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LETTER

Tri-trophic interactions affect density dependence of seed fate in a tropical forest palm

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Abstract

Natural enemies, especially host-specific enemies, are hypothesised to facilitate the coexistence of plant species by disproportionately inflicting more damage at increasing host abundance. However, few studies have assessed such Janzen–Connell mechanisms on a scale relevant for coexistence and no study has evaluated potential top-down influences on the specialized pests. We quantified seed predation by specialist invertebrates and generalist vertebrates, as well as larval predation on these invertebrates, for the Neotropical palm *Attalea butyracea* across ten 4-ha plots spanning 20-fold variation in palm density. As palm density increased, seed attack by bruchid beetles increased, whereas seed predation by rodents held constant. But because rodent predation on bruchid larvae increased disproportionately with increasing palm density, bruchid emergence rates and total seed predation by rodents and bruchids combined were both density-independent. Our results demonstrate that top-down effects can limit the potential of host-specific insects to induce negative-density dependence in plant populations.

Keywords

Attalea butyracea, Barro Colorado Island, enemies hypothesis, negative density dependence, seed predation, specialization, species coexistence, species diversity, trophic cascade, tropical forest.

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INTRODUCTION

Tropical forests are astonishingly rich in tree species, which in turn support an even more impressive diversity of animals and microbes. Ecologists have long sought to identify the mechanisms underlying the coexistence of so many competing plant species (reviewed in Leigh *et al.* 2004; Pennisi 2005). One of the most widely supported explanations holds that specialised natural enemies play a key role in making each species' per capita population growth negatively density dependent (Gillet 1962; Janzen 1970). The idea is that individuals in a population of a given tree species will become more susceptible to attack by natural enemies, as they become more common, reducing individual growth, survival and/or reproduction (Gillet 1962; Carson *et al.* 2008). Such effects are expected to be especially strong at early life stages, as seeds and seedlings are particularly vulnerable to attack (Wright 2002).

Janzen (1970) and Connell (1971) independently postulated that higher densities of host-specific seed predators, pathogens and herbivores near adult plants would suppress recruitment near conspecific adults, thereby providing an advantage to other species. Janzen (1970) recognised that this mechanism required population-level density dependence to facilitate coexistence: 'The percentage of seed mortality on a parent tree should be inversely correlated with its distance to other fertile adults of the same species ... [and] … the average seed mortality on these parents should be an inverse function of the density of reproducing adults'. This reduction in per capita success with increased density is crucial to most mechanisms of species coexistence (Chesson 2000). Nonetheless, empirical tests of the Janzen–Connell hypothesis have focused almost exclusively on the actions of natural enemies close to conspecific adults, hereafter the local spatial scale (see reviews by Clark & Clark 1984; Hammond & Brown 1998; Carson *et al.* 2008). Very few studies have examined enemy influences at the larger spatial scales, necessary to evaluate relationships between per capita recruitment and the density of reproductive plants, hereafter the population-level spatial scale (Schupp 1992).

Natural enemies differ in their potential to suppress plant recruitment at the local spatial scale near conspecific adults. Many insect herbivores are short-lived host specialists with high potential reproductive rates (Janzen 1970). In contrast, herbivorous vertebrates are long-lived, feed on many plant species and have low reproductive rates relative to insects. In addition, the movements of many herbivorous vertebrates are restricted to circumscribed home ranges or territories. For these reasons, insects might respond more strongly than vertebrates to the proximity of adult trees and aggregations of seeds and seedlings. Hammond & Brown (1998) review 46 studies that compare seed or seedling performance near and far from conspecific adults. Performance was lower near conspecific adults for 15 of

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19 populations whose principal herbivore was an insect, but for just two of 27 populations whose principal herbivore was a vertebrate ($P < 10^{-6}$, Fisher Exact Test). Thus, insect and vertebrate enemies apparently respond differently to the dense seed and seedling shadows near seed-bearing trees in tropical forests. Much less is known about natural enemy responses to population-level variation in the density of seed-bearing trees (Schupp 1992; Lewis & Gripenberg 2008, see Discussion).

Higher trophic levels also influence interactions between herbivores and plants (Terborgh & Estes 2010). It is well known that predators can indirectly alter plant species composition through their influences on herbivores (Schmitz 2006). Such trophic cascades are particularly strong for endothermic predators of insect herbivores (Borer *et al.* 2005). In tropical forests, in particular, insectivorous birds and bats have significant impacts on insect abundances and plant damage (Van Bael & Brawn 2005; Kalka *et al.* 2008). Predators of insect pests that focus their efforts where prey are more abundant (e.g. Oksanen *et al.* 1981) may especially limit the ability of insect pests to control host–plant populations. Furthermore, vertebrate seed predators that consume the larvae of insect seed predators serve as intraguild predators (Silvius 2002), and theoretical work has shown that intraguild predation can be particularly important in structuring communities (Holt & Polis 1997). Insectivores might thus influence the role that these insect pests play in promoting plant coexistence.

