Insect-Mediated Indirect Interactions in Tropical Forest Plant Communities



Harriet Downey

Merton College

University of Oxford

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Abstract

A key aim in ecology is to understand the mechanisms that contribute to structuring diverse communities such as tropical forest plant communities. Much focus has been on the role of highly host-specific natural enemies. With data on the diet breadths of plant enemies accumulating, it is becoming increasingly clear that enemies are rarely strict specialists. It is therefore necessary to broaden investigations to non-specialist enemies contributing to structuring plant communities.

In this thesis, I investigated the role of non-specialist insect enemies of seeds and seedlings in structuring plant communities in a forest in Panama, using a series of field investigations. Firstly, I used a quantitative food web approach to investigate the potential for indirect interactions between plant species in the Lauraceae family via their internally-feeding seed predators. I found high potential for enemy-mediated indirect interactions between species in this study system. These interactions are likely asymmetric and driven by the volume of fruit produced by each species. Secondly, using the same study system, I investigated patterns of seed predator attack across space and in response to the presence of conspecifics and alternative host species in the neighbourhood. I found that local and neighbourhood resource densities affected rates of insect attack and premature fruit abscission. Finally, using seedlings of *Cordia* trees and their associated insect herbivore I investigated the role of shared enemies at a later life stage. I detected negative conspecific density dependence as well as the potential for indirect interactions between plant species mediated by the shared herbivore.

Overall, I show that insects caused significant amounts of damage to early life stages of plants, and that they have the potential to link the regeneration dynamics of their host species via enemy-mediated indirect interactions. This work contributes to a better understanding of the factors influencing the reproduction and recruitment of plant species in tropical forests.

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Statement of authorship

Each chapter was written in collaboration with my supervisors, and in chapter 2 with an extra co-author. As stated in each manuscripts authorship statement ‘HD, SG, OL and MB conceived and designed the study. HD carried out data collection, analysed the data, interpreted the results and drafted the manuscript with input from SG, OL and MB’.

Format of chapters

This thesis is prepared as an integrated thesis. Each data chapter has been prepared in the style of the journal to which the manuscript has been submitted or is intended to be submitted to. As a result there is some overlap in information between chapters. Supplementary material associated with each chapter is presented at the end of that chapter, but references are presented together in one bibliography at the end of the thesis.

Chapter 1

General Introduction

Introduction

A key aim in community ecology is to understand what allows species to coexist and what processes determine the distribution and abundance of individual species. These questions are particularly relevant in species-rich communities such as those found at many tropical forest sites. For example, a single hectare of tropical forest can contain over 400 tree species (Valencia, Balslev, & Pazy Mino 1994) and a large number of liana species (Schnitzer et al. 2012). For decades, ecologists have been trying to understand how it is possible that so many plant species can co-occur locally. In recent years, much attention has been given to the role of interactions between plants and their enemies.

A large body of research has shown that biotic interactions can play an important role in shaping ecological communities. Although intraspecific processes are important for determining the abundance of populations, interspecific interactions shape the composition of species within communities and determine the functioning of ecosystems (Bascompte 2009; van Dam 2009). The strength, duration and direction of interspecific interactions may vary in time and space under the influence of abiotic conditions (Coulson et al. 2001; Forchhammer et al. 2005; Gilman et al. 2010).

Biotic interactions and the coexistence of species within communities

Classic competition theory predicts that species that share the same niche cannot coexist in space and time (Grinnell 1904; Gause 1934). In practice, species-rich ecological communities comprise many similar co-occurring species, leading ecologists to explore the mechanisms which make this possible. Modern coexistence theory (developed by Peter Chesson) suggests that species can coexist through two distinct mechanisms (Chesson 2000): firstly, through reduced average fitness differences between species (an equalising mechanism); and secondly, through niche differentiation between species (a stabilising mechanism). Put

simply, when niche differences outweigh fitness differences, coexistence is possible. Since its conception, modern coexistence theory has received a lot of attention, with studies showing how species can finely partition resources (e.g. Kartzinel et al. 2015) allowing coexistence. Theoretical studies have shown that biotic interactions such as predation can alter the mechanisms leading to coexistence in a similar manner to resource competition. Niche overlap can be extended to include how a prey species responds to their predator as well as to their resources. Whether coexistence or exclusion occurs, is then dependent on which process more strongly regulates the density of focal species (Chesson & Kuang 2008). Despite this, there has been less empirical work demonstrating how biotic interactions may affect average fitness differences and niche differences.

The role of biotic interactions in shaping ecological communities

Interspecific interactions can be direct (where one species directly interacts with another in a simple pair-wise manner) or indirect (where two species interact via an intermediary species) (Kéfi et al. 2012). Direct interactions (e.g. pollination, seed dispersal, parasitism and herbivory) are commonly observed and studied in nature and their importance in ecosystem functioning is well known (Millennium Ecosystem Assessment 2005).

Indirect interactions may be harder to observe directly than direct trophic interactions, and their importance in ecological systems is more difficult to predict. Several types of indirect interactions have been identified (Fig. 1) and it has been suggested that they could in some cases be as strong and prevalent as direct interactions (Montoya et al. 2009).

Enemy-mediated indirect interactions

Shared predation (two or more species being attacked by the same predator species) is widespread in nature (Chaneton & Bonsall 2000) and may affect the coexistence and

abundances of species within communities via enemy-mediated indirect interactions such as apparent competition and apparent mutualism (reviewed in Holt & Bonsall 2017).

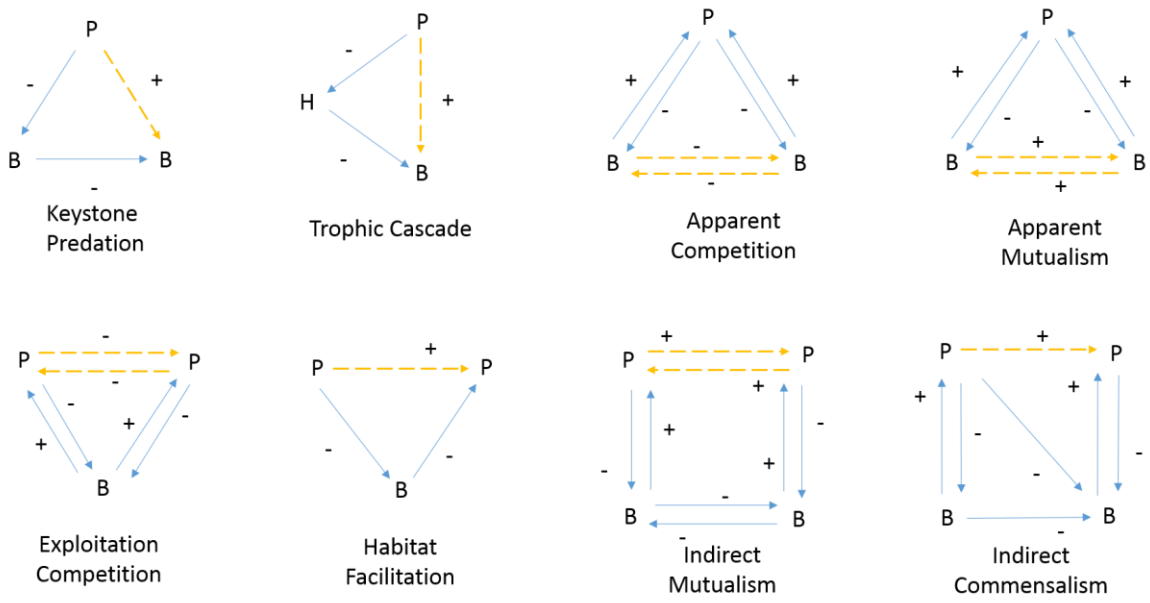


Figure 1- A schematic representation of some of the main indirect interaction pathways in ecological communities. Direct interactions are represented by solid blue lines and indirect interactions are represented by yellow dashed lines. The + and - symbols indicate positive and negative interactions. P, H, and B refer to predator, herbivore, and basal species, respectively. Based on Menge (1995).

Apparent competition

Apparent competition occurs where two or more species that share a natural enemy (for example a herbivore, parasite or pathogen) have a negative influence upon each other by increasing the numbers of their shared enemy when they occur together (Fig. 1). This may lead to a reduction in the abundance of one or all resource species (Holt 1977). Apparent competition may occur through density changes in the predator population (multiple prey species can jointly sustain a larger predator population than would be possible when any of the species occurred on their own). This is sometimes referred to as long-term apparent

competition. Mobile natural enemies may also respond behaviourally to changes in host densities by aggregating in patches where multiple prey species occur, causing a negative interaction between prey species, often referred to as short-term apparent competition (Holt & Kotler 1987). Apparent competition may be reciprocal (-/-) or asymmetrical (-/0) due to predator diet preference, prey nutritional value, population sizes or susceptibility to attack. Asymmetry can lead to exclusion of one species from the community (Bonsall & Hassell 1997), where the species that persists is the one that can tolerate the highest number of natural enemies (Bonsall & Hassell 1997; Chaneton & Bonsall 2000). Early theoretical studies (Holt 1977; Holt & Kotler 1987) on apparent competition have been bolstered by a growing number of empirical studies from, amongst others, rocky inter-tidal habitats (Menge 1995), temperate plant communities (Veech 2001), game bird communities (Tompkins, Draycott, & Hudson 2000) and tropical and temperate insect communities (Muller & Godfray 1999; Morris et al. 2004).

Apparent competition can play an important role in exotic species invasions (White et al. 2006), and studies have demonstrated that it can increase the likelihood of endangered native species going extinct as a result of invasion (Dangremond et al. 2010). The concept of apparent competition has also been explored as a potential biological tool for pest management (Chailleux et al. 2014). Since its original description, the concept of apparent competition has also been expanded to include the specific situation where a herbivore is constrained by its ‘nurse plant’ species (a plant used as a refuge from abiotic stress), increasing herbivory on other plant species within the proximity of the nurse plant (Connell 1990). Field experiments have demonstrated apparent competition inflicted by nurse plants can reduce the recruitment of heterospecific seedlings in certain environments (Chaneton et al. 2010).

Apparent mutualism

The interaction between prey species via their shared enemy does not necessarily lead to suppressed prey densities (Fig. 1). The concept of apparent mutualism provides an alternative scenario, where species with a shared enemy actually benefit from occurring together. Here, two or more prey species may jointly satiate their shared enemy by providing it with more resources than it can possibly consume (Holt 1977), allowing for the survival and coexistence of the prey species. This has been suggested as a mechanism that led to the evolution of synchronised fruiting in some tree species (Kelly & Sork 2002). 'Prey switching', a situation where the predator focuses its attacks on one of the prey species, thereby neglecting the other, can also lead to positive interactions between prey species, and provide a way for species to coexist. Prey switching can be passive, where the predator consumes food in proportion to their availability, or active, where there is a favouring of the more common or safer choice (Abrams & Matsuda 1996; Abrams 2010).

Whilst theoretical work provides scenarios under which apparent mutualism may occur, there is notably less empirical data explicitly exploring apparent mutualism than apparent competition (but see Long et al. 2012; Van Maanen et al. 2012). Apparent mutualism is often predicted to occur in populations that are not at equilibrium density. The cycling of populations allows for a temporary reduction in predation on one of the prey species which may be short-term, or if the population is continually cycling, long-term (Abrams et al. 1998). Both apparent competition and apparent mutualism may occur in the same system at different spatial and temporal scales when populations cycle (Abrams et al. 1998; Van Maanen et al. 2012), or when a new prey species invades a habitat (Holt & Kotler 1987). The exact ways in which fluctuations in population sizes affect the outcome of indirect interactions is still a topic of debate.

Enemy-mediated indirect interactions in plant communities

Plants are attacked by many types of natural enemies (e.g. pathogens, seed predators and herbivores) many of which are not restricted to a single host species. Whilst biologists have only recently starting to explore patterns of host associations of many plant pathogens (Gilbert & Webb 2007; Hersh et al. 2012) and their potential to structure plant communities, ecologists have long considered the potential for non-specialised herbivores to cause context-dependent patterns of herbivory and/or enemy-mediated interactions between host plant species. Tahvanainen & Root (1972) and Root (1973) suggested that the amount of herbivory a plant experiences depends on the local plant community composition. Plants in diverse communities may suffer from higher herbivore attack rates from *generalist* enemies than those in monocultures. This is known as associational susceptibility (AS) (Brown & Ewel 1987). The opposite may occur with *specialist* herbivores that are likely to stay in less diverse patches that contain multiple host species; a hypothesis referred to as the resource concentration hypothesis (Root 1973). Associational susceptibility (which could lead to apparent competition) may also occur when a plant species that is typically unattractive to enemies experiences greater levels of attack through spill-over of enemies from nearby preferred plant species or habitats (Frost et al. 2016). Many studies provide evidence for apparent competition between plant species mediated by a shared herbivore (Thomas 1986; Hails & Crawley 1991). In most cases the interactions have been asymmetric, with one plant species negatively affecting the other whilst experiencing negligible or undetectable effects of the other plant species themselves (Chaneton and Bonsall 2000).

Where a plant species experiences reduced levels of herbivory due to neighbour identity, an indirect positive interaction known as associational resistance (AR) may occur (Barbosa et al. 2009). There are several hypotheses as to why this phenomenon may occur, including less palatable species repelling herbivores, plant species emitting volatile chemicals that interfere

with the consumer's host finding abilities, or a more palatable plant species attracting the herbivore away from a less palatable species. Knowledge of this mechanism has been applied in agricultural systems in the form of 'trap crops' that lure insect herbivores away from the commercially grown crop species (Himanen et al. 2015).

Whilst patterns predicted by the hypotheses on associational resistance and associational susceptibility seem to hold true in many cases (Jactel & Brockerhoff 2007; Kaitaniemi et al. 2007), Plath et al. (2012) demonstrated that diverse tree stands experienced more damage by a specialist herbivore than monocultures, i.e. the opposite of what would be expected under AR and AS hypotheses. They concluded that the direction of association may be due to the herbivore's traits and life history strategies, not simply its feeding habit, and that information on these variables is crucial when predicting the effects of herbivores on plant community structure.

Enemy-mediated indirect interactions in tropical forest plant communities

The role of natural enemies in shaping and maintaining tropical forest plant diversity has long been an active area of research, with much focus on the Janzen-Connell hypothesis (Janzen 1970; Connell 1971). This hypothesis proposes that host-specific enemies act in a distance- or density-dependent manner, increasing mortality rates of hosts near parent trees and freeing up space for other species to colonise, thereby promoting higher overall levels of plant diversity.

Indeed, some studies have shown that even a small reduction in recruitment of conspecifics in areas of high seed density can increase local diversity (Harms et al. 2000). As the mechanism proposed by the Janzen-Connell hypothesis relies on enemies being host-specific, to date the vast majority of studies on the role of plant enemies in tropical forests have focussed on the effects of specialist plant enemies (Terborgh 2012; Comita et al. 2014). As more studies of insect herbivore diet breadths have been undertaken, it has been shown that many tropical

insect herbivores are not as specialised as originally thought (Novotny & Basset 2005; Sam et al. 2017). Despite this, the role of non-specialised enemies in shaping tropical plant communities through enemy-mediated indirect interactions has largely been overlooked. Observational studies have demonstrated that plant individuals (at early life stages) surrounded by closely related neighbours may be more likely to experience higher mortality rates than individuals surrounded by more distantly related neighbours. Since interspecific competition between seedlings tends to be weak in tropical forests (Svenning, Fabbro & Wright 2008; Paine et al. 2008), the observed patterns in mortality rates are thought to be due to natural enemies: when closely related species – likely to be attacked by the same species of enemy – grow together, enemy load will be higher (Webb et al. 2006; Bagchi et al. 2010). In a recent study, Garzon-Lopez et al. (2015) demonstrated that seed predation rates of tropical tree seeds by rodent predators were better explained by the combined densities of conspecific and congeneric seed densities, than by conspecific densities alone. They concluded that such heterospecific density effects could work in concert with, but also reduce, the contribution of conspecific density effects to maintain species diversity at the community level. The role of non-specialist insect herbivores in creating similar effects remains untested. As insects do not tend to have territories and tend to be less generalised in their diets than mammalian herbivores, their effects on their host plants are likely to be different.

