

Seed predation by insects across a tropical forest precipitation gradient

Article

Published Version

Creative Commons: Attribution 4.0 (CC-BY)

Open Access

Jeffs, C. T., Kennedy, P., Griffith, P., Gripenberg, S. ORCID: <https://orcid.org/0000-0002-8788-2258>, Markesteijn, L. and Lewis, O. T. (2018) Seed predation by insects across a tropical forest precipitation gradient. *Ecological Entomology*, 43 (6). pp. 813-822. ISSN 0307-6946 doi: 10.1111/een.12672 Available at <https://centaur.reading.ac.uk/80226/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

Published version at: <http://dx.doi.org/10.1111/een.12672>

To link to this article DOI: <http://dx.doi.org/10.1111/een.12672>

Publisher: Wiley-Blackwell

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Seed predation by insects across a tropical forest precipitation gradient

CHRISTOPHER T. JEFFS,^{1,2} PATRICK KENNEDY,^{1,3}
PHOEBE GRIFFITH,¹ SOFIA GRIPENBERG,¹
LARS MARKESTEIJN^{1,4,5} and OWEN T. LEWIS¹

¹Department of Zoology, University of Oxford, Oxford, U.K., ²Department of Life Sciences, London Natural History Museum, London, U.K., ³School of Biological Sciences, University of Bristol, Bristol, U.K., ⁴School of Environment, Natural Resources and Geography, Bangor University, Bangor, U.K. and ⁵Smithsonian Tropical Research Institute, Ancon, Republic of Panama

Abstract. 1. Pre-dispersal predation of seeds by insects can be an important source of plant mortality, with consequences for plant population dynamics and community diversity.

2. The intensity and host-specificity of natural enemies, including seed predators, is predicted to vary systematically across environmental gradients. These trends could contribute to large-scale patterns in diversity, such as the widely observed positive relationship between plant species richness and precipitation. However, fundamental information on inter- and intraspecific seed predation is lacking at the landscape scale.

3. We assessed the intensity of seed predation by insects at eight forest sites spanning a steep precipitation and plant species-richness gradient in Panama. We dissected freshly abscised fruits and seeds of woody plants to measure rates of predation, and assessed host-specificity by rearing insects from them.

4. On average, 22.6% of dissected seeds were predated across 31 host species. Species-specific and site-level seed predation rates and the number of seed predator species reared per host were not significantly associated with annual rainfall or dry season water deficit.

5. In total, 45 seed predator morphospecies were reared from seeds of 16 plant species. Host-specificity was high, with 91% of seed predator morphospecies associated with just one plant species.

6. Our results support evidence that pre-dispersal insect seed predators are highly host-specific and exhibit great intraspecific, interspecific and landscape-scale (i.e. between-site) variation in seed predation intensity. Host-specific seed predators, particularly those causing moderate to high seed predation rates, could influence plant population dynamics and local community structure.

Key words. community diversity, host-specificity, natural enemies, Panama.

Introduction

The survival of seeds and the initial establishment of seedlings are key stages determining plant population dynamics and community structure (Webb & Peart, 1999; Harms *et al.*, 2000; Green *et al.*, 2014). One potentially important source of seed

mortality is pre-dispersal predation by insects, particularly Coleoptera, Lepidoptera, Diptera and Hymenoptera (Janzen, 1971). These insects consume and kill seeds *in situ* on the plant, directly influencing the relative abundances of viable seeds across species that are dispersed into the community (Fenner & Thompson, 2005; Lewis & Gripenberg, 2008; Crawley, 2013a). For example, specialised insect seed predators may cause negative density-dependent seed survival, generating greater mortality as seed densities increase. This negative feedback mechanism provides a rare species advantage which can prevent competitive

Correspondence: Christopher T. Jeffs, Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, U.K. E-mail: christopher.jeffs@zoo.ox.ac.uk

exclusion and enhance local plant diversity (Janzen, 1970; Hammond *et al.*, 1998). More abundant plant species also may be associated with a greater number of specialised seed predators, further intensifying this density-limiting mechanism (Janzen, 1970). This could arise if specialisation on abundant hosts represents a stable ecological strategy, buffering against stochastic extinction (Futuyma & Moreno, 1988), or if generalists opportunistically use hosts in proportion with their relative availabilities (e.g. Singer & Stireman, 2001).

Variations in the intensity of mortality caused by and the host-specificity of natural enemies also could contribute to gradients in plant diversity in relation to latitude (Janzen, 1970) or rainfall (Givnish, 1999; Comita *et al.*, 2014). On local and global scales, rainfall is a strong predictor of plant species richness (Pyke *et al.*, 2001; Krefl & Jetz, 2007), although the mechanisms underlying this relationship are not fully understood. In principle, increased rainfall may amplify both the intensity and host-specificity of seed predation by insects, generating stronger negative density-dependence. First, insects are intolerant of desiccation and so may reach higher abundances in wetter and less seasonal forests (Coley & Barone, 1996; Givnish, 1999). More abundant insect populations would be expected to exert more intensive seed predation. Second, host-specificity may rise with rainfall (driven, for instance, by greater niche-partitioning within a more crowded insect community or by stronger plant defences). However, despite the potential importance of pre-dispersal insect seed predators to plant demography and structuring tropical forest communities (Janzen, 1970), assessments of their effects on seed survival, plant recruitment and host-specificity (Janzen, 1980; Nakagawa *et al.*, 2003; S. Gripenberg, unpublished), and density-dependence (Ashton, 1988; Jones & Comita, 2010; Bagchi *et al.*, 2011) are scarce.

In order to address these gaps in knowledge, we collected, reared, and dissected fruits and seeds from multiple woody plant species at sites spanning a steep rainfall and plant species-richness gradient across the Isthmus of Panama, Central America. We reared and identified insects from seeds and fruits to generate an Isthmus-wide food web to quantify seed predator specificity. We then tested whether seed predation rates and the number of seed predator species associated with a host species (seed predator load) increased with measures of humidity (annual rainfall and dry season soil water deficit) to assess whether pre-dispersal seed predators may contribute to a gradient in local plant diversity. We use our data to highlight research priorities for understanding the ecological role of pre-dispersal insect seed predators in tropical forests. To our knowledge, we provide the first assessment of within- and between-species trends in seed predation across a steep regional gradient of precipitation and species richness.

Materials and methods

Study sites and sample collection

From March to July 2014, we collected freshly abscised fruits, pods and seeds under all fruiting woody species (trees, shrubs and lianas) encountered at eight sites spanning a precipitation

and species-richness gradient across the Isthmus of Panama (Fig. 1; Table 1). Each study site was located around a 1-ha permanent forest plot (part of the CTFs-ForestGEO network; Condit *et al.*, 2013). To increase sample sizes, fruits were collected over larger areas around the plots, but typically within a 1.5 km radius of the plot. Wherever possible, we collected seeds from multiple individuals per species per site to account for inter-individual variation in predation rates. The vast majority of seeds collected were recently abscised (gauged by their fresh condition and location on the leaf litter layer surface in repeatedly sampled locations) and, therefore, most insects reared from seeds were likely to have infested them pre-dispersal, although we cannot discount the presence of post-dispersal seed predators in our samples.

