

Female Preference and Offspring Performance in the Seed Beetle *Gibbobruchus bergamini* Manfio & Ribeiro-Costa (Coleoptera: Chrysomelidae): A Multi-Scale Comparison

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Abstract

The search for and choice of oviposition sites are a key step in the life cycle of herbivorous insects. Theory predicts that natural selection should favor the discrimination ability of female insects to select between high- and low-quality oviposition sites. However, correlation between female preference and offspring performance is apparently lacking or even negative in some herbivore-plant systems. A possible explanation for this seeming failure is that most studies have focused on a single factor and spatial scale. Here, we investigated the preference-performance relationship in the seed beetle *Gibbobruchus bergamini* Manfio & Ribeiro-Costa (Coleoptera: Chrysomelidae). We took into account several potential factors affecting oviposition choices and larval survivorship through a multi-level approach. Hierarchical analysis that controlled for the non-independence of observations demonstrated that oviposition site choices were not related to the factors that most influenced larval survivorship. The apparent effects of other pod-feeding herbivores were greater at the plant and branch scales while at the pod level the most important factors were plant-related variables. Oviposition choices seemed to be time-constrained, meaning that females have little opportunity to further increase offspring performance through additional compensatory choices.

Introduction

The feeding activity of holometabolous insects occurs mostly during their larval stage (Boggs 2009). However, insect larvae usually have little mobility and thus are severely constrained in their ability to select their food and avoid natural enemies and potential competitors. Consequently, the search for and choice of oviposition sites by female insects are a key step in their life cycle (Bernays & Chapman 1994, Campbell & Runnion 2003, Lancaster *et al* 2010, Refsnider & Janzen 2010). This holds especially true for herbivore species in which the whole larval stage is spent in the specific resource selected by the female, such as galling insects (Price *et al* 1987), leaf-miners (Hespenheide 1991), and pre-dispersal seed predators (Janzen 1971). It is expected, therefore, that natural selection favors the discrimination ability of females

to select between high- and low-quality oviposition sites (Jaenike 1978, 1990, Thompson 1988), given the environmental and phylogenetic constraints to which their decisions are restricted.

A positive correlation between female preference and offspring performance has been reported in many studies (Brodbeck *et al* 2007, Santos *et al* 2008, Gripenberg *et al* 2010); however, for some species, this correlation is apparently lacking or even negative (Jaenike 1990, Mayhew 1997, 2001). A possible explanation for this apparent failure in the expectations of the optimal oviposition theory (Jaenike 1978) is that most studies have focused on a single factor and spatial scale (Mayhew 2001). Earlier studies, for instance, used to evaluate only the role of bottom-up processes, such as defensive compounds and plant nutritional quality in host plant selection. More recently, however, some studies have

also addressed interactions with predators and parasitoids (Fabrikschleischach 2005, Pöykkö 2011) as well as herbivore-herbivore interactions (Kaplan & Denno 2007).

In addition, the importance of different factors can be scale-dependent, meaning that the overall resource quality varies from patches (Gonz *et al* 1995, Janz *et al* 2005), to individual plants in these patches (Heisswolf *et al* 2005, Cornelissen & Fernandes 2008) and even between parts of an individual plant (Cornelissen & Fernandes 2008, Flaherty & Quiring 2008). Therefore, the decisions of a female insect can be influenced by the relative importance of resource aggregation at different scales (Rabasa *et al* 2005, Gripenberg *et al.* 2007).

For insects that feed and develop inside their host plants, the choice of a suitable resource is even more important, since their larvae usually cannot change a bad choice made by the female. Endophagous insects in flowers, fruits, or seeds, for example, are unable to avoid the negative effects of other herbivores confined to the same resource unit (Toquenaga & Fujii 1990, Fox *et al* 1996). This is the case for the seed beetle *Gibbobruchus bergamini* Manfio & Ribeiro-Costa, a seed beetle species of the *scurra* species group from Central Brasil (Ribeiro-Costa *et al* 2014). The larvae of this species feed exclusively on seeds of the genus *Bauhinia* (Manfio *et al* 2013), and often co-occur with other seed beetles and microlepidopteran larvae on their host plants (Bergamini pers. obs.). Females of *G. bergamini* lay their eggs in the pod walls, and the larvae penetrate the pod and feed inside the seeds. Each larva develops inside a single seed, where it spends its entire larval and pupal stages, emerging as an adult beetle (Bergamini pers. obs.). The pods are not a homogeneous resource, since their spatial aggregation and quality may vary at different spatial scales. The position of the pods in the inflorescence axis, for example, can be a good indicator of their probability of early abortion (Ostergård *et al* 2007). Besides the variation due to plant traits, abiotic factors, such as sunlight exposure, can also affect the quality of seeds to the beetles (Traveset 1991). Here, we consider the effects of various factors in different levels of organization, such as plant size and the presence of other herbivores on the same pods, on both oviposition patterns and offspring performance in this seed beetle species (a summary of the factors considered and their expected effects is presented in Table 1).