Herein, we quantified the responses of generalist vertebrate and specialised insect seed predators to population-level variation in density of the palm *Attalea butyracea* in a diverse moist tropical forest. Previous studies at the local scale show that the proportion of seeds that escaped bruchids and rodents increased with distance from the nearest seed-bearing *Attalea* (Wright 1983; Wright & Duber 2001). Herein, we evaluated levels of (1) seed predation by a host-specific insect and (2) seed predation by generalist vertebrates, (3) predation of insect larvae by vertebrates, and (4) the net effect on seed survival over a 20-fold range in population density of the host palm. We found important top-down influences that were stronger at higher host plant abundances, and that prevented the insect seed predator from increasing disproportionately with host–plant density, strongly limiting the potential to induce negative density-dependent seed survival at the population scale. Our study highlights the importance of considering feedbacks from higher trophic levels and population-level variation when evaluating natural enemy influences on plant communities.

MATERIAL AND METHODS

Study site and species

Barro Colorado Island (BCI) is a 1560-ha island in Lake Gatun, Panama (9°9′ N, 79°51′ W). Annual rainfall averages 2600 mm, there is a distinct 4-month dry season, and the vegetation is tall semi-deciduous forest (Leigh 1999).

The arborescent palm *Attalea butyracea* (Mutis ex L.f.) Wess.Boer is monoecious, grows to heights of 30 m and is abundant in central Panama (Foster 1982; De Steven *et al.* 1987). Canopy trees produce one to three infructescences with 100–600 large fruits (3–5 cm, Wright 1983) each year (Wright 1983). Fruits have a tough exocarp, soft mesocarp and a single hard 'stony' endocarp. Most endocarps enclose one seed, but 2% contain two or three seeds (Wright 1983). The endocarp remains on the forest floor for more than 3 years as it slowly decomposes (Wright 1983).

Two bruchid beetles, *Speciomerus giganteus* (Chevrolat) and *Pachymerus cardo* (Fahraeus) prey on *Attalea* seeds. Both species require *Attalea* seeds to complete their life cycles and have no other local host species (S. Pinzon, S. Gripenberg & O.T. Lewis, unpublished data). Both species lay eggs on *Attalea* fruit; larvae then drill through the endocarp and develop inside, consuming the endosperm (Wright 1983). Adults emerge at the onset of the following wet season (S.J. Wright, personal observation). An analysis of exit holes, which are substantially larger for the bigger *Speciomerus*, indicates that *Speciomerus* comprises more than 95% of the bruchids emerging from *Attalea* endocarps on BCI (S.J. Wright and K. Silvius, unpublished data). We have reared several thousand bruchids from *Attalea* endocarps in central Panama and have yet to encounter a parasitoid (S.J. Wright, unpublished data).

Many mammal species consume the soft mesocarp of *Attalea* fruits; however, just three rodents can open the endocarps to access the seeds (Wright & Duber 2001). The three rodents are red-tailed squirrel (*Sciurus granatensis*), Central American agouti (*Dasyprocta punctata*) and Central American spiny rat (*Proechimys semispinosus).* Endocarps with the characteristic scars left by the small teeth of spiny rats were not encountered in this study, and the spiny rat is not considered further. Agoutis and squirrels are generalist seed predators, known to feed on a wide variety of seeds and fruits, and both are important seed predators of *Attalea* on BCI (Heaney & Thorington 1978; Forget *et al.* 1994). The white-lipped peccary, which once inhabited BCI, cannot open *Attalea* endocarps (Kiltie 1982).

Mammal abundances on BCI are comparable to other Neotropical sites (Wright *et al.* 1994). Squirrel abundance is somewhat lower than at most Neotropical sites, and agouti abundances are at the 75th percentile of other Neotropical sites (Wright *et al.* 1994). Large mammalian predators are also present. BCI supports 25–30 Ocelots (*Leopardus pardalis*), and Jaguar (*Panthera onca*) and Puma (*Puma concolor*) are regular visitors (J. Giacalone and P.A. Jansen, unpublished camera-trap data). Agoutis comprise almost 20 and 15% of the diets of ocelots and puma on BCI, respectively, and squirrels are a minor diet item for both cats (Moreno *et al.* 2006).