Seed predator identification and food web construction

In order to generate insect material for host-specificity assessment, we reared collected mature seeds, pods and fruits within plastic pots with fine mesh lids, lined with absorbent paper, in a ventilated, shaded room at ambient environmental conditions. Seeds, pods and fruits were housed separately for each species, parent individual and collection date. Seed predators are thought to drive premature fruit abscission in *Oenocarpus mapora* (S. Gripenberg, pers. obs.), so we collected abscised immature fruit in addition to mature fruit for this species and reared them in separate containers to account for succession in seed predator attack (Hosaka *et al.*, 2009). We added small volumes of water to some containers periodically to maintain humidity. Samples were reared for three months after collection to allow insects to develop and emerge for later identification (see below).

Adult insects emerging during rearing and obtained via dissection were stored individually in vials of 95% ethanol or pinned and dried (Lepidoptera) for later morphospecies-level identification under a dissection microscope and using SG's reference collection for seed predators reared from woody plant species on Barro Colorado Island in Central Panama. We assessed seed predator host-specificity using a food web approach (Lewis & Gripenberg, 2008) with all identified seed predator morphospecies and the plant species from which they were reared presented, and the relative frequency of all host plant–insect interactions plotted. We assessed the number of host species consumed by each seed predator species as a measure of seed predator specificity. Without community-level data on seed availability, we were unable to estimate specificity in a way that takes into account the proportional availability of alternative hosts (e.g. Blüthgen *et al.*, 2006). We calculated the percentage of monophagous species as a measure of diet breadth or host-specificity within the food web. Because infrequently recorded seed predators will inevitably have small recorded diet breadths, we compared the percentage of monophagous species for the full dataset with data for morphospecies where five or more individuals were reared. We also compared the median sample size (the number of individuals reared per seed predator morphospecies) for monophagous versus polyphagous seed predators using a post-hoc Mann–Whitney *U*-test. The number

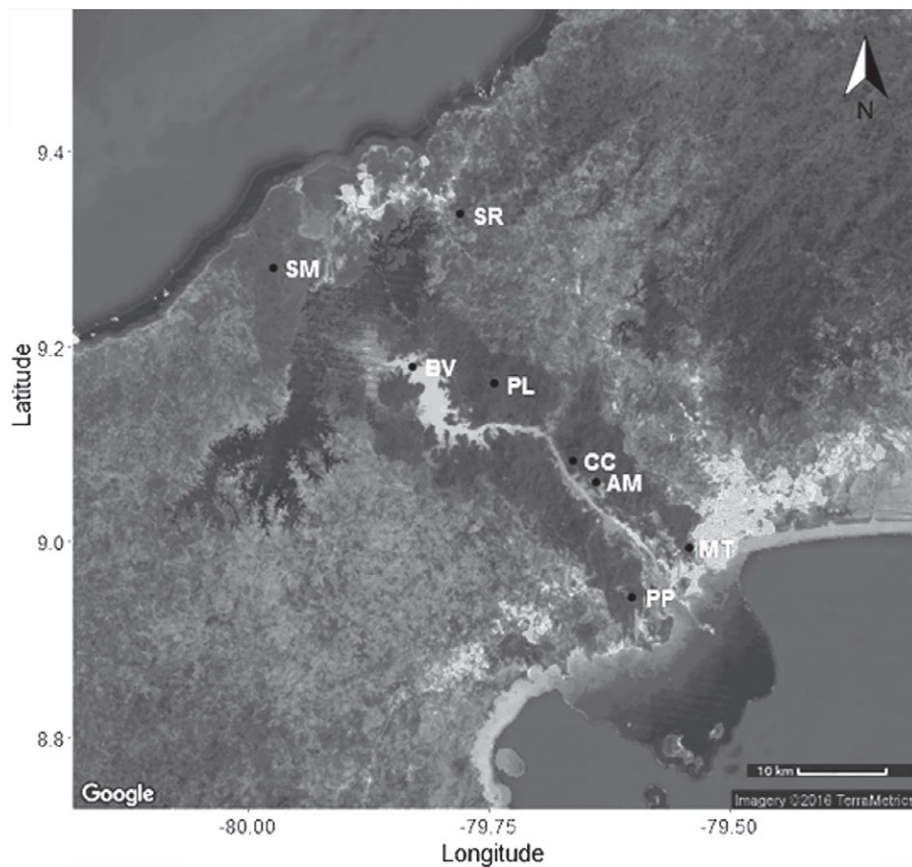


Fig. 1. Locations of forest sites spanning the Isthmus of Panama from which seeds and fruits were collected. From lowest to highest annual rainfall and species richness across the gradient, the sites are: PP, Panama Pacifico; MT, Parque Natural Metropolitano; AM, Parque Soberania, ANAM office; CC, Parque Soberania, El Charco; PL, Oleoducto 'Pipeline Road'; BV, Buena Vista Peninsula; SM, Fort Sherman; SR, Santa Rita. Image credit Google, ©2016 TerraMetrics.

Table 1. Locations and characteristics of study sites, as shown in Fig. 1. Data for annual rainfall (mm), dry season deficit (the peak volume of rain required to reach field capacity soil water content over the dry season) and species richness (the number of free-standing woody species >1 cm diameter at breast height) were collected within a central 1-ha plot at each site (Condit *et al.*, 2013; L. Comita, unpublished data).

Site	Latitude	Longitude	Annual rainfall (mm)	Dry season deficit (mm)	Species richness
PP	08.9435000	-079.6015333	1756	-571.688	69
MT	08.9945889	-079.5430000	1874	-574.926	49
AM	09.0619500	-079.6394000	2007	-579.902	34
CC	09.0840500	-079.6634000	2051	-579.148	84
PL	09.1617600	-079.7453000	2330	-549.423	132
BV	09.1793611	-079.8296000	2595	-510.470	85
SR	09.3355778	-079.7807417	3054	-487.530	170
SM	09.2808694	-079.9747000	3203	-491.669	161

PP, Panama Pacifico; MT, Parque Natural Metropolitano; AM, Parque Soberania, ANAM office; CC, Parque Soberania, El Charco; PL, Oleoducto 'Pipeline Road'; BV, Buena Vista Peninsula; SM, Fort Sherman; SR, Santa Rita

of plant species per site yielding reared insects was insufficient to produce site-specific food webs and assess variation in host-specificity across the humidity gradient. We therefore constructed a semi-quantitative food web of all insect material pooled across sites using the R package bipartite (Dormann *et al.*, 2008).

