RESEARCH ARTICLE

Evidence for insect seed predator dynamics mediated by vertebrate frugivores

Evidencias de dinámicas de insectos depredadores de semillas mediadas por vertebrados frugívoros

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ABSTRACT

Vertebrate frugivores have been suggested to reduce seed predation, indirectly controlling populations of insect seed predators (ISP) by means of consuming many individuals when feeding on fruits. The possibility has not been explored, however, that this 'frugivore predation' may differentially affect ISP according to species-specific differences in larval development time within the fruit. In the dry tropical forest trees *Acacia pennatula* and *Guazuma ulmifolia* we compared seed predation and the absolute and relative abundances of bruchid beetle species (Bruchinae) in two sites, one with large frugivores (cattle) and the other cattle-free for a considerable time. In the site with cattle we found a notable overall reduction in the proportion of seeds predated (ca. 15 %) with respect to cattle-free site, and changes in the ISP community as well, in particular a reduced absolute and relative abundance of the bruchid species with the longest larval development time. Our results suggest that the interplay between evolutionary processes (resulting in variation in insect life-histories) and complex ecological interactions (inadvertent consumption by larger animals) may contribute to the coexistence of different insect species feeding upon the same host plant.

Key words: frugivory, seed predation, trophic interactions, tropical dry forests.

RESUMEN

Se ha sugerido que los vertebrados frugívoros reducen la depredación de semillas, al controlar indirectamente las poblaciones de insectos depredadores de semillas (IDS) por medio del consumo de muchos individuos cuando se alimentan de los frutos. No obstante, no se ha explorado la posibilidad de que esta 'depredación frugívora' pueda afectar diferencialmente a los IDS de acuerdo a diferencias específicas en el tiempo de desarrollo larval dentro del fruto. En las especies del bosque seco tropical *Acacia pennatula* y *Guazuma ulmifolia* comparamos la depredación de semillas y las abundancias absolutas y relativas de las especies de escarabajos brúquidos (Bruchinae) en dos sitios, uno con grandes frugívoros (ganado) y el otro libre de ganado por un tiempo considerable. En el sitio con ganado encontramos una notable reducción general en la proporción de semillas depredadas (ca. 15 %) respecto al sitio sin ganado, y cambios en la comunidad de IDS, en particular una reducción en la abundancia absoluta y relativa de la especie de brúquido con el tiempo de desarrollo larval más largo. Nuestros resultados sugieren que la interacción entre procesos evolutivos (resultantes en variaciones de las historias de vida de los insectos) y las complejas interacciones ecológicas (consumo inadvertido por grandes animales) puede contribuir a la coexistencia de distintas especies de insectos alimentándose de la misma planta huésped.

Palabras clave: bosques tropicales secos, depredación de semillas, frugivoría, interacciones tróficas.

INTRODUCTION

Coexistence among species exploiting the same resources has long intrigued ecologists. In particular, coexistence among insect seed predators sharing the same host plant has often been attributed to trait-mediated effects resulting from ecological constraints based on differences in insect sizes (Espelta et al. 2009, Bonal et al. 2011). Vertebrates that consume the fruits whose seeds are being exploited by different species of insect predators, though, add another dimension to their coexistence. Indeed, the evolutionary triads of host plant, seed predators, and vertebrate frugivores may lead to complex and dynamic interactions. In

addition to direct two-way relationships with the plant -exploitative with seed predators and generally mutualistic with frugivores- there may be indirect interactions between frugivores and seed predators that end up benefiting the plant (Sallabanks & Courtney 1992). For example, once they are dispersed seeds are no longer available for pre-dispersal insect predators, in essence "escaping" in space (Janzen 1971). Additionally, frugivores may indirectly control the size of seed predators' populations by killing insects (i.e. larvae or pupae) still in the seeds when fruits are consumed (Herrera 1989, Hauser 1994, Gómez & González-Megías 2002, Bonal & Muñoz 2007).

If insect infestation does not deter vertebrate frugivores from consuming a fruit because of altered fruit characteristics (Herrera 1984), vertebrate-dispersed fruits may be 'risky places' for insects feeding inside them. Furthermore, the longer the time spent in the fruit the greater the probability of being ingested. Therefore, the guild of insects that predate the seeds of the same plant may experience different degrees of risk of death in frugivore mouths or guts depending on their residence time in fruits, that is, by species-specific differences in larval development time. This reasoning suggests that the presence of frugivores might not only reduce seed predation but also might modify the assemblage of insect seed predators, by reducing populations of species with longer development times more than those of species with more rapid development. Nevertheless, the possibility that insect seed predators with different life-history traits may have different vulnerabilities to frugivores, has not previously been explored.

