



**Universidade Federal de Goiás
Instituto de Ciências Biológicas
Programa de Pós-Graduação em
Ecologia e Evolução**



**SELEÇÃO DE RECURSOS POR DUAS ESPÉCIES DE BESOUROS
BRUQUÍNEOS DO GÊNERO *GIBBOBRUCHUS* NA PLANTA HOSPEDEIRA
BAUHINIA CURVULA Benth.**

Leonardo Lima Bergamini
Orientador: Mário Almeida-Neto

Goiânia - GO
2013



**Universidade Federal de Goiás
Instituto de Ciências Biológicas
Programa de Pós-Graduação em
Ecologia e Evolução**



**SELEÇÃO DE RECURSOS POR DUAS ESPÉCIES DE BESOUROS
BRUQUÍNEOS DO GÊNERO *GIBBOBRUCHUS* NA PLANTA HOSPEDEIRA
BAUHINIA CURVULA Benth.**

Leonardo Lima Bergamini
Orientador: Mário Almeida-Neto

Dissertação apresentada à Universidade Federal de Goiás como parte das exigências do Programa de Pós-Graduação em Ecologia e Evolução para a obtenção do título de Mestre.

Goiânia - GO
2013

**SELEÇÃO DE RECURSOS POR DUAS ESPÉCIES DE BESOUROS
BRUQUÍNEOS DO GÊNERO *GIBBOBRUCHUS* NA PLANTA HOSPEDEIRA
*BAUHINIA CURVULA Benth.***

Leonardo Lima Bergamini

Dissertação apresentada à Universidade Federal de Goiás como parte das exigências do Programa de Pós-Graduação em Ecologia e Evolução para a obtenção do título de Mestre.

Nome e assinatura do Presidente da Banca
Examinadora

Nome e assinatura do 2º membro da Banca
Examinadora

Nome e assinatura do 3º membro da Banca
Examinadora

AGRADECIMENTOS

Agradeço à CAPES e ao Programa de Pós Graduação em Ecologia e Evolução pelos auxílios financeiros,;

Agradeço ao meu orientador Prof.Mário Almeida-Neto, pela orientação, incentivo e companheirismo;

Aos membros da banca examinadora, pela disposição;

À Profª Viviane Gianluppi Ferro e a todos do laboratório de Ecologia de Insetos pela disponibilização da câmara climatizada e de espaço;

À Profª Cibele Stramare Ribeiro-Costa e a todos do laboratório de Bioecologia e Sistemática de Coleoptera, da UFPR pela ajuda na identificação dos insetos;

Aos meus colegas e amigos, pelas conversas, trabalhos e aprendizado;

Àqueles que me ajudaram nas coletas de dados, amigos e familiares;

A todos que, de uma forma ou de outra, contribuíram pra minha formação;

Agradeço especialmente à minha amada companheira Bárbara, pela ajuda e amparo em todos os momentos.

SUMÁRIO

1 ñ INTRODUÇÃO GERAL	3
2 ñ CAPÍTULO 1	7
Hierarchical patterns in female preference and offspring performance in the seed-beetle <i>Gibbobruchus cavillator</i>	8
Introduction	9
Material and Methods	12
Study system	12
Sampling	13
Data Analysis	18
Results	20
Discussion	28
References	32
3- CAPÍTULO 2	39
The mid-domain effect applied to fine-scale resource use: geometric constraints predict egg-laying pattern better than seed quality.....	40
Introduction	41
Material and Methods	43
Data Collection.....	43
Data Analysis	44
Results	46
Discussion	49
References	50
4 ñ CONSIDERAÇÕES FINAIS	54
5 ñ REFERÊNCIAS BIBLIOGRÁFICAS	54

RESUMO

O processo de seleção de plantas hospedeiras é a principal interface nas interações herbívoro-planta, com profundas consequências para a ecologia e evolução destes grupos. Neste trabalho investigamos algumas etapas da seleção de hospedeiras em duas espécies co-genéricas de besouros bruquíneos. No primeiro capítulo avaliamos o padrão de oviposição e de sobrevivência das larvas de *G. cavillator* para testar se as escolhas das fêmeas estão de acordo com as expectativas da teoria de oviposição ótima, considerando a estrutura espacialmente hierárquica do sistema. No segundo capítulo analisamos a seleção em fina-escala da posição dos ovos nas vagens pelas fêmeas de *G. speculifer*, e exploramos o papel da restrição geométrica da vagem em definir o padrão de distribuição dos ovos. Tomados em conjunto, os resultados dos dois capítulos exemplificam como pequenas diferenças em atributos-chave do processo de seleção de hospedeiras podem levar a importantes diferenças na interação entre insetos herbívoros e suas plantas hospedeiras.

ABSTRACT

The process of host-plant selection is the main interface in the herbivore-plant interactions, with deep consequences for the ecology and evolution of those groups. In this work, we investigate some steps of the host-plant selection in two congeneric species of seed beetles. In the first chapter, we assess the oviposition pattern and larval survival in the seed-beetle *G. cavillator* to evaluate whether oviposition site choices maximize offspring survival, accounting for the spatially hierarchic structure of the system. In the second chapter, we analyze the fine-scale positioning of *G. speculifer* eggs, and explore the role of geometric constraints in the egg distribution patterns. Altogether our results exemplify how small differences in key traits of the host-plant selection can lead to significant differences in the interaction between herbivore insects and their host plants.

1 ã INTRODUÇÃO GERAL

Insetos herbívoros, suas plantas hospedeiras e seus parasitoides compreendem a maior parte da diversidade terrestre conhecida (Gaston 1991; Ødegaard 2000; Scotland R.W. & Wortley A.H. 2003; Mayhew 2007). Essa enorme diversidade tem atraído a nossa atenção por muito tempo, e há evidências de que o hábito fitófago em si tenha promovido a diversificação dos insetos herbívoros (Mitter et al. 1988; Farrell 1998; Mitter, Farrell, & Wiegmann 1988). Uma característica importante nesses sistemas é a grande especialização dos insetos herbívoros (Bernays & Graham 1988; Jaenike 1990), o que tem sugerido várias hipóteses que relacionam essa especialização com a geração e manutenção de sua grande diversidade (Nosil & Ecology 2002; Janz & Nylin 2008; Nyman 2010).

Como os estágios imaturos da maioria dos insetos herbívoros são relativamente imóveis, principalmente em insetos endófagos cujas larvas se alimentam e se desenvolvem no interior de suas plantas hospedeiras, a seleção do local de oviposição pelas fêmeas de insetos herbívoros é um processo central nas interações inseto-planta (Bernays & Chapman 1994). Portanto, o sucesso de uma larva depende da escolha do local de oviposição pelas fêmeas adultas, e espera-se uma forte pressão seletiva sobre estas em favor da habilidade de escolher um recurso adequado para a prole. Essa correlação entre preferência da fêmea e o desempenho da prole tem encontrado um bom suporte empírico (Gripenberg et al. 2010); embora haja casos em que essa relação é inexistente ou mesmo negativa (Jaenike 1990; Mayhew 1997, 2001).

Neste trabalho investigamos algumas etapas do processo de seleção de hospedeiras em duas espécies co-genéricas de besouros bruquíneos (Chrysomelidae:

Bruchinae), *Gibbobruchus speculifer* (Gyllenhal, 1833) e *G. cavillator* (Fahreus, 1839), as quais consomem sementes de algumas espécies do gênero *Bauhinia* L.(Fabaceae)(Manfio et al. 2012). Em áreas de cerrado da região central do Estado de Goiás estas duas espécies de besouros têm sido encontradas nas vagens de *B. curvula* Benth., uma leguminosa de porte arbustivo muito comum em cerrado sentido restrito e pastagens abandonadas (Figura 1).

As fêmeas dos besouros depositam seus ovos na parede das vagens de *B. curvula* e as larvas penetram na vagem e se alimentam das sementes. Cada larva se desenvolve no interior de uma semente, no caso de *G. cavillator*, ou, ocasionalmente partes da semente adjacente, no caso de *G. speculifer*, até a sua emergência como um besouro adulto. Neste sistema, *G. speculifer* ovipõe em vagens ainda verdes, enquanto *G. cavillator* utiliza vagens quase ou já maduros (Bergamini, dados não publicados). Assim, mesmo pertencendo à mesma guilda de oviposição em uma classificação comumente utilizada para bruquíneos(Johnson et al. 2004), estas duas espécies apresentam diferenças importantes no comportamento de oviposição.