Intensity of seed predation

In order to quantify seed predation rates, after three months of seed rearing (as above), we dissected fruits and seeds under a stereomicroscope and calculated predation rates for each sample as the number of predated seeds (indicated by the presence of

exit holes, larvae/adults or frass, and feeding damage) divided by the total number of seeds in the dissected sample. Site-specific predation rates were calculated for each plant species, in addition to an average seed predation rate per species, based on the average of all samples pooled across sites. Insect-infested fruits may be prematurely abscised, desiccated but retained, or avoided by avian frugivores (Manzur & Courtney, 1984; Jones & Comita, 2008). These outcomes could affect the precision of absolute estimates of predation rate for individual species based on fruits collected from the forest floor. However, we do not expect these factors to introduce biases into our analyses by differing systematically across locations within species.

In order to test for changes in mean site-level predation rates with precipitation across the gradient, we initially modelled predation rates (weighted by sample size) as a function of either site-specific annual rainfall or dry season deficit, with tree species (as a random factor), in a generalised linear mixed effects framework. However, these models did not meet model assumptions of homogeneity of variance, normality of errors, or absence of influential outliers. As a conservative alternative analysis for landscape-level trends, we first calculated Spearman's ρ for the correlation between seed predation and two measures of site humidity for each plant species. Annual rainfall and dry season deficit (the peak volume of rain required to reach field capacity soil water content over the dry season) were both investigated as they capture distinct aspects of humidity relevant to insect performance. For example, dry season deficit might be expected to reflect risk of desiccation for insect pupae in the soil (Givnish, 1999). We then tested whether the median effect size, based on the correlation between predation rate and each humidity variable, was significantly different from zero, using a one-sample Wilcoxon signed rank test. Although each species spans a different section of the precipitation gradient (due to niche specialisation and changes in abiotic conditions), the diversity enhancing effect would still occur if within the natural range of each species there is increased predation pressure at the wetter end of that natural range. Results of species-specific analyses were scrutinised for the three plant species for which sufficient seed sample sizes (≥ 20 seeds dissected per site) were available at four or more sites.

Seed predator load (the number of seed predator species reared per host species) reflects a second measure of predation intensity, where we predict that a greater seed predator load increases total mortality experienced by the host due to complementary action. Therefore, using the same statistical methods as for dissection-based predation rates, parallel site-level and species-specific analyses also were conducted to assess whether seed predator load correlated with measures of humidity. Species present at a minimum of two sites and with ≥ 50 seeds reared per site were included in these analyses. Results of species-specific analyses were scrutinised for species with four or more sites with sufficient seed sample sizes (≥ 50 seeds reared per site) and at least one seed predator species reared across all sites. Alternative community and species-specific analyses also were conducted using the number of seed predator morphospecies reared per seed unit reared to account for potential sample size biases. We conducted a post-hoc univariate generalised linear model (GLM) with Poisson errors to test whether

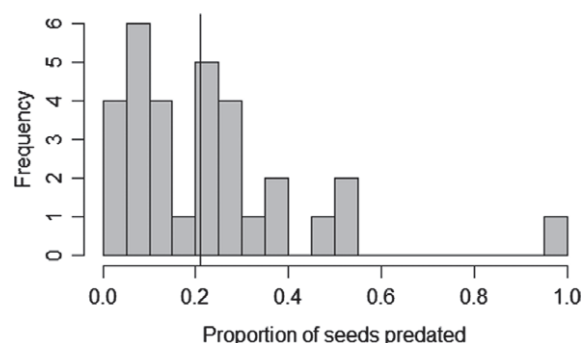


Fig. 2. Histogram of the proportion of seeds predated per plant species. The median proportion of seeds predated across species (21%) is indicated by the vertical black line. Seeds were pooled across sites for each species ($N = 31$). Only species with a minimum of 20 seeds dissected are included.

species-specific seed predator load correlated with sample size (the number of seed units reared). Only species with one seed per fruit were included due to the inability to compare multi-seeded fruits on the same scale.

In order to test whether the fraction of seeds predated was correlated with seed predator load, we used a post-hoc univariate GLM (with quasibinomial error structure), with seed predation modelled using the `cbind` function in R to combine predated and nonpredated seeds, so that proportions are weighted by sample size (Crawley, 2013a). The analysis was restricted to species with ≥ 20 seeds dissected, ≥ 50 seeds reared, and at least one seed predator morphospecies recorded.

For seed predation rate and load analyses, only data for immature *Oenocarpus mapora* seeds were included, because we only reared adult insects from immature fruits.

All analyses were carried out using R statistical software v3.1.3 (R Development Core Team, 2015).

Results

Intensity of seed predation and cross-gradient trends

A total of 12 866 seeds and fruits were dissected from 31 plant species with sufficient sample sizes to estimate predation rates. Mean seed predation rates varied greatly among species (mean 22.6%, ranging from 0–100%, median across species 21%, interquartile range 9–29%; Fig. 2, Table 2).

There were 15 plant species collected from a minimum of two sites (with ≥ 20 dissected seeds per site) available for testing the association between predation rate and humidity measures (Fig. 3, Table 2). The median effect size on predation across species was not significantly different from zero for annual rainfall ($N = 15$, $V = 63$, $P = 0.807$) or for dry season deficit ($N = 15$, $V = 93$, $P = 0.188$). Results did not differ qualitatively when mature *Oenocarpus mapora* fruits were analysed instead of immature fruits (data not presented). Species-specific tests for *Apeiba tibouria*, *Attalea butyraceae* and immature *Oenocarpus mapora* found no evidence for significant associations of seed predation rate with annual

Table 2. Plant species from which seeds and fruits were reared and dissected to calculate seed predator load (Load) and predation rates (PR). Liana species are indicated by '^', shrubs by '+', with all other species being trees. For Load, the number of seed units reared pooled across sites is provided in brackets, with an asterisk indicating the number of fruits reared for species with multiseeded fruits. For PR, the number of seeds dissected pooled across sites is provided in brackets. For each site (abbreviations given in Fig. 1/Table 1, ordered by increasing annual rainfall from left–right), the number of individual trees that seeds were reared from is listed. For each species, each site included in cross-gradient analyses are marked with 'a' for Load (sites excluded if <50 units reared), and 'b' for PR (sites excluded if <20 units dissected).