Here, we report on insect seed predation in two common species of trees of dry tropical forests, *Guazuma ulmifolia* L. (Malvaceae) and *Acacia pennatula* Benth. (Fabaceae). We quantified (1) frequency and intensity of seed predation by host-specific insects and (2) the species composition of seed predators for both plant species in two sites, one with and one without large vertebrate frugivores (cattle). The results suggest that vertebrate frugivores may not only reduce overall seed predation by insects but may also lead to changes in insect species composition by disproportionately

reducing the abundance of those species with longer larval development times.

METHODS

Study site and species

This study took place at El Limón Biological Field Station of the National Autonomous University of Nicaragua-Managua, in the Estelí valley of northwest Nicaragua (13º 03' 44" N - 86º 21' 57" W). The region's climate is typical of Central American dry tropical forests, with monthly temperatures ranging from 16 to 33 ºC and 90 % of the 830 mm of mean annual precipitation falling between November and May (Nicaraguan Institute of Territorial Studies/INETER, personal communication). The landscape is a mosaic of savannah-like pastures, secondary vegetation and remnant tropical dry forest patches, which the natives *A. pennatula* (Schltdl. & Cham.) Benth. and *G. ulmifolia* Lam. usually dominate (Somarriba 2012). Both species bloom during the dry season and fruits initiated remain immature until the end of the rainy season, when they rapidly mature and then gradually fall from February to May. Fruits of *A. pennatula* are indehiscent dry flat pods with mean length of 8.5 ± 0.06 cm (mean ± SE hereafter, n = 600), width of 2 ± 0.01cm and an average of 10 ± 0.14 seeds, each with a mass of 0.08 ± 0.002 g. Fruits of *G. ulmifolia* are hard spheroidal nuts averaging 2.3 ± 0.01 cm in maximum diameter (n = 600) and containing 44 ± 0.67seeds each with mass of 5.7 ± 0.3 mg (Peguero & Espelta, unpublished data). Once on the ground, both types of fruits are actively consumed by domestic cattle, the modern day substitute for the Pleistocene mega-herbivores with which these plants probably evolved (Janzen & Martin 1982), as well as by extant native herbivores such as *Odocoileus virginianus* Zimm., *Pecari tajacu* L. and *Tayassu pecari* Palmer, virtually absent from this fragmented and defaunated landscape.

Seeds of both species suffer frequent predation by the larvae of several bruchid species (Fig. 1): *Mimosestes anomalus* Kingsolver & Johnson (1978) and *Mimosestes humeralis* Gyllenhal in *A. pennatula*, and *Amblycerus cistelinus* Gyllenhal and *Acanthoscelides guazumae* Johnson & Kingsolver (1971) in *G. ulmifolia*. It should be noticed that, while larvae of *M. anomalus*, *M. humeralis* and *A. guazumae* require a single seed to complete their development, the larvae of *A. cistelinus* must eat almost all seeds of a single fruit to become an adult (Janzen 1982, Traveset 1992). Therefore in *A. cistelinus* only one adult can emerge from each infested fruit after a time length of about 6 weeks after oviposition, whereas in the other species in 3-4 weeks as many adults may emerge as sound seeds in the fruit (Janzen 1982, Traveset 1992). Accordingly, *A. cistelinus* is fundamentally a univoltine species whereas *Mimosestes* spp. and *A. guazumae* are multivoltine and produce several generations during a given fruiting season (Janzen 1982, Traveset 1992).