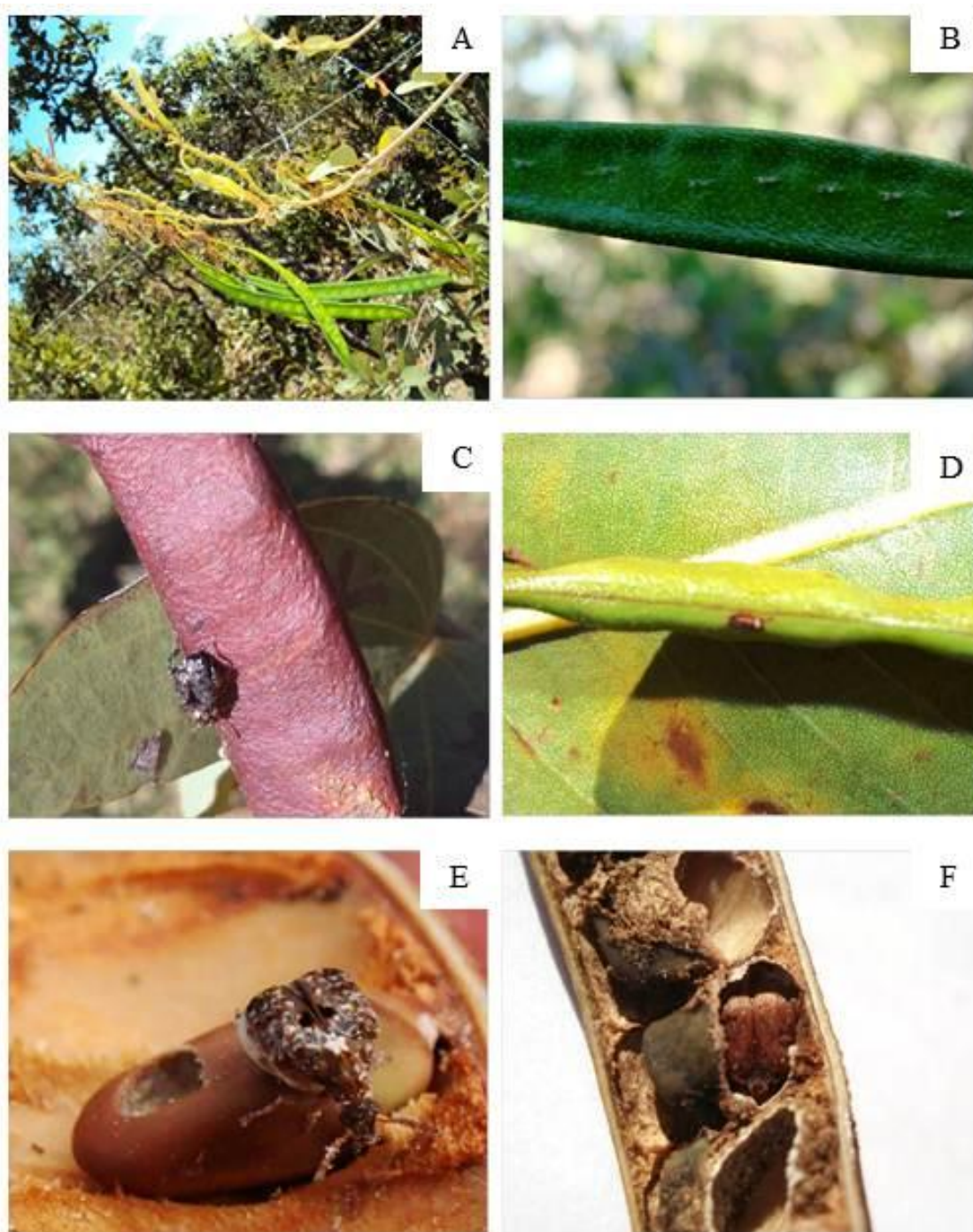


Figura 1 ãA) Ramo de *Bauhinia curvula* com vagens jovens; B) Ovos de *Gibbobruchus speculifer* sobre uma vagem jovem, com sementes ainda não expandidas; C) Fêmea de *Gibbobruchus cavillator* sobre vagem já madura, prestes a ovipor; D) Ovo de *G. cavillator* sobre área danificada em uma vagem verde, porém com as sementes já desenvolvidas; E) Adulto de *G. scurra* recém-emergido de uma semente de *B. rufa*, tanto o besouro como o tipo de dano à semente são muito similares aos causados por *G. cavillator* em *B. curvula*; F) Adulto de *G. speculifer* ainda na câmara pupal em semente de *B. curvula*.

No primeiro capítulo avaliamos o padrão de oviposição e de sobrevivência das larvas de *G. cavillator* para testar se as escolhas das fêmeas estão de acordo com as expectativas da teoria de oviposição ótima (Jaenike 1978; Gripenberg et al. 2010), considerando a estrutura espacialmente hierárquica do sistema. No segundo capítulo analisamos a seleção em fina-escala da posição dos ovos nas vagens pelas fêmeas de *G. speculifer*. Nossos resultados demonstram como o simples comportamento de botar ovos em fileira, aliado às restrições geométricas da vagem, pode gerar uma distribuição de incidência com mais ovos na região central da vagem, devido a um efeito de domínio central.

2 - CAPÍTULO 1

PADRÕES HIERÁRQUICOS NA PREFERÊNCIA DAS FÊMEAS E DESEMPENHO DAS LARVAS NO BRUQUÍNEO *GIBBOBRUCHUS CAVILLATOR**

*Submetido à revista *Journal of Insect Science* em 25 de outubro de 2012

HIERARCHICAL PATTERNS IN FEMALE PREFERENCE AND OFFSPRING PERFORMANCE IN THE SEED-BEETLE *GIBBOBRUCHUS CAVILLATOR*

Leonardo Bergamini^{1,2a*} and Mário Almeida-Neto^{1b}

¹ Departamento de Ecologia, ICB, Universidade Federal de Goiás (UFG), Goiânia, GO, 74001-970, Brazil

² Programa de pós-graduação em Ecologia e Evolução, ICB, UFG, Brazil

Correspondence: ^allbergamini@gmail.com, ^b marioeco@gmail.com, *Corresponding author

Keywords: Bruchidae, Coleoptera, herbivory, resource aggregation, seed predation, scale dependence

ABSTRACT

The search for and choice of oviposition site is 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, positive 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. This study aimed to investigate this relationship in the seed-beetle *Gibbobruchus cavillator* by taking into account several potential factors affecting oviposition choices and larval survivorship through a multi-level approach. The study was conducted in an area of Cerrado vegetation in the municipality of Hidrolândia, State of Goiás, Brazil. Through hierarchical analysis that controlled for the non-independence of observations, this study showed 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 severely time-constrained, meaning that females have little opportunity to further increase offspring performance through compensatory additional 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 site by

female insects is a key step in their life cycle (Bernays and Chapman 1994; Lancaster et al. 2010; Refsnider and 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 (e.g., 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 seeming 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 (White, 2009). More recently, however, some studies have also addressed interactions with predators and parasitoids (Heisswolf et al. 2005; Pöykkö 2011) as well as herbivore-herbivore interactions (Kaplan and 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 and Fernandes 2008) and even between parts of an individual plant (Cornelissen and Fernandes 2008; Flaherty and Quiring 2008). Therefore, the decisions of the 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 and Fujii 1990; Fox et al. 1996). This is the case for the seed-beetle *Gibbobruchus cavillator* (Fåhraeus, 1839), whose larvae feed exclusively on seeds of the genus *Bauhinia* (Manfio et al., *in press*), and often co-occur with other seed-beetle species and microlepidoptera larvae on their host plants (Bergamini, pers. obs.). The *G. cavillator* females 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, also can affect the quality of seeds to the beetles (Traveset et al. 1991).

The aim of this study was to assess the oviposition pattern and larval survival in the seed-beetle *G. cavillator* 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 those factors that mostly affect larval survival will also be the most important ones for oviposition

site choice by females of *G. cavillator*. 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 of the type Aw in the Köppen 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, mainly 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 the Fabaceae is the largest plant family.

A plot of 2025 m² (45 x 45 m) was established in a small hill, within which all individuals of *Bauhinia curvula* (Fabaceae) (> 1 m of height) were mapped in a Cartesian coordinate system. Since *B. curvula* presents an underground stem growth, only stems more than 45cm apart were counted as separate individuals. *Bauhinia curvula* (Fabaceae) is a common host plant of the beetle *G. cavillator* in cerrado areas of the central region of the State of Goiás (Bergamini, unpublished data). This plant species is a shrub with flowering peak between May and June and fruiting period lasting until the middle of August in the studied region. The eggs of *G. cavillator* and *G. speculifer* have very different morphologies, while the former are round and laid scattered over the pod, *G. speculifer* eggs are fusiform, with attaching filaments on

its ends and laid usually in lines on the pod sides. While most insect species lay their eggs at the beginning of the fruiting period of *B. curvula*, eggs of *G. cavillator* are mostly found when pods are mature at the end of the fruiting period. Therefore, *G. cavillator* females may be able to choose pods and plants using cues 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. cavillator* females assess host plant quality for their offspring, possible determinants of plant quality we 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 scale, the variables recorded were: (1) 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 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. We used the R package *deldir* to calculate 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.

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 lepidopterous 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 in the branch; (2) pod development stage; (3) the number of *G. speculifer* eggs; (4) the presence of Lepidoptera holes; and (5) the presence of other signs of herbivory. The pod position within 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 2 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 two 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 'empty' aspect; 3) healthy seeds, with no signs of damage; 4) seeds attacked by *Gibbobruchus speculifer*, with beetle puparia; 5) seeds attacked by *G. cavillator*, 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, in order to estimate egg and larval survival rates, as a measure of beetle performance.

Table 1. Measured variables and their expected and recorded effects on oviposition site choices by *G. cavillator* females and on offspring performance. ↓ = negative effect, ↑ = positive effect, none = not expected to have any effect, --- = not tested, null = no effect recorded.

Level	Variable	Effects on egg distribution		Effects on offspring survivorship	
		Expected	Observed	Expected	Observed
Plant	Isolation	↓	↑	none	---
	Reproductive branch number	↑	↑	none	---
	Pod number	↑	null	none	---
	Density of other pod-feeding herbivores	↑/↓	↑	↑/↓	↑
	Sunlight exposure	↓	null	↓	↓
Branch	Pod number	↑	null	none	↑
	Presence of other pod-feeding herbivores	↑/↓	↑	↑/↓	null
Pod	Pod position	↓	↓	↓	null
	Development stage	↑	↑	none	---
	Presence of other pod-feeding herbivores	↑/↓	null	↑/↓	↓

Table 2. Description of the expected effects of different potential factors affecting oviposition site choices and offspring performance in the seed beetle *G. cavillator*.

Levels	Variable	Description	Expected effects
Plant	Isolation	The weighted area of the polygon encompassing all points nearer to the focal point than any other plant. The greater the area, the more isolated the plant is.	More isolated plants should be chosen less often if females move more between closer plants, and also if plant conspicuity increases with plant number (Root 1973).
	Reproductive branch number	Number of inflorescence-bearing branches	If females are attracted by pod- or flower-related cues (Solomon 1981), then plants with more reproductive branches should be chosen more often.
	Pod number	Number of pods	Plants with more pods should be chosen more often if they are more attractive (Silva et al. 2007) or if females lay eggs in many pods in the same plant.
	Density of other pod-feeding herbivores	Proportion of pods with signals of lepidopterous larvae, <i>Gibbobruchus speculifer</i> or chewing marks	Already attacked plants may be more or less attractive due to changes in their volatile profile caused by induced responses (Delphia et al. 2007). Other seed-feeding insects might respond to the same quality cues as <i>G. cavillator</i> .
	Sunlight exposure	A binary variable indicating whether the focal plant is totally exposed to sunlight or if it is at least partially covered by another plant's canopy	High temperatures or desiccation may cause mortality of bruchid eggs or larvae (Traveset et al. 1991), so sunlight exposed plants should be avoided.
Branch	Pod number	Number of pods on the branch	Same as at the plant level

	Presence of other pod-feeding herbivores	Presence of pods with signals of lepidopterous larvae, <i>Gibbobruchus speculifer</i> or chewing marks in the same branch	Same as at the plant level
Pod	Pod position	Position of the flower originating the pod within the raceme; more basal pods with lower position numbers	More apical pods may have lower quality seeds due to the resource distribution along the raceme (Vallius 2000), so more basal ones should be preferred.
	Development stage	A categorical variable indicating pod development stage with four levels: 0 ñ unexpanded seeds, less than 5 cm; 1 ñ unexpanded seeds, greater than 5 cm; 2 ñ expanded seeds, green tegument; 3 ñ ripe pod	Bruchids often oviposit only in pods at a specific stage (Johnson et al. 2004); secondary compound composition changes with development (De Menezes et al. 2010); hardness of pod and seed coat increase with age, which can be a barrier to bruchid larvae penetration (Souza et al. 2011).
	Presence of other pod-feeding herbivores	Presence of pods with signs of lepidopterous larvae or chewing marks in the same pod	Chewing and drilling by other insects may facilitate bruchid larvae penetration (Ribeiro-Costa and Costa 2002); previous herbivory may reduce seed quality (De Menezes et al. 2010).
	Number of <i>G. speculifer</i> eggs	Number of <i>G. speculifer</i> eggs	Seeds consumed by <i>G. speculifer</i> larvae may not be available, reducing pod quality (De Menezes et al. 2010).