Species	Family	Load (units)	PR (seeds)	PP	MT	AM	CC	PL	BV	SR	SM
<i>Albizia adinocephala</i>	Fabaceae	3 (1010)	13.8 (770)		2						
<i>Anacardium excelsum</i>	Anacardiaceae	0 (1568)	11.3 (249)		3 ^{a, b}	7 ^{a, b}	10 ^{a, b}				1 ^a
<i>Apeiba membranacea</i>	Malvaceae	8 (85*)	36.7 (1852)				1 ^b	2 ^b			
<i>Apeiba tibourbou</i>	Malvaceae	0 (143*)	36.4 (940)		1 ^b	1 ^b		5 ^b			1 ^b
<i>Astrocaryum standleyanum</i>	Arecaceae	1 (667)	29.3 (41)				6 ^a	4 ^a	2 ^a		1
<i>Attalea butyracea</i>	Arecaceae	4 (1956)	29.2 (431)	10 ^{a, b}		3 ^{a, b}	14 ^{a, b}	2 ^{a, b}	1 ^b		
<i>Bixa orellana</i> ⁺	Bixaceae	0 (67)	–								1
<i>Callichlamys latifolia</i> [^]	Bignoniaceae	1 (559)	21 (419)	2 ^{a, b}			2 ^{a, b}	6 ^{a, b}			
<i>Cavanillesia platanifolia</i>	Malvaceae	–	8.8 (34)	1	2						1
<i>Combretum decandrum</i> [^]	Combretaceae	0 (1826)	–	3 ^a	2 ^a	1 ^a	1 ^a	8 ^a			
<i>Cupania sylvatica</i>	Sapindaceae	0 (428)	29.6 (226)			2 ^a	2 ^{a, b}	2 ^{a, b}			
<i>Elaeis oleifera</i>	Arecaceae	1 (915)	100 (76)	6 ^a		1 ^a		1 ^a			
<i>Enterolobium cyclocarpum</i>	Fabaceae	9 (888*)	9.1 (1165)			4 ^b	1 ^b	1 ^b			
<i>Enterolobium schomburgkii</i>	Fabaceae	8 (2482*)	50.5 (2722)		1 ^a	4 ^{a, b}	1 ^{a, b}	1 ^a			
<i>Fissicalyx fendleri</i>	Fabaceae	2 (187)	21.1 (38)		4	1					
<i>Inga</i> sp.	Fabaceae	1 (184*)	14.7 (95)	1				3 ^a			4 ^a
<i>Luehea seemannii</i>	Tiliaceae	0 (928)	5.2 (582)	1	3 ^{a, b}			1 ^{a, b}	1 ^a		
<i>Machaerium milleflorum</i> [^]	Fabaceae	1 (484)	10.3 (262)		4 ^a	1		2 ^a			
<i>Machaerium riparium</i> [^]	Fabaceae	–	22.7 (22)					2			
<i>Mucuna mutisiana</i> [^]	Fabaceae	2 (61)	–				2				3
<i>Oenocarpus mapora</i> (immature)	Arecaceae	3 (3597)	50.0 (567)	5 ^{a, b}	3 ^{a, b}	1 ^b	5 ^{a, b}	5 ^{a, b}	4 ^{a, b}	4 ^a	1 ^{a, b}
<i>Oenocarpus mapora</i> (mature)	Arecaceae	0 (1571)	25.8 (686)	2	3		3	3	3	3	
<i>Ormosia macrocalyx</i>	Fabaceae	–	0 (30)								1
<i>Paullinia fibrigera</i> [^]	Sapindaceae	1 (138)	–				1				
<i>Platypodium elegans</i>	Fabaceae	3 (459)	15.2 (66)					2			
<i>Pourouma bicolor</i>	Cecropiaceae	0 (366)	9.1 (176)					3			1
<i>Sapindus saponaria</i>	Sapindaceae	1 (344)	26.4 (227)	1 ^{a, b}	3 ^{a, b}						
<i>Schizolobium parahyba</i>	Fabaceae	0 (167)	3.1 (131)			1		1			
<i>Serjania cornigera</i> [^]	Sapindaceae	0 (153)	31.6 (155)								1
<i>Serjania decapleuria</i> [^]	Sapindaceae	0 (235)	21.2 (33)					2			
<i>Serjania mexicana</i> [^]	Sapindaceae	–	21.1 (20)					2			
<i>Socratea exorrhiza</i>	Arecaceae	0 (294)	1.8 (218)					1 ^{a, b}			6 ^{a, b}
<i>Terminalia oblonga</i>	Combretaceae	0 (794)	7.6 (721)	2 ^b			1 ^b				
<i>Thinouia myriantha</i> [^]	Sapindaceae	0 (82)	52.5 (59)				1 ^b	2 ^b			
<i>Triplaris cumingiana</i>	Polygonaceae	0 (322)	4.6 (394)	1	1						
<i>Vantanea depleta</i>	Humiriaceae	0 (134)	6.9 (145)							1 ^b	1 ^b

rainfall or dry season deficit (all $P > 0.05$), although site-level replication was low for most species, limiting statistical power.

Across the 13 plant species with reared seed predators present at two or more sites (Table 2), no association between site-level seed predator load (either raw counts or sample size adjusted measures) and annual rainfall or dry season deficit was observed ($P > 0.05$). However, site-level replication was low for most species, limiting statistical power. Species-specific tests on *Anacardium excelsum*, *Attalea butyracea*, *Enterolobium schomburgkii* and immature *Oenocarpus mapora* did not find significant associations between predator load and either annual rainfall or dry season deficit ($P > 0.05$ for both seed predator load measures), although again site-level replication was low for most species, limiting statistical power.

Host-specificity and species-specific seed predator load

A total of 311 adult individuals of 45 insect seed predator morphospecies were obtained from 16 plant species by rearing or dissection of 14 866 seed and fruit units. Of the 45 seed predator morphospecies, the majority were Coleoptera (27) and Lepidoptera (15), with a few species of Diptera (3). The food web constructed based on data pooled across sites revealed high host-specificity (Fig. 4). Of the 45 seed predator morphospecies, 41 (91%) were monophagous, one was associated with two congeneric host species (*Enterolobium cyclocarpum* and *E. schomburgkii*, Fabaceae), two were associated with pairs of host plant species within the same family (one Arecaceae, one Fabaceae), and one was associated with hosts in two different families (Bignoniaceae and Fabaceae). Based on site-specific