The aim of this study was to assess the oviposition pattern and larval survival in the seed beetle *G. bergamini* to evaluate whether oviposition site choices maximize offspring survival. Both egg distribution and larval survival were analyzed with respect to the influence of multiple potential determinants of resource quality at different levels of resource aggregation. Specifically, this study tested the hypothesis that the factors that mostly affect larval survival will also be the most important ones for oviposition site choice by females of

G. bergamini. If this hypothesis is correct, then egg distribution and larval survival must be similarly influenced by the selected explanatory variables.

Material and Methods

Study system

This study was conducted in an area of Cerrado vegetation in the municipality of Hidrolândia, state of Goiás, Brazil (16°59' 29"S, 49°15'10"W). This region has a climate type Aw according to Köppen's classification, with a well-defined rainy season from October to April and a dry season from May to September. The local vegetation comprises a mosaic of savanna-like vegetation (Cerrado *sensu stricto*), riparian forests, and dry forest fragments embedded in an agricultural matrix, consisting mostly of cattle pasture. The Brazilian Cerrado harbors the most diverse flora among the world's savannas, with more than 11,000 vascular plant species, of which Fabaceae is the largest plant family.

A plot of 2025 m² (45×45 m) was established on a small hill, within which all individuals of *Bauhinia curvula* (Fabaceae) (>1 m of height) were mapped in a Cartesian coordinate system. *B. curvula* is a common host plant of the beetle *G. bergamini* in Cerrado areas of the central region of the state of Goiás. This plant species is a shrub with a flowering peak between May and June and a fruiting period lasting until the middle of August in the studied region. Each female can lay up to 50 eggs, which are laid singly and spread among several pods (Bergamini pers. obs.) and are distinctly round and with no visible ornamentations. Besides *G. bergamini*, unidentified lepidopterans and the seed beetle species *Gibbobruchus speculifer* (Gyllenhal) are also commonly encountered in *B. curvula* pods. While most insect species lay their eggs at the beginning of the fruiting period of *B. curvula*, the eggs of *G. bergamini* are mostly found when the pods are mature at the end of the fruiting period. Therefore, *G. bergamini* females are able to choose pods and plants with more or less clear signs of their vigor (e.g., branch number, number of pods), including those that were previously chosen by other herbivorous insects and have signs of herbivory.

Sampling

In order to evaluate whether *G. bergamini* females assess host plant quality for their offspring, possible determinants of plant quality were recorded at three levels of resource aggregation: individual plants within patches, branches from the same plants, and pods from the same branches, hereafter, "plant level," "branch level," and "pod level," respectively (Table 1). At the plant level, the variables recorded were: (1)

Table 1 Measured variables and their expected and recorded effects on oviposition site choices by *Gibbobruchus bergamini* females and on offspring performance.

Levels	Variables	Description	Expected and recorded effects on oviposition site choice		Expected and recorded effects on offspring survivorship	
Plant	Isolation	The weighted area of the polygon encompassing all points nearer to the focal point than to any other plant	↓	Null	None	—
	Reproductive branch number	Number of inflorescence-bearing branches	↑	↑	None	—
	Pod number	Number of pods	↑	Null	None	—
	Density of other pod-feeding herbivores	Proportion of pods with signs of lepidopterous larvae, <i>Gibbobruchus speculifer</i> , or chewing marks	↑/↓	↑	↑/↓	↑
	Sunlight exposure	A binary variable indicating whether the focal plant is entirely exposed to sunlight (1) or at least partially covered by another plant's canopy (0)	↓	Null	↓	↓
Branch	Pod number	Number of pods on the branch	↑	Null	None	—
	Presence of other pod-feeding herbivores	Presence of pods with signals of lepidopterous larvae, <i>Gibbobruchus speculifer</i> , or chewing marks on the same branch	↑/↓	↑	↑/↓	Null
Pod	Pod position	Position of the flower originating the pod within the raceme; more basal pods with lower position numbers	↓	↓	↓	Null
	Development stage	A categorical variable indicating pod development stage with four levels: 1—unexpanded seeds, less than 5 cm; 2—unexpanded seeds, greater than 5 cm; 3—expanded seeds, green tegument; 4—ripe pod	↑	↑	None	—
	Presence of other pod-feeding herbivores	Presence of signs of lepidopteran larvae, chewing marks, or <i>G. speculifer</i> eggs in the same pod	↑/↓	Null	↑/↓	↓