Plot selection

We selected locations for 10 square 4-ha plots using BCI-wide maps of canopy *Attalea* (C.X. Garzon-Lopez *et al.*, unpublished data) developed from high-resolution aerial photographs (Jansen *et al.* 2008). These maps allow identification of a wide range of adult *Attalea* densities including the most dense stands on BCI. We chose 4-ha plots because the seed predators function at this scale. Agouti and squirrel home ranges on BCI are typically 1.34–2.45 ha (Aliaga-Rossel *et al.* 2008) and 0.83–2.15 ha (Heaney & Thorington 1978), respectively. Bruchids oviposit on *Attalea* endocarps at similar high levels within 16 m of fruiting palms and at just 16% of this level at 100 m from fruiting palms (Wright 1983). Each plot was laid out to have a reproductive *Attalea* at its centre, and the ten plots included a wide range of adult *Attalea* densities. To minimise confounding factors, all 10 plots were located in a secondary forest, avoiding streams, steep slopes (> 30%) and lake edge (> 200 m from the lake). *Attalea* reaches its peak abundance on BCI in secondary forests that are about 130 years old and cover roughly half of BCI (Svenning *et al.* 2004).

Adult census

We mapped the position of every *Attalea* palm with a bole height (ground to lower crown) greater than 1.3 m for the ten 4-ha plots between October and December 2007, using a precision compass (Suunto KB-14 precision, Vantaa, Finland) and an ultra-sonic rangefinder (Hagloff DME-201 cruiser, Långsele, Sweden). The presence of infructescenses and inflorescences (these remain on palms for a year) identified reproductive individuals (Wright 1983). Reproductive *Attalea* density ranged from 1.25 to 23.25 individuals per hectare (Table S1 Supporting information).

Endocarp census

We investigated seed fate for endocarps collected from the forest floor and topsoil between January and August 2008. We collected endocarps from thirty-two 1-m^2 quadrats for each 4-ha plot (320 quadrats total). We located quadrats in a stratified random manner, with two qudrats in each of the sixteen 25 × 25 m subplots in the central hectare of each 4-ha plot. If a rock, tree or debris covered a randomly selected point, the quadrat was placed as close as possible to the randomly selected point in a randomly generated direction. We collected endocarps from the surface to a depth of *c.* 5 cm using a small rake. This depth ensures that scatter-hoarded endocarps were recovered; rodents typically hoard *Attalea* endocarps at depths of 2–4 cm on BCI (Smythe 1989; P.A. Jansen, personal observation). Endocarps that could be crushed by hand were excluded because decomposition could obscure bruchid and rodent scars (*sensu* Wright & Duber 2001). The remaining endocarps were up to 3 years old.

Seed fates

Bruchids and rodents leave species-specific scars on *Attalea* endocarps that can be distinguished by their size, location and shape (Silvius 2002). Bruchid larvae leave pin-sized entrance holes when they initially drill into the endocarp. Adult bruchids leave large circular emergence holes [diameter 6.6 ± 0.86 mm (mean ± SD) for *Speciomerus*]. Agoutis hold endocarps horizontally and use their lower jaws (Silvius 2002) to gnaw holes in the middle of the endocarp, and leave broad tooth marks. Red-tailed squirrels leave long gashed tooth marks and triangular openings at one end of the endocarp. We opened unscarred endocarps with a vice to determine whether seeds were viable.

Rodents make large openings in *Attalea* endocarps to extract seeds and significantly smaller openings to extract bruchid larvae (Gálvez & Jansen 2007). We used this difference to identify rodent predation of larvae, based on the size of the openings made by rodents. The size of the openings assigned to larval predation (mean ± SD; 39.4 ± 20.5 mm^2, $n = 26$) and seed extraction (115 ± 51.6 mm^2, $n = 69$) closely matched the values for larval predation (38.1 ± 17.5 mm^2, $n = 12$) and seed extraction (116 ± 31.8 mm^2, $n = 10$) reported by Gálvez & Jansen (2007).

We distinguished eight seed fates. When a bruchid larva enters an *Attalea* endocarp, this can lead to the successful development of a bruchid (BB), the consumption of the larva by an agouti (BA) or a squirrel (BS) or the death of the larva inside the endocarp for unknown reasons (BX). Fate BB included both endocarps with live larvae and endocarps with emergence holes left by an adult beetle. For endocarps that escaped bruchids, the seed may be eaten by agoutis (A) or squirrels (S), may escape predation and die for unknown reasons (X) or remain viable (V). We pooled endocarps collected from the 32 quadrats for each 4-ha plot, and calculated the proportion of endocarps for each of the eight observed seed fates, so that BB + BA + BS + BX + A + S + X + V = 1 for each 4-ha plot.

Analyses

We used logistic regression with binomial errors to evaluate relationships between palm density and 14 indices of seed fate. Model slope values ($\hat{a}_1$) were tested against zero with the z statistic (normal approximation of the binomial). Model fits were compared with null models containing only the intercept ($\hat{a}_0$, indicating no relationship with palm density) using likelihood ratio tests (Table S2). We used a Bonferroni correction across our 14 analyses. To maintain a family wise error rate of $\alpha = 0.05$, we considered an effect significant if $P \leq 0.0036$.