Thesis aim

In this thesis I use field experiments and observations to explore the potential for non-specialist insect herbivores to contribute to shaping species abundances and distributions in tropical tree communities via enemy-mediated indirect effects. I focus upon the earliest stages in the plant life cycle (seeds and seedlings) as these have been shown to be the stages where biotic interactions have the greatest effect upon forest structure and diversity (Green, Harms & Connell 2014). My work aims to assess the overall prediction that including enemy-

mediated indirect interactions into studies of tropical plant communities can improve our understanding of the role of natural enemies in structuring hyperdiverse tropical forest plant communities.

Study systems

To investigate the potential for enemy-mediated indirect interactions in tropical forests, I conducted a series of observational and experimental studies on Barro Colorado Island (BCI), Panama, during 2015 and 2016. BCI is a 15.6 km² island located in Gatun Lake (Lat: 9.1543, Long:-79.846). The island is covered with tropical semi-deciduous forest. The northeast half experienced widespread clearing during the late 1800s, whilst the other half has been little disturbed for up to 1500 years. The climate on BCI is seasonal. The dry season occurs from December to April/May and the average yearly rainfall is 2612mm (Leigh 1999). In addition to its famous 50 ha forest dynamics plot BCI has several additional mapped forest plots where the investigations presented in this thesis were carried out. These plots were established in the mid-1990s and every free-standing woody stem over 20cm diameter at breast height (dbh) has been tagged, measured (dbh in mm) and identified to species level. These plots were last censused in 2013 and 2014.

I used two study systems for my investigations:

1. *The Lauracaeae family and its associated pre-dispersal insect seed predators*

The Lauraceae family is a common and widespread family in tropical and subtropical forests and includes economically important species such as cinnamon (*Cinnamomum* sp.), Bay laurel (*Laurus nobilis*) and avocado (*Persea americana*). There are nine naturally occurring Lauraceae species on BCI, and a very small number of *P. americana* that were likely introduced there by humans. Preliminary collections (S. Gripenberg, unpublished data) showed that members of the Lauraceae family share some pre-dispersal insect seed predators.

For this reason they were chosen for further investigation into the effects of shared predators. In addition, pre-dispersal seed predators are an excellent system for studying the impacts of natural enemies: since they kill seeds, it is straightforward to quantify their impacts on offspring production.

2. The genus Cordia *and their associated herbivore* Ischnocodia annulus

The genus *Cordia* (Boraginaceae) consists of trees, shrubs, lianas, vines and erect herbs. Three tree species occur on BCI. All three *Cordia* species on BCI overlap in their reproductive phenology, with the majority of seeds germinating in May and June. One species (*Cordia lasiocalyx*) was too rare to be included in our studies. Preliminary studies at our field site showed that the other two species, *Cordia alliodora* and *Cordia bicolor*, are attacked by the tortoise beetle *Ischnocodia annulus* at the early seedling stage (immediately following germination). *Ischnocodia annulus* leaves distinctive feeding marks on the leaves, allowing for easy identification of seedling attack.

Thesis outline

This thesis consists of three data chapters:

In Chapter 2, I use a quantitative food web approach (e.g. Lewis et al. 2002; van Veen, Morris & Godfray 2006; Tack, Gripenberg & Roslin 2011) to detect the potential for enemy-mediated indirect interactions between plant species within the same family (Lauraceae) caused by internally feeding pre-dispersal insect seed predators. I also assess whether the potential for indirect interactions between pairs of Lauraceae species depends on similarities in morphological traits (if it does, this might be helpful when predicting the likelihood for these types of interactions across the wider plant community).

In Chapter 3 (which also focuses on the Lauraceae seed predator system), I explore how the spatial setting of species sharing the same enemies may affect their reproductive dynamics. I investigate how connectivity to other alternative host species affects premature fruit abscission rates, seed predator attack rates and successful fruit production.

Having explored the potential for enemy-mediated indirect interactions at the pre-dispersal stage, in Chapter 4 I explore these interactions at a later life stage (seedlings). Chapter 4 focuses on the tree genus *Cordia* and its seedling herbivore *Ischnocodia annulus*. Using this system I test for distance- and density-dependence in herbivory rates both within and *across* species, covering a range of spatial scales.

I conclude the thesis with a general discussion (Chapter 5).

Chapter 2

Assessing the potential for indirect interactions between tropical tree species via shared seed predators

Status: In review, Oikos

Abstract

Pre-dispersal insect seed predators have the potential to influence the dynamics of plant populations, and the structure of plant communities. In diverse tropical forests, research has largely focused on the effects of specialised insect enemies of plants, despite many insects having broader diet breadths. Enemies with broader diets have the potential to connect the dynamics of their host plant species via mechanisms such as apparent competition and apparent mutualism. We investigated the community of insect seed predators interacting with seven tree species in the family Lauraceae on Barro Colorado Island (Panama). We present one of the first quantitative food webs for pre-dispersal insect seed predators and their host plants, and use information in the web to assess the potential for indirect interactions between the tree species. Our data suggest that there is potential for indirect interactions between Lauraceae species via their shared seed predators (notably the beetle *Pagiocerus frontalis*). The strength and direction of these interactions are likely governed by the volume of fruit produced by each host species.

Keywords: apparent competition, food webs, indirect interactions, Janzen-Connell hypothesis, tropical forest, seed predator

Introduction

The interactions between plants and their natural enemies are among the commonest and most ecologically important interactions observed in nature. Natural enemies including herbivores and pathogens have the potential to affect the reproductive output of individual plants, the dynamics of plant populations, and the structure of plant communities. Of the many guilds of insects that could affect plant fitness, pre-dispersal seed predators have received comparably little attention. By killing mature and immature seeds *in situ*, pre-dispersal insect seed predators have the potential to drastically reduce plant fecundity. Although individual plants may lose up to 90% of their seed crop to pre-dispersal seed predators (Crawley 2000), the impact of reduced seed crops on plant population dynamics are often not known. Some studies have suggested that the effect of seed predators is minimal (Andersen 1989). However, Maron & Crone (2006) showed that pre-dispersal seed predators can reduce plant population growth as much as any other insect guild.

The potential community-level effects of pre-dispersal seed predation by insects will largely depend on their diet breadth. Few studies have investigated the host-specificity of pre-dispersal insect seed predators. Existing studies focusing on internally-feeding seed predators (i.e. 'seed parasitoids') suggest that insect seed predators tend to be relatively host-species specific, but there are also cases where individual seed predator species are associated with multiple plant species, typically within the same genus or family (Janzen 1980; Ctvrtecka et al. 2016; Sam et al. 2017). Such non-specialised seed predators could potentially link the regeneration dynamics of their host plant species via enemy-mediated indirect interactions such as apparent competition (Holt 1977). When plant species sharing seed predator species co-occur in space or time, larger populations of seed predators could build up than would be the case in any single-host scenario. This could cause reductions in the abundance of one or more of the plant species and provide a mechanism structuring plant communities. An

alternative scenario, where co-occurring hosts satiate natural enemies with more resources than they can consume, is known as ‘apparent mutualism’ (Abrams & Matsuda 1996) and is thought to be one of the explanations for mast fruiting seen in the tropical forests of Asia (Kelly & Sork 2002).

In hyper-diverse tropical tree communities, substantial effort has been invested in investigating how host-specific enemies contribute to structuring and maintaining plant diversity (Wright 2002; Bagchi et al. 2014). While it seems likely that indirect interactions mediated by non-specialist enemies occur, few studies have explicitly assessed the importance of enemy-mediated indirect interactions in structuring tropical plant communities (but see Garzon-Lopez et al. 2015), or investigated the extent to which plant species relatedness and plant traits influence the incidence and magnitude of indirect interactions (Lewis & Gripenberg 2008).

In this study we investigated the potential for seed-predator mediated indirect interactions between tree species within the Lauraceae family on Barro Colorado Island (BCI) in Panama. Specifically, we (1) construct a quantitative food web describing the interactions between Lauraceae and their internally-feeding insect seed predators; (2) use the web to assess the potential for indirect interactions between Lauraceae tree species, mediated by shared seed predators; and (3) assess whether closely related Lauraceae species and Lauraceae species with similar traits are more likely to share enemies than more distantly related species and those with greater trait dissimilarity. In each case we also quantify inter-annual variation in the interactions between the plants and their seed predators.

Materials and Methods

Study Site

Barro Colorado Island (BCI) is a 15.6 km² island located in Gatun Lake, Panama, (Lat: 9.1543, Long: -79.846). It was isolated from the surrounding mainland in 1914 when the Rio Chagres was dammed to form part of the Panama Canal. The island is covered with tropical semi-deciduous forest. The northeast half of the island experienced widespread clearing during the late 1800s, whilst the other half has been little disturbed for up to 1500 years. The climate of BCI is seasonal, with a dry season from December to April or May, and a mean yearly rainfall of 2612mm (Leigh 1999).

Most investigations presented here were carried out in four mapped forest plots (one ten hectare, one 25 hectare and two six hectare; Supplementary Fig. 1) that were established in the mid-1990s. In these plots, every freestanding woody stem over 20cm diameter at breast height (dbh) has been tagged, measured (dbh) and identified to species level. These plots were last censused in 2013 and 2014.

Study System

The Lauraceae is one of the commonest and ecologically most significant woody plant families in tropical and subtropical forests (Gentry 1988). Lauraceae fruits are an important food source for many bird (Wheelwright 1983) and mammal species (Tabarelli & Peres 2002). The family also includes species that provide products of economic value including avocado, bay leaf, cinnamon and timber. Nine species of Lauraceae representing four genera occur on BCI (see Table 1 for individual species descriptions) (Croat 1978). All of these species have single-seeded fleshy fruits that are animal-dispersed, but there is substantial variation among species in terms of fruit size and shape. In addition to the species listed in

Table 1, the avocado (*Persea americana*) also occurs on BCI, but was likely introduced there by humans and is too rare to be included in this study.

Pre-dispersal insect seed predators are commonly Coleoptera, Lepidoptera and Hymenoptera. Adults lay eggs on fruit surfaces and the larvae hatch and feed on the seeds before boring their way out of the fruit. Often, multiple seed predators can develop in a single fruit (Basset et al. 2018). Preliminary investigations in 2011-2013 showed that insects damage a substantial proportion of Lauraceae seeds, and that some of them appear to share multiple pre-dispersal seed predator species.

Data Collection

Constructing a quantitative food web describing the interactions between Lauraceae and their internally feeding seed predators- To assess the potential for seed predator-mediated indirect interactions between the Lauraceae species on BCI we constructed a quantitative food web. Quantitative food webs have been used in past studies of indirect interactions (Morris et al. 2004; Tack et al. 2011) and allow the incidence and strength of indirect interactions to be predicted (van Veen et al. 2006, Lewis and Gripenberg 2008).

To construct a quantitative plant-seed predator food web data are needed on a) host densities (individuals m^{-2}) for fruits of each Lauraceae species in the mapped plots on BCI; and b) the frequencies of interactions between each seed predator species and each Lauraceae species (interactions m^{-2}).

Table 1- Lauraceae species found on BCI and investigated in this study. Descriptions of tree and fruit attributes are based on information in Croat (1978) (tree size, reproductive phenology), our own measurements taken over the first year of data collection (mean fruit weights (g), lengths (mm) and widths (mm)), and from trait data collected within the ForestGeo 50 ha forest dynamics plot (Wright et al. 2013).

Species	**Adult tree description**	**No. of individuals in mapped plots**	**Fruiting phenology**	**Immature fruit weight**	**Mature fruit weight**	**Immature fruit length**	**Mature fruit length**	**Immature fruit width**	**Mature fruit width**
Beilschmedia pendula (Sw.) Hemsl. 1882	13-40 m tall, to 75 cm dbh	56	February - June	2.19	6.80	23.5	37.0	9.88	17.89
Ocotea oblonga (Meisn.) Mez 1889	To 30 m tall, to 45 cm dbh.	35	February - June	0.72	0.94	16.25	18.60	8.53	10.07
Ocotea puberula (Rich.) Nees 1920	To 27 m tall, to 50 cm dbh.	13	January - July	0.12	0.78	5.72	12.23	4.87	7.69
Ocotea cernua (Nees) Mez 1889	To 12 m tall	11	April - September.	0.26	0.56	10.15	13.28	6.46	7.46
Ocotea whitei Woodson 1945	15-30 m tall	21	February - July	0.33	0.70	14.17	14.5	6.23	9.33
Nectandra cissiflora Nees 1836	To 27 m tall, to 30 cm dbh	40	March - June	0.42	0.90	10.66	11.53	10.70	11.68
Nectandra lineata (Kunth) Rohwer		10	February - June	0.35	0.42	8.89	9.33	0.29	0.35

Estimating densities of fruits of each species of Lauraceae in mapped plots on BCI – In February 2015 all adult trees of the nine Lauraceae species occurring on BCI were visited in four mapped plots (Supplementary Fig. 1). From these trees, up to six individuals of each species were selected to quantify fruit abundances. The criteria for selection were that the tree was reproductive (presence of flowers or fruits), the canopy was reasonably clear of lianas, and the terrain was open enough to allow us to place seed traps (described below) underneath its canopy. It was not possible to find six reproducing individuals for all nine species based upon these criteria, and in total 41 trees were selected. In 2016, eight additional trees were selected, bringing the total number of trees with traps to 49 (Table 2).

Table 2- Numbers of individuals of each species of Lauraceae monitored using seed traps. Note 4 trap trees are not listed in the table as no fruit collections were ever made from them.

Species	**Number of trap trees 2015**	**Additional trap trees 2016**	**Total number of trap trees**
B. pendula	6	8	14
O. oblonga	6	0	6
O. puberula	6	0	6
O. cernua	4	0	4
O. whitei	5	0	5
N. cissiflora	5	0	5
N. lineata	5	0	5

Four 0.5m² seed traps (Fig. 1) were established in scattered locations underneath the canopy of each selected tree. All trees were visited monthly to assess when a fruiting period began, and traps were visited weekly throughout the fruiting period of each individual. Fruit collections took place between 26th February 2015 and 26th January 2017. Upon each visit, all

fruits were collected and debris emptied from each trap. Fruits were taken back to the laboratory where abundance, total mass and maturity status of fruit samples were recorded for each trap. From each trap and collection date, three fruits were randomly selected and their mass (g), width (mm) and length (mm) measured.

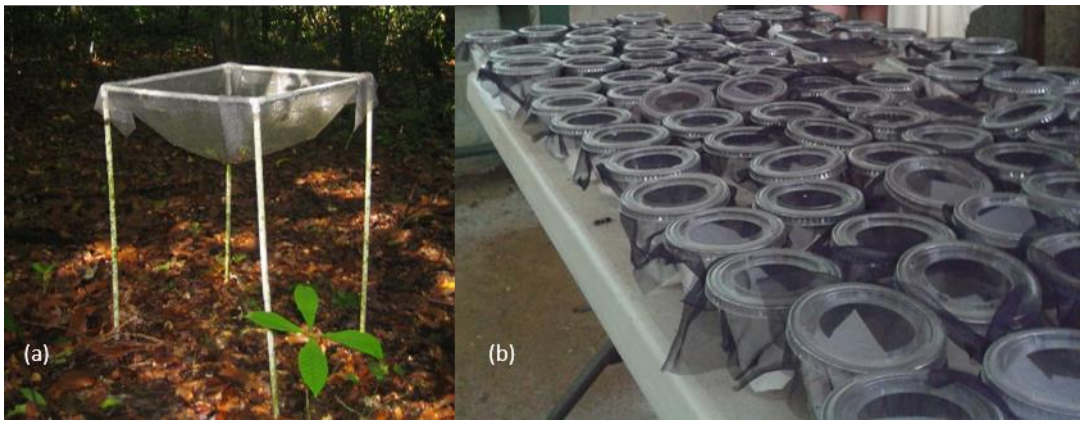


Figure 1- (a) a seed trap in the forest of Barro Colorado Island, (b) insect rearing pots.