↓ = negative effect; ↑ = positive effect; none = not expected to have any effect; — = not tested; null = no effect recorded.

reproductive branch number, (2) pod number, (3) isolation from conspecific plants, and (4) the direct exposure of the plant to sunlight. Plant isolation was measured through the standardized weighted area of the Dirichlet polygon (i.e., a polygon in the Cartesian plan containing all the points that are closer to the focal plant than to any other plant). The greater the number and proximity of neighbors a plant has, the smaller the area of the polygon. The R package *deldir* was used to calculate the Dirichlet tessellation. The plants were classified in two broad categories according to the presence or absence of a canopy above them: (1) entirely exposed and (2) partially exposed to sunlight. Sunlight exposure was determined by the presence of larger trees near the plants. The size of the plants had no influence on the probability of being entirely exposed to sunlight (logistic regression: z value = 0.992, $p = 0.32$).

At the branch level, the following variables were recorded: (1) the presence of pods with the congeneric seed beetle *G. speculifer*, (2) the presence of pods with signs of lepidopteran larvae attack (seen as a hole in the pod wall with silk and frass), (3) the presence of pods with other signs of herbivory (such as scars and chewing marks), and (4) the number of pods. At the pod level, the variables taken were: (1) pod position on the branch, (2) pod development stage, (3)

the number of *G. speculifer* eggs, (4) the presence of lepidopteran holes, and (5) the presence of other signs of herbivory. The pod position on the branch was determined by counting the number of flower scars or pods, starting at the base of the inflorescence. The pod development stage was categorized according to the pod length and aspect (see Table 1 for further details).

All pods of the mapped individuals were marked and had the above-cited variables measured on two occasions, one in the middle (May 23, 2011) and the other at the end of the fruiting period (June 26, 2011). All mature pods were collected on July 1 and August 3. These pods were measured and kept in the laboratory. After 2 months, when all insects had emerged, the pods were dissected and all seeds were sorted into six categories: (1) aborted seeds and/or unfertilized ovules, characteristically small; (2) undeveloped seeds, expanded seeds with a “shrunk” aspect; (3) healthy seeds, with no signs of damage; (4) seeds attacked by *G. speculifer*, with the characteristic beetle puparia; (5) seeds attacked by *G. bergamini*, with the exit holes; and (6) seeds attacked by lepidopteran larvae, totally consumed and with frass and silk. Pod walls were inspected for the number of eggs and signs of larval penetration and adult emergence holes. The condition of the

pod wall under oviposition was also noted, since eggs were sometimes laid on damaged areas on the pod surface.

Data analysis

The importance of the above-cited explanatory variables for oviposition site selection was estimated through hierarchical models that controlled for non-independence of observations from the same plant individual or branch. These models were built to mimic the sequence of events that supposedly occur during the selection of oviposition sites; i.e., females first locate an individual plant within a patch, and then proceed to choose a landing branch within a plant, and finally a specific pod for oviposition. Therefore, the hierarchical models were structured according to the adequate spatial scale, including the pertaining variables.

At the plant level, a hurdle model that first estimates the probability of occupation and then estimates the number of ovipositions in a given plant was adjusted. This hurdle model takes into account the fact that even suitable plants may not be used if they were not found by *G. bergamini* females. Also, it was assumed that some plant traits affect the probability of at least one oviposition event for each individual plant, while the others affect the number of oviposition events in those plants where ovipositions do occur. In this way, the model comprises a binomial process related to plant conspicuity and a counting process related to host plant quality. Since each egg-bearing pod is the result of at least one oviposition event, the response variable in this model is the number of pods with eggs, i.e., the minimum number of oviposition events. The effect of the difference in pod numbers between the plants was accounted for by the inclusion of the pod number as an offset variable in the model. For the binomial process, the variables chosen to represent differences in the conspicuity of the plant were: number of reproductive branches, plant exposure to sunlight, and plant isolation. For the count process, a negative binomial link function was used due to the great number of plants with no oviposition. The predictor variables were the plant's exposure to sunlight and the proportion of pods with other herbivores: the congeneric seed beetle *G. speculifer*, lepidopterans larvae, and other signs of herbivory.

At the branch level, a generalized linear mixed model (GLMM) with the number of *G. bergamini* eggs per branch as the response variable was adjusted. The number of pods and the presence of other herbivores were treated as fixed variables, whereas plant identity was adjusted as a random variable in order to control for the non-independence of branches on the same individual plant. Since the response variable is a counting process, the model was adjusted with a Poisson distribution function and a log link function.