Sampling design

To evaluate the potential effect of frugivores on seed predation and on the relative abundances of the four bruchid species, we selected two adjacent woodlands, both on flat terrain and with similar management histories (in particular, long-term control of anthropogenic fires) and both with abundant *A. pennatula* and *G. ulmifolia* trees. One woodland of ca.
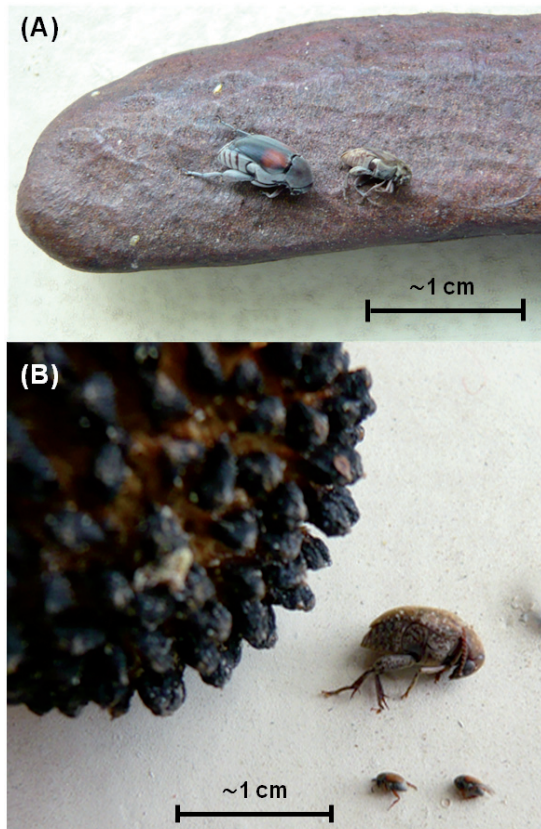
Fig. 1: Insect seed predators of (a) *Acacia pennatula*: *Mimosestes humeralis* (length pronotum-elytra, 3.6–6.0 mm) and *M. anomalus* (3.0–3.6 mm); and (b) *Guazuma ulmifolia*: *Amblycerus cistelinus* (7.0–8.5 mm) and *Acanthoscelides guazumae* (1.7–2.2 mm).

Insectos depredadores de semillas de (a) *Acacia pennatula*: *Mimosestes humeralis* (longitud pronoto-elitro, 3.6-6.0 mm) y *M. anomalus* (3.0-3.6 mm); y (b) *Guazuma ulmifolia*: *Amblycerus cistelinus* (7.0–8.5 mm) y *Acanthoscelides guazumae* (1.7–2.2 mm).

15 ha has abundant cattle, which consume most or all fallen fruits of those two species. The other tract, 17 ha of secondary forest managed by El Limón Biological Field Station, is a former ranch from which cattle have been excluded for 13 years and where there is little or no consumption of fallen fruits by any vertebrate frugivore (Peguero, personal observation).

During March 2009, at the peak of the fruiting season of both species, we selected ten randomly distributed trees of each species within each site. We measured projected crown area of each tree, and estimated its fruit crop size by making two independent counts of fruits over a 30 second period. Then we gently shook the tree's branches and collected 30 fallen ripe fruits, for a total of 1200 fruits across the two species and the two sites. In the laboratory, each fruit was tagged and packed into an inflated plastic-bag until adults ceased to emerge. All adult beetles were collected for species determination. Finally, we dissected each fruit and first classified it as infested or not, from which we calculated the proportion of infested fruits per tree. We then counted the number of predated and healthy seeds within the fruit, from which we calculated the proportion of predated seeds per fruit, averaged across the 30 fruits per tree as a measure of seed predation intensity.

Data analysis

Differences in fruit crop sizes and seed predation intensity among sites were analyzed by general linear models after confirming the normality of the response variables (GLM procedure, SAS Institute 1996). When analyzing fruit crop size we included projected crown area as a covariate in order to take into account differences in tree size between the two sites. To analyze seed predation intensity we included fruit crop size as a covariate to account for the potential effect of predator satiation. Differences in the frequency of infestation among sites were analyzed through Kruskal-Wallis tests (NPAR1WAY procedure, SAS Institute 1996). Changes in the relative species composition of the seed predators assemblage among sites were tested at tree level (i.e., considering all sampled fruits per tree) by comparing the absolute number of adults of each beetle species and the relative number (proportion with respect to total). After confirming normality, absolute counts of adults were analyzed by means of a general linear model with site as explanatory variable and tree as a random factor to control for individual-level variation (MIXED procedure, SAS Institute 1996), whereas proportional abundances were analyzed with Kruskal-Wallis tests (one per beetle species) using site as the grouping variable (NPAR1WAY procedure, SAS Institute 1996).