To allow us to estimate total fruit abundances based on fruit numbers in the traps, we estimated the canopy area of each Lauraceae individual that had associated seed traps. For each individual, eight cardinal points were marked at the canopy edge from the canopy centre (N, S, E, W, NE, NW, SE, and SW). The distances from the canopy centre to these points were measured and were used to estimate canopy size as the area of the convex polygon formed by these points. The fruit counts in the traps and the estimated canopy area allowed us to estimate tree-specific fruit abundances, assuming that fruit densities are reasonably uniform within each canopy. As dbh and canopy size are correlated (see Supplementary information), and dbh is known for every Lauraceae individual in the mapped plots, estimates of overall fruit numbers of each tree could be calculated. Of the 49 trees with traps, we were unable to measure the canopy area of 12 because of high liana loads in the canopy, difficult terrain or treefalls. The canopy area for these trees was estimated based on their dbh.

Lauraceae individuals without traps also had canopy areas estimated based on their dbh, and were included in the species-specific fruit estimates.

Assessing frequencies of interactions between each seed predator species and each Lauraceae species – The fruits collected from the seed traps along with Lauraceae fruits collected opportunistically from the ground around the trap trees and from various other locations on BCI were used to estimate interaction frequencies between Lauraceae and their internally feeding insect seed predators.

Once fruits had been collected and traits measured (see below), they were placed in transparent plastic pots covered with a mesh lid (Fig. 1). Fruits from different collection dates and traps were kept separately, as were mature and immature fruits. Pots were checked twice weekly for emerging adult insects for up to three months after collection, after which fruits were opened and any adult insects or larvae removed. Emerged Coleoptera, Hymenoptera and Diptera and all larvae were stored in 95% ethanol in a freezer. Lepidoptera were killed by freezing and pinned to facilitate morphological identification. All species were morphotyped and compared to a seed predator reference collection kept at the insect collections of the Smithsonian Tropical Research Institute in Panama City.

We constructed a set of quantitative foods webs to depict graphically the interactions between Lauraceae seeds and their internally feeding seed predators. The webs were constructed using data from fruits collected from traps (ground fruits excluded).

We calculated the mean fruit density (fruits m^{-2}) for each Lauraceae species in the part of the forest that comprises the mapped plots (Supplementary Fig. 1). To do this we first estimated the number of fruits produced by each trap tree by multiplying the number of fruits produced in $1m^2$ (the sum of the four $0.5m^2$ traps) by the tree's canopy area (m^2).

Fruit crop increased significantly with dbh (Pearson correlation coefficient r=0.59, df = 40, P<0.001). We used this relationship to estimate the fruit crops of all Lauraceae trees on the basis of their dbh. Predicted fruit numbers from trap and non-trap trees for each Lauraceae species were combined to get total estimated fruit numbers for each Lauraceae species. We divided this by the area of mapped forest (470,000 m^2) to calculate fruits per m^2 of forest for each Lauraceae species.

We found the mean density of each seed predator species (seed predators m^{-2}) by calculating the average number of seed predator individuals per fruit (number seed predators/number of fruits attacked by that species) for each seed predator species and Lauraceae species combination. We multiplied the average seed predators per fruit (for each seed predator and Lauraceae species combination) by the number of fruits m^{-2} of each Lauraceae species to get seed predators m^{-2}

The numbers of seed predators m^{-2} were represented in the upper trophic level in the web, and the fruits m^{-2} were represented as the lower trophic level, allowing the inclusion of both attacked and unattacked fruits. We present a summary web (data from 2015 and 2016 combined), as well as webs displaying 2015 and 2016 separately. As the dry season begins in late December at our study site, wet and dry seasons are combined in the webs.

To provide a quantitative description of web structure, several commonly used metrics (weighted connectance, linkage density, interaction evenness, H2', generality and vulnerability (van Veen et al. 2006) were calculated for each web using the R package bipartite (Dormann, Gruber & Fruend 2008). Overall, low values of connectance, linkage density, generality and vulnerability indicate high specialisation in the network (with generality and vulnerability focusing on the specialisation of the top level and bottom level, respectively). These metrics are all scale-dependent, with smaller networks appearing to be

more specialised than larger networks (Blüthgen et al. 2006). Conversely, high values of H2' (ranging from 0 to 1) imply a high degree of specialisation in the network. H2' is scale independent.

Is there potential for indirect interactions between Lauraceae species mediated by shared insect seed predators?

We assessed the potential for host species to interact indirectly with each other using the equation set out in Müller et al. (1999). For each host plant species, we calculate the probability (d_{ij}) that a seed predator attacking species *i* developed on species *j* (where *j* is the same species d_{ii}).

$$d_{ij=} \sum_{k} \left[\frac{\alpha_{ik}}{\sum_{l} \alpha_{il}} \frac{\alpha_{jk}}{\sum_{m} \alpha_{mk}} \right]$$

Here α_{ik} is the interaction between host *i* and seed predator *k*, the sums of *j* and *m* include all seed predators, and the sum of *m* includes all hosts. The first term in the brackets represents the fraction of seed predators of host *i* that belong to species *k* and the second term represents the fraction of seed predators of species *k* that develops on host species *j*. d_{ij} equals zero when no seed predators are shared between a pair of host species (*i* and *j*) and equals 1 when all seed predator species on host species *i* develop on host species *j*. Values of d_{ij} depend on host densities, so we calculated values for the two years combined and for 2015 and 2016 separately. The d_{ij} values were calculated using the 'PAC' (potential for apparent competition) function in the R package 'bipartite'.

Species with low d_{ii} and species pairs with low d_{ij} values are expected to experience low levels of density-dependent predation because of low specificity of seed predators. Species pairs with low d_{ij} values are not expected to respond strongly to the densities of heterospecific seeds because of the lack of shared enemies. Species with high d_{ii} and d_{ij}

values should have a density-dependent effect upon themselves, and an interspecific density dependent effect when surrounded by species with which they share common seed predators. To investigate whether plants are likely to respond more strongly to the density of conspecific seeds than to the density of seeds of other species with which they share seed predators, we compared d_{ii} and d_{ij} values. Using a paired t-test, we assessed whether the d_{ii} value for each plant species was significantly different to the sum of its d_{ij} values.

Are closely related Lauraceae species and Lauraceae species with similar traits more likely to share enemies than more distantly related species/ species with greater trait dissimilarity?

We investigated factors hypothesised to affect the web structure and determine the potential for indirect interactions between species in our study system. For each of the webs (both years combined, 2015 and 2016) we assessed how traits of species *j* affect the d_{ij} values. We hypothesised that species with similar seed and fruit morphologies and highly overlapping fruiting phenologies would be most likely to share seed predators (i.e. have high d_{ij} values), and that the potential for apparent competition would also be highest for pairs of closely related Lauraceae species.

Because many fruit morphology measurements were highly correlated (Supplementary Table 4) we selected one trait, fruit mass (g), as a representative fruit morphology variable in our analyses. We calculated pairwise fruit size differences by calculating fruit mass ratios (mass of species *i*/mass of species *j*).

Following Fleming and Partridge (1984) we calculated pairwise overlap (O_{jk}) in fruiting phenology using Pianka's (1974) niche overlap equation:

$$O_{jk} = \frac{\sum_{i=1}^{n} P_{ij} P_{ik}}{\sqrt{\sum P_{ij}^2 \sum P_{ik}^2}}$$

Here, P_{ij} and P_{ik} are the proportion of species *j* and *k* fruiting at time interval *i.* The time interval here refers to the time between phenological surveys (one month). O_{jk} equals 0 when there is no phenological overlap, and 1 when there is complete overlap between any given pair of species. Phenological overlap was calculated separately for 2015 and 2016.

Data on phylogenetic distances between pairs of Lauraceae species were obtained from a phylogeny of woody plant species of BCI provided by David Erickson (Smithsonian Institution). The method used to construct the phylogeny was similar to that presented by Kress et al. (2009). Pairwise phylogenetic distances were obtained using the ape package in R (Paradis et al. 2004).

Assessing whether closely related Lauraceae species and Lauraceae species with similar traits are more likely to share enemies than more distantly-related species/species with greater trait dissimilarity - To assess whether Lauraceae species-specific traits were correlated with d_{ij} values, we performed three separate Mantel tests using the R package vegan (Oksanen et al. 2017) to assess correlation between d_{ij} values and phenological overlap, genetic distance and fruit size difference. Data were analysed for both years combined and separately for 2015 and 2016. In addition, we tested whether the abundance of species *j* (m^{-2}) affected the magnitude of d_{ij} values using logistic generalised linear models (GLM).

All analyses were carried out in Rstudio version 3.3.1 (2016-06-21) -- "Bug in Your Hair" using the packages bipartite, ape and vegan.

Results

During 2015 and 2016 we collected 18424 fruits of eight species of Lauraceae: 15938 from the traps and another 2490 fruits opportunistically from the ground (Table 1). We reared a total of 18595 seed predator individuals from these fruits (14540 individuals from fruits

collected from traps, 4055 from fruits collected from the ground). The seed predators represented six species: two weevils (Curculionoidea: Curculionidae) and four moths (Lepidoptera). A further 129 Lepidoptera adults and 22 larvae could not be assigned to morphospecies due to their poor condition and these were excluded from further analyses. All seed predator species were represented by more than one individual. Seed predators attacked both immature and mature fruits of all Lauraceae species.

Table 1- Summary of total fruits collected from traps and total seed predators reared from these fruits over two years.

Tree species	**Number of fruits collected**	**Number of seed predators**	***Pagiocerus frontalis* (Coleoptera)**	***Heilipus draco* (Coleoptera)**	**Histura (Lepidoptera)**	**Pyra (Lepidoptera)**	***R. ancyloides* (Lepidoptera)**	**Tortricidae sp. (Lepidoptera)**
B. pendula	3404	6082	5850	116	14	84	16	2
O. oblonga	1125	2212	2191	2	0	5	14	0
O. puberula	8778	5481	5442	5	7	17	9	1
O. cernua	273	5	0	5	0	0	0	0
O. whitei	622	159	156	0	0	0	3	0
N. cissiflora	389	27	22	3	0	1	0	1
N. lineata	1181	61	29	11	0	1	19	1

(1) Constructing a quantitative food web describing the interactions between Lauraceae and their internally feeding seed predators

The quantitative food webs are depicted in Figure 2. The summary web combining data from 2015 and 2016 (Fig. 2a) comprises six seed predator species, seven Lauraceae species and 29 feeding links. Fruits of an eighth species (*Nectandra purpurea*) were collected, but in such small quantities (eight fruits from which no seed predators were reared) that this host was excluded from further analyses. The 2015 web (Fig. 2b) has six species of seed predator, seven Lauraceae species and 19 links. Seed predators were reared from six of the sampled Lauraceae species. The 2016 (Fig. 2c) web contains six species of seed predator, seven species of host plants and 22 links. Seed predators were reared from six Lauraceae species.

Between 2015 and 2016 there was a marked change in the density of Lauraceae fruits. Total host density was over three times higher, and total seed predator density was six times higher in 2016 than in 2015. There was also an increase in the connectance (proportion of realised links) from 0.53 to 0.61. This is likely due to sampling as the number of seeds collected in 2016 was around three times higher than in 2015 (Table 4).

Table 2- Quantitative food web metrics calculated using network level function in the R package bipartite. The metrics were calculated using the total number of each seed predator species to emerge from each host plant species.

	Both years	**2015**	**2016**
Connectance	0.69	0.53	0.61
Linkage Density	2.14	1.72	2.06
Interaction Evenness	0.34	0.28	0.34
H2’	0.17	0.22	0.17
Vulnerability	1.13	1.14	1.09
Generality	3.16	2.30	3.03

Pagiocerus frontalis (a scolytid weevil) was the most abundant seed predator in all three webs, comprising over 90% of the seed predator individuals and killing around 93% of all the predated fruits. Overall abundances of the weevil *Heilipus draco* (Hylobiinae) were approximately six times higher in 2015 than in 2016, and the fraction of seeds attacked by *H. draco* decreased from 30% to 2% over the same period. Of the Lepidoptera species the numbers all stayed fairly low and only one new species (a pyralid moth; morphospecies code Pyra in SG's seed predator reference collection) not recorded in 2015 appeared in 2016.

(2) Assessing the potential for indirect interactions between Lauraceae tree species, mediated by shared seed predators

The quantitative food webs (Figure 2) show that most seed predator species were associated with multiple Lauraceae species. The PAC plot for 2015 (Fig. 3) and the corresponding d_{ij} values show the potential for indirect interactions between eight species, with four of these being high potential (d_{ij}> 0.5). The PAC plot for 2016 (Fig. 3) and corresponding d_{ij} values show the potential for indirect interactions between nine species, with one of these being high potential (d_{ij}>0.5).

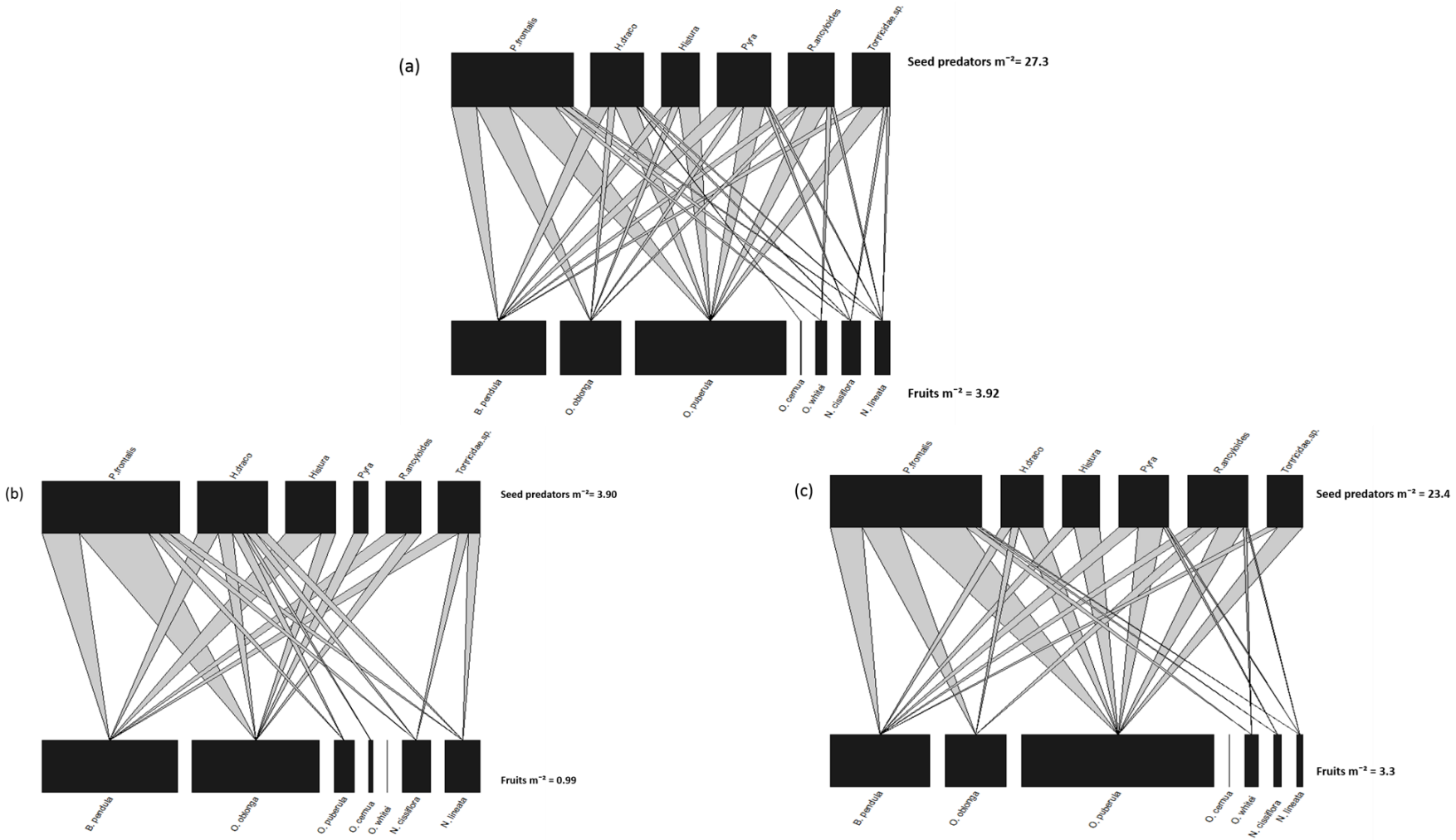


Figure 2- Quantitative plant-seed predator food web for (a) 2015 and 2016 combined, (b) 2015 and (c) 2016. The lower bars represent the availability of each host plant species and the upper bars represent seed predator abundance. The width of the links represent the frequency of interactions.