Eight of the indices of seed fate were simple sums of the proportion of endocarps over subsets of the eight observed seed fates. This included total seed predation (A + S + BA + BS + BB + BX), total rodent attack (A + S + BA + BS), total bruchid attack (BA + BS + BB + BX), agouti attack (A + BA), squirrel attack (S + BS), seed predation by rodents (A + S), larval predation by rodents (BA + BS) and seed predation by bruchids (BB). As these eight indices of seed fate are proportions, significant slopes indicate disproportionate changes in seed fate with palm density.

The six remaining indices of seed fate provide additional information concerning the rodent's preference for larval infested and uninfested endocarps. Three indices concern the proportion of endocarps attacked by bruchids that were also attacked by agoutis, squirrels or rodents [BA/(BA + BS + BB + BX), BS/(BA + BS + BB + BX) or (BA + BS)/(BA + BS + BB + BX) respectively]. The final three indices concern the proportion of endocarps that escaped bruchids, but were attacked by agoutis, squirrels and rodents [A/(A + S + X + V), S/(A + S + X + V) and (A + S)/(A + S + X + V), respectively]. Significant slopes for these six indices indicate that rodents' preference for larval infested and uninfested endocarps changed with palm density.

RESULTS

We determined the fates of 2154 *Attalea* endocarps across 320 m^2 of samples. Palm density was a strong predictor of endocarp density among the 4-ha plots (Table S1; Spearman $\rho = 0.9$, $P = 0.00039$).

Of the recovered endocarps, 72.5% had scars from rodent feeding, with many more scarred by squirrels (83.2%) than by agoutis (16.9%); 40.3% had been attacked by bruchids; and 23.2% were unscarred of which just 0.8% were viable. Viable seeds all appeared to be from the most recent fruiting season. These percentages sum to more than 100% because 89.3% of the endocarps attacked by bruchids were also attacked by rodents providing evidence of larval predation. Just 4.3% of endocarps had living bruchid larvae or exit holes indicative of successful bruchid emergence.

The proportion of endocarps attacked by rodents (A + S + BA + BS) was independent of palm density (Fig. 1a); however, agoutis and squirrels showed distinct responses to palm density. The proportion of endocarps attacked by agoutis (A + BA) decreased with palm density (Fig. 1b; $\hat{a}_1 = -0.055$, $P < 0.001$), whereas the propor-

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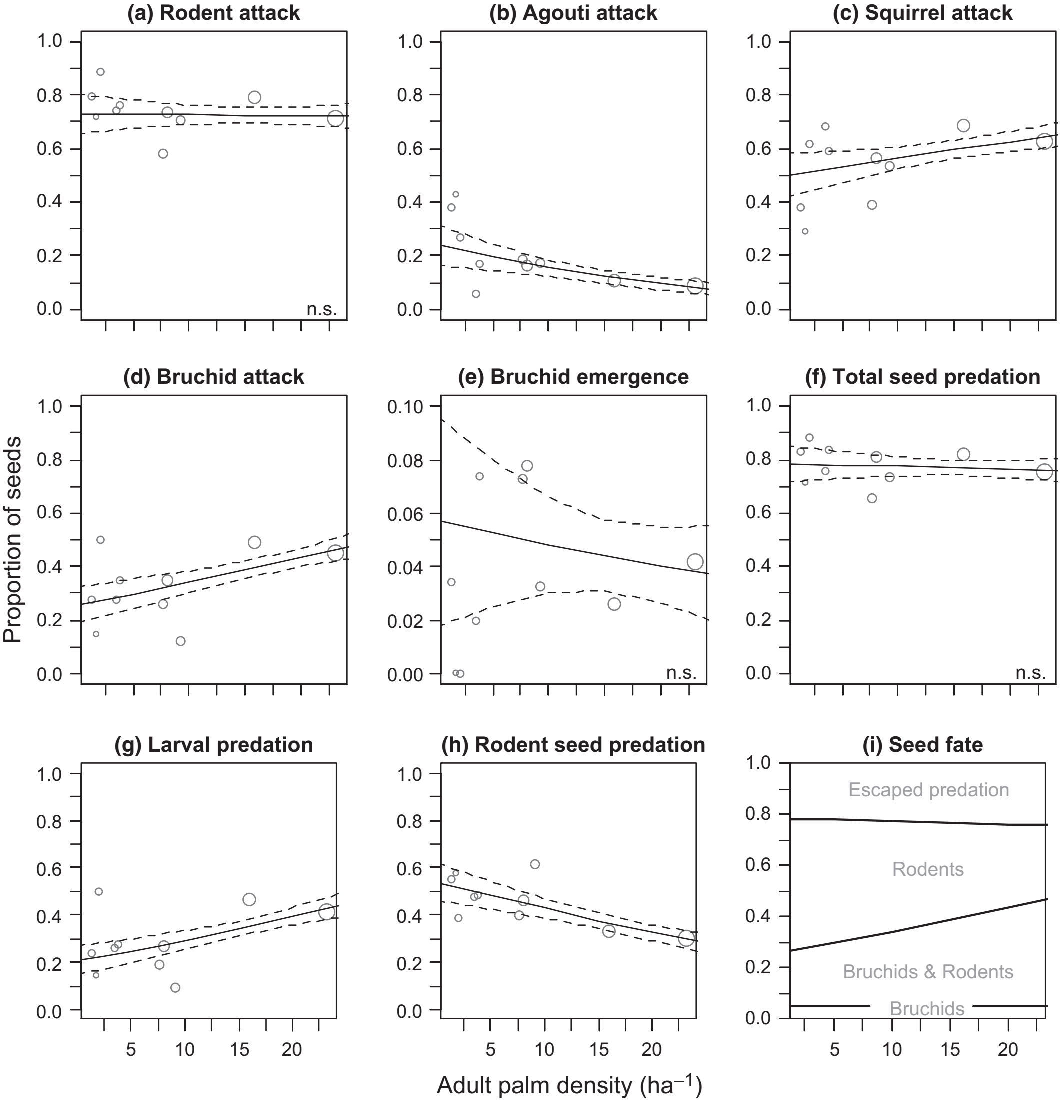