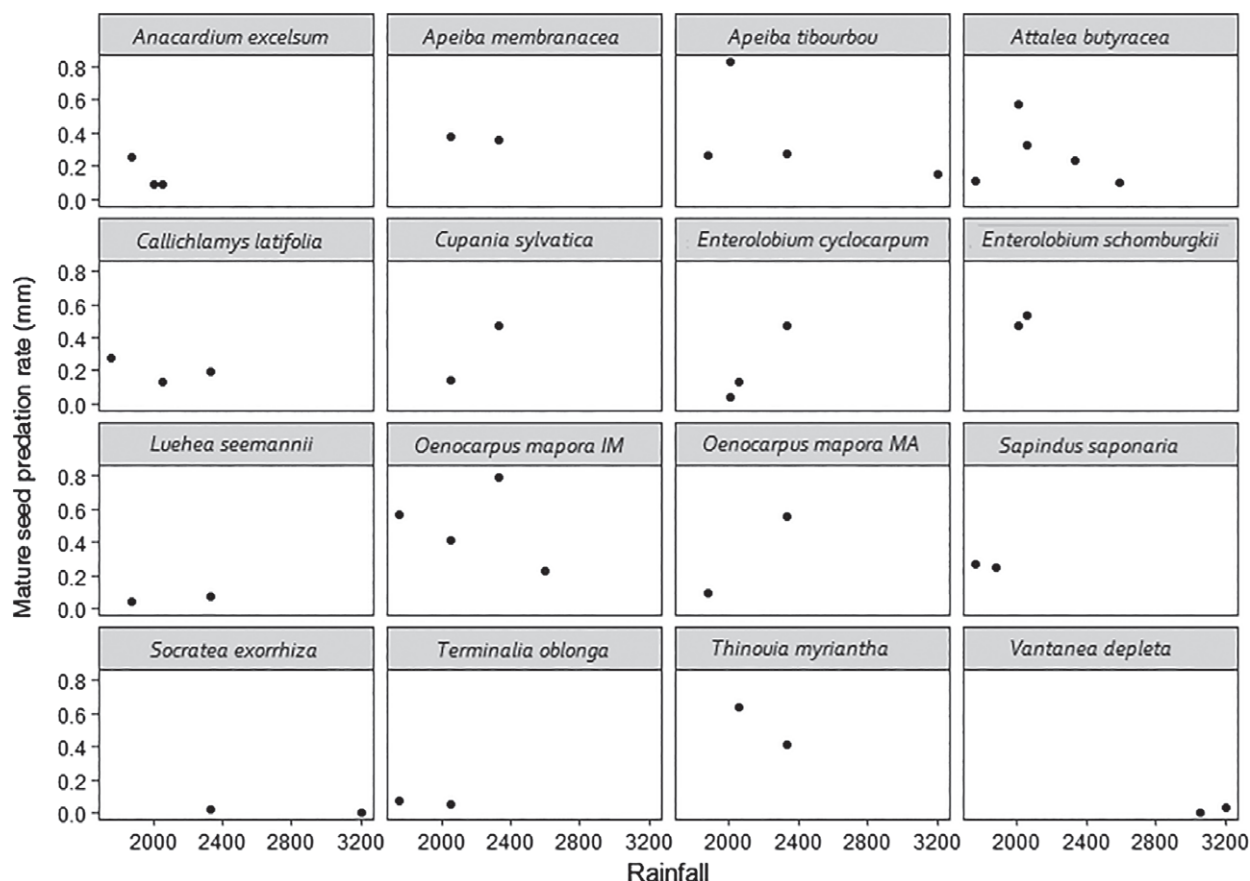


Fig. 3. Scatterplots of the proportion of seeds predated per species against annual rainfall (mm). Only species with ≥ 20 seeds dissected per site from a minimum of two sites are included ($N = 15$). For *Oenocarpus mapora*, data for both immature and mature *Oenocarpus mapora* seeds are shown to reflect potential differences in predation rates between development stages even though only immature data were analysed. See Table 2 for seed and tree sample sizes.

rearing data, there was only one instance of co-occurring tree species sharing a seed predator (*Attalea butyracea* and *Elaeis oleifera*, both Arecaceae, at site PP). The percentage of monophagous seed predator morphospecies appeared consistent when only including seed predator morphospecies with five or more reared individuals (92.3%, one of 13 morphospecies feeding on multiple host species). The median number of individuals reared per seed predator morphospecies associated with one host plant species did not differ significantly from the median number of individuals reared per seed predator morphospecies associated with two host plant species (Mann–Whitney $U = 43.5$, $z = -1.5887$, $P = 0.112$).

When samples were pooled across all sites per species, of the 45 host plant species, most were associated with 0 or 1 seed predator morphospecies (48.4% and 22.6% of species respectively) (Table 2). The number of seed predator morphospecies reared per host species increased with the sample size of seeds reared for single-seeded species ($N = 26$, $\chi^2 = 6.188$, $P = 0.013$).

There was no significant relationship between species-specific seed predation rate and seed predator load ($N = 14$, d.f. = 13, $F = 0.558$, $P > 0.05$).

Discussion

Variation in the intensity of seed predation among species

The intensity of seed predation varied widely among species, suggesting the potential importance of pre-dispersal seed predators in determining species-specific seedling recruitment may differ. Whilst predation rates were occasionally very high on particular individuals (frequently $> 70\%$), most individuals and species had modest or low mean predation rates (for example, 24 of 31 species had predation rates of $< 30\%$). Although several previous studies of pre-dispersal insect seed predators have documented levels of predation of 90% or higher (Janzen, 1971; Fenner & Thompson, 2005; Crawley, 2013b; Chen *et al.*, 2017), low and modest predation rates appear to be more typical (Jones & Comita, 2010; Crawley, 2013a; Civrtecka, *et al.* 2014; Xu *et al.*, 2015; Chen *et al.*, 2017). However, many plant species in central Panama are seed-limited (Svenning & Wright, 2005), meaning that even modest seed predation rates may reduce seedling recruitment. For instance, our predation rate estimates for *Cupania sylvatica* (29.6%) and *Oenocarpus mapora* (50% and 25.8% for immature and mature seeds, respectively) could