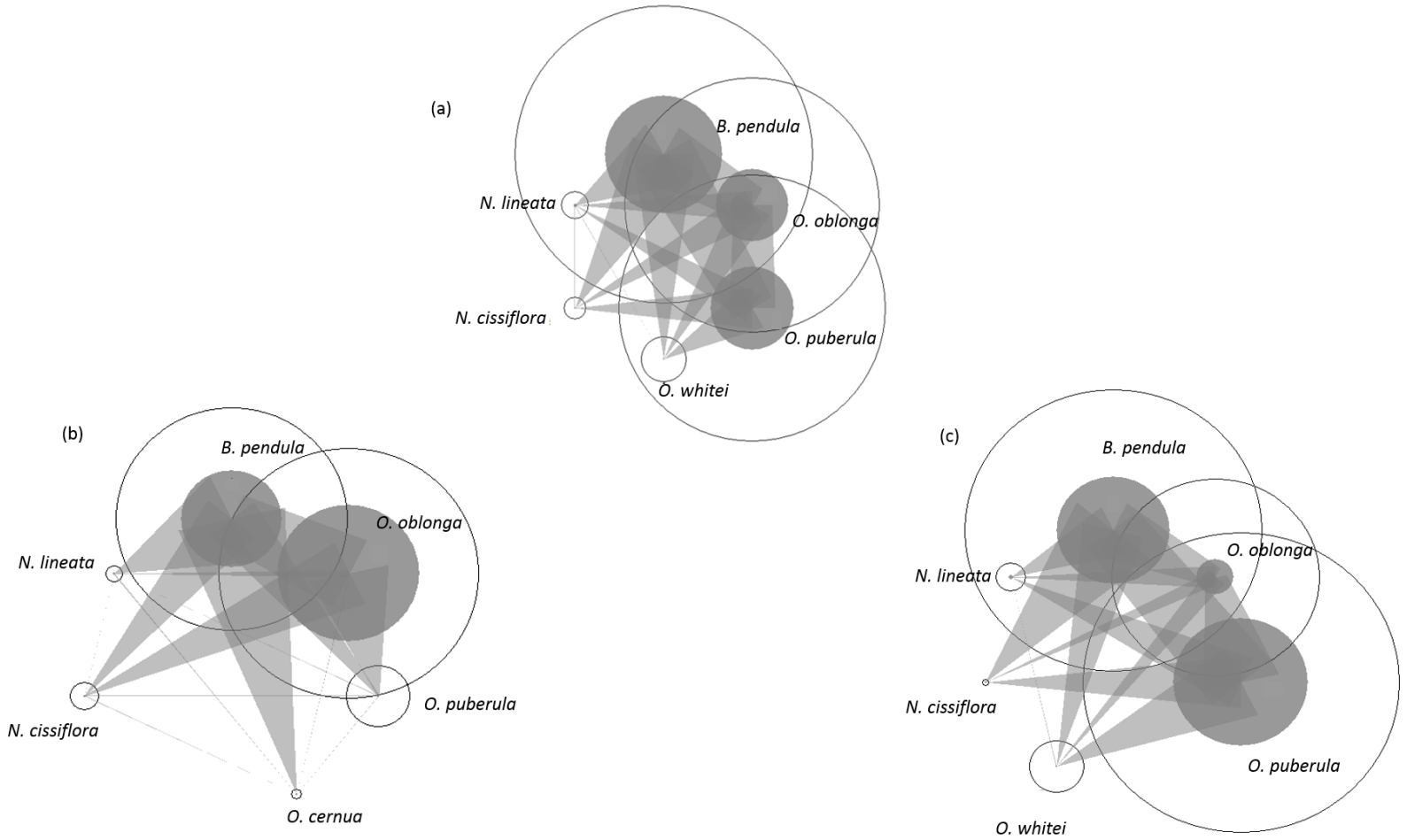


Figure 3- Potential for Apparent Competition (PAC) plots for (a) 2015 and 2016 combined (b) 2015 and (c) 2016, drawn using the PlotPAC function in the R package bipartite. The area of circles is proportional to host species' abundance. The amount to which they are shaded is proportional to self-loops (seed predators emerging from this species and then attacking it again). The width of connecting lines is proportional to the 'export' of seed predators.

For each of the three webs we explored what factors determine the potential for indirect interactions. In all of the three data sets (both years, 2015, 2016) there was a significant difference between d_{ii} and d_{ij} values. In all instances the mean of d_{ij} was significantly higher than the mean of d_{ii} (t-tests; both years t=-7.61, P<0.001, df= 12, mean d_{ii}= 0.16, mean d_{ij}= 0.83; 2015 t= -4.61, P<0.001, df=10, mean d_{ii}= 0.19, mean d_{ij} = 0.81; 2016 t= -5.91, P<0.001, df=10, mean d_{ii}= 0.19, mean d_{ij}= 0.81), suggesting that attack by natural enemies is likely to be influenced more by heterospecific densities than conspecific densities.

(3) Assessing whether closely related Lauraceae species and Lauraceae species with similar traits are more likely to share enemies than more distantly-related species and those with greater trait dissimilarity

In two data sets (both years combined and 2015) there was no significant correlation between magnitude of d_{ij} and phenological overlap (o_{ij}) (Mantel tests; P>0.05). In 2016, there was a significant positive correlation between o_{ij} and magnitude of d_{ij} values (r =0.65, P=0.04). There were no significant correlations between the magnitude of d_{ij} values and genetic distance in any of the three data sets (Mantel test; all P>0.05). In the combined data set and 2016, there was no significant correlation between magnitude of d_{ij} and difference in fruit size (Mantel tests; P>0.05). In the 2015 data, there was a non-significant trend for d_{ij} to increase with increasing fruit size difference (r= 0.44, P=0.056).

In two data sets (2015 and 2016) there was a significant effect of species *j* abundance upon the magnitude of d_{ij}. As the abundance of species *j* increased, d_{ij} increased (2015 β= 25.13, SE=12.13, z=2.04, P=0.04; 2016, β= 1.82, SE= 0.86, z=2.12, P=0.03). In the combined years data set there was a non-significant trend for d_{ij} to increase with increasing species *j* abundance (β=1.51, SE=0.80, z=1.88, P=0.06).

Discussion

Our study is one of the first to construct a quantitative seed predator-host web using techniques identical to those that have commonly been used in the study of insect host-parasitoid communities (Lewis et al. 2002; Morris et al. 2004) . Quantitative food webs are useful tools for exploring community structure and provide a basis for predicting indirect interactions between species, although these predictions can only be validated experimentally (Morris et al. 2004; van Veen et al. 2006, Lewis & Gripenberg 2008; Tack et al. 2011). Analyses of the Lauraceae-seed predator webs showed that there is potential for indirect interactions between host plant species sharing pre-dispersal seed predators. These potential interactions appear to be driven by the abundance of host plant species, with more common species negatively affecting less common ones by acting as a source of seed predators.

Structure of the quantitative food web

Numerically, our webs are dominated by three host plant species (*Beilschmiedia pendula*, *Ocotea puberula* and *Ocotea oblonga*) and one seed predator species (*Pagiocerus frontalis*). All seed predator species in the webs attacked more than one host plant species, and web analyses suggest that there is the potential for indirect interactions between multiple species. Host use patterns appeared not to be influenced by host genus and flexibility in resource use occurred between years, suggesting that these species are not specialised on individual species or genera within the Lauraceae, but are specialised at the family level. This contrasts with other studies that generally show seed predators specialised at lower taxonomic levels (i.e. species or genus level, Janzen 1980).

The changes in the relative abundances of seeds of different species between the two study years are reflected in the web metrics (Table 2). For example, seed predators were more generalised in their diets and there was a higher number of feeding links in 2016 compared to

2015. From just two years of data, we cannot know whether this substantial shift in fruit production is a normal feature of the Lauraceae or whether it is due to the unusually strong El Niño event in 2015. If the seed abundances of individual Lauraceae species do indeed tend to cycle this could lead to temporal variation in both the strength and direction of any indirect interactions between host species (Abrams et al. 1998). Interaction evenness was low in all networks, likely due to the dominance of some species in the web. H2' is also low in all webs, indicating a low level of specialisation in the networks, again contrasting findings of other seed predator networks (Janzen 1980; Ctvrtecka et al. 2016; Sam et al. 2017).

Overall attack rates of fruits sampled from traps were lower in 2016 (36%) than in 2015 (49%). This could be due to the higher densities of fruit available in 2016 (around three times as many) leading to predator satiation (Janzen 1971). Here, during periods of high fruit production, predators are satiated with more resources than they can consume, leading to individual seeds being less likely to be attacked than in periods of lower resource abundance. Various studies have documented this phenomenon (e.g. Sork et al. 1993; Cannon et al. 2007; Norden et al. 2007), and it is a possible explanation for the evolution of mass fruiting events (Kelly & Sork 2002).

Assessing the potential for indirect interactions between Lauraceae species mediated by shared seed predators

To our knowledge, our study is the first study that explicitly assesses the potential for indirect interactions between trees mediated by shared insect seed predators. Despite early theoretical work suggesting large implications of indirect interactions between plant species sharing a common enemy (Holt 1977; Holt & Kotler 1987) studies explicitly testing for apparent competition in plant-herbivore systems are still scarce. Some studies have shown that plants experience increased herbivory rates when surrounded by heterospecifics that share a common enemy (Root 1973; White & Whitham 2000; Barbosa et al. 2009) but few explicitly

test for indirect interactions and subsequent impacts upon community structure (but see Rand 2003). Studies that have tested for indirect interactions tend to report asymmetric interactions (one host plant experiencing a strong negative effect, but the other species experiencing only a weak or neutral effect), although many also fail to uncover reciprocal effects by focusing only upon the effect on one species (see Chaneton & Bonsall 2000). Many studies specifically investigating apparent competition in plants have done so from an applied perspective, for example investigating the effect of these indirect interactions in the control of invasive species or weeds (Orrock et al. 2008; Dangremond et al. 2010; Chailleux et al. 2014). While these studies demonstrate the potential for indirect interactions to significantly alter plant-based systems, they may not reflect processes operating in other systems.

We are only aware of one previous study of indirect interactions between plant species in the tropics (Garzon-Lopez et al. 2015), and none focusing on insect enemies as the mechanism. In hyperdiverse tropical plant communities, even identifying feeding relationships can be difficult, which may explain the scarcity of investigations of this kind. Detailed studies of feeding relationships of tropical insect herbivores have revealed that insects diet breadths are more generalised than previously thought (Novotny et al. 2010). This opens the possibility that indirect interactions between plant species may be widespread, and affects our assumptions about how insect herbivores are shaping the plant communities in which they exist. In theory, any plant species that share one or more enemy species and that co-occur in space and time could experience indirect interactions via shared enemies. In order to test this possibility, experimental manipulation of the system is required. This could involve, for example, removing either the enemy or one of the host species, or introducing one of the host species where it does not normally occur (e.g. Morris et al. 2004). In our Lauraceae-seed predator system such experiments would be very challenging, requiring manipulation of entire forest canopies (Lewis & Gripenberg 2008). However, experiments of this kind may be

more feasible for systems involving seedlings or saplings, and for enemies such as folivores, gallers or post-dispersal seed predators.

The abundance of insect herbivores can be influenced by the presence of other herbivore species with which they share a common enemy (Morris et al. 2004), even where the herbivore species are separated in space (attacking different host plant species) and therefore do not compete directly for food resources. Apparent competition could operate similarly in tropical trees. Although competition for resources between tree seeds and seedlings appears to be weak in the understorey of tropical forests (Svenning et al. 2008; Paine et al. 2008), it is still possible that seeds and seedlings will be negatively influenced by the presence of heterospecifics with which they share enemies.

Most studies of apparent competition in plant-herbivore systems investigate short-term effects (Holt & Kotler 1987) (aggregative responses by the enemy) as opposed to long-term effects (demographic responses of the enemy) of resource availability. The demographic response of seed predators to high and low crop densities could have led to the evolution of synchronised mast fruit events (Kelly & Sork 2002). In our study we are only able to comment on the short-term responses of enemies to resource availability, which appears to influence d_{ij} values. However, without data collected over multiple years it would be difficult to untangle whether a demographic response would also be apparent. Very little is known about the life cycles of these seed predators, so the number of insect generations observed over the course of our study is uncertain.

The potential for apparent competition between Lauraceae species, calculated using the approach introduced by Müller et al. (1999), was high, with five of 36 pairwise d_{ij} values greater than 0.5. However, whilst these calculations can indicate the potential for indirect interactions between host species, they do rely on a number of assumptions. One important

assumption is that there is no spatial or temporal segregation of hosts from the perspective of their enemies. Although different host plant species may share a common enemy, they may be isolated in space or time (e.g. if species produce fruit at different times of the year). Tree individuals that grow close together and/or overlap in their temporal fruiting patterns are most likely to interact indirectly via shared enemies. To assess the potential for indirect interactions across space, it would be useful to know the dispersal ability of the enemies. For seed predators, where resource trees are essentially analogous to islands, the potential for geographical separation of resources is high if the predator has a low dispersal ability.

A further assumption in Müller et al.'s potential for apparent competition index is that there is no tendency for adult seed predators to be more likely to attack the species on which they developed. The phenomenon of insects showing a preference for their natal host plants, known as Hopkin's host selection principle (Barron 2001), could arise if insects recognize and respond positively to odour cues experienced during their development. The phenomenon has been documented in other plant-insect systems (Takasu & Lewis 1995; Saadat et al. 2014) but we are not aware of studies investigating its occurrence in insect seed predators. If seed predators do respond strongly to seeds of the species in which they developed, over time this could lead to the formation of cryptic host races (e.g. Blair et al. 2005), where populations of a species only use one host species as a resource. This phenomenon has been documented in other insect-host systems, and would mean that any potential indirect interactions between host plant species would not occur if host races have become reproductively isolated from each other. These assumptions demonstrate that snapshots of communities captured by quantitative food webs may not be sufficient to predict indirect interactions (Tack et al. 2011).

