested primary fruit (see “Materials and methods”). Data are mean \pm SE; SE was calculated on family means. Asterisk indicates significant (generalized linear mixed models; $P < 0.05$) differences between selection lines or between non-aborted and aborted fruits

Table 3 GLMM models for the effects of selection line, fruit abortion and covariates (fruit provisioning traits) on mass and age at emergence of two *H. bicurris* larvae per plant

Trait	Larva	<i>n</i>		Minimal adequate model			
		Plants	Families	Fixed effects	<i>df</i>	<i>F</i>	<i>P</i>
Mass	First	43	15	Selection line (SF vs. LF)	1, 13	13.15	0.003
				First fruit (aborted vs. not aborted)	1, 27	36.28	<0.001
	Second	35	15	Selection line (SF vs. LF)	1, 13	9.36	0.009
				Second fruit (aborted vs. not aborted)	1, 17	11.14	0.004
				First fruit (aborted vs. not aborted)	1, 17	8.59	0.009
				Seed C/N	1, 17	6.3	0.023
Age	First	43	15	First fruit (aborted vs. not aborted)	1, 27	25.79	<0.001
				Second fruit (aborted vs. not aborted)	1, 19	28.8	<0.001

Minimal adequate models are given after stepwise reduction. For abbreviations, see Table 1

Effect of selection line on infestation failure and predictors of fruit abortion

For the establishment of the first larvae, significantly more infestation attempts were needed (i.e., more flowers had to be infested with an egg until we observed a fruit with extruding excrements, as a sign of successful infestation) in SF than LF plants [Fig. 2; LLRT: deviance difference between a model with selection line and the constant-only model = -2.92 , $P(\chi_1^2 > 5.83) = 0.02$]. In addition, for the first infested fruit, plants that aborted their fruit also needed more attempts until there was a successful infestation [Fig. 2; LLRT: deviance difference between a model with fruit abortion and the constant-only model = -5.33 , $P(\chi_1^2 > 10.66) = 0.001$]. However, there was no significant

difference in number of attempts between plants aborting versus not aborting their second infested fruit.

In complete GLMMs, abortion of the first fruit was significantly explained only by the larval mass gain (slowly growing larvae in aborted fruits, fast-growing larvae in non-aborted fruits, either because fast-growing larvae were more likely to escape abortion, or because abortion reduces the intake of resources and slows down growth). The probability of abortion of the first fruit increased marginally with increasing C/N ratio in seeds (Table 4). Selection line and all other covariates (including pollen germination) did not significantly affect the probability of abortion of the first fruit and were eliminated during model simplification. Abortion of the second fruit was significantly affected by the interaction between selection line and abortion of the

Table 4 Minimal adequate GLMM models for the predictors of fruit abortion in response to infestation with *H. bicurris* eggs

Response	<i>n</i>		Model comparisons						
	Plants	Families	Model	Fixed effects	Effect size	Deviance	<i>df</i>	Deviance difference	Likelihood ratio test
Abortion first fruit	43	15	Minimal model (III)	Mass gain first larva	-0.44			I-III: -10.56	$P(\chi_1^2 > 21.14) < 0.001$
				Seed C/N	0.62	-16.91	4	II-III: 1.87	$P(\chi_1^2 > 3.75) = 0.052$
			Model (II)	Mass gain first larva	-	-18.78	3		
			Model (I)	Seed C/N	-	-27.47	3		
			Null model (0)	Constant only	-	-27.52	2	0-III: -10.61	$P(\chi_2^2 > 21.23) < 0.001$
Abortion second fruit	35	15	Minimal model (II)	Selection line (SF)	1.85	-22.94	5		
				First fruit (aborted)	2.69				
				Line × first fruit (aborted)	-3.65			I-II: 2.59	$P(\chi_1^2 > 5.17) = 0.023$
			Model (I)	Selection line (SF)	-	-20.35	4		
				First fruit (aborted)	-				
Null model (0)	Constant only	-	-24.13	2	0-II: -3.78	$P(\chi_3^2 > 7.56) = 0.056$			

Factor levels for which the estimates are given are shown in parentheses. For abbreviations, see Table 1

first fruit (Table 4). When the analysis was repeated separately for LF and SF plants to dissect the interaction, in LF plants the second fruit was significantly more likely to be aborted if the first fruit had been aborted [LLRT: deviance difference = -2.73 , $P(\chi_1^2 > 5.46) = 0.019$], but not significantly so in SF plants [LLRT: deviance difference = -0.39 , $P(\chi_1^2 > 0.78) = 0.38$].