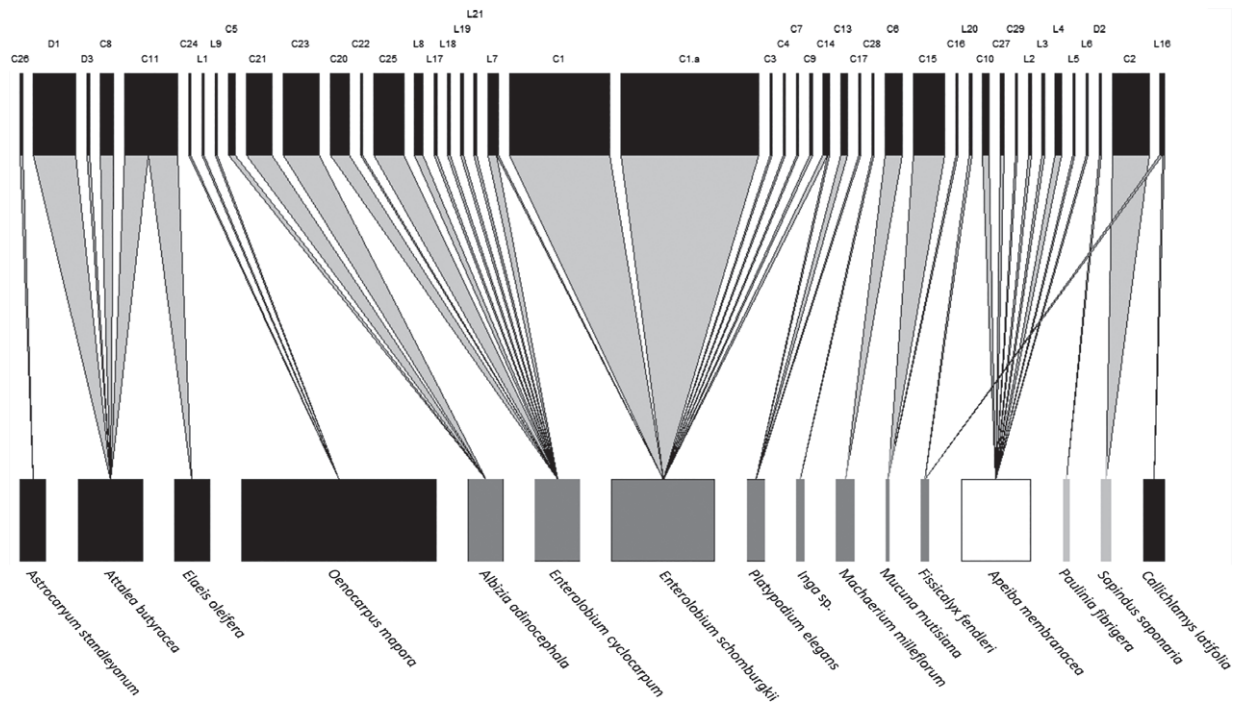


Fig. 4. Food web of seed predator morphospecies and host plant species. Bar widths for seed predators reflect their abundance from rearing across all samples; bar widths for hosts reflect the summed abundances of all seeds reared per plant species (i.e. 'sampling effort'). For plant species with multi-seeded fruits, the number of seeds dissected was used as an estimate of sampling effort as true seed number was unknown. Width of linkages indicate the relative frequencies of interactions. Insect seed predator morphospecies are as follows: Coleoptera: unknown family C1, C1a, C3, C4, C5, C7, C8, C9, C10, C11, C12, C13, C14, C24, C25, C26, C27, C28, C29; Curculionidae: Apioninae C6, C21; Chrysomelidae: Bruchinae C2, C15, C16, C23; Cerambycidae C17, C20, C22. Diptera: unknown family D2; Richardiidae D1; Diptera, unknown family near Calliphoridae D3. Lepidoptera: unknown family L1, L16, L18, L19, L20, L21; Pyralidae: Phycitinae L3, L6, L17; Gelechiidae L4, L7, L8; Blastobasidae L5, L9. Plant species are arranged by family left-right: Arecaceae (black), Fabaceae (dark grey), Malvaceae (white), Sapindaceae (light grey), Bignoniaceae (striped).

substantially reduce seedling recruitment, considering that these species have been shown to experience significant seed limitation in Panama (Svenning & Wright, 2005).

We did not find evidence that plant species with larger seed predator assemblages experienced greater seed predation rates overall. Seed predator species do not therefore appear to be acting in an additive fashion in determining overall seed predation rates. However, our subsample of the plant community that yielded reared seed predator morphospecies was limited, and larger sample sizes will be necessary to confirm such results.

Host-specificity of seed predators

Monophagy in our food web was high, in agreement with other studies documenting high host-specificity for insect seed predators (Janzen *et al.*, 1980; Novotny & Basset, 2005; Pinzón-Navarro *et al.*, 2010; Civrtecka *et al.*, 2014; S. G. unpublished). High host-specificity is consistent with the possibility that pre-dispersal insect seed predators contribute to enhancing community diversity if they generate negatively density-dependent survival. In a review of insect herbivore and granivore guilds by Novotny and Basset (2005), seed predators were found to be the most host-specific guild. Our results also demonstrate that host-specificity of

pre-dispersal insect seed predators is comparable to the most host family-specialised guilds of tropical herbivores (Novotny *et al.*, 2010).

Our results support observations that monophagy is common in insect seed predators (Janzen *et al.*, 1980; Pinzón-Navarro *et al.*, 2010; Civrtecka *et al.*, 2014). In cases where seed predator morphospecies were reared from multiple plant species the hosts were typically from the same genus or family, in line with previous studies (Novotny *et al.*, 2005; Pinzón-Navarro *et al.*, 2010; Civrtecka *et al.*, 2014). However, without sequencing samples reared from closely related hosts it is not possible to rule out the possibility that cryptic, host-associated seed predator species may be present (Pinzón-Navarro *et al.*, 2010). Where congeneric host species occur sympatrically, seed predators may still generate frequency-stabilising negative density-dependence if they attack hosts differentially (e.g. Hosaka *et al.*, 2009). Alternatively, multi-host seed predator species associated with multiple plant species may link host population dynamics through apparent competition, as observed in vertebrate post-dispersal seed predators (Garzon-Lopez *et al.*, 2015), with potential consequences for community diversity (Lewis & Gripenberg, 2008). Despite high host-specificity, low and even modest seed predation intensities may be insufficient to generate frequency-stabilising Janzen–Connell effects (Civrtecka *et al.*, 2014).

Trends in seed predation intensity across the precipitation gradient

Contrary to our predictions of higher pest pressure in wetter sites, we found no tendency for the intensity of pre-dispersal seed predation by insects to increase with measures of site-level humidity. However, our analyses are conservative as the number of species included in analyses was limited. In addition, as many of the host species included in analyses were restricted to two neighbouring sites, the magnitude of environmental differences may not have been great enough to affect seed predation. Although plant species are present at different sections across a precipitation gradient (e.g. due to environmental specialisation), increased predation pressure at the wetter end of species' natural ranges collectively would still generate a positive gradient in local plant diversity with increased precipitation.

Working in Australia, Chen *et al.* (2017) found that pre-dispersal seed predation at the community level was lower at tropical latitudes relative to more temperate latitudes. They found that only 30% of species assessed at multiple latitudes experienced higher predation at tropical latitudes, with a similar percentage of species showing the reverse trend. Although precipitation was not examined explicitly by Chen *et al.* (2017), their data are not consistent with the prediction that higher predation at wetter, low-latitude sites will contribute to latitudinal trends in biodiversity (Janzen, 1970). This mirrors the lack of a consistent relationship across species between seed predation rates and precipitation in our study. Basset *et al.* (2018) compared three tropical forest sites on different continents and found that the driest site (Barro Colorado Island in Panama) had lowest seed predation. One possible explanation is the high prevalence of dry-fruited plant species in Panama: these are easier for seed predators to attack than the fleshy fruits that are more prevalent at wetter sites. Seed and fruit traits may therefore be an important factor influencing seed attack rates (Basset *et al.* 2018) that could be assessed when investigating community-level and regional variation in seed predation rates. Across a wider absolute latitudinal range (0–60.3°), Moles and Westoby (2003) did not find a significant relationship across plant species between latitude and seed predation at either the pre-dispersal (122 plant species) or post-dispersal (205 species) stages, again failing to support the hypothesis that a positive relationship between precipitation and seed predation generates latitudinal gradients in biodiversity.