In studying a single family of host plants and their associated enemies, we are analysing a module within a wider network of plant-enemy interactions, which has been documented in a

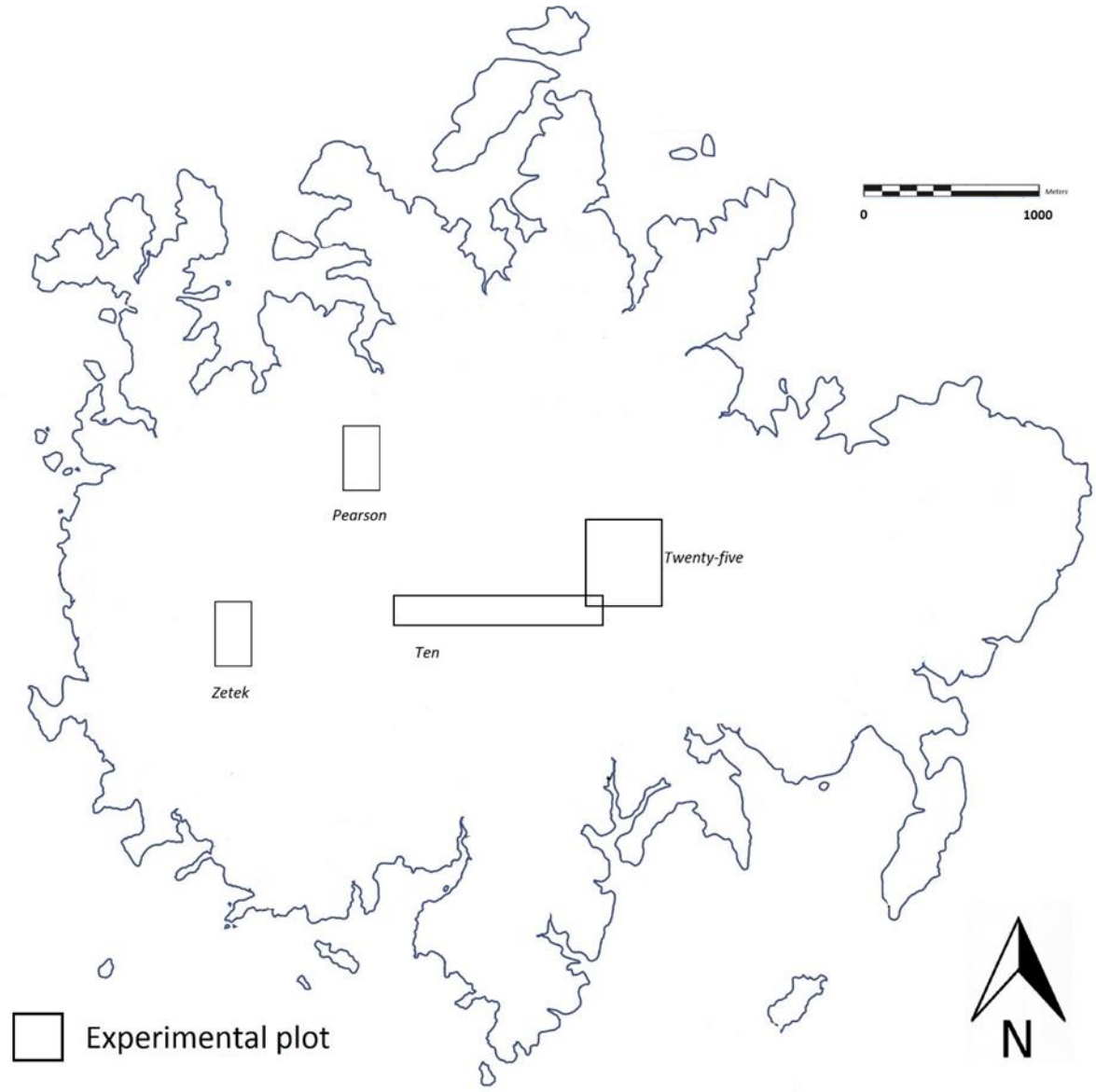
separate study. On Barro Colorado Island, most internally feeding insect seed predators appear to be highly specialised (S. Gripenberg, unpublished data), and the Lauraceae module of the larger network that is the target of our study appears to be one of the most likely candidate systems for seed predator-mediated indirect interactions in the plant community of our study site. Given that patterns of host use (including the degree of seed predator specialisation) might differ between study sites (Basset et al. 2018), studies assessing the role of seed predator mediated indirect interactions would be interesting.

Indirect interactions such as apparent competition have been suggested to be of importance in species-rich tropical forests because individuals of any given pair of plant species are unlikely to grow in close proximity and are therefore unlikely to be competing for resources (Novotny et al. 2010). Mobile natural enemies can play a role by linking the population dynamics of these species (Connell 1990). Understanding where theses interactions take place will be fundamental to understanding the structure of hyper-diverse tropical plant communities and how plant species may respond to disturbances such as selective logging or other disturbances that increase or decrease the abundance of potential apparent competitors.

Supplementary Information

Chapter 2

Supplementary Information Chapter 2



Supplementary Figure 1- Map of Barro Colorado Island with mapped plots shown.

Supplementary Table 1 - PAC outputs for (a) both years, (b) 2015 and (c) 2016 - those highlighted in red indicate a high potential for indirect interactions (d_{ij}>0.5) and those in yellow a medium potential for indirect interactions (d_{ij}>0.3).

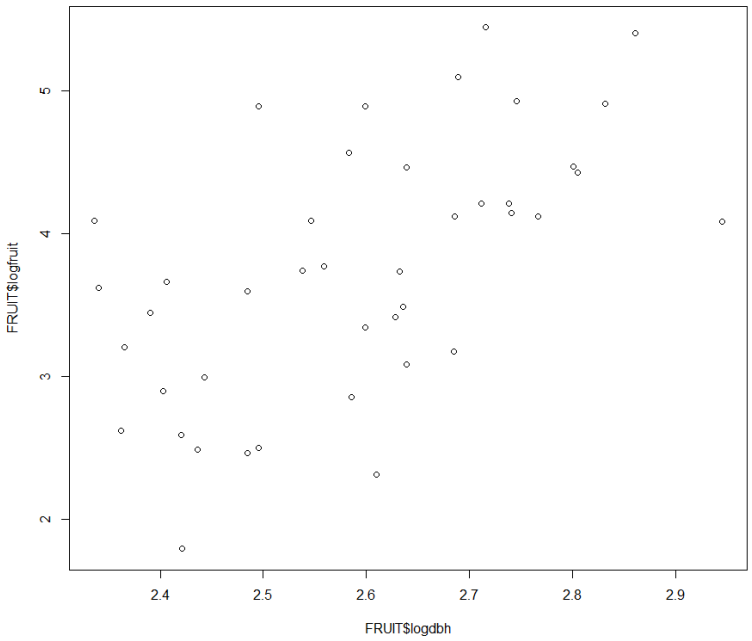
(a)	*B. pendula*	*O. oblonga*	*O. puberula*	*O. cernua*	*O. whitei*	*N. cissiflora*	*N. lineata*
B. pendula	0.398	0.280	0.307	NA	0.009	0.002	0.004
O. oblonga	0.383	0.289	0.315	NA	0.009	0.002	0.003
O. puberula	0.384	0.288	0.315	NA	0.009	0.002	0.002
O. cernua	NA	NA	NA	NA	NA	NA	NA
O. whitei	0.380	0.288	0.313	NA	0.010	0.002	0.007
N. cissiflora	0.442	0.247	0.279	NA	0.008	0.009	0.013
N. lineata	0.438	0.216	0.207	NA	0.019	0.009	0.112

(b)	*B. pendula*	*O. oblonga*	*O. puberula*	*O. cernua*	*O. whitei*	*N. cissiflora*	*N. lineata*
B. pendula	0.436	0.518	0.032	<0.001	0	0.007	0.004
O. oblonga	0.413	0.549	0.032	<0.001	0	0.006	<0.001
O. puberula	0.422	0.536	0.032	<0.001	0	0.007	0.002
O. cernua	0.829	0.007	0.027	0.003	0	0.027	0.075
O. whitei	0	0	0	0	0	0	0
N. cissiflora	0.456	0.473	0.031	<0.001	0	0.018	0.018
N. lineata	0.759	0.048	0.026	0.003	0	0.049	0.089

(c)	*B. pendula*	*O. oblonga*	*O. puberula*	*O. cernua*	*O. whitei*	*N. cissiflora*	*N. lineata*
B. pendula	0.383	0.180	0.420	0	0.013	<0.001	0.003
O. oblonga	0.370	0.185	0.428	0	0.013	<0.001	0.004
O. puberula	0.371	0.185	0.429	0	0.013	<0.001	0.003
O. cernua	0	0	0	0	0	0	0
O. whitei	0.368	0.186	0.424	0	0.013	<0.001	0.009
N. cissiflora	0.555	0.123	0.304	0	0.009	0.004	0.005
N. lineata	0.324	0.195	0.305	0	0.028	<0.001	0.148

Supplementary table 2 - Correlation scores for morphological fruit traits.

	Mean mature fruit fresh mass (g)	Mean mature fruit dry mass (g)	Mean diaspore fresh mass (g)	Mean diaspore dry mass (g)	Mean width (mm)	Mean Length (mm)
Mean mature fruit fresh mass (g)	-	0.9997519	0.9995539	0.9996168	0.5234609	0.3702989
Mean mature fruit dry mass (g)		-	0.9992277	0.9997711	0.5299780	0.3743665
Mean diaspore fresh mass (g)			-	0.9997194	0.5351655	0.3738549
Mean diaspore dry mass (g)				-	0.5352621	0.3740395
Mean width (mm)					-	0.8407796
Mean length (mm)						-



Supplementary figure 2- Relationship between dbh and fruit crop of trap trees

Chapter 3

Spatial patterns of enemy attack: the effects of conspecific and heterospecific neighbours on premature fruit abscission and pre-dispersal insect seed predation of tropical tree seeds

Status: In preparation, Biotropica

Abstract

The spatial patterns of attack by insect consumers and other natural enemies of plants can dictate the reproductive output of individual plants and the structure of plant communities. In hyper-diverse tropical forests, biotic explanations for high biodiversity tend to focus on the Janzen-Connell hypothesis, where plant individuals surrounded by conspecific neighbours have an increased risk of enemy attack. Studies investigating the effects of non-specialist enemies and their potential to link the regeneration dynamics of their host species are much rarer. In addition, patterns of attack are often not investigated across space, making predictions of community-wide patterns more difficult. Here we investigate spatial patterns of attack by non-specialist seed predators feeding on multiple Lauraceae species in the forest of Barro Colorado Island, Panama. Seed predation rates were highest on trees with large seed crops, in line with the Janzen-Connell hypothesis. At a larger spatial scale, increased proximity to congeneric tree species decreased seed predation on focal trees in a year when seed production was low. This pattern was not observed in a year when seed production was higher, consistent with predator satiation across multiple resource species. Fruit abscission rates were also affected by densities of congenerics and confamilials at a larger spatial scale.

Key-words: indirect interactions, Janzen-Connell hypothesis, plant-herbivore interaction, predator satiation.

Introduction

Insect herbivores and other plant enemies such as seed predators and pathogens can have marked effects on their host populations (Maron & Crone 2006) and on the wider plant communities of which these hosts are part. For example, plant enemies might promote plant species coexistence if they tend to disproportionately attack species that are locally abundant (Janzen 1970; Connell 1971), and the spatial patterns of attack by natural enemies can influence the likelihood of plant survival with potential consequences for the structure of the plant community. Since enemies are often drawn to areas of high resource abundance (Root 1973), individuals surrounded by conspecifics can be under increased risk of attack by specialist enemies (Janzen 1970; Connell 1971). This mechanism can free up space in the local plant community for other species to colonise. Where enemies have broader diets, individuals surrounded by heterospecific neighbours attacked by the same species of enemy can also experience increased rates of attack (Holt 1977). This can lead to exclusion of the species least able to cope with the enemy load (Bonsall & Hassell 1997; Holt & Bonsall 2017) or may allow for the coexistence of species if predator-defined niche space is partitioned (Chesson & Kuang 2008). Many studies (typically focusing on seeds or seedlings of single plant species) have shown indirect evidence of such enemy-mediated conspecific density and distance effects on plant survival (synthesised in Comita et al. 2014), but the mechanisms driving these effects are only starting to be uncovered (Gilbert et al. 1994; Bagchi et al. 2014; Fricke, Tewksbury, & Rogers 2014).

Enemies that kill fruits whilst they are still attached to the mother plant, such as internally feeding insect seed predators, can significantly affect the reproductive output of plants (Louda 1982; Rose, Louda & Rees 2005). As well as killing fully developed seeds prior to dispersal, these enemies may cause plants to abscise fruits prematurely, further reducing the output of viable offspring (Stephenson 1981; Andersen 1988). If pre-dispersal seed predators

tend to kill a large proportion of seeds in areas of high resource density, they could potentially contribute to structuring and maintaining plant diversity via the mechanisms described above. However, if species produce more fruits than needed to sustain the plant population, or synchronise fruit production (Kelly & Sork 2002) to account for potential losses to pre-dispersal seed predators, high rates of pre-dispersal seed predator attack might not lead to suppressed reproductive output. This mechanism (also referred to as seed predator satiation) has been shown to reduce the effects of species-specific natural enemies on plant reproduction in years of high seed abundance (Xiao et al. 2017).

In the context of tropical forest ecology, the role of pre-dispersal enemies in maintaining and structuring plant diversity has yet to be thoroughly investigated (Gripenberg 2018). In his seminal paper on the role of host-specific enemies in promoting local plant diversity, Janzen (1970) suggests that host-specific pre-dispersal insect seed predators could promote the coexistence of plant species if they tend to kill a larger proportion of seeds in parts of the forest where multiple fruiting conspecifics grow in close proximity. This prediction has rarely been tested (but see Jones & Comita 2010) although it seems plausible that seed-eating insects could respond to the spatial distribution of their host trees in a way similar to that of insects associated with patchily distributed host plants in temperate systems (e.g. Tscharntke & Brandl 2004; Gripenberg & Roslin 2005). Where seed predators have diets spanning multiple species, both the density of conspecifics and heterospecifics attacked by the same enemy could dictate the patterns of pre-dispersal seed predator attack on focal trees.

Using the Lauraceae family and its associated pre-dispersal insect seed predators as a study system, we investigated spatial patterns in seed predator attack in the forest of Barro Colorado Island (Panama). The aim of our study was to investigate how seed predators respond to local densities of seeds and to seed crops of neighbouring trees, and to distinguish between effects of conspecific and heterospecific neighbours. Specifically, we ask: (1a) do

rates of seed predation vary between individual canopies and do tree-specific seed predation rates depend on local seed crop sizes? (1b) Do trees experience higher attack rates by insect seed predators when surrounded by fruiting conspecifics/congenerics/confamilials known to be attacked by the same species of pre-dispersal insect seed predators? (2a) do rates of premature fruit abscission vary between individual canopies and are they dependent on local fruit crop sizes? (2b) Do trees experience higher premature fruit abscission rates when surrounded by fruiting conspecifics/congenerics/confamilials known to be attacked by the same species of pre-dispersal insect seed predators? (3) Do individuals with high levels of pre-dispersal insect seed predation produce fewer mature seeds than individuals with low levels of seed predation? Through these questions we aim to uncover how pre-dispersal seed predators affect the seed production of focal trees, and how the presence of conspecifics or heterospecifics attacked by the same enemy species affect seed predator attack rates and the seed production of focal trees.

Methods

Study Site

Barro Colorado Island (BCI) is a 15.6 km² island located in Gatun Lake, Panama, (Lat: 9.1543, Long:-79.846). The island is covered with tropical semi-deciduous forest. The northeast half experienced widespread clearing during the late 1800s, whilst the other half has been little disturbed for up to 1500 years. The climate on BCI is seasonal. The dry season occurs from December to April/May and the average yearly rainfall is 2612mm (Leigh 1999).

We carried out investigations in four mapped forest plots (one ten hectare, one 25 hectare and two six hectare; Supplementary Fig. 1). These plots were established in the mid-1990s and every free-standing woody stem over 20cm diameter at breast height (dbh) has been tagged,

measured (dbh in mm) and identified to species level. These plots were last censused in 2013 and 2014.