Figure 1 Observed seed fates (open circles with area proportional to sample size) and fitted logistic regression models of seed fate (black solid lines) as a function of adult palm density in *Attalea butyracea*, with 99% model confidence intervals (grey dashed lines). Seed fates are grouped as (a) total rodent attack (A + S + BA + BS, see methods for definitions), (b) agouti attack (A + BA), (c) squirrel attack (S + BS), (d) bruchid attack (BA + BB + BS + BX), (e) bruchid emergence (BB; note the different *y*-axis scale in this panel), (f) total seed predation (A + S + BA + BS + BB + BX), (g) bruchid larval predation by rodents (BA + BS) and (h) seed predation by rodents (A + S). The letters 'n.s.' in the bottom right of panels (a, e and f) indicate that the fitted model slopes are not significantly different from zero in these cases. The final panel (i) shows overall seed fate as a function of adult palm density. Attack of the same seeds by both bruchids and rodents results in larval predation by rodents and seed death.

tion attacked by squirrels (S + BS) increased (Fig. 1c; $\hat{a}_1 = 0.025$, $P < 0.001$).

The proportion of endocarps attacked by bruchids (BA + BS + BB + BX) also increased with palm density (Fig. 1d; $\hat{a}_1 = 0.039$, $P < 0.001$). However, the proportion of endocarps with viable bruchids (BB) was unrelated to palm density (Fig. 1e, note the unique vertical scale; $\hat{a}_1 = -0.018$, $P = 0.196$). Total seed predation by rodents and bruchids combined (A + S + BA + BS + BB + BX) was also unrelated to palm density (Fig 1f, $\hat{a}_1 = -0.0057$, $P = 0.411$).

Given that the proportion of endocarps attacked by rodents was similar for all palm densities (Fig. 1a) and the proportion of endocarps attacked by bruchids increased with palm density (Fig. 1d), it is surprising that the total seed predation by rodents and bruchids was similar for all palm densities (Fig. 1f). This discrepancy reflects an increase in larval predation by rodents (BA + BS) with palm density (Fig. 1g; $\hat{a}_1 = 0.045$, $P < 0.001$) and a decrease in seed predation by rodents (A + S) with palm density (Fig. 1h; $\hat{a}_1 = -0.044$, $P < 0.001$).

To determine whether the increase in larval predation by rodents with palm density (Fig. 1g) is a passive consequence of the increase in bruchid attack with palm density (Fig. 1d), we examined the proportion of endocarps attacked by bruchids that were also attacked by rodents [(BA + BS)/(BA + BS + BB + BX)]. This last proportion increased with palm density (Fig. 2a, $\hat{a}_1 = 0.048$, $P = 0.0012$). Squirrels attacked endocarps containing larvae [BS/(BA + BS + BB + BX)] at more than twice the level they attacked uninfested endocarps [S/(A + S + X + V)] for all palm densities (Fig. 2e,f), and were entirely responsible for the increase in the proportion of bruchid attacked endocarps that were also attacked by rodents (Fig. 2e, $\hat{a}_1 = 0.042$, $P = 0.003$). In contrast, agoutis attacked a similar and very low proportion of bruchid infested endocarps [BA/(BA + BS +