RESULTS

There were no significant differences, statistically or biologically speaking, in fruit crop size per tree among sites for *A. pennatula* (143 ± 4.4 vs. 137 ± 12.2 mean number ± 1 SE of fruits per tree; $F_{1,17} = 1.0$, $P = 0.3$) or for *G. ulmifolia* (174 ± 9.7 vs. 184 ± 4.3; $F_{1,17} = 1.2$, $P = 0.3$). Likewise, in neither of the two species was fruit production influenced by crown area (covariate effect $F_{1,17} = 1.9$, $P = 0.2$ for *A. pennatula*, and $F_{1,17} = 0.07$, $P = 0.8$ for *G. ulmifolia*). The frequency of fruits infested was notably higher for *G. ulmifolia* trees located in the site without frugivores than in the site with cattle (0.65 ± 0.06 vs. 0.44 ± 0.06; $\chi^2_{1,20} = 6.5$, $P = 0.01$) but no significant differences were observed in *A. pennatula* (0.87 ± 0.03 vs. 0.92 ± 0.03; $\chi^2_{1,20} = 2.3$, $P = 0.13$). In contrast, for both species average seed predation intensity per fruit was notably greater among trees in the cattle-free site than in the site with cattle (84.3 ± 4 % vs. 69.5 ± 4 % of seeds predated per fruit, $F_{1,17} = 7.8$, $P = 0.01$ for *A. pennatula* and 37.8 ± 4 % vs. 11.5 ± 4 %, $F_{1,17} = 19.1$, $P = 0.0004$ for *G. ulmifolia*; Fig. 2). These predation rates were not significantly influenced by fruit crop size
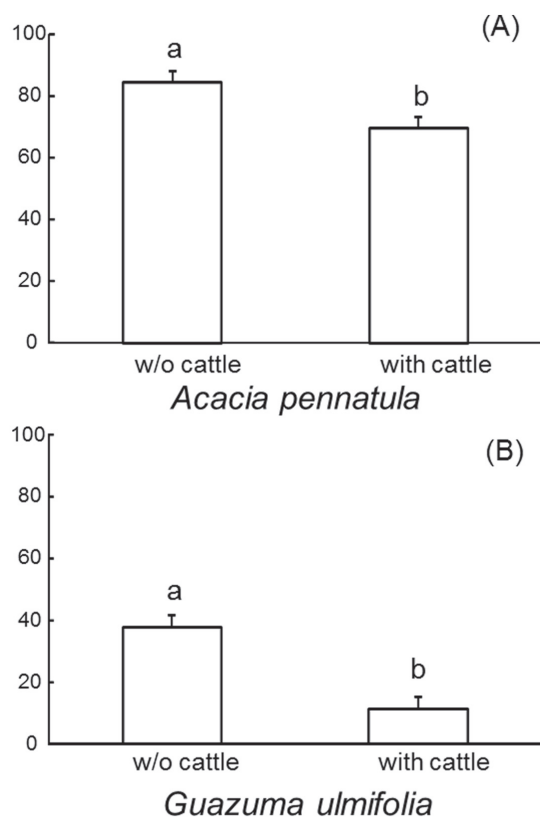
Fig. 2: Differences between the two sites (with and without cattle) in the proportion of seeds predated. Different letters indicate significant differences according to the LSMEANS test.

Diferencias entre los dos sitios (con y sin ganado) en la proporción de semillas depredadas. Letras distintas denotan diferencias significativas de acuerdo al test LSMEANS.

per tree (covariate effect $F_{1,17} = 0.26$, P = 0.2 and $F_{1,17} = 0.07$, P = 0.8, respectively).

In the laboratory, numerous adult beetles emerged from the bagged fruits but we did not encounter any parasitoid. The two beetles preying on *A. pennatula* displayed no statistically significant between-site differences either in absolute numbers of adult beetles collected per tree (6.8 ± 2.7 vs. 8.2 ± 2.7, $F_{1,9} = 0.13$, P = 0.73 for *M. humeralis*, and 24.4 ± 5.5 vs. 25.2 ± 5.5, $F_{1,9} = 0.01$, P = 0.92 for *M. anomalus*; Fig. 3a) or in relative abundances ($\chi^2_{1,20} = 0.57$, P = 0.45; Fig. 4a). In contrast, the seed predators of *G. ulmifolia* displayed marked differences between sites. Absolute abundance of the large *Amblycerus cistelinus* in the cow-free site was nearly three times greater than in the site with cattle (5.5 ± 0.9 vs. 2.0 ± 0.9 beetles

collected per tree, $F_{1,9} = 7.63$, P = 0.02; Fig. 3b) while numbers of *Acanthoscelides guazumae* were exponentially lower in the cow-free site than in the other (0.1 ± 3.8 vs. 15.9 ± 3.8 beetles collected per tree, $F_{1,9} = 8.87$, P = 0.016; Fig. 3b). In consequence, the relative abundances of the two beetles showed a dramatic shift (Fig. 4b): i.e. in the cow-free site almost all beetles collected (98 %) were *A. cistelinus*, whereas in the site with cattle only 28 % were of that species ($\chi^2_{1,9} = 12.9$, $P = 0.0003$).