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. cavillator* 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 differences 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 (Lindén et al. 2011). The predictor variables were the plant's exposure to sunlight and the proportion of pods with other herbivores: the congeneric seed-beetle *G. speculifer*, lepidopterous larvae and other signs of herbivory.

At the branch level, a generalized linear mixed model (GLMM) with the number of *G. cavillator* 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. cavillator* 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 at two stages: from egg to larvae and from larvae to the adult stage. We adjusted one model to each survivorship stage. For the egg to larvae we used a quasipoisson model where the response variable was the number of penetration holes, with the number of eggs as an offset variable. The proportion of *G. cavillator* 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 lepidopterous 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 in this model the number of potential predictor variables was high, we performed a model simplification procedure and present the minimal adequate model. All statistical analyses were performed in the software R (R Core Team 2012), using the following packages: *pscl* for the hurdle model; *lme4* for the GLMMs; *stats* for the GLM, and *step* for the stepwise model simplification.

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 SD), totaling 1096 pods. Among the sampled plants, 39 (34%) had eggs of *G. cavillator* (mean: 1.7; SD: 4.2; range: 0–34). The incidence of the congeneric seed-beetle *G. speculifer* was smaller, with 27 (24%) 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 eggs of *G. cavillator* were collected (mean number of pods with eggs per plant: 1.1; SD: 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 aborted seeds), and *G. cavillator* larvae accounted for 22% of the damaged seeds.

Oviposition patterns across the levels of resource aggregation

At the individual plant level, only isolation and the number of reproductive branches showed a significant and positive effect on the incidence of *G. cavillator* eggs (Figures 1a,b). In this first result, we only contrasted plants with no sign of oviposition by *G. cavillator* and plants with at least one sign of oviposition by this seed-beetle (Table 3). Although not significant, sunlight exposure had a moderate positive effect on *G. cavillator* incidence (z-value = 1.83; P = 0.068; Table 3). Among the plant individuals used by *G. cavillator*, those with more signs of lepidopterans attacking pods also had more pods bearing *G. cavillator* eggs (z-value = 2.55; P = 0.011; Table 3).

Table 3. The effects of potential explanatory variables of oviposition site choice at the plant level by the seed beetle *G. cavillator* according to the hurdle model. Count model coefficients: truncated negative binomial with log link. Zero hurdle model coefficients: binomial with logit link.

Model	Parameter	Estimate	Std. Error	z value	P
Count model coefficients	(Intercept)	-2.680	0.201	-13.311	< 0.001
	Lepidoptera density	2.006	0.788	2.545	0.011
Zero hurdle model coefficients	Intercept	-2.495	0.629	-3.965	< 0.001
	Sunlight exposure	0.817	0.447	1.827	0.068
	Reproductive branch number	0.304	0.01	3.044	0.002
	Plant isolation	90.893	44.784	2.03	0.042

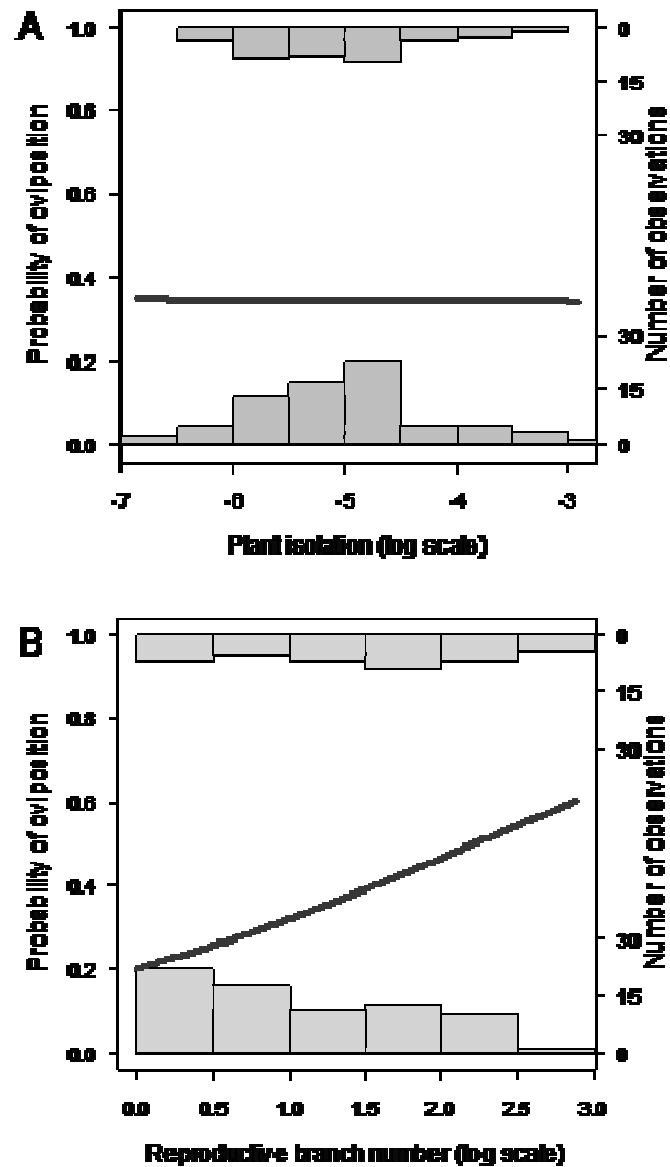


Figure 1. The effects of plant isolation from conspecifics (A) and the number of reproductive branches (B) on the probability that at least one *G. cavillator* female laid eggs on individual plants. Lower and higher bars represent the relative frequencies of plants in each data interval without and with *G. cavillator* eggs.

At the level of branches, only the presence of lepidopteran species showed a significant effect on the number of eggs of *G. cavillator* (Table 4). Contrary to our expectation, females of *G. cavillator* laid more eggs on branches that were used by seed-feeding lepidopterans (z-value = 2.19; P = 0.028; Table 4).

Finally, at the level of the pods, the most important variables were the development stage and the position of the pod along the branch (Figures 2a,b; Table 5). Although a few eggs were deposited on mature but green pods, most oviposition occurred on dry pods (z-value = 5.55; P < 0.001). Also, there were more eggs on more basal pods (Figure 2b). The number of eggs varied more among branches from the same plant than between individual plants (Table 5). Therefore, within-plant variation in the quality and aggregation of the resource (pods) was more important for female oviposition choice than among-plants differences.

Table 4. The effects of potential explanatory variables of oviposition site choice at the branch level by the seed beetle *G. cavillator*. Plant individual as random effect: Variance = 1.402; Std. Dev.= 1.184.

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

Table 5. The effects of potential explanatory variables of oviposition site choice at the pod level by the seed beetle *G. cavillator*. Plant individual as random effect: Variance = 2.287; **Std. Dev.** = 1.512. Branch nested in plants as random effect: Variance = 1.691; **Std. Dev.** = 1.30.

Parameter	Estimate	Std.Error	z value	P
(Intercept)	-7.030	0.868	-8.103	0.000
Development stage	1.896	0.341	5.552	0.000
Pod position	-0.126	0.042	-2.984	0.003
G. speculifer 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

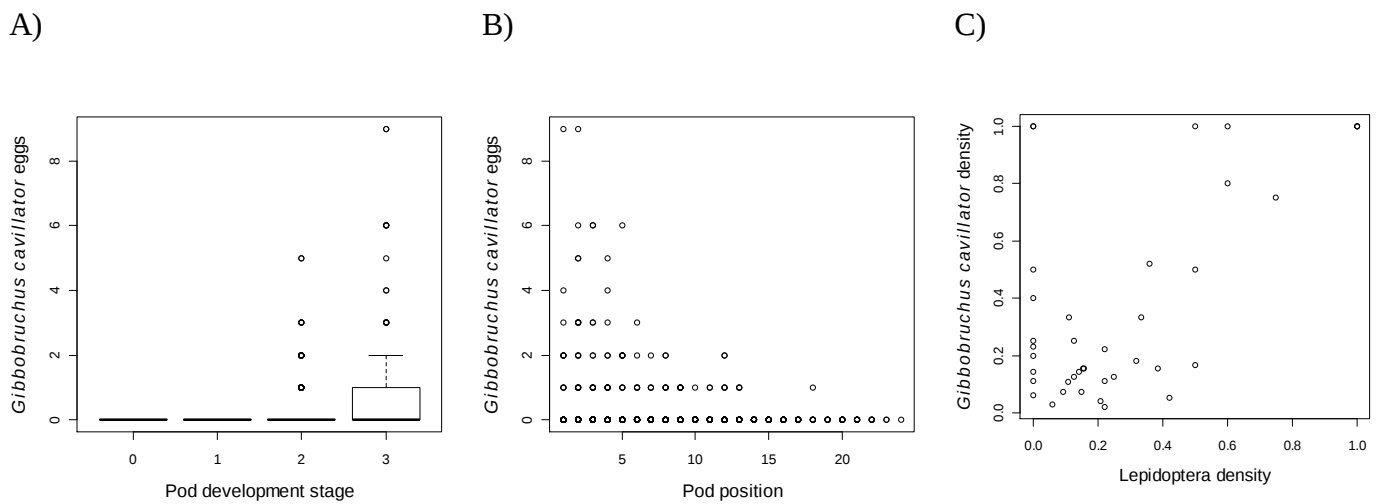


Figure 2. The distribution of eggs laid by the seed beetle *G. cavillator* in four developmental stage classes (see Table 2 for details) (A) and according to pod position (B) and density of herbivorous Lepidoptera (C).