Previous studies have investigated other categories of plant enemy in relation to rainfall and humidity measures, with a variety of results. Pathogen-related mortality of understory seedlings for multiple plant species has been documented to be higher at wetter sites across the Panama rainfall gradient (Brenes-Arguedas *et al.*, 2009; Spear *et al.*, 2015). However, insect herbivores have been documented to show different relationships between pest pressure and humidity, with observations of greater damage in wetter sites (Brenes-Arguedas *et al.*, 2009), and observations showing no trend (Gaviria & Engelbrecht, 2015) or more damage in dry sites (Baltzer & Davies, 2012; Weissflog *et al.*, 2017). Current evidence therefore suggests that natural enemy groups may differ in their response to precipitation. Humidity and soil moisture can directly affect

fungal activity and infection (Griffin, 1963; Martin & Loper, 1999; Swinfield *et al.*, 2012; Hersh *et al.*, 2012), whereas similar direct effects of natural variation in humidity on the demography and activity of insects are less obvious. More intense and more extended dry seasons may be insufficient to decrease insect population sizes (Coley & Barone, 1996). In addition, plant species at wetter forest sites may be better protected against enemies if high pest densities have selected for defence. Because tolerance to enemies and drought may be traded off, this could be facilitated by reduced investment in drought tolerance at wetter sites (Coley & Barone, 1996; Gaviria & Engelbrecht, 2015). Lower seed predation rates could occur within species and at the community level if plants invest more heavily in chemical seed defences at wetter sites.

Conclusion

We provide evidence that intra- and interspecific variation in seed predation in tropical woody plant species is highly variable. Additionally, we find that the host-specificity of pre-dispersal insect seed predators is high. This supports a potential diversity-enhancing role of this enemy guild if it generates negative density-dependent seed survival. However, we did not find evidence supporting a positive relationship between humidity measures and the intensity of seed predation across sites which could generate a gradient in local plant diversity.

Longer-term studies with increased host plant coverage, as well as assessments across additional rainfall gradients, are required to test robustly whether insect seed predators may contribute to regional patterns of plant diversity such as positive precipitation–plant-richness relationships. Testing for density-dependent pre-dispersal seed predation and its variation in strength with precipitation will be challenging, requiring data collection at multiple spatial scales to identify the distances at which seed predators respond to the abundance of resources (e.g. Schupp, 1992; Visser *et al.*, 2011). Due to the densities of tropical tree species typically being very low, assessments of the densities of reproductive-sized trees will typically need to be made in multi-hectare plots. The complementary or compensatory action of pre- and post-dispersal mortality agents also needs greater attention (Crawley, 2013a). Even when acting in a density-independent manner, pre-dispersal seed predators may still have important population dynamic consequences for seed-limited species (Turnbull *et al.*, 2000; Clark *et al.*, 2007), with wider implications for local community composition.

Acknowledgements

We thank Joe Wright for valuable discussions, Marleny Rivera for assistance in insect identification, and Tom Lewis, Joe Lewis and Lily Lewis for assistance in seed dissections. Liza Comita kindly provided 1-ha plot data. Lindsay Turnbull, Martijn Bezemer and two anonymous reviewers provided helpful comments on earlier versions of the manuscript. This work was supported by a Varley-Gradwell Travelling Fellowship in Insect Ecology to CJ and NERC Standard Grant NE/J011169/1 to OL. SG is a Royal Society University Research Fellow. LM acknowledges

the financial support provided by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 663830, and the Welsh Government through the Sêr Cymru National Research Network for Low Carbon, Energy and Environment (NRN-LCEE).

Contribution of authors

Project design by OL, LM, SG, PK and CJ; data collection by PK, PG, LM and OL; database construction by PK, PG and CJ; data analysis by CJ; manuscript written by CJ, with extensive input from all co-authors.