Discussion

In nursery pollination, the pollinator acts as a seed predator during its larval stage (Dufay and Anstett 2003; Kephart et al. 2006; Westerbergh 2004). This should lead to plant defenses to reduce the costs imposed by seed predation. A potential defense is the ability to abort attacked fruits, as in yucca/yucca moth (Addicott 1986; Pellmyr and Huth 1994; Richter and Weis 1995) or senita cactus/senita moth (Holland and Fleming 1999; Holland et al. 2004a). The *Silene latifolia/Hadena bicruris* interaction is not obligate and is potentially less specialized than that between yucca/yucca moth and senita cactus/senita moth (Dufay and Anstett 2003). However, here too it was recently found that experimental infestation with seed predator eggs significantly increased the probability of fruit abortion (Jolivet and Bernasconi 2006), and that under natural pollination and infestation, individually marked fruits which had been dropped by the plant were significantly more likely to have been infested compared to fruits of the same age that had not been dropped (J.A. Elzinga and G. Bernasconi, unpublished data). These findings suggest that fruit abortion may also be an effective control mechanism in this non-obligate system. However, to be effective as a defense, abortion of infested fruits needs to reduce the costs of predation to the plants, and lower the profitability to the larvae, so as to ultimately limit survival and reproductive rates of the seed predator (Shapiro and Addicott 2004; Holland et al. 2004b).

Our results clearly demonstrate that fruit abortion reduces larval mass and age at emergence, and thus strongly suggest that it is effective in imposing a reduction in fitness of the seed predator that is likely to benefit the plant. Enemy attack (e.g., predators, parasitoids) may be more likely (and thus larval survival lower) if the larva emerges at an earlier age from the primary fruit (where the egg was laid), because larvae that emerge at a smaller size and earlier age likely need to locate a larger number of secondary fruits to complete development than larvae that can develop further in a non-aborted, primary fruit. In our study, larval mass at emergence from aborted fruits was less than half the mass of larvae emerging from non-aborted fruits. Leaving earlier and at smaller size from the primary fruit implies increased risks, since the primary fruit

grants protection from parasitoids (Awmack and Leather 2002; Benrey and Denno 1997; Biere et al. 2002). At least 14 parasitoid species have been described from *H. bicruris* larvae (Elzinga et al. 2007), most of which attack the larvae at instar L4 or L5 (Elzinga et al. 2007). Lower larval survival as a consequence of fruit abortion may be reinforced by the fact that aborted fruits are dropped away from the plant, thus reducing the probability of secondary attack for other fruits on the same plant. In this study we measured larval performance and did not directly address effects on plant fitness. However, it seems reasonable to assume that the plant loses fewer resources in aborted, infested fruits (producing a larva with a smaller mass at emergence) than in non-aborted, infested fruits. A low mass at emergence may reduce larval survival but also adult fecundity. Indeed, larval growth rate is positively correlated with adult fecundity in several insect species (e.g., Awmack and Leather 2002; Honek 1993; Kause et al. 1999). It would thus be very interesting in future studies to quantify how fruit abortion translates into reduced energetic costs and risk of secondary attack for the plant, and into lower moth survival and fecundity, and as a result lower abundance of the seed predator (Holland and DeAngelis 2002; Holland et al. 2004b; Westerbergh and Westerbergh 2001).

We found that infesting larvae were influenced by previous abortion: if the first larva had been aborted, the second larva was significantly smaller (compared to second larvae on plants that did not abort their first larva). This suggests a difference in defense or allocation in plants that were previously exposed to attack and aborted their fruit, compared to plants that were exposed but did not abort the fruit containing the first larva. A previous investigation asked whether infestation with *H. bicruris* eggs induced plant responses for fruit-wall thickness, seed mass and C/N content (as measures of allocation), but no significant differences were found between induced and non-induced plants (Jolivet and Bernasconi 2006). Thus the reduced larval growth observed in the present study on plants that previously aborted attacked fruits may be mediated by other mechanisms, including the production of chemical defenses that may be toxic, or a reduction in the digestibility of seeds (Mattson 1980). Both allocation of resources to developing fruits (Klüber and Eckert 2004) and how this is modified by abortion in response to attack may change in the flowering sequence and with increasing levels of attack. In our study, we examined one non-infested control fruit and analyzed larval performance in two additional successfully infested fruits per plant, which required an attempted infestation of up to nine flowers per plant. However, during one season *S. latifolia* females will usually produce more than three fruits. In a study exposing plants from the same selection lines as in this experiment (see “Plant material and rearing conditions”) to natural