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 (Figure 3a; Table 6). Larval survivorship was greatly affected by the number of 'empty' seeds in the pod (z -value = -3.14, $p < 0.01$; Table 6), with pods with more than 45% empty seeds presenting zero survivorship (Figure 3b), probably due to the added effects of other mortality factors. 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 (z -value = -3.23, $p < 0.01$; Figure 3c; Table 5), but a positive effect at the plant level (z -value = 2.66, $p < 0.01$; Figure 3d; Table 5), consistent with the effects upon female preference.

Table 6. The effects of potential explanatory variables of offspring survivorship in the seed beetle *G. cavillator*. Egg survivorship: dispersal parameter for quasipoisson = 0.709.

Survivorship	Variables	Estimate	Std.Error	t value	P
From eggs to larvae	(Intercept)	0.185	0.246	0.751	0.456
	<i>G. cavillator</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
	óEmptyô seeds	-0.578	0.210	-2.753	0.006
	Pod length	0.534	0.260	2.058	0.040
	Pod number in the branch	0.547	0.239	2.289	0.022
	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

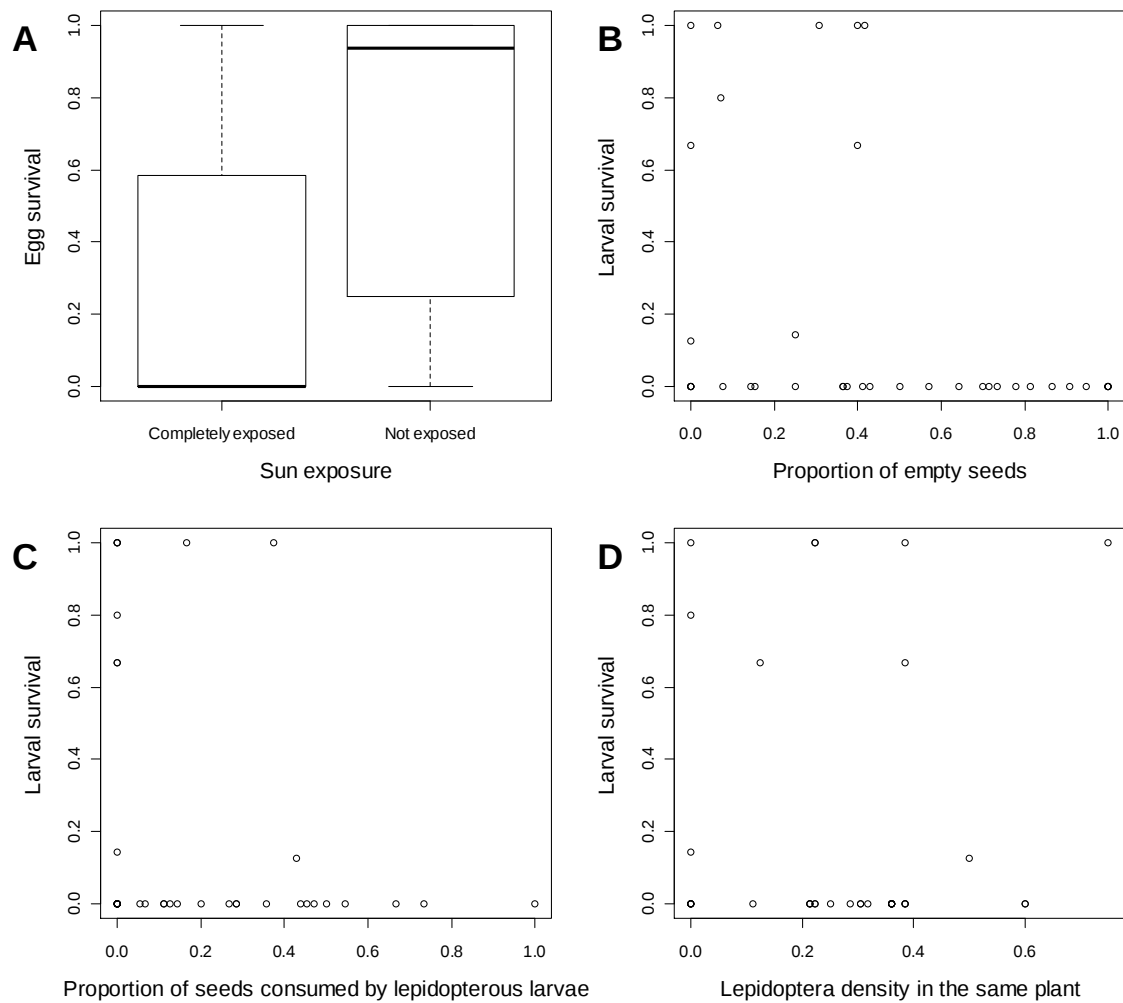


Figure 3. Differences in egg survival between host plants completely or partially exposed to sunlight (A). The relationships of larval survival (as the proportion of larvae that developed to the adult stage on each individual plant) to the proportion of empty seeds (B), proportion of seeds consumed by pod-feeding Lepidoptera (C) and the density of herbivorous Lepidoptera on each plant (D).

DISCUSSION

The results of this study are not in agreement with the hypothesis that female oviposition choices maximize offspring performance. Overall, oviposition site choices by the seed beetle *G. cavillator* 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 other pod-feeding herbivores had the same effect on egg distribution and larval survival. 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 patterns

At the plant level, the oviposition choices of *G. cavillator* females were mostly related to the number of reproductive branches, plant isolation from conspecifics and density of Lepidoptera. Larger and more isolated host plants were most often used by *G. cavillator*. Interesting, those host plants with a greater density of Lepidoptera were more likely to be chosen by at least one *G. cavillator* female. Since the ovipositions of *G. cavillator* occur mainly at the end of the fruiting season, the positive effect of the number of reproductive branches indicates that females can be attracted to pod related cues. The positive effect of plant isolation, in turn, could result from the spreading of oviposition events, leading to a dilution effect between more aggregated plants. Finally, the apparent positive herbivore-herbivore interaction related to the fact that plants more used by herbivorous Lepidoptera were also more likely to be used by *G. cavillator* could be the result of similar cues used by the seed beetles and the moths. The female

insects from different lineages could be responding to common factors such as plant genotype (Cronin and Abrahamson 2001), nutritional state (Seagraves et al. 2011) or simply plant phenology (Boege and Domínguez 2008). Since the positive correlation between *G. cavillator* and lepidopterans was evident at the plant and branch level, but not at the pod level, the only likely explanations are shared cues or induced facilitation at the plant and branch levels only, as with a greater attractiveness of attacked plants or even a positive effect of moth-induced defense compounds on beetle larvae performance.

At the pod level, however, the presence of other herbivores had no detectable effect on the density *G. cavillator* eggs. The avoidance of undeveloped pods may be a way to avoid possible plant defenses such as constitutive or induced resistance (De Menezes et al. 2010) and pod abortion (Ostergård et al. 2007). The effect of pod position, on the other hand, might 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 for 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 exact mechanisms of egg mortality could not be tested, but the most likely are the direct effects of temperature and desiccation. Another possible mechanism is the increased

volatile emission from more UV exposed plants, which could attract more egg parasitoids (Foggo et al. 2007).

The main factors affecting larval survivorship were the number of empty seeds at the pod level and the contrasting effects of herbivorous Lepidoptera. Empty seeds are apparently unsuitable for *G. cavillator* development. Candidate explanations for empty seeds are seed herbivorous Hemiptera (Krugman and 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 did not have an 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 and Giliomee 1987; Mustart et al. 1995; Traveset 1993) 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 tested.

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. 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 than within plants. Such among-plants variation in resource quality could be perceived by the moths as well; this could explain

the apparent positive effect of lepidopteran density at the plant level on oviposition site choice.

Concluding remarks

In this study, the main factors explaining oviposition site choice by the seed beetle *G. cavillator* were phenology-related traits: pod position and pod development stage. Oviposition by *G. cavillator* females is apparently severely time-constrained, and probably the females have little opportunity to further increase offspring success through compensatory better future choices. 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.

Acknowledgements

Leonardo Bergamini was supported by a grant from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

Avidov Z, Berlinger MJ, Applebaum SW (1965) Physiological aspects of host specificity in the Bruchidae: III. Effect of curvature and surface area on oviposition of *Callosobruchus chinensis* L. *Animal Behaviour* 13:178–180. doi: 10.1016/0003-3472(65)90089-8

- Bernays EA, Chapman RF (1994) Host plant selection by phytophagous insets. Springer
- Boege K, Domínguez CA (2008) Pre-dispersal seed predation reduces the reproductive compensatory advantage of thrum individuals in *Erythroxylum havanense* (Erythroxylaceae). *Evolutionary Ecology* 22:675–687. doi: 10.1007/s10682-007-9190-8
- Boggs CL (2009) Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology* 23:27–37. doi: 10.1111/j.1365-2435.2009.01527.x
- Brodbeck B, Andersen P, Oden S (2007) Preference-performance linkage of the xylem feeding leafhopper, *Homalodisca vitripennis* (Hemiptera Cicadellidae). *Environmental Entomology* 36:1512–1522. doi: 10.1603/0046-225X(2007)36
- Brody AK (1997) Effects of pollinators, herbivores, and seed predators on flowering phenology. *Ecology* 78:1624–1631.
- Coetzee JH, Giliomee JH (1987) Seed predation and survival in the infructescences of *Protea repens* (Proteaceae). *South African Journal of Botany* 53:61:64.
- Cornelissen T, Fernandes GW (2008) Size does matter : variation in herbivory between and within plants and the plant vigor hypothesis. *Oikos* 112:1121–1130. doi: 10.1111/j.2008.0030-1299.16588.x
- Cronin JT, Abrahamson WG (2001) Goldenrod stem galler preference and performance: effects of multiple herbivores and plant genotypes. *Oecologia* 127:87–96. doi: 10.1007/s004420000561
- Delphia CM, Mescher MC, De Moraes CM (2007) Induction of plant volatiles by herbivores with different feeding habits and the effects of induced defenses on host-plant selection by thrips. *Journal of Chemical Ecology* 33:997–1012. doi: 10.1007/s10886-007-9273-6