Finally, at the pod level, a GLMM with the number of *G. bergamini* eggs as the response variable was adjusted. In

this analysis, pod position, pod development stage, Lepidoptera presence, herbivory signs, and number of *G. speculifer* eggs were treated as fixed effects. The identity of branches, nested by plants, was used as a random effect. For the same reasons listed above, a Poisson distribution function and a log link function were used here.

The effects of the predictor variables on the survivorship of the larvae were estimated with GLMs, since the number of replications obtained was not sufficient for the estimation of plant and branch random effects (pods per branch = 1.289 ± 0.727 , mean \pm SD; pods per plant = 2.231 ± 1.681). The survivorship was measured in two stages: from egg to larvae and from larvae to the adult stage. One model was adjusted to each survivorship stage. For the egg to larvae survivorship, a quasi-Poisson model was used, where the response variable was the number of penetration holes, with the number of eggs as an offset variable. The proportion of *G. bergamini* eggs laid in damaged pod areas and sunlight exposure were used as predictor variables. For the larvae to adult model, the response variable was the number of adult emergence holes, with the number of penetration holes as an offset variable. The predictor variables tested were the number of "empty" (undeveloped) seeds, the number of aborted seeds, the number of seeds consumed by lepidopteran larvae, pod length, pod position, presence of pod-feeding Lepidoptera in the same branch, and density of pod-feeding Lepidoptera in the same plant. Since the number of potential predictor variables was high in this model, a model simplification procedure was performed and the minimal adequate model is presented here (the only model with Δ AIC smaller than 3). All statistical analyses were performed in the software R (R Core Team 2014), using the following packages: *pscl* for the hurdle model (Zeileis *et al* 2008), *lme4* for the GLMMs (Bates *et al* 2014), *stats* for the GLM, and the step-wise model simplification (R Core Team 2014).

Results

A total of 113 individuals of the host plant *B. curvula* were sampled and mapped. The mean number of pods per plant was 9.7 ± 10.4 , totaling 1096 pods. Among the sampled plants, 39 (34%) had *G. bergamini* eggs (mean number of eggs 1.7 ± 4.2 ; range 0–34). The incidence of the congeneric seed beetle *G. speculifer* was smaller, with 27 (24%) of plants bearing *G. speculifer* eggs. Lepidopteran signs and other herbivory signs were present in 67 (60%) and 97 (87%) of the plants, respectively. In the two surveys at the end of the fruiting season, 55 pods with *G. bergamini* eggs were collected (mean number of pods with eggs per plant 1.1 ± 2.1 ; range 0–12), from which 36 adult beetles emerged. Individual pods of *B. curvula* had, on average, 25% of their seeds directly damaged by herbivorous insects (58% of undeveloped and

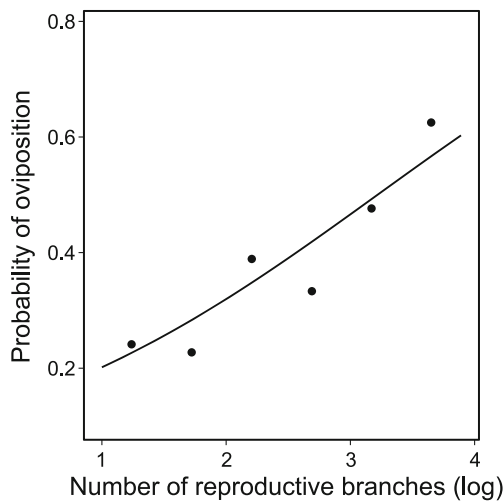


Fig 1 Relationship between the number of reproductive branches and the probability that at least one *Gibbobruchus bergamini* female laid eggs on an individual plant. Points are the mean incidence of *Gibbobruchus bergamini* ovipositions in six class intervals of branch number (one branch, two branches, three or four branches, five or six branches, 7 to 11 branches, and 12 to 18 branches). The solid line represents model prediction.

aborted seeds), and *G. bergamini* larvae accounted for 22% of the damaged seeds.

Oviposition patterns across the levels of resource aggregation

At the individual plant level, only the number of reproductive branches showed a significant and positive relation with the number of *G. bergamini* ovipositions (Fig 1). In this first result, only plants with no sign of oviposition by *G. bergamini* and plants with at least one sign of oviposition by this seed beetle were contrasted (Table 2). Although not significant, sunlight exposure had a moderate positive relationship with *G. bergamini* incidence (z value=1.7; $p=0.088$; Table 2). Among the plant individuals used by *G. bergamini*, those with more signs of lepidopteran attack on pods also had more pods bearing *G. bergamini* eggs (z value=2.68; $p=0.007$; Table 2 and Fig 2c).

Table 2 Effects of potential explanatory variables of oviposition site choice at the plant level by the seed beetle *Gibbobruchus bergamini* according to the hurdle model.