pollination and seed predation, each plant produced 4.5 ± 0.4 (mean \pm SE) flowers per week in the LF line, and 6.9 ± 0.5 in the SF line. Of these flowers, a mean of 12.4% (i.e., roughly one flower per week) were primarily attacked in the LF, and 15.1% in the SF lines (difference between lines not significant; A. Burkhardt, L. D. Delph, G. Bernasconi, unpublished data). Thus, although the number of infested fruits we examined in our study is within the range of natural infestation levels, future work is required to investigate the effects of abortion on larval performance over the entire season or plant life cycle.

Plants more likely to abort fruits also exhibited significantly lower egg survival, as reflected in the fact that significantly more attempted infestations were needed to successfully infest the first fruit. This suggests that plants with a higher propensity to fruit abortion are also more likely to prevent eggs from developing, or very young larvae from establishing themselves. Since our experimental plants stemmed from controlled crosses under greenhouse conditions, it is possible that the association between the number of attempted infestations and fruit abortion may reflect at least some genetic variation in defense; however, the mechanistic basis for this remains to be elucidated.

We found no significant difference between SF and LF lines in their propensity for fruit abortion. However, the difference was in the direction of abortion and also failed infestation being more likely, albeit not always significantly so, in the SF line, possibly reflecting the fact that abortion of the smaller SF fruits may lead to lower costs to the plant (Wright and Meagher 2003).

We found strong evidence that LF plants, which produced fewer, larger fruits with twice as many seeds, provided a better food source for larvae (larvae growing on LF fruits reached twice the mass at emergence compared to larvae growing on SF fruits). Since food quantity and quality affect larval growth (Mattson 1980; Wheeler and Halpern 1999) and adult fitness in insects (Awmack and Leather 2002), moths may be selected to oviposit in plants that offer better resources to their offspring (Thompson and Pellmyr 1991). Oviposition choice based on food quality or quantity has been shown in the Lepidoptera *Tyria jacobaeae* (Vandermeijden et al. 1989) and *Euphydryas editha monoensis* (Singer et al. 1988). In our study system, oviposition choice for more profitable flowers may be reinforced because a larva growing in a larger primary fruit may need fewer secondary fruits to complete its development. Indeed, oviposition was found to decrease with decreasing flower and ovary size in naturally occurring *S. latifolia* (Biere and Honders 2006). Similarly, *Hadena compta* moths prefer to lay eggs in *Dianthus sylvestris* plants with large perfect flowers compared to plants with small pistillate flowers (Collin et al. 2002). However,

although such preferences may clearly be important under natural conditions, by using experimental infestation, our design specifically highlights the effects of fruit size and abortion on larval performance and avoids confounding by choice behavior—i.e., if the insect would choose specific plants for oviposition (for instance those with low propensity to abort) this would mask the effects.

On the other hand, flower size may evolve in response to selection imposed by the behavior of the seed predator, which deserves further investigation. In particular, flower size is known to trade off with flower number in *S. latifolia* (Delph et al. 2004). Large floral displays attract more pollinators, but they also bring in more pollinator-borne pathogens (Shykoff and Bucheli 1995) and may increase the risk of receiving eggs (e.g., Collin et al. 2002; Biere and Honders 2006). Hence, interactions with pollinators, pathogens, and seed predators, as well as the positive relationship between seed number and flower size, are likely to interact in terms of selecting for or against large or small flowers in females. Moreover, in dioecious species like *S. latifolia*, male and female plants may evolve sexually dimorphic responses to biotic interactions.

In conclusion, this study demonstrates that fruit size is an important determinant of larval growth, and that fruit abortion reduces the mass and age at emergence of juvenile seed predators. From the lower mass of larvae in aborted than non-aborted fruits we can infer that abortion likely reduces the amount of resources that the plant invests in attacked fruits and thus the costs it suffers. Since the smaller larvae emerging from aborted fruits are most likely to perish in the search for additional fruits to complete development, this strongly suggests that fruit abortion is effective both as resistance and defense in this system, and may contribute to the maintenance of balanced costs and benefits also in this non-obligate, less specialized association.

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