- Flaherty L, Quiring D (2008) Plant module size and dose of gall induction stimulus influence gall induction and galler performance. *Oikos* 117:1601–1608. doi: 10.1111/j.1600-0706.2008.16555.x
- Foggo A, Higgins S, Wargent JJ, Coleman RA (2007) Tri-trophic consequences of UV-B exposure: plants, herbivores and parasitoids. *Oecologia* 154:505–512. doi: 10.1007/s00442-007-0857-1
- Fox CW, Martin JD, Thakar MS, Mousseau T a. (1996) Clutch size manipulations in two seed beetles: consequences for progeny fitness. *Oecologia* 108:88–94. doi: 10.1007/BF00333219
- Gonz G-RH, Grez AA, González RH (1995) Resource concentration hypothesis : effect of host plant patch size on density of herbivorous insects. *Ecology* 103:471–474.
- Gripenberg S, Mayhew PJ, Parnell M, Roslin T (2010) A meta-analysis of preference-performance relationships in phytophagous insects. *Ecology Letters* 13:383–393. doi: 10.1111/j.1461-0248.2009.01433.x
- Gripenberg S, Morriën E, Cudmore A, et al. (2007) Resource selection by female moths in a heterogeneous environment: what is a poor girl to do? *The Journal of Animal Ecology* 76:854–865. doi: 10.1111/j.1365-2656.2007.01261.x
- Heisswolf A, Obermaier E, Poethke HJ (2005) Selection of large host plants for oviposition by a monophagous leaf beetle: nutritional quality or enemy-free space? *Ecological Entomology* 30:299–306. doi: 10.1111/j.0307-6946.2005.00706.x
- Hespenheide HA (1991) Bionomics of Leaf-Mining Insects. *Annual Review of Entomology* 36:535–560. doi: 10.1146/annurev.en.36.010191.002535
- Jaenike J (1990) Host Specialization in Phytophagous Insects. *Annual Review of Ecology and Systematics* 21:243–273. doi: 10.1146/annurev.es.21.110190.001331
- Jaenike J (1978) On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology* 14:350–356. doi: 10.1016/0040-5809(78)90012-6

- Janz N, Bergström A, Johansson J (2005) Frequency Dependence of Host Plant Choice within and Between Patches: A Large Cage Experiment. *Evolutionary Ecology* 19:289–302. doi: 10.1007/s10682-005-6078-3
- Janzen DH (1971) Seed Predation by Animals. *Annual Review of Ecology and Systematics* 2:465–492. doi: 10.1146/annurev.es.02.110171.002341
- Johnson CD, Romero J, Romero-Nápoles J (2004) A review of evolution of oviposition guilds in the Bruchidae (Coleoptera). *Revista Brasileira de Entomologia* 48:401–408. doi: 10.1590/S0085-56262004000300017
- Kaplan, I., & Denno, R. F. (2007). Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, 10(10), 977–994. doi:10.1111/j.1461-0248.2007.01093.x
- Krugman SL, Koerber TW (1969) Effect of Cone Feeding by *Leptoglossus occidentalis* on Ponderosa Pine Seed Development. *Forest Science* 15:104:111.
- Lancaster J, Downes BJ, Arnold A (2010) Environmental constraints on oviposition limit egg supply of a stream insect at multiple scales. *Oecologia* 163:373–384. doi: 10.1007/s00442-010-1565-9
- Lindén A, Mantyniemi S, Mäntyniemi S (2011) Using the negative binomial distribution to model overdispersion. *Ecology* 92:1414–1421.
- Manfio, D., Ribeiro-costa, C. S., & Caron, E. (2013). Phylogeny and revision of the New World bruchine genus *Gibbobruchus* Pic, 1913 (Coleoptera: Chrysomelidae). *Invertebrate Systematics*, 27, 1-37.
- Mayhew PJ (2001) Herbivore host choice and optimal bad motherhood. *Trends in ecology & Evolution (Personal edition)* 16:165–167.
- Mayhew PJ (1997) Adaptive Patterns of Host-Plant Selection by Phytophagous Insects. *Oikos* 79:417. doi: 10.2307/3546884

- De Menezes LCCRCR, Klein J, Kestring D, Rossi MN (2010) Bottom-up and top-down effects in a pre-dispersal seed predation system: are non-predated seeds damaged? *Basic and Applied Ecology* 11:126–134. doi: 10.1016/j.baae.2009.11.004
- Mustart PJ, Cowling RM, Wright M. (1995) Clustering of fertile seeds in infructescences of serotinous *Protea* species: Anti-predation mechanism? *African Journal of Ecology* 33:224:229.
- Nakamura RR (1988) Seed abortion and seed size variation within fruits of *Phaseolus vulgaris*: Polen donor and resource limitation effects. *American Journal of Botany* 75:1003:1010.
- Ostergård H, Hambäck PA, Ehrlén J (2007) Pre-dispersal seed predation: the role of fruit abortion and selective oviposition. *Ecology* 88:2959–65.
- Price PW, Fernandes GW, Waring GL (1987) Adaptive Nature of Insect Galls. *Environmental Entomology* 16:10.
- Pöykkö, H. (2011). Enemy-free space and the host range of a lichenivorous moth: a field experiment. *Oikos*, 120(4), 564–569. doi:10.1111/j.1600-0706.2010.18882.x
- R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Rabasa SG, Gutiérrez D, Escudero A (2005) Egg laying by a butterfly on a fragmented host plant: a multi-level approach. *Ecography* 28:629–639. doi: 10.1111/j.2005.0906-7590.04229.x
- Refsnider JM, Janzen FJ (2010) Putting Eggs in One Basket: Ecological and Evolutionary Hypotheses for Variation in Oviposition-Site Choice. *Annual Review of Ecology, Evolution and Systematics* 41:39–57. doi: 10.1146/annurev-ecolsys-102209-144712
- Ribeiro-Costa CS, Costa ADS (2002) Comportamento de oviposição de Bruchidae (Coleoptera) predadores de sementes de *Cassia leptophylla* Vogel

- (Caesalpinaceae), morfologia dos ovos e descrição de uma nova espécie. *Revista Brasileira de Zoologia* 19:305 ã 316.
- Root RB (1973) Organization of a Plant-Arthropod Association in Simple and Diverse Habitats: The Fauna of Collards (*Brassica oleracea*). *Ecological Monographs* 43:95ñ124.
- Santos JC, Silveira FAO, Fernandes GW (2008) Long term oviposition preference and larval performance of *Schizomyia macrocapillata* (Diptera: Cecidomyiidae) on larger shoots of its host plant *Bauhinia brevipes* (Fabaceae). *Evolutionary Ecology* 22:123ñ137. doi: 10.1007/s10682-007-9162-z
- Seagraves MP, Riedell WE, Lundgren JG (2011) Oviposition Preference for Water-Stressed Plants in *Orius insidiosus* (Hemiptera: Anthocoridae). *Journal of Insect Behavior* 132ñ143. doi: 10.1007/s10905-010-9242-8
- Silva LA, Maimoni-Rodella RCS, Rossi MN, et al. (2007) A Preliminary Investigation of Pre-Dispersal Seed Predation by *Acanthoscelides schrankiae* Horn (Coleoptera: Bruchidae) in *Mimosa bimucronata*(DC.) Kuntze Trees. *Neotropical Entomology* 36:197ñ202.
- Solomon BP (1981) Response of a Host-Specific Herbivore to Resource Density, Relative Abundance and Phenology. *Ecology* 62:1205ñ1214.
- Souza AJ, Santos PO, Pinto MST, et al. (2011) Natural seed coats provide protection against penetration by *Callosobruchus maculatus* (Coleoptera: Bruchidae) larvae. *Crop Protection* 30:651ñ657. doi: 10.1016/j.cropro.2010.12.014
- Thompson JN (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata* 47:3ñ14.
- Toquenaga Y, Fujii K (1990) Contest and scramble competition in two bruchid species, *Callosobruchus analis* and *C. phaseoli* (Coleoptera: Bruchidae) I. Larval competition curves and interference mechanisms. *Researches on Population Ecology* 32:349ñ363. doi: 10.1007/BF02512569

- Traveset A (1993) Deceptive fruits reduce seed predation by insects in *Pistacia terebinthus* L.(Anacardiaceae). *Evolutionary Ecology* 7:357:361.
- Traveset A, Rosa S, Park N, Rica C (1991) Pre-dispersal seed predation in Central American *Acacia farnesiana*: factors affecting the abundance of co-occurring bruchid beetles. *Ecological Entomology* 87:570ñ576.
- Vallius E (2000) Position-dependent reproductive success of flowers in *Dactylorhiza maculata* (Orchidaceae). *Functional Ecology* 14:573ñ579. doi: 10.1046/j.1365-2435.2000.t01-1-00450.x
- White, T. C. R. (2009). Plant vigour versus plant stress : a false dichotomy. *Oikos*, (January), 2ñ3. doi:10.1111/j.1600-0706.2009.17495.x

3 - CAPÍTULO 2

**O EFEITO DO DOMÍNIO CENTRAL APLICADO AO USO
DE RECURSOS EM FINA ESCALA: PADRÃO DE
OVIPOSIÇÃO É MAIS BEM EXPLICADO POR
RESTRIÇÕES GEOMÉTRICAS DO QUE PELA
QUALIDADE DAS SEMENTES***

*Em submissão à revista *Biology Letters*

THE MID-DOMAIN EFFECT APPLIED TO FINE-SCALE RESOURCE USE: GEOMETRIC CONSTRAINTS PREDICT EGG-LAYING PATTERN BETTER THAN SEED ABORTION RISK

Leonardo Bergamini^{1,2a*} and Mário Almeida-Neto^{1b}

¹ Departamento de Ecologia, Universidade Federal de Goiás (UFG), Goiânia, GO,
74001-970, Brazil

² Programa de Pós-Graduação em Ecologia e Evolução, UFG, Brazil

Correspondence: ^allbergamini@gmail.com, ^b marioeco@gmail.com

*Corresponding author

Keywords: Brazilian Cerrado, Coleoptera, herbivory, Leguminosae, null model,
resource selection, seed abortion