Model	Parameter	Estimate	Std. error	z value	P
Count model coefficients	(Intercept)	-2.768	0.350	-7.920	<0.001
	Reproductive branch number	2.644	0.782	3.383	<0.001
Zero hurdle model coefficients	Intercept	-1.742	0.424	-4.111	<0.001
	Sunlight exposure	0.734	0.432	1.697	0.088
	Lepidoptera density	0.152	0.057	2.677	0.007
	Plant isolation	0.113	0.213	0.532	0.595

Count model coefficients: truncated negative binomial with log link. Zero hurdle model coefficients: binomial with logit link.

At the branch level, only the presence of lepidopteran species showed a significant relationship with the number of eggs of *G. bergamini* (Table 3). Contrary to expectations, females of *G. bergamini* laid more eggs on branches that were used by seed-feeding lepidopterans (z value=2.19; $p=0.028$; Table 3).

Finally, at the pod level, the most important variables were the development stage and the position of the pod along the branch (Fig 2a, b; Table 4). Although a few eggs were deposited on mature but green pods, most ovipositions occurred on dry pods (z value=5.55; $p<0.001$). Also, there were more eggs on more basal pods (Fig 2b). The number of eggs varied more among branches from the same plant than between individual plants (Table 4). Therefore, within-plant variation in the quality and aggregation of the resource (pods) was more important for female oviposition choice than plant-to-plants differences.

Larval survivorship

The egg to larvae survivorship was reduced in plants exposed to sunlight, but the proportion of eggs laid in damaged areas did not affect the survivorship (Fig 3a and Table 5). Larval survivorship was greatly affected by the number of “empty” seeds in the pod (z value=-2.75, $p=0.006$; Table 5), with pods with more than 45% empty seeds presenting zero survivorship (Fig 3b). The lepidopterans had contrasting effects on the different levels of resource aggregation, showing a strong negative relationship with larval survivorship at the pod level, as depicted by the number of seeds consumed by lepidopterans (z value=-2.57, $p=0.013$; Fig 3c and Table 5), but a positive relationship at the plant level (z value=2.38, $p=0.017$; Fig 3d and Table 5), consistent with the patterns of female preference.

Discussion

Our data do not support the hypothesis that female oviposition choices maximize offspring performance. Overall,

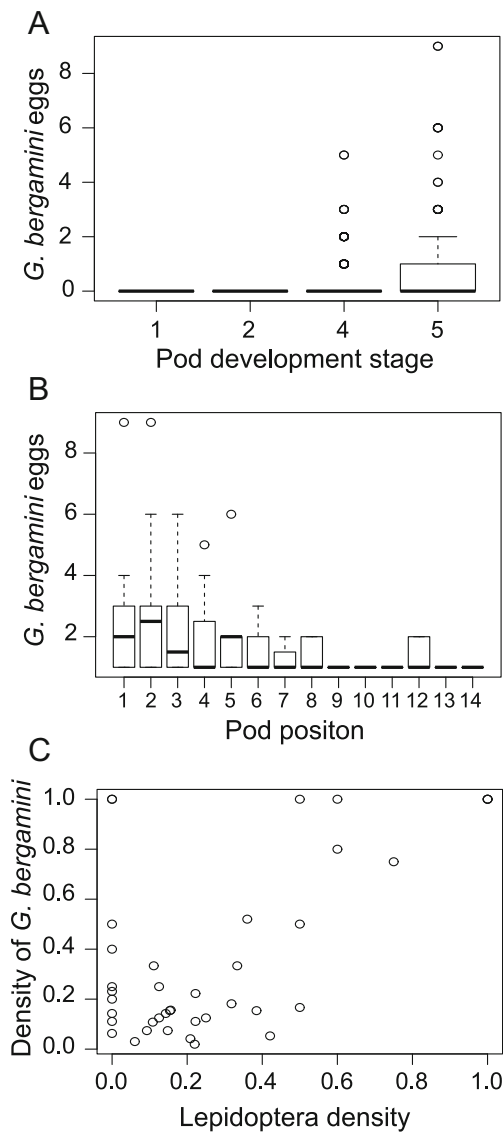


Fig 2 Distribution of eggs laid by the seed beetle *Gibbobruchus bergamini* among pods and plants. **a** Distribution at the pod scale according to the four developmental stage classes: 1 unexpanded seeds, less than 5 cm; 2 unexpanded seeds, greater than 5 cm; 3 expanded seeds, green tegument; and 4 ripe pod. **b** Distribution at the pod scale according to pod position from 1 the most basal pod in the raceme to 14—most apical pod. Horizontal lines represent the median, boxes the second and third quartiles, whiskers 1.5 times the interquartile range, and circles represent the outliers. **c** Distribution at the plant scale according to the density of herbivorous Lepidoptera on the same plant.

oviposition site choices by the seed beetle *G. bergamini* did not seem to be influenced by the factors that most influence larval survivorship. In fact, among the seven explanatory variables chosen for their potential effects on female preference and offspring performance, only the density of lepidopteran larvae at the plant and branch scales had the same effect on egg distribution and larval survival. The apparent effects of

Table 3 Effects of potential explanatory variables of oviposition site choice at the branch level by the seed beetle *Gibbobruchus bergamini*.