DISCUSSION

The results of this study provide preliminary evidence to support the proposal that frugivores may affect the populations of seed-feeding insects through incidental digestion (Herrera, 1989, Hauser 1994, Gómez & González-Megías 2002) and that this may result in a significant reduction in seed predation (Bonal & Muñoz 2007). The results also suggest a hitherto unreported corollary: that "frugivore effects" may differ among different species of insect seed predators exploiting the same host species, according to differences in insect life history traits such as their larval development time.

We acknowledge that these results must be cautiously taken since the lack of comparable tracts suitable as replicates made true replication impossible. Clearly, spatial replication would have greatly strengthened these arguments, given that the observed differences between the two tracts could also be related to tract-specific features other than cattle presence or absence (see Ramage et al. 2012). Nevertheless, the frugivory explanation for the observed between-tract changes in seed predation levels and seed predator species composition is the most parsimonious. It is supported by two additional results: (i) the parallel reduction of seed predation intensity in trees of both *A. pennatula* and *G. ulmifolia* with cows present and (ii) the marked contrast between the drastic reduction in numbers of the bruchid species with the longest larval development time (*A. cistelinus*) and the much less conspicuous reduction in numbers of the three species whose larvae spend less time in fruits (*A. guazumae*, *M. anomalus*, *M. humeralis*).

The lack of grass during the dry season means that cattle highly desire the fallen ripe

fruits of these two tree species and that fruits are likely to be consumed whether they are sound or insect-infested (Janzen 1982, Traveset 1992, Peguero & Espelta unpublished data). Cows ingesting fruits directly mediate 'seed escape' of viable seeds but also inadvertently kill larvae or pupae infesting other seeds (Herrera 1989, Hauser 1994). Indeed, once ingested by a vertebrate frugivore a larva of *A. cistelinus* has no chance of survival given that it does not have any protection against the cow digestive process (i.e. acids and bacteria). Though the larvae of the other species are
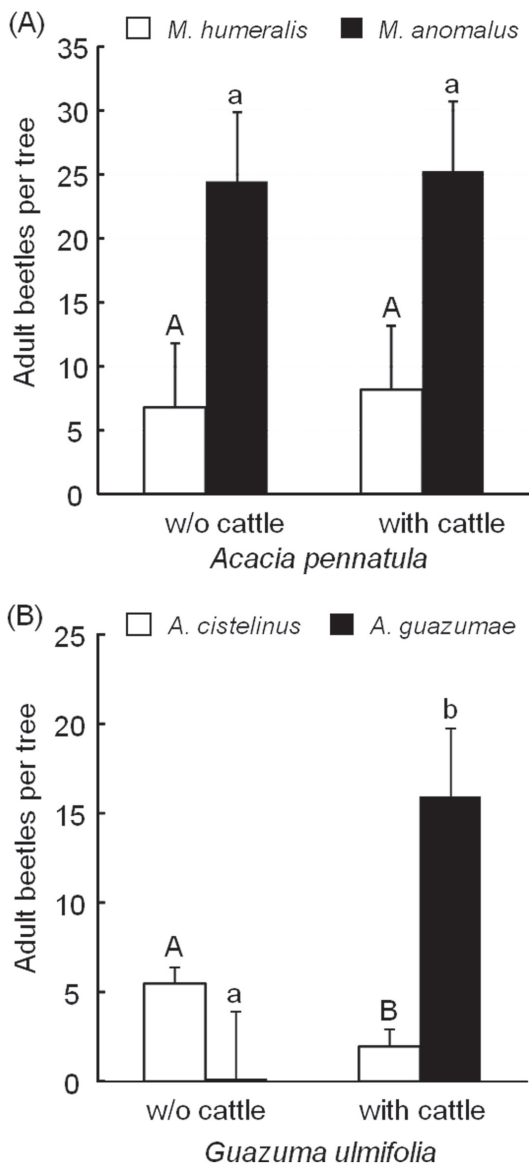
Fig.3: Differences between the two sites (with and without cattle) in the mean number of adult beetles emerged per tree. Different letters indicate significant differences according to the LSMEANS test. Upper-case and lower-case letters indicate the between-sites comparison of each insect species.