Study System

The Lauraceae family is one of the most common and ecologically significant woody plant families in tropical and subtropical forests. Lauraceae fruits provide an important food source for many bird and mammal species (Wheelwright 1983; Tabarelli & Peres 2002), and are a source of many products of economic value such as avocado, bay leaf, cinnamon and timber. Nine species of Lauraceae representing four genera occur naturally on BCI (Table 1). All nine species have single-seeded fleshy fruits that are animal dispersed, but there is substantial variation in fruit size and shape. In addition to the species listed in Table 1, the avocado (*Persea americana*) also occurs on BCI, but since it was likely introduced there by humans (Croat 1978) and is very rare, it was not included in this study. The nine Lauraceae species fruit throughout the year, with considerable overlap in fruiting times. Species were observed to have immature fruits as early as January to as late as September (Table 1).

Previous work showed that species in the Lauraceae family on BCI share multiple seed predators and that ~40% of all seeds showed signs of attack by insect seed predators (Chapter 2 of this thesis), the majority of which are likely to be pre-dispersal seed predators (i.e. attack their host seeds in the canopy).

Survey of fruit production, insect seed predation, premature fruit abscission, and the production of intact mature seeds

To assess spatial variation in fruit production, rates of attack by pre-dispersal insect seed predators, premature fruit abscission and the production of healthy seeds, we visited all Lauraceae trees >20cm dbh within each of the four above-mentioned mapped plots (Supplementary Fig. 1) in February 2015. We selected up to six individuals of each species as

'focal individuals' for our studies. Criteria for selection were the tree being reproductive (presence of flowers or fruits), the terrain being open enough to allow placement of seed traps (described below) underneath the canopy, and the canopy being reasonably clear of lianas. We failed to find six reproducing individuals for all nine Lauraceae species; in total we selected 41 trees. In 2016, we selected eight additional trees, bringing the total number of focal trees (i.e. trees with seed traps) to 49 (Table 2).

Table 1- Lauraceae species found on BCI and investigated in this study. Descriptions of tree and fruit attributes are based on information in Croat (1978) (tree size, reproductive phenology), our own measurements taken over the first year of data collection (mean fruit weights (g), lengths (mm) and widths (mm)), and from trait data collected within the ForestGeo 50 ha forest dynamics plot (Wright et al. 2013).

Species	**Adult tree description**	**No. of individuals in mapped plots**	**Fruiting phenology**	**Immature fruit weight**	**Mature fruit weight**	**Immature fruit length**	**Mature fruit length**	**Immature fruit width**	**Mature fruit width**
Beilschmedia pendula (Sw.) Hemsl. 1882	13-40 m tall, to 75 cm dbh	56	February - June	2.19	6.80	23.5	37.0	9.88	17.89
Ocotea oblonga (Meisn.) Mez 1889	To 30 m tall, to 45 cm dbh.	35	February - June	0.72	0.94	16.25	18.60	8.53	10.07
Ocotea puberula (Rich.) Nees 1920	To 27 m tall, to 50 cm dbh.	13	January - July	0.12	0.78	5.72	12.23	4.87	7.69
Ocotea cernua (Nees) Mez 1889	To 12 m tall	11	April - September.	0.26	0.56	10.15	13.28	6.46	7.46
Ocotea whitei Woodson 1945	15-30 m tall	21	February - July	0.33	0.70	14.17	14.5	6.23	9.33
Nectandra cissiflora Nees 1836	To 27 m tall, to 30 cm dbh	40	March - June	0.42	0.90	10.66	11.53	10.70	11.68
Nectandra lineata (Kunth) Rohwer		10	February - June	0.35	0.42	8.89	9.33	0.29	0.35

We set up four 0.5m² seed traps (Fig. 1a) in arbitrary locations underneath the canopy of each selected tree. We visited all trees monthly to assess when a fruiting period began, and we visited traps weekly throughout the fruiting period of each individual. Upon each visit, we collected all fruits and emptied debris from each trap. We took all fruits to the laboratory and recorded the abundance, total weight and maturity status (i.e. immature = prematurely abscised, mature = fully developed) of fruit samples. Fruit collections took place from 26/02/2015 to 26/01/2017. To estimate tree-specific fruit production (mature and immature fruits combined) we first estimated the canopy area of each focal Lauraceae individual. For each individual, we marked eight cardinal points at the canopy edge from the canopy centre (N, S, E, W, NE, NW, SE, and SW). We measured the distance from the canopy centre to each of these points and used these distances to estimate canopy size as the area of the convex polygon formed by these points. The fruit counts in the traps and the estimated canopy area allowed us to estimate tree-specific fruit abundances, assuming that fruit densities are reasonably uniform within each canopy. We were unable to measure the canopy area of 12 of the 49 focal trees because of high liana loads, difficult terrain or treefalls. As dbh and fruit crop size are correlated (see Supplementary Fig. 2), estimates of overall fruit numbers on these trees as well as on non-focal Lauraceae trees in the mapped plot could be calculated. In addition to collections from traps, fruits were opportunistically collected from the ground whilst surveying the forest. The ground-collected fruits were not included when estimating fruit abundances or fruit abscission rates (see below), but were used alongside the fruits collected from the traps – to assess overall seed predation rates and patterns of attack by individual seed predator species.

We stored fruits in transparent plastic pots lined with tissue paper and covered with a mesh lid (Fig. 1b). Fruits collected from the ground were kept separately from trap-collected fruits and fruits from different traps and collection dates were kept separately, as were mature and

immature fruits. We checked pots twice a week for emerging adult insects up to three months after collection. After this we opened the fruits and removed any remaining adult insects or larvae. We stored emerged Coleoptera, Hymenoptera and Diptera and all larvae in 95% ethanol in a freezer. All Lepidoptera were frozen and pinned to facilitate morphological identification. We morphotyped all specimens and compared them to a seed predator reference collection kept at the insect collections of the Smithsonian Tropical Research Institute in Panama City.

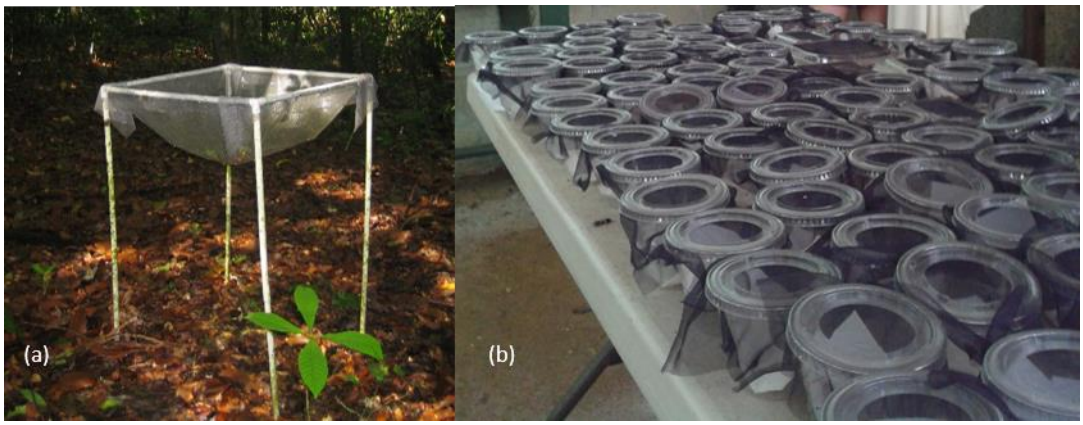


Figure 1 - (a) A seed trap in the forest of Barro Colorado Island, (b) insect rearing pots.

Before fruits were disposed of, we checked them for signs of pre-dispersal insect seed predation. This included exit holes, frass or presence of insects upon opening. This allowed us to assess the number of intact seeds and the number of seeds that were attacked by insect seed predators on each focal tree.

Tree-specific premature fruit abscission rates were taken as the proportion of all fruits collected in the traps that were immature. Tree-specific production of healthy mature seeds was calculated as the number of mature seeds that experienced no sign of insect attack (exit holes, frass or presence of insects) at the end of the study period.

Table 2- Numbers of individuals of each species of Lauraceae monitored using seed traps and summary of data on seed predation and premature fruit abscission (summed across 2015 and 2016). Note for four trap trees we did not collect any fruits and these were excluded from analyses.

Species	**Total number of trap trees**	**Total number of fruits**	**No. of fruits attacked**	**Prop. of fruits attacked**	**No. of fruits abscised**	**Prop. of fruits abscised**	**No. of prematurely abscised fruits attacked**	**Prop. of fruits prematurely abscised attacked**	**No. of mature fruits**	**No. of mature fruits attacked**	**Prop. of mature fruits attacked**
B. pendula	14	4281	2859	0.67	2805	0.66	1435	0.51	2016	470	0.23
O. oblonga	6	2033	1054	0.52	1009	0.50	473	0.47	1024	559	0.55
O. puberula	6	9569	2528	0.26	8540	0.89	2151	0.25	1029	440	0.43
O. cernua	4	274	22	0.08	17	0.06	3	0.18	257	19	0.07
O. whitei	5	616	121	0.20	565	0.92	93	0.16	51	27	0.53
N. cissiflora	5	391	335	0.86	355	0.91	304	0.86	36	34	0.94
N. lineata	5	1215	305	0.25	1165	0.96	298	0.23	50	7	0.14

Data analysis

For each study question, we conducted analyses separately for data collected in 2015 and 2016, to account for differences in overall fruit density between the two study years.

(1a) Do rates of seed predation vary between individual canopies and do tree-specific seed predation rates depend on local seed crop sizes?

To assess whether tree individuals experience higher attack rates when the local (tree-specific) fruit crop is large we used a generalized linear mixed effects model (GLMM) with binomial errors. The ratio of predated to intact seeds (assessed using the cbind function in R) was modelled as a function of the log-transformed estimated fruit crop size with tree species as a random factor.

(1b) Do trees experience higher attack rates by insect seed predators when surrounded by fruiting conspecifics/congenerics/confamilials known to be attacked by the same species of pre-dispersal insect seed predators?

To assess whether tree individuals experience higher attack rates by enemies when surrounded by conspecific/congeneric/confamilial adults than elsewhere in the landscape, we used a GLMM with binomial errors. The ratio of attacked to not attacked seeds (using the cbind function in R) was modelled as a function of scaled connectivity (see below) to conspecifics (including self-contribution), connectivity to congenerics (excluding conspecifics) and connectivity to confamilials (excluding conspecifics and congenerics), with tree species as a random factor. 15 of the focal trees were excluded from these analyses because of small sample sizes (fewer than ten fruits collected from the traps).

To calculate the connectivity values of each focal tree, we use the connectivity kernel set out in Hanski (1994)

$$S_i = \sum \exp(-\alpha d_{ij}) A_j^b$$

which calculates the predicted in-flow of insect seed predators to each focal tree *i*, summed across all patches *j* (trees). The value of S_i takes into account the size (*A*, i.e. dbh) of each neighbouring conspecific, congeneric or confamilial tree (*j*, n= 152), and its distance (*d*) in metres from the focal tree, and assumes a negative exponential insect dispersal kernel. Data on the typical dispersal kernels for our seed predator species are not available, so α was set at 0.01 (corresponding to a migration range of 100m). Connectivity values have previously been shown to be robust to the chosen value of α (Moilanen & Nieminen 2002). Nevertheless, to assess how the values of the connectivity index were affected by changes in α in the context of our study, we ran calculations at a series of α values (0.01 – 0.1) corresponding to migration ranges from 10 to 1000 m. The value of S_i was remained similar across the whole range of α values (Supplementary Table 1).

For each seed predator species reared from the Lauraceae fruits (n=6) we also assessed whether the likelihood of detecting the species on a tree was dependent on connectivity to conspecifics, congenerics or confamilials using a logistic regression model. Sample size (number of fruits used for insect rearing from each tree) was included as a covariate in the model.

(2a) Do rates of premature fruit abscission vary between individual canopies and are they dependent on local fruit crop sizes?

To assess whether tree individuals experience higher levels of premature fruit abscission when the local fruit crop is large, we used a GLMM with binomial errors. The ratio of immature (prematurely abscised) to mature fruits (using the cbind function in R) was

modelled as a function of the log-transformed estimated fruit crop size, with tree species as a random factor.

(2b) Do trees experience higher premature fruit rates when surrounded by fruiting conspecifics/congenerics/confamilials known to be attacked by the same species of pre-dispersal insect seed predators?

To assess whether focal individuals experience higher premature fruit abscission rates when surrounded by conspecific/congeneric/confamilial adults than elsewhere in the landscape, we used a GLMM with binomial errors. The ratio of immature (prematurely abscised) to mature fruits (using the cbind function in R) was modelled as a function of scaled connectivity to conspecifics (including self-contribution), connectivity to congenerics (excluding conspecifics) and connectivity to confamilials (excluding conspecifics and congenerics), with tree species as a random factor.

(3) Do individuals with high levels of pre-dispersal insect seed predation produce fewer healthy fruits than individuals with low levels of seed predator attack?

To assess whether tree individuals experiencing high rates of seed predation produced fewer healthy seeds (i.e. mature seeds not attacked by insects) than individuals with low seed predation rates we used a GLMM with Poisson errors. The number of healthy fruits produced on each tree was modelled as a function of the proportion of seeds attacked with tree species as a random factor.

All analyses were run in RStudio, R version 3.5.1 (2018-07-02) -- "Feather Spray" using the packages lme4 (Bates et al. 2015) for statistical analyses, and ggplot2 (Wickham 2009) for figures.

Results

A total of 18379 fruits representing seven species of Lauraceae were collected from the seed traps between February 2015 and August 2016. There was considerable overlap in fruiting phenology between individual species (Supplementary Fig. 3). The timing of the peak fruit fall of individual species was similar in both years (Supplementary Fig. 3), but fruit abundance was over three times higher in 2016 compared to 2015. 14540 seed predator individuals were reared from the collected fruit samples. The seed predators comprised six species: two weevils (Curculionoidea: Curculionidae) and four moths (Lepidoptera) (Table 3) and all species were represented by more than one individual. Seed predators attacked both immature and mature fruits of all Lauraceae species, and fruits of even the smallest size categories experienced predation by seed predators so fruits of all sizes were included in the final analyses.

Table 3- Seed predator species reared from seed samples of each of the studied Lauraceae species. Shown are the total numbers of individuals reared from each species.

Tree species	**Number of fruits collected**	**Total number of seed predators**	***Pagiocerus frontalis* (Coleoptera)**	***Heilipus draco* (Coleoptera)**	**Histura sp, (Lepidoptera, Tortricidae)**	**Total Unknown Pyralidae (Lepidoptera, Pyralidae)**	**Total *Riculorampha ancyloides* (Lepidoptera, Tortricidae)**	**Total Unknown Tortricidae (Lepidoptera, Tortricidae)**
B. pendula	3404	6082	5850	116	14	84	16	2
O. oblonga	1125	2212	2191	2	0	5	14	0
O. puberula	8778	5481	5442	5	7	17	9	1
O. cernua	273	5	0	5	0	0	0	0
O. whitei	622	159	156	0	0	0	3	0
N. cissiflora	389	27	22	3	0	1	0	1
N. lineata	1181	61	29	11	0	1	19	1

In total, 14456 (~ 78%) of fruits were prematurely abscised (i.e. dropped from the tree before having completed development). Species specific premature fruit abscission rates ranged from 6 to 96% (Table 2). The proportion of fruits showing signs of insect attack varied across species (8-86%). The proportion of intact vs. attacked fruits differed significantly between mature and immature fruits for five of the seven species (Table 4), and the direction of the relationship between maturity status and insect attack varied between species (Fig. 2). Tree-specific predation rates ranged from 0% to 96%.

Table 4- Outputs from tests conducted to assess differences in seed predation rates (assessed through visual examination of seed samples) between mature and immature fruits. Differences in ratios of predated to intact seeds were assessed using chi squared tests (where all cells in the contingency table had >5 observations) or Fisher's exact test (if one or more cells had fewer than 5 observations).