Parameter	Estimate	Std. error	z value	P
(Intercept)	-3.349	0.328	-10.222	<0.001
Pod number	0.109	0.072	1.511	0.131
Herbivory signs	0.405	0.261	1.550	0.121
Presence of Lepidoptera	0.557	0.254	2.193	0.028
Presence of <i>G. speculifer</i>	0.303	0.358	0.846	0.398

Plant individual as random effect: Variance=1.402; Std. Dev.=1.184.

other pod-feeding herbivores were greater at the plant and branch scales, while at the pod level the most important factors were plant-related variables.

Oviposition patterns

At the plant level, the oviposition choices of *G. bergamini* females were mostly related to the number of reproductive branches, and density of Lepidoptera. Larger host plants were most often used by *G. bergamini*. Since the ovipositions of *G. bergamini* occur mainly at the end of the fruiting season, the positive relationship with the number of reproductive branches might indicate that females can be attracted to pod-related cues (Solomon 1981, Silva *et al* 2007) or, on the other hand, it might be a consequence of plant size per se due to preference of females for larger resource patches, as predicted by the resource concentration hypothesis (Root 1973, Hambäck & Englund 2005). However, the absence of an effect of plant isolation indicates that the effect of plant size might not be mediated only by plant conspicuity.

Interestingly, those host plants with a greater density of Lepidoptera were more likely to be chosen by at least one *G. bergamini* female. This apparently positive herbivore-herbivore interaction could be the result of similar cues used by the seed beetles and the moths. The female insects from

Table 4 Effects of potential explanatory variables of oviposition site choice at the pod level by the seed beetle *Gibbobruchus bergamini*.

Parameter	Estimate	Std. error	z value	P
(Intercept)	-7.030	0.868	-8.103	<0.001
Development stage	1.896	0.341	5.552	<0.001
Pod position	-0.126	0.042	-2.984	0.003
<i>G. speculifer</i> eggs	-0.071	0.071	-1.001	0.317
Herbivory signs	0.346	0.243	1.420	0.156
Presence of Lepidoptera	0.116	0.247	0.469	0.639

Plant individual as random effect: Variance=1.691; Std. Dev.=1.30. Branch nested in plants as random effect: Variance=2.287; Std. Dev.=1.512.

Table 5 Effects of potential explanatory variables of offspring survivorship in the seed beetle *Gibbobruchus bergamini*.

Survivorship	Variables	Estimate	Std. error	t value	P
From eggs to larvae	(Intercept)	0.185	0.246	0.751	0.456
	<i>G. bergamini</i> eggs laid in damaged pod areas (%)	-0.371	0.234	-1.587	0.119
	Sunlight exposure	-0.257	0.113	-2.275	0.027
From larvae to adult	(Intercept)	-4.087	2.222	-1.839	0.066
	Seeds consumed by Lepidoptera	-0.822	0.320	-2.565	0.013
	"Empty" seeds	-0.578	0.210	-2.753	0.006
	Presence of Lepidoptera on the same branch	-0.359	1.295	-0.277	0.782
	Density of Lepidoptera on the same plant	6.854	2.883	2.378	0.017

Egg survivorship: dispersal parameter for quasi-Poisson family=0.709.

different lineages could be responding to common factors such as plant genotype (Cronin & Abrahamson 2001), nutritional state (Seagraves *et al* 2011), plant phenology (Boege & Domínguez 2008), or plant size (Root 1973, Hambäck & Englund 2005). Another explanation would be that previously attacked plants may be more attractive due to changes in their volatile profile caused by induced responses (Delphia *et al* 2007). Since the positive correlation between *G. bergamini* and lepidopterans was evident at the plant and branch level, but not at the pod level, the likely explanations are shared cues or induced facilitation at the plant and branch levels only.