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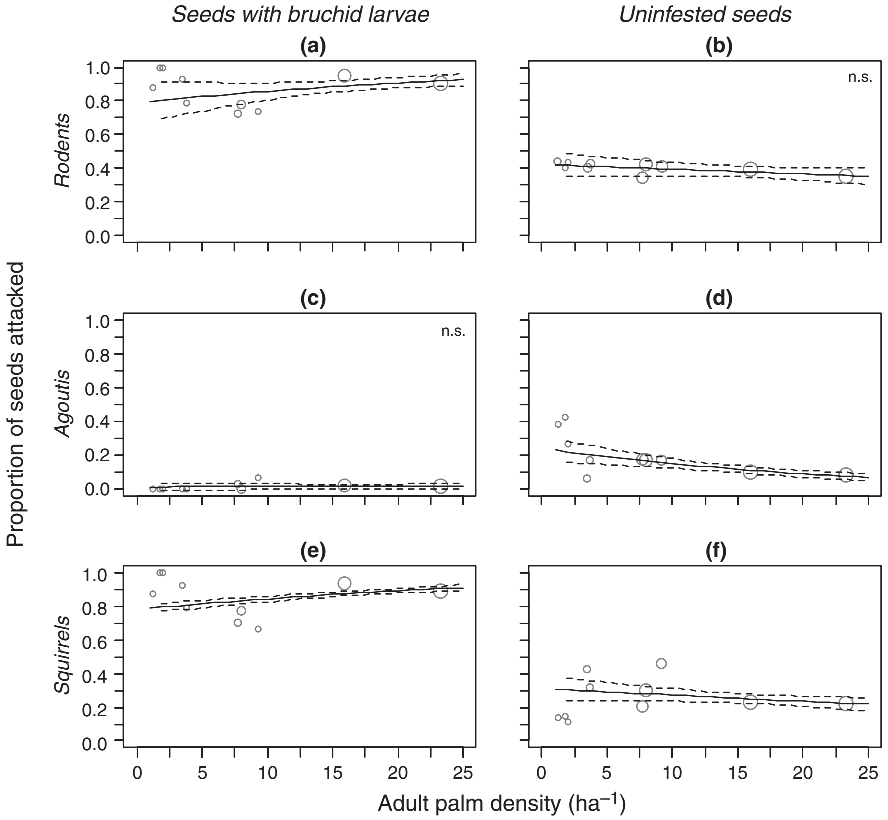

Figure 2 Rodent attack rates on seeds with (a, c and e) and without (b, d and f) bruchid larvae; the former leads to larval predation by rodents, whereas the latter leads to seed predation by rodents. Results are shown for all rodents combined (a and b), just agoutis (c and d) and just squirrels (e and f). Open circles represent plots, with circle area proportional to sample size. Fitted logistic regression models are shown as black solid lines, with 99% confidence intervals shown as dashed lines.The letters 'n.s.' in the top right of panels b and c indicate that the fitted model slopes are not significantly different from zero.

BB + BX)] at all palm densities (Fig. 2c, $\hat{a}_1 = 0.017$, $P = 0.71$) and attacked uninfested endocarps [A/(A + S + X + V)] at more than twice this level for all palm densities (Fig. 2c,d).

To determine whether the decline in seed predation by rodents with palm density (Fig. 1h) is a passive consequence of the increase in bruchid attack with palm density (Fig. 1d), which leaves a smaller proportion of uninfested endocarps to be attacked, we examined the proportion of the endocarps that escaped bruchids and were attacked by agoutis [A/(A + S + X + V)] (Fig. 2d) or by squirrels [S/(A + S + X + V)] (Fig. 2f). Both proportions decreased significantly with palm density ($\hat{a}_1 = -0.059$, $P < 0.001$ and $\hat{a}_1 = -0.019$, $P = 0.003$ for agoutis and squirrels respectively).This decline was only marginally significant with both rodents combined [(A + S)/(A + S + X + V)] (Fig. 2b, $\hat{a}_1 = -0.012$, $P = 0.047$).

DISCUSSION

We found that the density dependence of seed predation at the population scale differed from what was expected based on distance-dependence at the local scale, and that trophic interactions were critical to explaining the population-level patterns. The proportion of seeds attacked by bruchid beetles increased with palm density, whereas the proportion attacked by rodents held steady, as expected based on their degree of specialisation and responses to local variation in seed density around individual fruiting trees (Hammond & Brown 1998). However unexpectedly, rodent predation of bruchid larvae increased disproportionately with increasing palm density, completely negating the increase in bruchid infestation, and causing bruchid emergence rates to be very low and density-independent (with a trend towards a *decrease* with palm density). These results demonstrate that top-down influences can limit the potential of host-specific insects to regulate the populations of their host plants.