Diferencias entre los dos sitios (con y sin ganado) en el número promedio de escarabajos adultos emergidos por árbol. Letras distintas denotan diferencias significativas de acuerdo al test LSMEANS. Letras mayúsculas y minúsculas indican la comparación entre sitios para cada una de las especies de insectos.
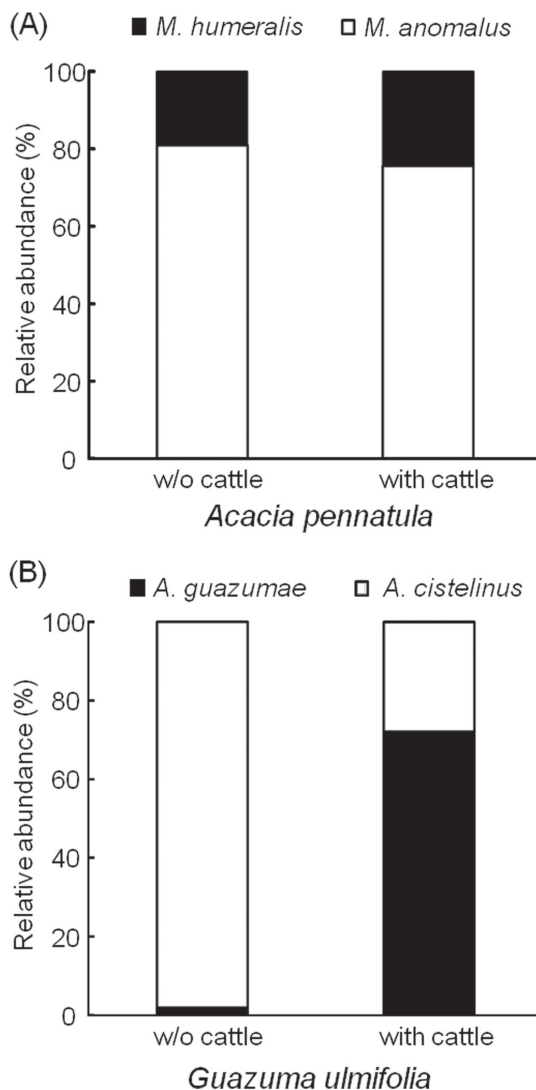

Fig. 4: Differences between the two sites (with and without cattle) in the relative abundances of insect seed predators.

Diferencias entre los dos sitios (con y sin ganado) en las abundancias relativas de los insectos depredadores de semillas.

somewhat sheltered within single seeds, their survival probability should be similarly low taking into account that the seed coat was previously breached by the first instar larvae at the time of infestation.

Thus, at least in the case of *A. pennatula* and *G. ulmifolia* in our study site, fallen fruits are likely to be risky places for all insect seed predators but apparently more risky for some species than for others. In the case of *G. ulmifolia*, the long absence of frugivores (no cattle for 13 years) was associated with a great increase in numbers of *A. cistelinus* and conspicuous decrease in the tiny *A. guazumae*. It is possible that larvae of the former species devour the single-seed infesting larvae of the latter when multi-species infestation of the same fruit occurs (see Janzen 1975, 1982). Such kind of intra-guild predation seems to be commonplace between pairs of bruchids sharing the same host plant (Johnson & Kingsolver 1971, Janzen 1982), and taking into account the high levels of fruit infestation frequency this competitive interaction should be particularly intense between the two *Mimosestes* species. The lower vulnerability of *A. guazumae* to vertebrate frugivory could arise from its life-history strategy: small body size and multivoltinism associated with short development time and therefore less time spent within the fruit exposed to hungry cows. Interestingly, the two Mimosestes species exploit the seeds of the same host through similar life histories (i.e. multivoltinism, short larval development times and small body size) and apparently are equally (and little) vulnerable to ingestion by cattle.

Larger body size generally confers several competitive advantages such as higher fecundity (Honěk 1993), though it also has drawbacks such as increased resource requirements (Bonal & Muñoz 2009, Espelta et al. 2009). On the other hand, taking advantage of the favorable season (i.e., multivoltinism) appears to be correlated with reductions in development time and body size (Nylin & Gothard 1998). Our results suggest that the coexistence of insect species exploiting the seeds of the same host plant may result in part from the interplay between evolutionary processes resulting in variations on life-history traits (e.g. larval development times) and ecological interactions (e.g. trophic interactions) (Bonsall et al. 2004). Nonetheless, the results provide only initial support for this hypothesis, as our study design lacked spatial replicates and we examined a limited number of bruchid species infesting just two of the many tree species experiencing seed predation in dry tropical forests. We suggest that future studies on this intriguing three-way interaction (plants, frugivores and seed predators with different life history strategies) be extended to additional species.

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