At the pod level, however, the presence of other herbivores had no detectable effect on the density of *G. bergamini* eggs. Although chewing and drilling by other insects may facilitate bruchid larvae penetration (Ribeiro-Costa & Costa 2002), we did not find evidences that the females prefer to oviposit on damaged pods. The main effects found at the pod level were instead those related to development stage and pod position. The avoidance of undeveloped pods may be a way to avoid possible plant defenses such as induced resistance (De Menezes *et al* 2010) and pod abortion (Ostergård *et al* 2007). Bruchids often oviposit only on pods at a specific stage (Johnson *et al* 2004). Such developmental specialization may be associated to important chemical and morphological changes during pod development, such as secondary compound composition (De Menezes *et al* 2010) and increase in the hardness of pod and seed coat with age, which can be a barrier to bruchid larvae penetration (Souza *et al* 2011). The relationship with pod position can also be mediated by pod quality, since more apical pods may have lower quality seeds due to the resource distribution along the raceme (Vallius 2000). Alternatively, this pattern might also be a byproduct of a phenological trend, where the first pods to mature (i.e., the most basal pods) were the ones to get more ovipositions simply by being available in a period of high oviposition activity (Solomon 1981). If this is true, beetle

oviposition could act as a selective force favoring later flowering (Brody 1997).

Offspring performance

Although egg survivorship was greatly reduced in plants fully exposed to sunlight, this variable had no significant effect on egg distribution. The mechanisms responsible for egg mortality could not be directly tested in our system. We hypothesize that they are associated to direct effects of temperature and desiccation. Both high temperatures and desiccation may reduce the survivorship of bruchid eggs or larvae (Traveset 1991). Therefore, bruchid mortality could be higher in plants fully exposed to sunlight. This negative effect of sunlight exposure should be especially strong on eggs because they are attached to the surface of the pod. Another possible mechanism is the increased volatiles emitted by plants exposed to higher UV levels, which could attract more egg parasitoids (Foggo *et al* 2007), or other predators. The lack of response to the sunlight exposure level by the adult females could be due to opposite effects of sunlight on the adult seed beetles. It has been shown that fully exposed plants may be more attractive to beetles (Rowe & Potter 2000) and, although our data do not show a preference for exposed plants, this attractiveness effect may counterbalance the negative effect on egg survivorship. The main factors affecting larval survivorship were the presence of herbivorous Lepidoptera and the number of empty seeds at the pod level. However, the presence of lepidopteran larvae had contrasting effects depending on the scale at which the variables are measured. For instance, despite the positive effects on female preference at the plant and branch scales, the presence of lepidopteran larvae at the same pod had a negative effect on larval survival. On the other hand, Lepidoptera density at the plant level showed a positive relationship with larval survival. Since previous herbivory may reduce seed quality (De Menezes *et al* 2010) and the direct effects of resource consumption by other insects are presumably