Variation among natural enemies

The two vertebrate seed predator species exhibited contrasting responses to population-level variation in seed densities: agouties were satiated at higher seed densities, whereas squirrels exhibited increasing attack rates. These different responses are consistent with the different roles of *Attalea* seeds in their diet. *Attalea* seeds (or the larvae inside) comprise more than half of the diet of squirrels (Heaney & Thorington 1978), whereas agoutis clearly prefer seeds of other species, particularly the palm *Astrocaryum standleyanum* and the legume *Dipteryx panamensis* (Smythe 1978; P.A. Jansen, unpublished data). The focus of squirrels on *Attalea* essentially makes them behave more like specialist than generalist predators. More generally, the role of a particular plant species in the diet of a generalist seed predator may be expected to affect how that generalist responds to spatial variation in the plant's abundance, and thus whether its seed predation is positively or negatively density dependent. Social systems and specifically territoriality may also influence vertebrate responses. Both squirrels and agoutis have similar small home range sizes on BCI (see

Methods: Plot selection), and would be expected to experience similar associated limits on their ability to respond to population-level variation in tree density.

The observed increase in the proportion of bruchid attack with adult palm density is consistent with the expectation that specialist insects have the potential to respond disproportionately to host density (Hammond & Brown 1998; Lewis & Gripenberg 2008). However, the increased attack cannot be explained by a disproportionate numerical response as adult beetle emergence was proportional with palm density. The increased attack rates may reflect increased oviposition efficiency. Increased seed densities in plots with high adult palm density should improve the ability of female beetles to locate seeds (Wilson & Janzen 1972). Furthermore, where a tree species is more abundant, seed dispersers may become satiated (Hampe 2008), which might cause seed removal and dispersal to decline (Forget & Jansen 2007; Klinger & Rejmánek 2009) and expose more seeds for longer periods to bruchid attack. Therefore, increased per-beetle oviposition efficiency, a functional response caused by greater seed availability and exposure time, likely combined with a proportional numerical response in adult beetle densities lead to increased infestation rates.

Tri-trophic interactions

Though bruchids attacked seeds at an increasing rate at higher densities of their host palm, they also suffered a compensatory increase in larval predation by rodents, and thus adult emergence rates did not change with palm density. This increase in intraguild predation of bruchid larvae has a passive and an active component. The passive component arises because the proportion of endocarps attacked by bruchids increased with palm density, thus increasing the opportunity to attack larvae (compare Fig. 1d, g). The active component arises because squirrels, which prefer infested seeds (compare Fig. 2e, f), exhibited an increase in preference for infested seeds as palm density increased (Fig. 2e). Had rodents attacked seeds with larvae in the same proportion they attacked seeds without larvae, bruchid emergence, seed predation by bruchids and total seed predation would all have increased with increasing palm density. Together, satiation of agoutis (Fig. 1b) and increasing intraguild predation of bruchid larvae by squirrels at high palm densities (Fig. 2e) combine to neutralise increases in the proportion of endocarps attacked by bruchids and squirrels at high palm densities, so that overall levels of seed predation are independent of density (Fig. 1f).

This unexpectedly strong intraguild predation of bruchid larvae raises the question of how common such top-down control of pests (*sensu* Gillet 1962) might be in tropical forests. Insect larval predation on seeds is high in many tropical plant species (e.g. Herrera 1989). Bruchid predation on palm seeds is particularly well-documented (e.g. Delobel *et al.* 1995). Vertebrates often consume seeds of species that have insect larval predators, and commonly feed on infested fruit (Silvius 2002; Gálvez & Jansen 2007). Silvius (2002) argued that vertebrate granivores feeding on palm nuts should prefer larvae to seeds, as they require less effort to extract and are likely to be more nutritious than seeds. We found that squirrels indeed consumed a higher proportion of infested than uninfested fruit, consistent with such preference. This finding suggests abundant potential for intraguild predation and associated top-down control of insect seed predators, whenever vertebrates and insects prey on the same seeds.

Comparing density dependence at local and population scales

The interaction among *Attalea* seeds and their predators illustrates the problem posed by the spatial scale used to evaluate the Janzen–Connell hypothesis (Schupp 1992). In earlier studies of the *Attalea*-bruchid-rodent interaction, the proportion of seeds that escaped bruchids and rodents and the number of seedlings recruited per seed all increased with distance from the nearest seed-bearing *Attalea* (Wright 1983; Wright & Duber 2001). The conditions emphasised by Janzen (1970) and Connell (1971) are present at the local spatial scale. Nonetheless, seed predation is independent of variation in conspecific density at the larger spatial scale used in this study (Fig. 1f). The two mechanisms that neutralise population-level density dependence of seed survival in our system are unlikely to operate at the small spatial scales around single fruiting trees. Agoutis are unlikely to be satiated by variation in endocarp density around single fruit trees because extra seeds are avidly scatter hoarded for future use (Forget *et al.* 1994). The preference of squirrels for bruchid infested seeds is also unlikely to vary spatially around individual *Attalea* trees because most *Attalea* fruit are dispersed away from fruiting trees on BCI (Wright *et al.* 2000; Wright & Duber 2001). Thus, the mechanisms that neutralise population-level density dependence of seed survival are unlikely to operate at the small spatial scales around single fruiting trees, emphasised by the Janzen–Connell hypothesis. The result is a disconnect between the local spatial signature of the Janzen–Connell mechanism and population-level density dependence.