Species	**Test**	**χ^2**	**Odds ratio**	**P value**
B. pendula	χ^2	379.36	NA	<0.001
O. oblonga	χ^2	11.786	NA	<0.001
O. puberula	χ^2	142.73	NA	<0.001
O. cernua	Fishers exact	NA	5.686	<0.001
O. whitei	χ^2	37.397	NA	<0.001
N. cissiflora	Fisher's exact	NA	2.847	0.200
N. lineata	Fisher's exact	NA	1.479	0.224

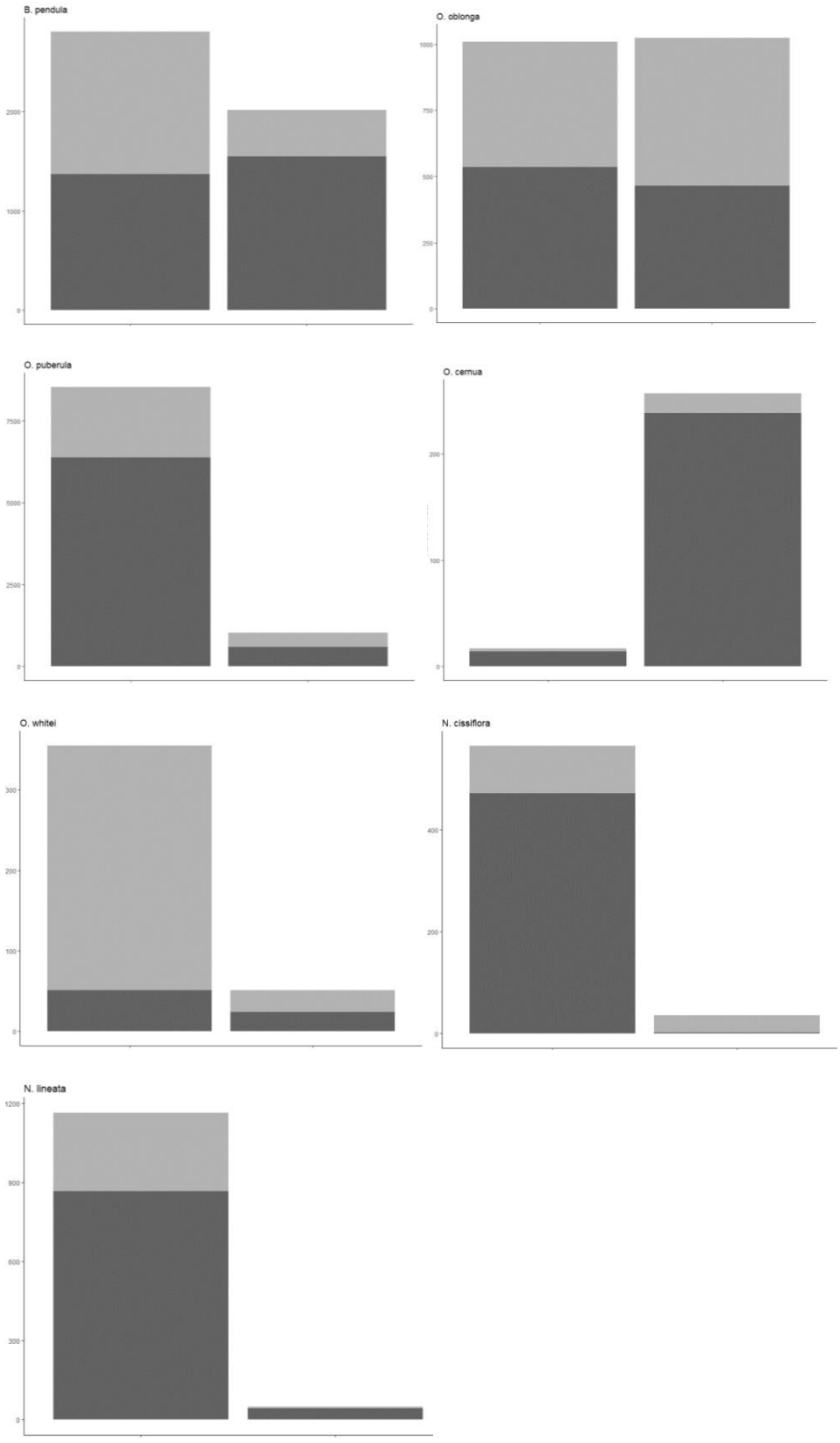


Figure 2 – Numbers of immature (left bars) and mature fruits (right bars) scored as attacked or not attacked by insect seed predators (data combined for 2015 and 2016). Attacked fruits are shaded in light grey and intact fruits shaded in dark grey.

(1a) Do rates of seed predation vary between individual canopies and do tree-specific seed predation rates depend on local seed crop sizes?

In both 2015 and 2016, as the local fruit crop increased in size, attack rates significantly increased (2015: Est. = 0.152, Std. Error= 0.025, z= 6.049, P<0.001; 2016: Est. = 0.086, Std. Error= 0.023, z= 3.721, P= <0.001) (Fig. 3).

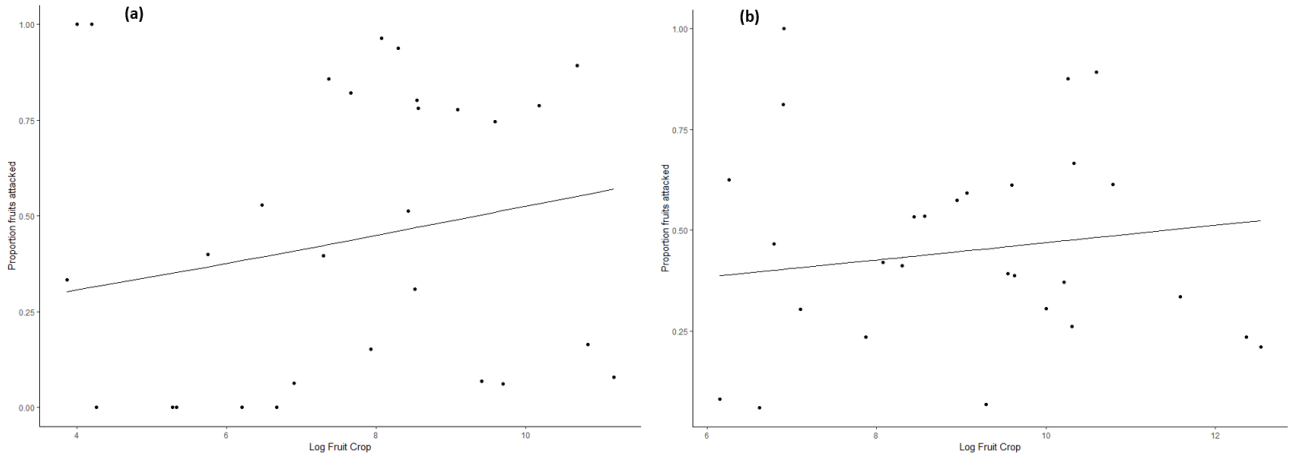


Figure 3- In both 2015 (a) and 2016 (b), seed predator attack rates increased with the log size of the local (tree-specific) fruit crop.

(1b) Do trees experience higher attack rates by insect seed predators when surrounded by fruiting conspecifics/congenerics/confamilials known to be attacked by the same species of pre-dispersal insect seed predators?

In 2015, as the connectivity to conspecifics increased, the proportion of seeds attacked on focal trees significantly increased, but as connectivity to congenerics increased, attack rates significantly decreased (species: Est. = 1.372, Std. Error = 0.080, z = 17.010, P <0.001; genus: Est. = -0.318, Std. Error= 0.036, z = -8.807, P <0.001) (Fig. 4).

In 2016, as the connectivity to conspecifics increased, attack rates of focal trees decreased, but as connectivity to congeneric increased, attack rates increased (species: Est. = -0.122, Std. Error = 0.059, z= -2.062, P =0.039; genus: Est.= 0.089, Std. Error =0.019, z= 4.692, P<0.001) (Fig. 4).

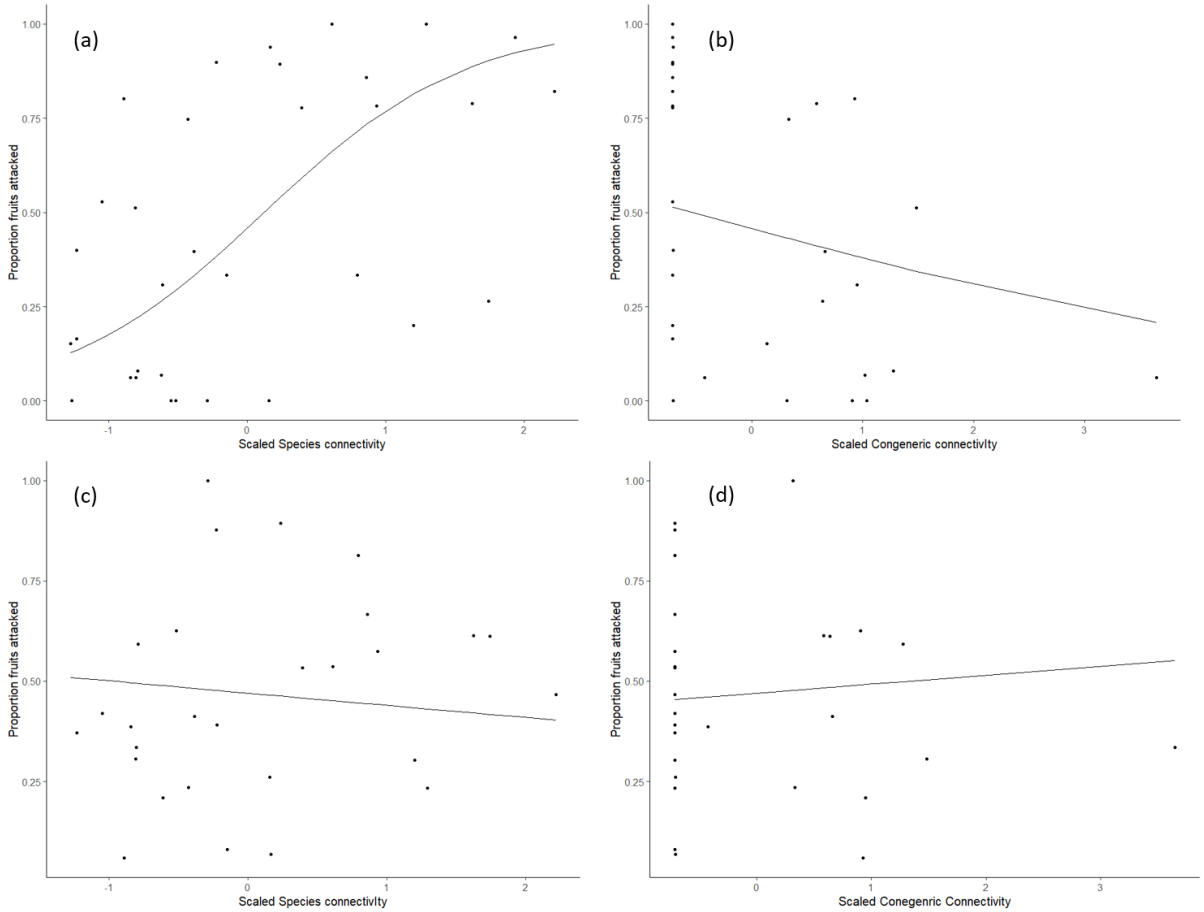


Figure 4- In 2015 seed predation rates on focal trees increased with increasing connectivity to conspecifics (a) and decreased with increasing connectivity to congenerics (b). In 2016 both connectivity to conspecifics (c) and congenerics (d) significantly affected seed predation rates of focal trees.

There were no statistically significant relationships between the incidence of individual seed predator species and the connectivity of the host tree to conspecific, congeneric, and confamilial trees (Supplementary Table 2, Supplementary Fig. 4). For one species of seed

predator (*Histura* sp.) as sample size increased, the likelihood of detecting the species on a tree increased, although this effect size was small.

(2a) Do rates of premature fruit abscission vary between individual canopies and are they dependent on local fruit crop sizes?

In 2015, as log fruit crop increased, the number of prematurely aborted fruits increased (Est. = 0.07336, Std. Error = 0.02705, z= 2.712, P = 0.007), although the effect size was small (Fig. 5). In 2016, premature fruit abscission rates were unrelated to local fruit crop sizes (2015: Est. = 0.058, Std. Error = 0.044, z = 1.317, P= 0.187; 2016: Est = 0.014, Std. Error = 0.043, z= 0.326, P= 0.744).

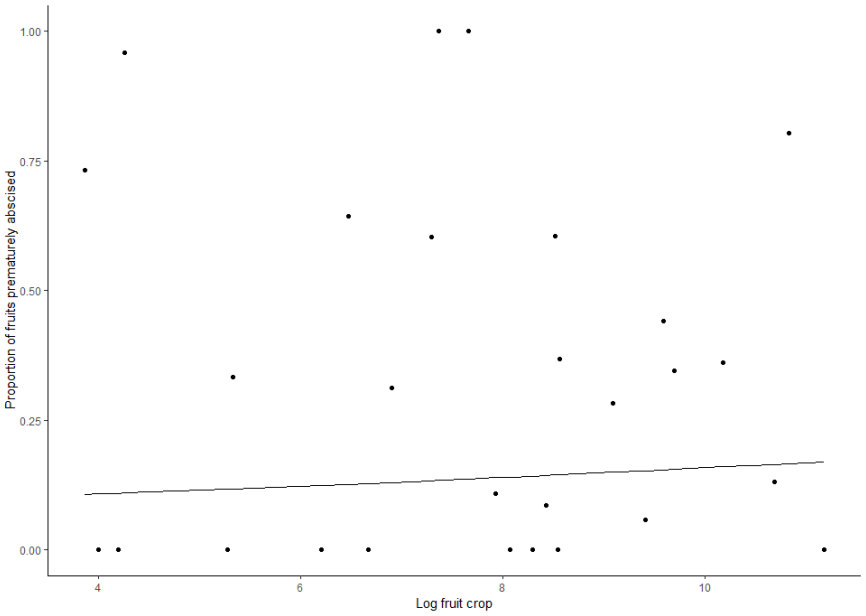


Figure 5- In 2015, premature fruit abscission rates increased with the log size of the local (tree-specific) fruit crop.

2b) Do trees experience higher premature fruit abscission rates when surrounded by fruiting conspecifics/congenerics/confamilials known to be attacked by the same species of pre-dispersal insect seed predators?

In both 2015 and 2016 premature abortion rates significantly decreased with increasing connectivity to conspecifics and congenerics, and increased with increasing connectivity to confamilials (2015: Species: Est. = -0.202, Std. Error = 0.075, z=-2.700, P= 0.006, Genus: Est. = -0.659, Std. Error = 0.043, z = -15.074, P <0.001; Family: Est. = 0.352, Std. Error = 0.061, z= 5.707, P<0.001; 2016:Species: Est. =-0.396, Std. Error = 0.055, z = -7.120, P<0.001; Genus: Est. =-0.686, Std. Error = 0.038, z = -18.027, P<0.001; Family: Est. = 1.292, Std. Error = 0.059, z= 21.585, P<0.001) (Fig. 6).