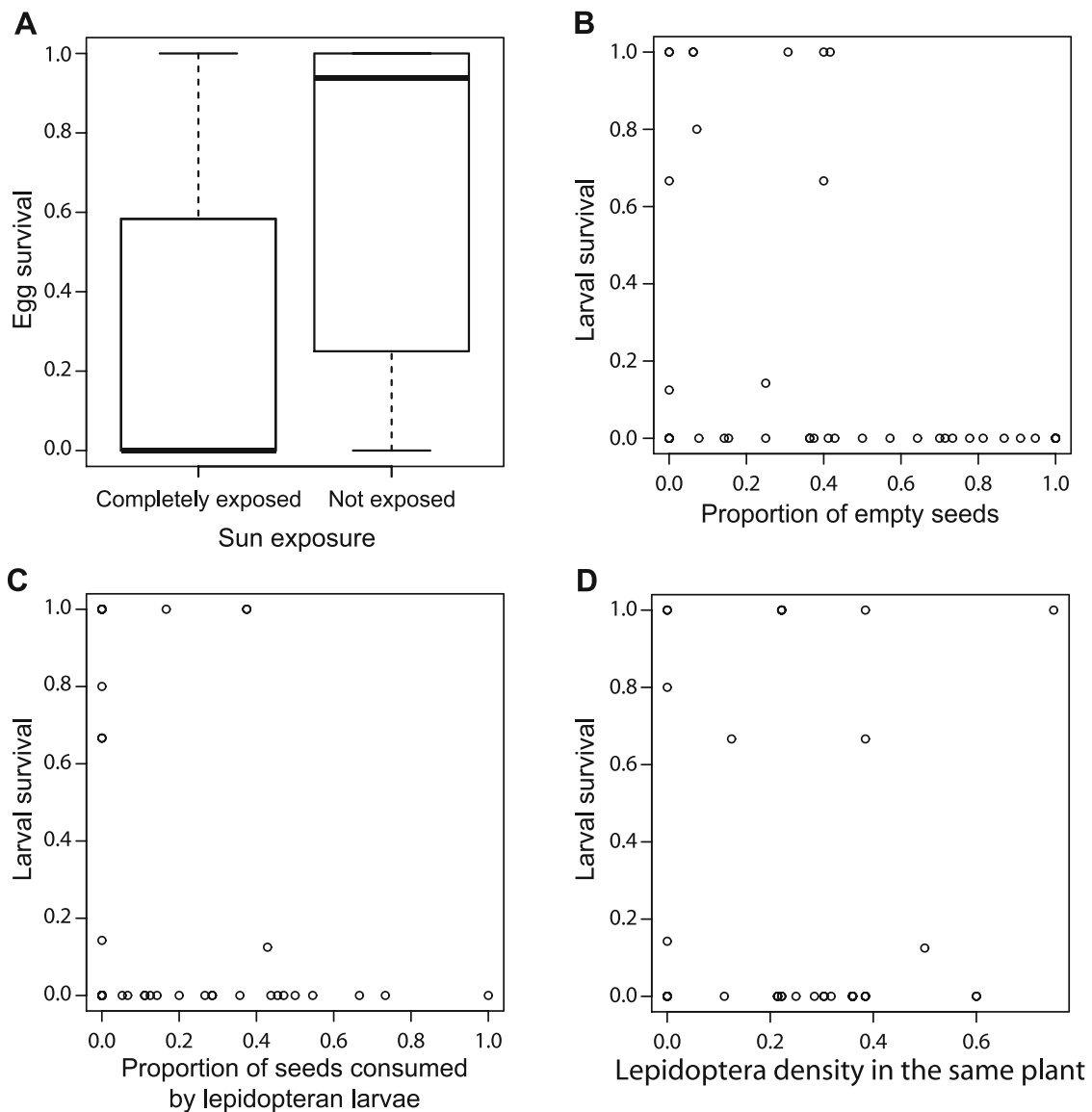


Fig 3 Effects of sun exposure (a), proportion of empty seeds in the pod (b), proportion of seeds consumed by lepidopteran larvae (c), and density of Lepidoptera in the same plant (d) on offspring survival (egg or larval survival). Horizontal lines represent the median, boxes the second and third quartiles, whiskers 1.5 times the interquartile range, and circles represent the outliers. Larval survival was measured as the proportion of larvae that developed to the adult stage on each individual plant).

stronger, positive systemic effects of the presence of other herbivores on larval survival are unlikely. A plausible explanation for this relationship is a concordance in the responses to plant quality cues between moth and beetle females.

Empty seeds are apparently unsuitable for *G. bergamini* development. Possible causes for empty seeds are their consumption by seed-feeding Hemiptera (Krugman & Koerber 1969), physiological failure due to resource shortage (Nakamura 1988), or even late seed abortion (Nakamura 1988). In contrast to the empty seeds, however, the aborted ones had no evident effect on larval survivorship. This could be an indication that the females are able to discriminate small aborted seeds, but incapable of recognizing grown but empty seeds. Some studies demonstrate that female

seed beetles may use the surface curvature as a cue for seed size (Avidov *et al* 1965) and the same might happen in a female assessing the surface of the pods. There are many examples of seed predators incapable of discriminating between healthy and empty seeds (Coetzee & Giliomee 1987, Traveset 1993, Mustart *et al* 1995) and a possible defensive role of undeveloped seeds has been suggested by some authors (Mustart *et al* 1995). Nonetheless, the real cause of the empty seeds in *Bauhinia* pods has yet to be determined.

The differences in the relative importance of the variables to oviposition site choice between the branch and pod levels did not result in similar differences for larval performance. Thus, even though some variables had a great impact on offspring performance, these variables showed no consistent

effects on female preference. This discordance could result from the lack of female capacity to detect certain differences in resource quality, such as the presence of other herbivores or the proportion of empty seeds. This also could mean that the most important plant traits affecting larval performance vary more among conspecific neighbors than within individual plants. Such among-plants variation in resource quality could be perceived by the moths as well, and this could explain the apparent positive effect of lepidopteran density at the plant level on oviposition site choice.

Despite the presence of considerable effects of some variables on offspring survival, such as sunlight exposure and the number of viable seeds, the main factors explaining oviposition site choice by the seed beetle *G. bergamini* were phenology-related traits: pod position and pod development stage. Oviposition by *G. bergamini* females is apparently severely time-constrained, and probably the females have little opportunity to further increase offspring success through better compensatory choices in the future. In this scenario, the best strategy would be to spread the risk by laying eggs on many pods and on many different individual plants (Gripenberg *et al* 2007). This study shows the importance of taking into account the multi-level effects at which insect-plant interactions occur in order to better understand these systems, and better clarify the role of different selective agents in the evolution of insect-host plant associations.

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