References

- Ashton, P.S. (1988) Dipterocarp biology as a window to the understanding of tropical forest structure. *Annual Review of Ecology and Systematics*, **19**, 347–370.
- Bagchi, R., Philipson, C.D., Slade, E.M. *et al.* (2011) Impacts of logging on density-dependent predation of dipterocarp seeds in a South East Asian rainforest. *Philosophical Transactions of the Royal Society B*, **366**, 3246–3255.
- Baltzer, J.L. & Davies, S.J. (2012) Rainfall seasonality and pest pressure as determinants of tropical tree species' distributions. *Ecology and Evolution*, **2**, 2682–2694.
- Basset, Y., Dahl, C., Civrtecka, R. *et al.* (2018) A cross-continental comparison of assemblages of seed- and fruit-feeding insects in tropical rain forests: faunal composition and rates of attack. *Journal of Biogeography*, **00**, 1–13. <https://doi.org/10.1111/jbi.13211>.
- Blüthgen, N., Menzel, F. & Blüthgen, N. (2006) Measuring specialization in species interaction networks. *BMC Ecology*, **6**, 9.
- Brenes-Arguedas, T., Coley, P.D. & Kursar, T.A. (2009) Pests vs. drought as determinants of plant distribution along a tropical rainfall gradient. *Ecology*, **90**, 1751–1761.
- Chen, S.C., Hemmings, F.A., Chen, F. & Moles, A.T. (2017) Plants do not suffer greater losses to seed predation towards the tropics. *Global Ecology and Biogeography*, **26**, 1283–1291.
- Clark, C.J., Poulsen, J.R., Levey, D.J. & Osenberg, C.W. (2007) Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. *The American Naturalist*, **170**, 128–142.
- Coley, P.D. & Barone, J.A. (1996) Herbivory and plant defences in tropical forests. *Annual Review of Ecology and Systematics*, **27**, 305–335.
- Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M. *et al.* (2014) Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *Journal of Ecology*, **102**, 845–856.
- Condit, R., Engelbrecht, B., Pino, D., Turner, B. & Pérez, R. (2013) *Panama Tree Distribution Database*. DOI <https://doi.org/10.5479/data.bci.20130204>.
- Crawley, M.J. (2013a) Seed predators and plant population dynamics. *Seeds: The Ecology of Regeneration in Plant Communities* (ed. by M. Fenner), pp. 167–182. CAB International, Wallingford, U.K.
- Crawley, M.J. (2013b) *The R Book*. John Wiley & Sons, West Sussex, U.K.
- Civrtecka, R., Sam, K., Brus, E., Weiblen, G.D. & Novotny, V. (2014) Frugivorous weevils are too rare to cause Janzen–Connell effects in New Guinea lowland rain forest. *Journal of Tropical Ecology*, **30**, 521–535.
- Dormann, C.F., Gruber, B. & Fründ, J. (2008) Introducing the bipartite package: analysing ecological networks. *Interaction*, **1**, 0-2413793.
- Fenner, M. & Thompson, K. (2005) *The Ecology of Seeds*. Cambridge University Press, Cambridge, U.K.
- Futuyma, D.J. & Moreno, G. (1988) The evolution of ecological specialization. *Annual Review of Ecology and Systematics*, **19**, 207–233.
- Janzen, D.H. (1980) Specificity of seed-attacking beetles in a Costa Rican deciduous forest. *Journal of Ecology*, **68**, 929–952.
- Garzon-Lopez, C.X., Ballesteros-Mejia, L., Ordoñez, A., Bohlman, S.A., Olf, H. & Jansen, P.A. (2015) Indirect interactions among tropical tree species through shared rodent seed predators: a novel mechanism of tree species coexistence. *Ecology Letters*, **18**, 752–760.
- Gaviria, J. & Engelbrecht, B.M.J. (2015) Effects of drought, pest pressure and light availability on seedling establishment and growth: their role for distribution of tree species across a tropical rainfall gradient. *PLoS One*, **10**, e0143955.
- Givnish, T.J. (1999) On the causes of gradients in tropical tree diversity. *Journal of Ecology*, **87**, 193–210.
- Green, P.T., Harms, K.E. & Connell, J.H. (2014) Nonrandom, diversifying processes are disproportionately strong in the smallest size classes of a tropical forest. *Proceedings of the National Academy of Sciences, U.S.A.*, **111**, 18 649–18 654.
- Griffin, D.M. (1963) Soil moisture and the ecology of soil fungi. *Biological Reviews*, **38**, 141–166.
- Hammond, D.S., Brown, V.K., Newbery, D.M., Prins, H.H.T. & Brown, N.D. (1998) Disturbance, phenology and life-history characteristics: factors influencing distance/density-dependent attack on tropical seeds and seedlings. *Dynamics of Tropical Communities: The 37th Symposium of the British Ecological Society* (ed. by D. M. Newberry, H. H. T. Prins & N. Brown), pp. 51–78. Blackwell Science, Oxford, U.K.
- Harms, K.E., Wright, S.J., Calderón, O., Hernández, A. & Herre, E.A. (2000) Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature*, **404**, 493–495.
- Hersh, M.H., Vilgalys, R. & Clark, J.S. (2012) Evaluating the impacts of multiple generalist fungal pathogens on temperate tree seedling survival. *Ecology*, **93**, 511–520.
- Hosaka, T., Yumoto, T., Kojima, H., Komai, F. & Noor, N.S.M. (2009) Community structure of pre-dispersal seed predatory insects on eleven *Shorea* (Dipterocarpaceae) species. *Journal of Tropical Ecology*, **6**, 625–636.
- Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist*, **104**, 501–528.
- Janzen, D.H. (1971) Seed predation by animals. *Annual Review of Ecology and Systematics*, **2**, 465–492.
- Janzen, D.H. (1980) Specificity of seed-attacking beetles in a Costa Rican deciduous forest. *Journal of Ecology*, **68**, 929–952.
- Jones, F.A. & Comita, L.S. (2008) Neighbourhood density and genetic relatedness interact to determine fruit set and abortion rates in a continuous tropical tree population. *Proceedings of the Royal Society*, **275**, 2759–2767.
- Jones, F.A. & Comita, L.S. (2010) Density-dependent pre-dispersal seed predation and fruit set in a tropical tree. *Oikos*, **119**, 1841–1847.
- Kreft, H. & Jetz, W. (2007) Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences, U.S.A.*, **104**, 5925–5930.
- Lewis, O.T. & Gripenberg, S. (2008) Insect seed predators and environmental change. *Journal of Applied Ecology*, **45**, 1593–1599.
- Manzur, M.I. & Courtney, S.P. (1984) Influence of insect damage in fruits of hawthorn on bird foraging and seed dispersal. *Oikos*, **43**, 265–270.
- Martin, F.N. & Loper, J.E. (1999) Soilborne plant diseases caused by *Pythium* spp: ecology, epidemiology, and prospects for biological control. *Critical Reviews in Plant Sciences*, **18**, 111–181.
- Moles, A.T. & Westoby, M. (2003) Latitude, seed predation and seed mass. *Journal of Biogeography*, **30**, 105–128.

- Nakagawa, M., Itioka, T., Momose, K. *et al.* (2003) Resource use of insect seed predators during general flowering and seeding events in a Bornean dipterocarp rain forest. *Bulletin of Entomological Research*, **93**, 455–466.
- Novotny, V., Miller, S.E., Baje, L. *et al.* (2010) Guild-specific patterns of species richness and host specialization in plant–herbivore food webs from a tropical forest. *Journal of Animal Ecology*, **79**, 1193–1203.
- Novotny, V. & Basset, Y. (2005) Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society B*, **272**, 1083–1090.
- Pinzón-Navarro, S., Barrios, H., Múrria, C., Lyal, C.H. & Vogler, A.P. (2010) DNA-based taxonomy of larval stages reveals huge unknown species diversity in neotropical seed weevils (genus *Conotrachelus*): relevance to evolutionary ecology. *Molecular Phylogenetics and Evolution*, **56**, 281–293.
- R Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria [WWW document]. URL <https://www.R-project.org/> [accessed on 7 November 2003].
- Pyke, C.R., Condit, R., Aguilar, S. & Lao, S. (2001) Floristic composition across a climatic gradient in a neotropical lowland forest. *Journal of Vegetation Science*, **12**, 553–566.
- Schupp, E.W. (1992) The Janzen-Connell model for tropical tree diversity: population implications and the importance of spatial scale. *The American Naturalist*, **140**, 526–530.
- Singer, M. & Stireman, J. (2001) How foraging tactics determine host-plant use by a polyphagous caterpillar. *Oecologia*, **129**, 98–105.
- Spear, E.R., Coley, P.D. & Kursar, T.A. (2015) Do pathogens limit the distributions of tropical trees across a rainfall gradient? *Journal of Ecology*, **103**, 165–174.
- Svenning, J.C. & Wright, S.J. (2005) Seed limitation in a Panamanian forest. *Journal of Ecology*, **93**, 853–862.
- Swinfield, T., Lewis, O.T., Bagchi, R. & Freckleton, R.P. (2012) Consequences of changing rainfall for fungal pathogen-induced mortality in tropical tree seedlings. *Ecology and Evolution*, **2**, 1408–1413.
- Turnbull, L.A., Crawley, M.J. & Rees, M. (2000) Are plant populations seed-limited? A review of seed sowing experiments. *Oikos*, **88**, 225–238.
- Visser, M.D., Muller-Landau, H.C., Wright, S.J., Rutten, G. & Jansen, P.A. (2011) Tri-trophic interactions affect density dependence of seed fate in a tropical forest palm. *Ecology Letters*, **14**, 1093–1100.
- Webb, C.O. & Peart, D.R. (1999) Seedling density dependence promotes coexistence of Bornean rain forest trees. *Ecology*, **80**, 2006–2017.
- Weissflog, A., Markesteijn, L., Lewis, O.T., Comita, L.S. & Engelbrecht, B.M. (2017) Contrasting patterns of insect herbivory and predation pressure across a tropical rainfall gradient. *Biotropica*, **0**, 1–10.
- Xu, Y., Shen, Z., Li, D. & Guo, Q. (2015) Pre-dispersal seed predation in a species-rich Forest Community: patterns and the interplay with determinants. *PLoS One*, **10**, e0143040.

Accepted 24 July 2018

Associate Editor: Shannon Murphy