Manuscript length: 2539 words

ABSTRACT

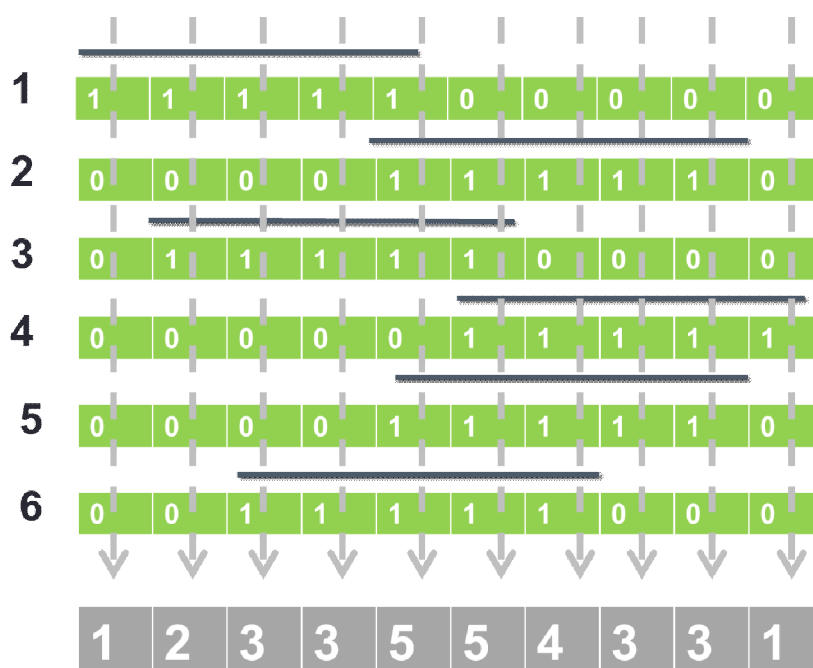
The ability to discriminate host plant quality and to select suitable resources for their offspring is widespread among herbivorous insects. However, the avoidance of poor-quality resources does not necessarily imply that insects are using sophisticated clues or complex behavioral rules to assess resource quality. Here, we investigated whether the preference-performance hypothesis or, alternatively, a mid-domain effect predict the egg-laying distribution in the seed beetle *Gibbobruchus speculifer*. Although the risk of abortion increases from apical to basal seeds, we found no preference for apical seeds. Actually, we showed that laying eggs contiguously along the pod is sufficient to explain the higher incidence of eggs in the middle portion of the pods. Such egg-laying behavior resulted in a higher escape from aborted seeds if compared to the random placement of eggs along the pod. This study highlights the importance of accounting for the effect of random processes and geometric constraints to explain and predict patterns in the distribution of organisms even at very small scales.

INTRODUCTION

The ability to discriminate host plant quality for their offspring is common among herbivorous insects [1–3]. With endophagous larvae and a high degree of feeding specialization [4,5], seed-beetles are a good model to investigate the mechanisms of resource selection in herbivorous insects. Seed-beetles species that lay their eggs on immature fruits, for instance, have to deal with the risk of seed abortion. A common feature in the Fabaceae, the main family used by seed-beetles, is an increase in the proportion of aborted seeds from the apical to the basal portions of the fruits [6–8], probably due to insufficient pollination in the ovules farthest away from the stigma [6,7,9].

Here, we investigate possible mechanisms underlying the egg laying pattern in the bruchid *Gibbobruchus speculifer* (Bruchinae, Chrysomelidae), a specialist seed-beetle whose females lay eggs on pods of a few species in the genus *Bauhinia* (Fabaceae) [10]. Since aborted and non-aborted seeds have similar sizes on immature pods and the seed-beetle larvae are unable to develop in the aborted ones (pers. obs), we might predict that adult females should avoid laying their eggs in the basal portions of the pods according to the preference-performance hypothesis [1,11]. However, previous field observations indicate that this seed beetle lay one egg per seed (pers. obs.) and also that the eggs tend to be laid contiguously (i.e. aggregated). Based on these observations, we devised a straightforward alternative hypothesis for the egg-laying behavior in *G. speculifer* that could also reduce the frequency of eggs laid on aborted seeds without requiring any complex behavior beyond two basic rules: one egg per seed and eggs laid contiguously. This hypothesis is basically the mid-domain effect [12] applied to the distribution of eggs on pods, in which eggs are predicted to be more frequently laid on middle positions than on the basal and apical portions of the pods (Box 1).

Before testing whether egg distribution on pods fits to the preference-performance hypothesis (hereafter PPH) or to the mid-domain effect (hereafter MDE), we investigated the prediction of the pollination failure hypothesis [6,8,9] that there is an increase in the proportion of aborted seeds from apical to basal portions of the pods. Then we tested the prediction of the PPH that females should prefer to lay their eggs on those positions of pods with lower risk of seed abortion, which ultimately would lead to higher larval survival. Likewise, we tested the prediction of the MDE that there should be a unimodal distribution of eggs, with a marked peak at intermediate positions of the pods. Finally, we evaluate whether the empirical distribution of eggs on pods reduces or increase the risk of larval mortality due to seed abortion compared to a random distribution of eggs on pods.



Box 1 ñ Applying the concept of MDE to eggs in pods: The numbered green rows represent the pods, with the cells representing each seed and a possible place for a beetle egg. If the ovipositions are contiguous the number of eggs in the middle positions will be greater than in the pod extremities, even if placed randomly along the pod.

MATERIAL AND METHODS

Data collection

We selected nine individuals of *Bauhinia curvula* (Fabaceae) in an old pasture located in the Brazil's central high plains region, in the State of Goiás (16°36'15"S, 49°4'3"W). During the fruiting season (May to July) of 2012, we sampled two to six young pods with *G. speculifer* eggs from each individual plant. In each pod, we ranked the seeds according to their position with the number 1 for the most basal seed, number 2 for the second most basal and so on until the most apical seed. Then we inspected all seeds for the presence of *G. speculifer* eggs and categorized the seeds as aborted or non-aborted.

In order to evaluate the effect of seed position on the probability of seed abortion we used additional data collected from a sample of 277 dry pods from 74 *B. curvula*

individuals. This sampling was undertaken at eight different sites during the fruiting season of 2010. Likewise, each pod had its seeds indexed and categorized as aborted or non-aborted.

Data analysis

To control for the effect of pod size (i.e. number of seeds per pod) we replaced the absolute position of seeds by their relative position in the analyses. This relative position varied from 0 (most basal) to 1 (most apical). The effect of seed position on the risk of seed abortion was tested by adjusting a binomial GLM with presence of an aborted seed as the response variable and the relative position of the seed as the explanatory variable.

Because a contiguous distribution (i.e. with few or no gaps) is a necessary condition for the MDE, we tested whether females laid their eggs contiguously by calculating the standard deviation of the absolute position indexes of the egg bearing seeds. Because this statistics is intrinsically dependent on the number of eggs, we rescaled it in proportion to the minimum possible value for a pod with the same number of eggs. The contiguity index has a maximum value of 1, which is observed when all the eggs are laid contiguously. Then we compared the mean value of the contiguity index with a distribution of simulated values generated by shuffling egg placement within each pod.

The MDE in the distribution of eggs on pods was accessed through two null models: i) each pod was assigned a number of eggs and a number of gaps between eggs randomly drawn from the observed distribution of egg number and gap number, and then eggs and gaps were randomly placed in the pod; ii) the entire ovipositions were reallocated within the pod by a random number of positions and random direction, within the limits of the pods. We also used a completely random null model in which egg placement was

shuffled within each pod in order to evaluate the effect of laying eggs contiguously on expected larval mortality (i.e. eggs placed directly above aborted seeds).

The effect of seed position on the probability of oviposition on that seed was tested with a binomial GLM with presence of eggs as the response variable and the second degree effect of the relative seed position, the status of the seed (aborted or not) and the predicted probability from each of the null models as explanatory variables.

All statistical analyses were performed in the software R [13], using the package *stats* for the GLM, and original code for the null model analysis (data and code available under request).

RESULTS

The mean number of seeds (aborted and non-aborted) perpod was 15.8 ($\pm$ 2.6 SD; range: 5 to 28). As predicted by the pollination failure hypothesis, there was a marked increase in the probability of abortion from apical to basal seeds (figure 1a; second degree coefficient = 14.8, z-value = 6.2, $p < 0.0001$).

Females laid on average 6.5 eggs per pod ($\pm$ 3.9 SD; range: 1 to 15). Confirming previous field observations, the eggs were laid contiguously, with an observed contiguity index of 0.6 against a simulated value of 0.49 in the 97.5 percentile. Therefore, the MDE is a plausible explanation for egg distribution in this system.

We found a hump-shaped relationship between seed position and egg incidence (figure 1b), indicating that females of the seed-beetle *G. speculifer* did not avoid aborted seeds by selecting apical portions of the pods. Actually, the empirical frequency distribution of eggs along the relative positions of seeds did not differ from the null models used to mimic the MDE (figure 2b for null model i and figure S1.1a in the electronic supplementary material S1 for null model ii). Furthermore, in the GLMs including the mean egg occurrence predicted by those models, the MDE alone was capable of predicting egg occurrence (table 1 and figure S1.1b in the electronic supplementary material S1). The simple behavior of laying eggs continuously reduced potential larval mortality at least by 7% compared to a random egg-laying behavior.