A few other studies have found contrasting patterns of density- and distance-dependence at local and population scales. Schupp (1992) reports increasing seed survival with distance to the nearest adult conspecific at the local spatial scale and *positive* density dependence at the population-level scale for a second BCI plant species, *Faramea occidentalis*. In both *Faramea* and *Attalea*, the local spatial signature of the Janzen–Connell mechanism is present, but the negative density dependence necessary to regulate populations is absent. Studies of the dipterocarp *Shorea laxa* find a disconnect in the opposite direction: in this case, seed survival decreases with distance from the nearest seed-bearing tree (Takeuchi & Nakashizuka 2007) and, at larger spatial scales, with the density of conspecific adults (Takeuchi *et al.* 2010). The local spatial signature of the Janzen–Connell mechanism is reversed, yet the negative density dependence necessary to regulate populations and promote coexistence is present at population-level scales. Norghauer *et al.* (2008) present a final example from Brazil in which the local spatial signature of the Janzen–Connell mechanism and negative density dependence at the population-level are both present in *Swietenia macrophylla*. A reasonable tentative conclusion is that the presence or absence of the spatial signature of the Janzen–Connell mechanism at local spatial scales provides no insight into the presence or absence of negative density dependence and the facilitation of species coexistence at population-level scales.

New studies will be needed to explore the responses of natural enemies to population-level variation in the density of tropical trees, not only for seed predators but also for seedling herbivores, seedling pathogens, etc. Studies now document negative-density dependence in plant recruitment, growth and/or survival at appropriate spatial scales, but do not evaluate the impacts of natural enemies (or any other mechanism; reviewed by Wright 2002). Schupp (1992) speculated that territoriality limited rodent responses to plant density, contributing to positive density-dependent seed escape. Takeuchi *et al.*

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(2010) and Chauvet *et al.* (2004) speculated that variation in community-level seed production overwhelmed the responses of generalist seed predators to spatial variation in the density of individual tree species. The record of mortality agents recorded in scars on the stony endocarps of *Attalea butyraceae* provides the first opportunity to evaluate responses of natural enemies to population-level variation for a tropical tree.

Conclusions and implications

Specialised natural enemies are widely believed to be one of the most important agents contributing to the maintenance of plant species diversity in tropical forests (Wright 2002; Leigh *et al.* 2004). Attack by specialised pests is expected to increase disproportionately with increasing host plant abundance (Gillet 1962; Janzen 1970; Connell 1971; Hammond & Brown 1998; Leigh *et al.* 2004; Lewis & Gripenberg 2008), leading to negative density dependence of plant species vital rates and giving each species an advantage when rare, a crucial feature of stable species coexistence (Chesson 2000). Classic papers by Janzen (1970) and Connell (1971) stimulated a huge volume of tropical research testing for negative density dependence in general and density dependence of enemy attack in particular on local spatial scales (Wright 2002). However, in all this literature about the importance of natural enemies to plants, there is virtually no mention of the potential impact of the enemies of those enemies. That predators affect herbivores and thereby herbivory on plants, and that predators respond to herbivore abundance, has been abundantly demonstrated, in tropical forests as well as in many other systems (Terborgh & Estes 2010). However, the potential for top-down influences to interact with Janzen–Connell effects has been neglected. This study provides evidence that top-down influences can critically alter population-level patterns of density dependence, and must be considered. We also show that local scale studies are not a reliable basis for inferring population-level patterns.

A key question for future research concerns the generality and importance of this phenomenon: Do top-down influences often prevent natural enemies from exerting increasingly negative effects at increasing abundances of the focal plant species? A better understanding of which enemies are likely to exert negative density-dependent effects on plant vital rates could illuminate which interactions are most crucial for the maintenance of plant diversity. In general, we expect that top-down influences of predators and parasitoids may be important for many invertebrate enemies (Van Bael & Brawn 2005; Kalka *et al.* 2008). Because pathogens, in contrast to insects, are rarely subject to top-down control from predators (Agrios 2005), microbes may be a fundamentally more powerful force for inducing negative density dependence at the population scale than other natural enemies (Mangan *et al.* 2010).

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AUTHORSHIP

MDV, PAJ, and SJW designed the study; MDV and GR collected the data; MDV, HCM and SJW analysed the data; HCM, SJW, MDV and PAJ interpreted the results; MDV wrote the first draft of the article; all authors revised the article.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 The densities of adult palms and of recovered endocarps.
Table S2 Model fit statistics.

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