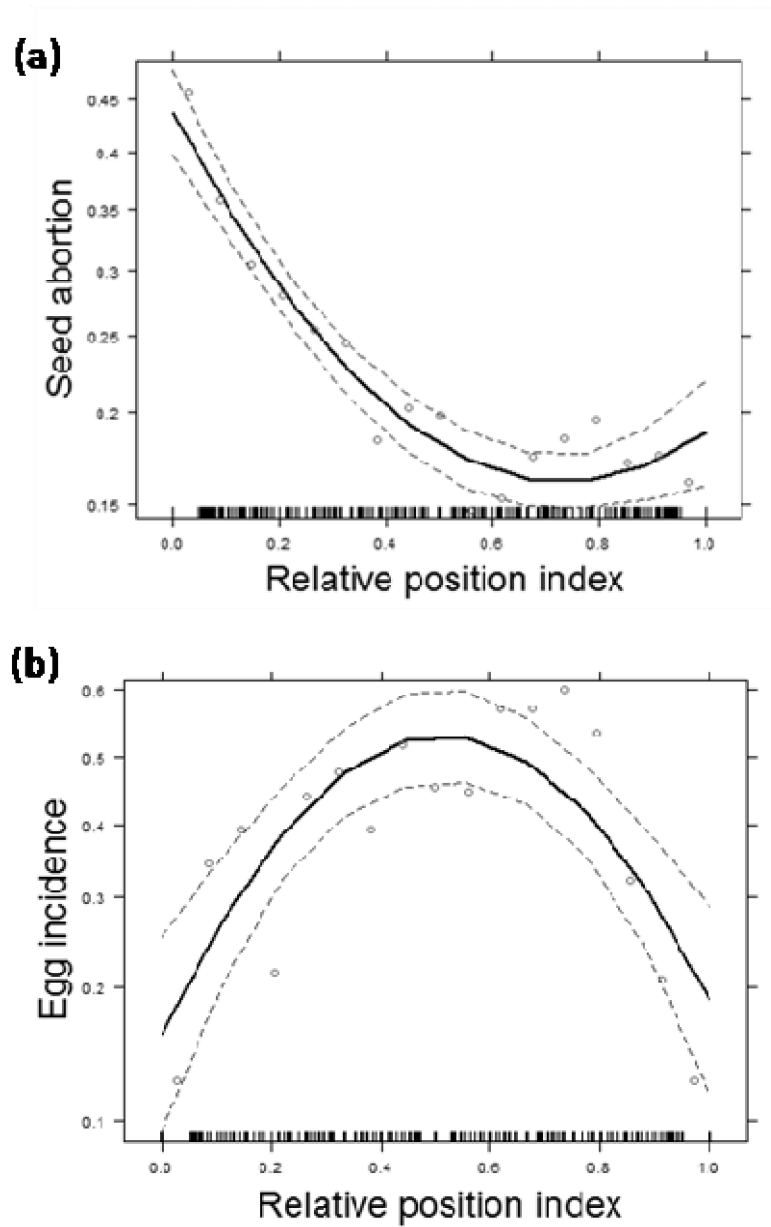


Figure 1. (a)Seed abortion and (b) egg incidence by seed position. Solid lines: model estimates; dashed lines: 95% CI; points: observed values; axis markings: data points. Observed values are expressed as mean seed abortion in 17 relative position intervals. Each interval has 271.8 ± 26.2 observations for seed abortion and 27.2 ± 3.3 observations for egg incidence.

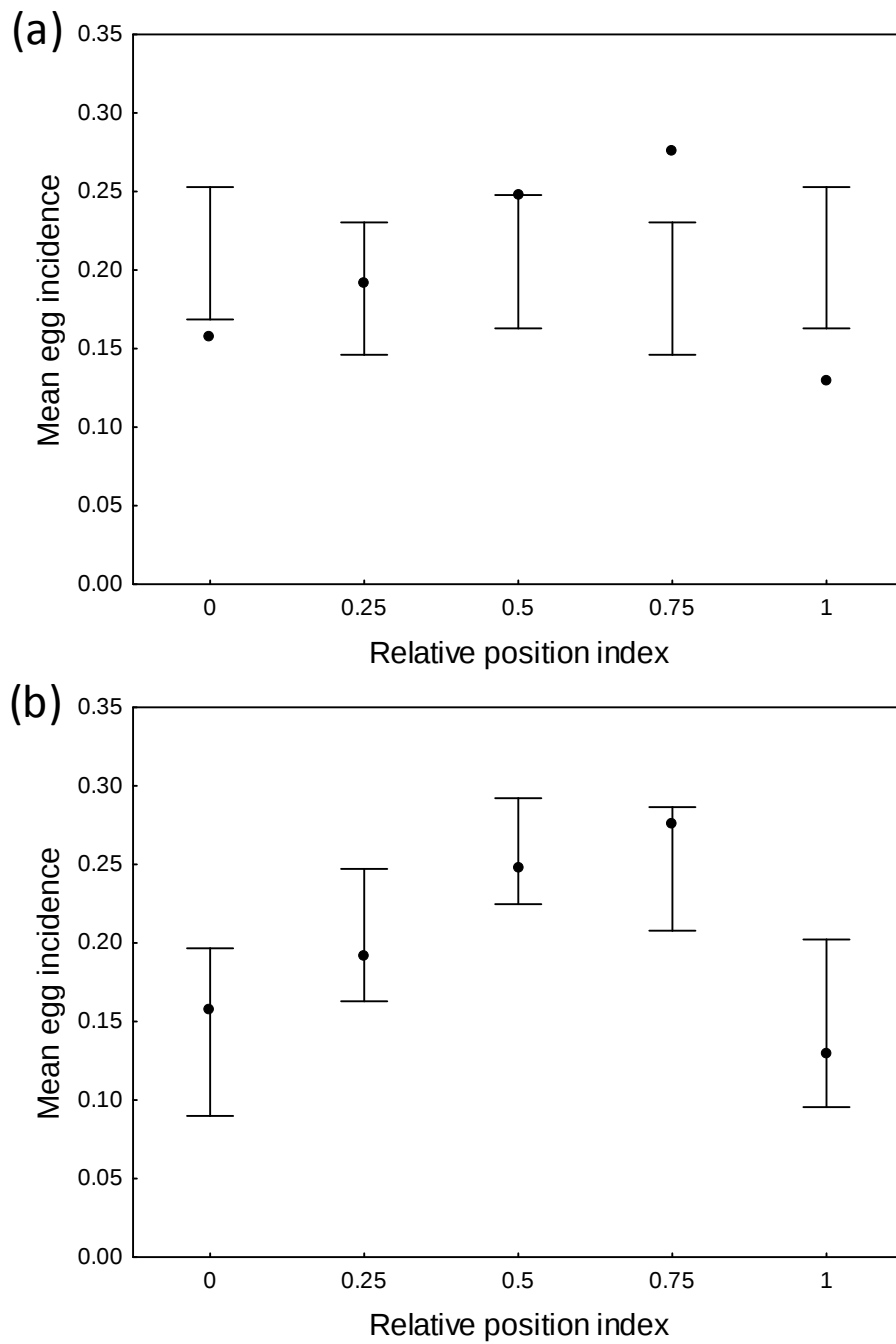


Figure 2. Mean egg incidence by seed position interval from the (a) random null model and the (b) displacement model (MDE-model). Whiskers: 95% CI; points: observed values. Observed values and null model CI are expressed as mean egg incidence in 5 relative position intervals.

Table 1 The effects of abortion status and seed position (represented by the mean egg occurrence predicted by the null model i in 5 position intervals) on egg presence.

Variable	Estimate	Std. Error	Z value	p
Intercept	-2.1424	0.4596	-4.662	<0.0001
Abortion status	-0.4998	0.2732	-1.829	0.0673
Null Model Prediction	8.7567	2.1471	4.078	<0.0001

DISCUSSION

The egg laying pattern of *G. speculifer* did not support the hypothesis that the females of this species discriminate resource quality (i.e. risk of seed abortion) for their offspring within pods. However, because the eggs were laid contiguously and the egg-laying occurs within a one-dimensional space with a geometric constraint, a random accumulation of eggs on middle portions of the pods was expected to occur as a kind of mid-domain effect. Our findings support a MDE on egg distribution in linear fruits, a parsimonious hypothesis that explains the empirical egg-laying pattern in *G. speculifer*.

The most interesting consequence of the MDE in this study is that it accounted for a reduction of 7% in offspring mortality compared to what would be expected due to seed abortion if the eggs were laid randomly along the pod. Such reduction occurred because the accumulation of eggs on middle portions of the pods caused a reduction of eggs on the basal seeds, which have higher risk of abortion. We hypothesize that the MDE might also represent a selective effect in clutch size because range size (here represented by clutch size) has a consistent effect in the strength of the MDE [12,14]. Thus, there should be an optimum clutch size for each pod size that leads to maximum MDE and, consequently, maximum avoidance of the risky aborted basal seeds (for an example of clutch size manipulation, see [15]).

On the other hand, laying eggs too contiguously might represent some risk of intraspecific competition, since about 1 in 5 larvae also eat parts of the adjacent seed (unp. data). If there is such trade-off, the degree of egg aggregation on pods might be under a stabilizing selection around an optimal value. Further studies detailing the costs of increased intraspecific competition might shed some light on these hypotheses.

We have shown that simple behavioral rules operating on a constrained one-dimensional space are sufficient to explain the fine-scale distribution of seed-beetle eggs. Even in the absence of any apparent resource assessment mechanism at the fruit level, there was a reduction in larval mortality due to seed abortion compared to a random oviposition scenario. As extensively discussed in other study areas of ecology [16,17] stochastic processes should be considered in the interpretation of observed biological to avoid misinterpretations of the real mechanisms generating those patterns.

ACKNOWLEDGEMENTS

L. B. was supported by a grant from CAPES, M.A.N was supported by CNPQ. We thank Marcos Vieira for his constructive comments and helpful suggestions.

REFERENCES

- 1 Gripenberg, S., Mayhew, P. J., Parnell, M. & Roslin, T. 2010 A meta-analysis of preference-performance relationships in phytophagous insects. *Ecology Letters* **13**, 383–393. (doi:10.1111/j.1461-0248.2009.01433.x)
- 2 Brodbeck, B., Andersen, P. & Oden, S. 2007 Preference-performance linkage of the xylem feeding leafhopper, *Homalodisca vitripennis* (Hemiptera Cicadellidae). *Environmental Entomology* **36**, 1512–1522. (doi:10.1603/0046-225X(2007)36)
- 3 Santos, J. C., Silveira, F. A. O. & Fernandes, G. W. 2008 Long term oviposition preference and larval performance of *Schizomyia macrocapillata* (Diptera :

- Cecidomyiidae) on larger shoots of its host plant *Bauhinia brevipes* (Fabaceae). *Evolutionary Ecology***22**, 123–137. (doi:10.1007/s10682-007-9162-z)
- 4 Janzen, D. H. 1980 Specificity of seed-attacking beetles in a costa rican deciduous forest. *Journal of Ecology***68**, 929–952.
 - 5 Kato, T., Bonet, A., Yoshitake, H., Romero-Nápoles, J., Jinbo, U., Ito, M. & Shimada, M. 2010 Evolution of host utilization patterns in the seed beetle genus *Mimosestes* Bridwell (Coleoptera: Chrysomelidae: Bruchinae). *Molecular Phylogenetics and Evolution***55**, 816–832. (doi:10.1016/j.ympev.2010.03.002)
 - 6 Mohan Raju, B., Uma Shaanker, R. & Ganeshaiah, K. N. 1996 Intra-fruit seed abortion in a wind dispersed tree, *Dalbergia sissoo* Roxb: Proximate mechanisms. *Sexual Plant Reproduction***9**, 273–278.
 - 7 Mena-Alí, J. I. & Rocha, O. J. 2005 Selective Seed Abortion Affects the Performance of the Offspring in *Bauhinia ungulata*. *Annals of Botany***95**, 1017–1023. (doi:10.1093/aob/mci109)
 - 8 Mena-Alí, J. I. & Rocha, O. J. 2005 Effect of Ovule Position within the Pod on the Probability of Seed Production in *Bauhinia ungulata* (Fabaceae). *Annals of Botany***95**, 1017–1023. (doi:10.1093/aob/mci109)
 - 9 Obeso, J. 2004 A hierarchical perspective in allocation to reproduction from whole plant to fruit and seed level. *Perspectives in Plant Ecology, Evolution and Systematics***6**, 217–225. (doi:10.1078/1433-8319-00080)
 - 10 Manfio, D., Ribeiro-costa, C. S. & Caron, E. 2012 Phylogeny and revision of the New World bruchine genus *Gibbobruchus* Pic, 1913 (Coleoptera: Chrysomelidae). *Invertebrate Systematics***Accepted**.
 - 11 Jaenike, J. 1978 On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology***14**, 350–356. (doi:10.1016/0040-5809(78)90012-6)
 - 12 Colwell, R. K. & Lees, D. C. 2000 The mid-domain effect: geometric constraints on the geography of species richness. *Trends in Ecology & Evolution***15**, 70–76.
 - 13 R Development Core Team 2011 R: A language and environment for statistical computing.
 - 14 Arita, H. T. 2005 Range size in mid-domain models of species diversity. *Journal of Theoretical Biology***235**, 119–126.
 - 15 Fox, C. W., Martin, J. D., Thakar, M. S. & Mousseau, T. a. 1996 Clutch size manipulations in two seed beetles: consequences for progeny fitness. *Oecologia***108**, 88–94. (doi:10.1007/BF00333219)

- 16 Williams, R. J. 2011 Biology, methodology or chance? The degree distributions of bipartite ecological networks. *PloS One***6**, e17645. (doi:10.1371/journal.pone.0017645)
- 17 Gotelli, N. J. 2001 Research frontiers in null model analysis. *Global Ecology and Biogeography***10**, 337–343.

Electronic Supplementary Material 1

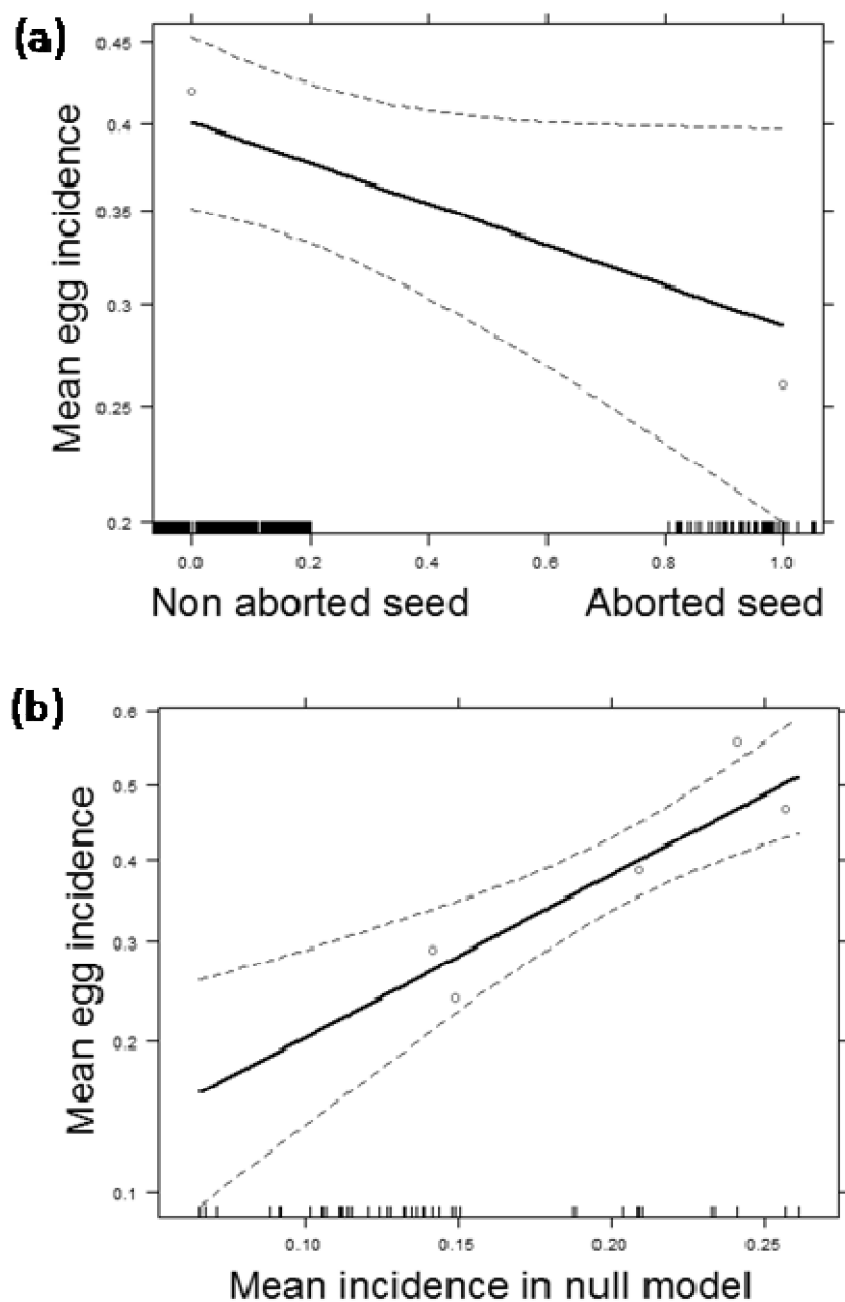


Figure S1.1. Effect of a) seed abortion and b) mean egg incidence predicted by the displacement null model on mean egg incidence. Solid line: Model estimates; dashed lines: 95% CI; points: observed values; axis markings: data points. Observed values are expressed as mean values in each of the position intervals.

4 ã CONSIDERAÇÕES FINAIS

Em cada capítulo, investigamos alguns aspectos do processo de seleção de hospedeiras de *Gibbobruchus cavillator* e *G. speculifer*. Apesar de serem duas espécies cogenéricas e simpátricas que compartilham a mesma planta hospedeira, notam-se claras diferenças nas estratégias de seleção de plantas hospederias de cada uma. Enquanto as decisões das fêmeas de *G. cavillator* podem parecer inadequadas se analisadas na escala incorreta, a aparente habilidade de seleção em fina-escala das fêmeas de *G. speculifer* pode ser um subproduto de comportamentos simples em um sistema geometricamente restrito. Este tipo de diferenças comportamentais entre espécies que, de outra forma, seriam ecologicamente muito parecidas, podem ter grandes consequências evolutivas(Kato et al. 2010). Portanto, entender como atributos-chave são capazes de modular a forma como os organismos filogeneticamente próximos respondem a pressões seletivas comuns é um passo importante para compreender os processos que levam à diferenciação e consequentemente à diversificação destes grupos.

5 ã REFERÊNCIAS BIBLIOGRÁFICAS

- Bernays, E. & Graham, M. (1988). On the Evolution of Host Specificity in Phytophagous. *Ecology*, 69, 886ã892.
- Farrell, B.D. (1998). óInordinate Fondnessô Explained: Why Are There So Many Beetles? *Science*, 281, 555ã559.
- Gaston, K.J. (1991). The Magnitude of Global Insect Species Richness. *Conservation Biology*, 5.

- Gripenberg, S., Mayhew, P.J., Parnell, M. & Roslin, T. (2010). A meta-analysis of preference-performance relationships in phytophagous insects. *Ecology letters*, 13, 383–393.
- Jaenike, J. (1978). On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology*, 14, 350–356.
- Jaenike, J. (1990). Host Specialization in Phytophagous Insects. *Annual Review of Ecology and Systematics*, 21, 243–273.
- Janz, N. & Nylin, S. (2008). The Oscillation Hypothesis of Host-Plant Range and Speciation. In: *Specialization, Speciation, and Radiation: the Evolutionary Biology of Herbivorous Insects* (ed. Tilmon, K.J.). University of California Press, Berkeley, California, pp. 203–215.
- Johnson, C.D., Romero, J. & Romero-Nápoles, J. (2004). A review of evolution of oviposition guilds in the Bruchidae (Coleoptera). *Revista Brasileira de Entomologia*, 48, 401–408.
- Kato, T., Bonet, A., Yoshitake, H., Romero-Nápoles, J., Jinbo, U., Ito, M., et al. (2010). Evolution of host utilization patterns in the seed beetle genus *Mimosestes* Bridwell (Coleoptera: Chrysomelidae: Bruchinae). *Molecular phylogenetics and evolution*, 55, 816–832.
- Manfio, D., Ribeiro-costa, C.S. & Caron, E. (2012). Phylogeny and revision of the New World bruchine genus *Gibbobruchus* Pic, 1913 (Coleoptera: Chrysomelidae). *Invertebrate Systematics*, Accepted.
- Mayhew, P.J. (1997). Adaptive Patterns of Host-Plant Selection by Phytophagous Insects. *Oikos*, 79, 417.
- Mayhew, P.J. (2001). Herbivore host choice and optimal bad motherhood. *Trends in ecology & evolution (Personal edition)*, 16, 165–167.
- Mayhew, P.J. (2007). Why are there so many insect species? Perspectives from fossils and phylogenies. *Biological reviews of the Cambridge Philosophical Society*, 82, 425–454.
- Mitter, C., Farrell, B. & Wiegmann, B. (1988). The Phylogenetic Study of Adaptive Zones : Has Phytophagy Promoted Insect Diversification ? *American Naturalist*, 132, 107–128.
- Nosil, P. & Ecology, B. (2002). Transition rates between specialization and generalization in phytophagous insects. *Evolution*, 56, 1701–1706.
- Nyman, T. (2010). To speciate , or not to speciate ? Resource heterogeneity , the subjectivity of similarity , and the macroevolutionary consequences of niche-width shifts in plant-feeding insects. *Biological Reviews*, 85, 393–411.

Scotland R.W. & Wortley A.H. (2003). How many species of seed plants are there? *Taxon*, 52, 101–104.

Ødegaard, F. (2000). How many species of arthropods? Erwin's estimate revised. *Biological Journal of the Linnean Society*, 71, 583–597.