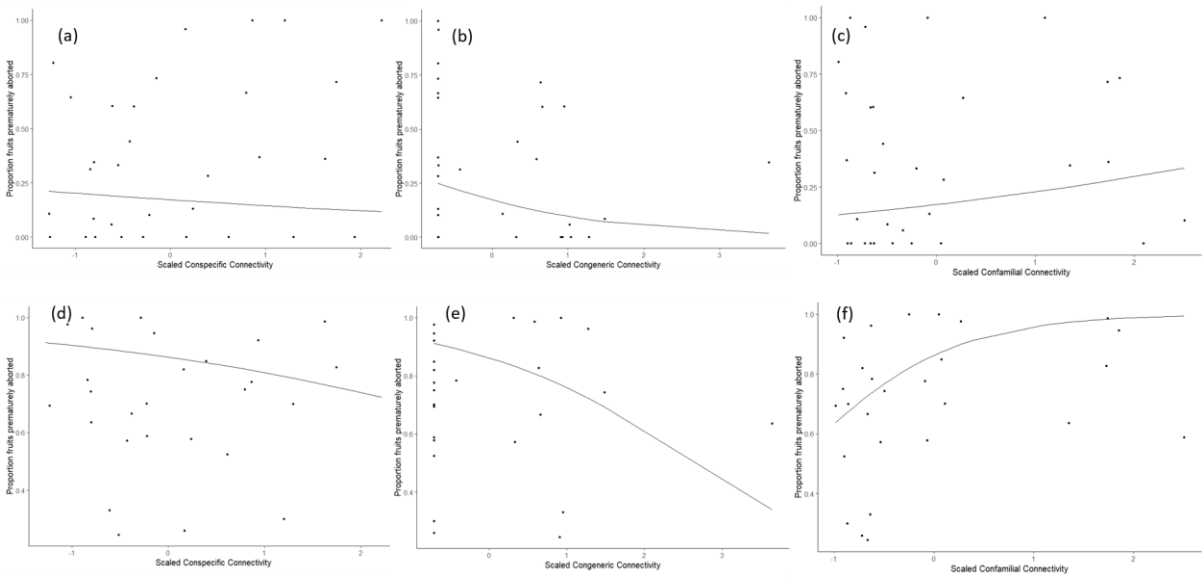


Figure 6- In 2015 (a-c) and 2016 (d-f), as the connectivity to conspecifics and congenerics increased, premature abscission rates decreased. As connectivity to confamilials increased, premature abscission rates significantly increased.

(3) Do individuals with high levels of pre-dispersal insect seed predation produce fewer healthy fruits than individuals with low levels of seed predator attack?

In 2015 individuals with higher rates of seed predation produced fewer intact mature seeds (Est. = -0.658, Std. Error = 0.072, z= -9.044, P<0.001). In 2016, the reverse pattern was observed (Est. = 0.664, Std. Error = 0.085, z= 7.832, P<0.001).

Discussion

Our study is one of few studies to have examined how pre-dispersal insect seed predators respond to spatial variation in resource abundances. The results suggest that levels of pre-dispersal seed predation inflicted by insects can be very high: up to 96% of the seed crop of individual trees was found to be destroyed by insects. Our analyses also showed that seed predation rates on individual trees were associated with local seed crop size (higher predation rates under high local seed abundances), and this pattern was consistent across years. This finding, which suggests that the seed predators in our system respond to local resource densities supports findings by Xiao et al. (2017) who found positive density-dependent seed predation for individual seed crops.

In 2015, attack rates on focal tree individuals significantly increased as their connectivity to conspecifics increased, in line with predictions of the Janzen-Connell hypothesis (Janzen 1970; Connell 1971) and supporting results found by Jones & Comita (2010) and Xiao et al. (2017), who found evidence for density-dependent seed predation at the population scale in their study systems. Contrary to our original prediction, seed predator attack rates significantly decreased as connectivity to congenerics increased. This could be due to prey switching: if a predator preferentially attacks one species the alternative host species could experience a short term positive effect when the two species co-occur (Allen 1988).

In 2016, we observed the opposite pattern, with seed predation rates decreasing as connectivity to conspecifics increased. One possible explanation for the discrepancy in results between the two years could be temporal variation in overall resource densities. In 2016 fruit densities were over three times higher than in 2015, but overall attack rates of fruits sampled from traps were lower in 2016 (36%) than in 2015 (49%). The predator satiation hypothesis posits that in years of high seed abundance, levels of seed attack are reduced (Janzen 1971; Bagchi et al. 2011). Our results are consistent with this idea.

We suggest that predator satiation may be one reason for why we did not observe positively density-dependent seed predation rates at scales larger than individual trees in 2016. Another reason could be the influence of other seed predators such as vertebrates. Visser et al. (2011) showed that the top-down influences of vertebrate seed predators on insect-infested seeds prevented insect seed predators from increasing with increasing host plant density at the population level. This limits the ability of insect seed predators to cause negative density dependent seed survival at this larger scale. It has also been argued that detecting density-dependence across larger spatial scales may be most likely when including higher taxonomic levels (e.g. genus or family) in addition to assessing the effects of conspecifics in the neighbourhood (Queenborough et al. 2009). This might be one reason for the lack of conspecific density-dependence across larger spatial scales in some studies (Comita et al. 2014). Here we investigated patterns at several taxonomic levels (species, genus and family level), and found that connectivity to congenerics and conspecifics best explained patterns of attack by pre-dispersal seed predators, although the strength and direction of these neighbour effects differed between years.

In addition to the effects of local and larger-scale seed abundances on rates of insect attack, patterns of premature fruit abscission were also associated with spatial variation in resource abundances. Premature fruit abscission rates of focal trees increased with increasing fruit

crop size one year, and in both years the connectivity of focal trees to conspecifics, congenerics and confamilials all affected abscission rates.

In some of our study species, the proportion of fruits with signs of insect attack was significantly higher in immature than mature fruit samples, suggesting that pre-dispersal seed predator attack may be responsible for at least some of the premature fruit abscission. Previous studies have documented high rates of insect infestation in abscised seeds (Stephenson 1981), but there are relatively few studies on pre-dispersal insect seed predation in tropical tree species. Studies in which seed predators have been experimentally excluded from developing fruits in the canopy have reported significantly increased fruit set for some, but not all, species studied (Beckman & Muller-Landau 2011), in line with our findings.

In cases where there was no association between premature fruit abscission rates and pre-dispersal seed predation rates, the observed spatial variation in fruit abscission rates might be due to factors such as spatial variation in pollination success. For example, if a tree individual is in an area of low flower density, cross-pollination might be less likely and this might have consequences for fruit set and/or maturation.

In 2015, as the proportion of fruits experiencing seed predation by insects increased on trees, the number of healthy mature seeds produced decreased. In contrast, in 2016, as the proportion of fruits experiencing predation increased on trees, the number of healthy mature fruits produced also increased; an observation that may be attributed to seed predator satiation under high resource densities. The relationship between predation rates and seed production documented in 2015 suggests that the reproductive success of individual trees may – at least under some circumstances – be reduced by pre-dispersal seed predation. Based on information collected in this study, we do not know how many of these seeds would establish as seedlings and therefore what the overall effect on reproductive success is.

However, if our study species are seed limited as opposed to microsite limited, seed predation could have a significant effect. Seed addition experiments (Turnbull et al. 2000) or seed predator exclusion experiments followed up by seedling monitoring (Harms et al. 2000; Beckman et al. 2017; postscript of this thesis) would be useful to uncover this possibility.

Conclusion

Our work supports previous findings of spatiotemporal variation in pre-dispersal seed predation rates (Xiao et al. 2017), but is the first to examine the role of heterospecific seed abundances. We found evidence that predation rates were affected by the density of both conspecific and congenerics. This finding supports previous suggestions that non-specialist plant enemies may contribute to structuring tropical tree communities (Lewis & Gripenberg 2008; Sedio & Ostling 2013).

Premature fruit abscission was associated with high seed predation rates for some species in our study, and in one year of the study the number of healthy seeds produced by focal trees decreased with increased rates of seed attack. These findings suggest that pre-dispersal insect seed predators may play an important role in the regeneration dynamics of tree species in our study system, although the fitness-related effects of seed predators on their hosts may only be apparent in years of relatively low seed abundances.

As both the strength and direction of conspecific and congeneric effects changed between years, it is difficult to predict the long-term effects of insect seed predation in our system, especially with only two years of monitoring. It is known that apparent competition and apparent mutualism may occur in the same system at different spatial and temporal scales when populations cycle (Abrams, Holt & Roth 1998). Uncovering the functional and numerical responses of the predator to the prey species could aid in predicting the strength and direction of indirect interactions over population cycles.

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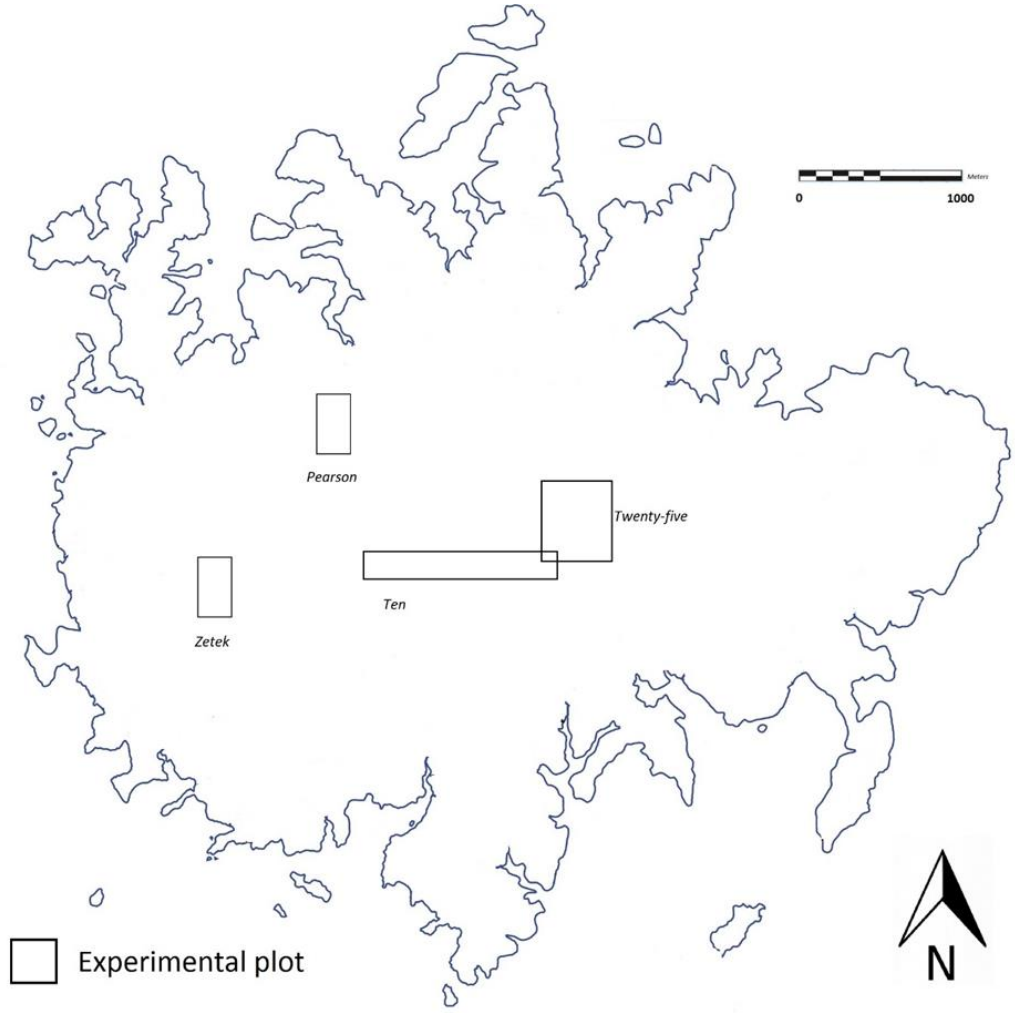
Author Contributions

HD, SG, OL and MB conceived and designed the study. HD carried out data collection, analysed the data, interpreted the results and drafted the manuscript with input from SG, OL and MB.

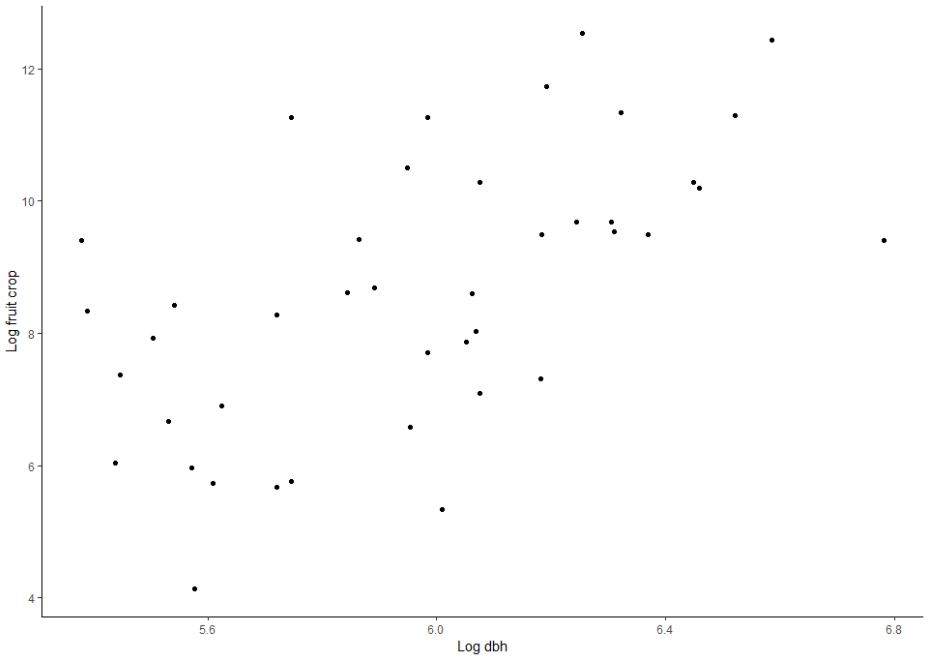
Supplementary Information

Chapter 3

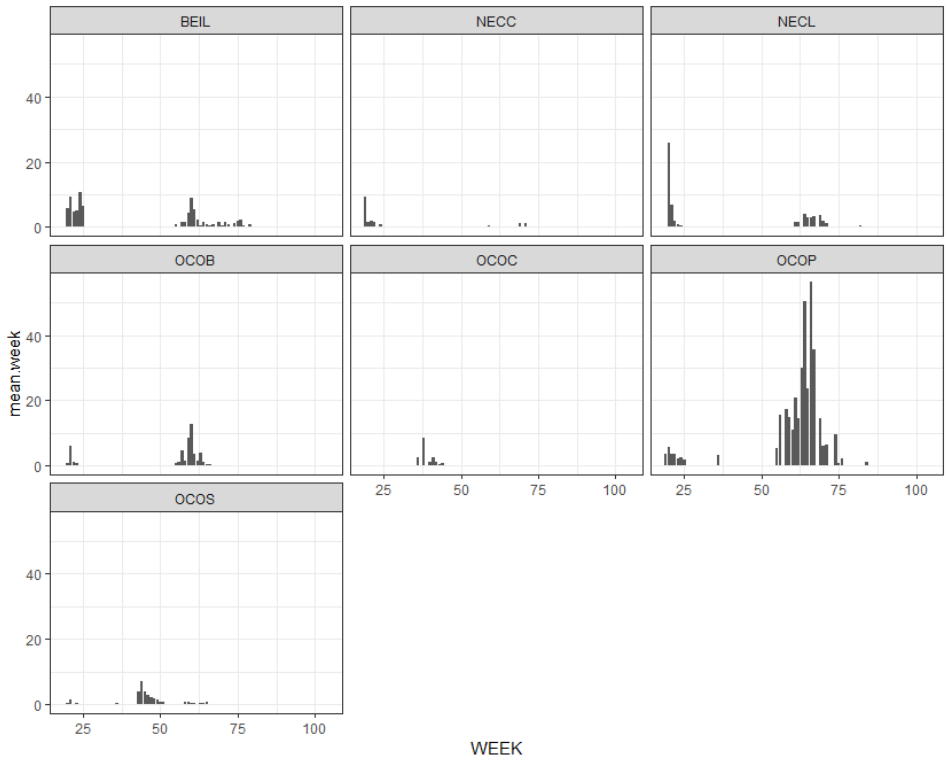
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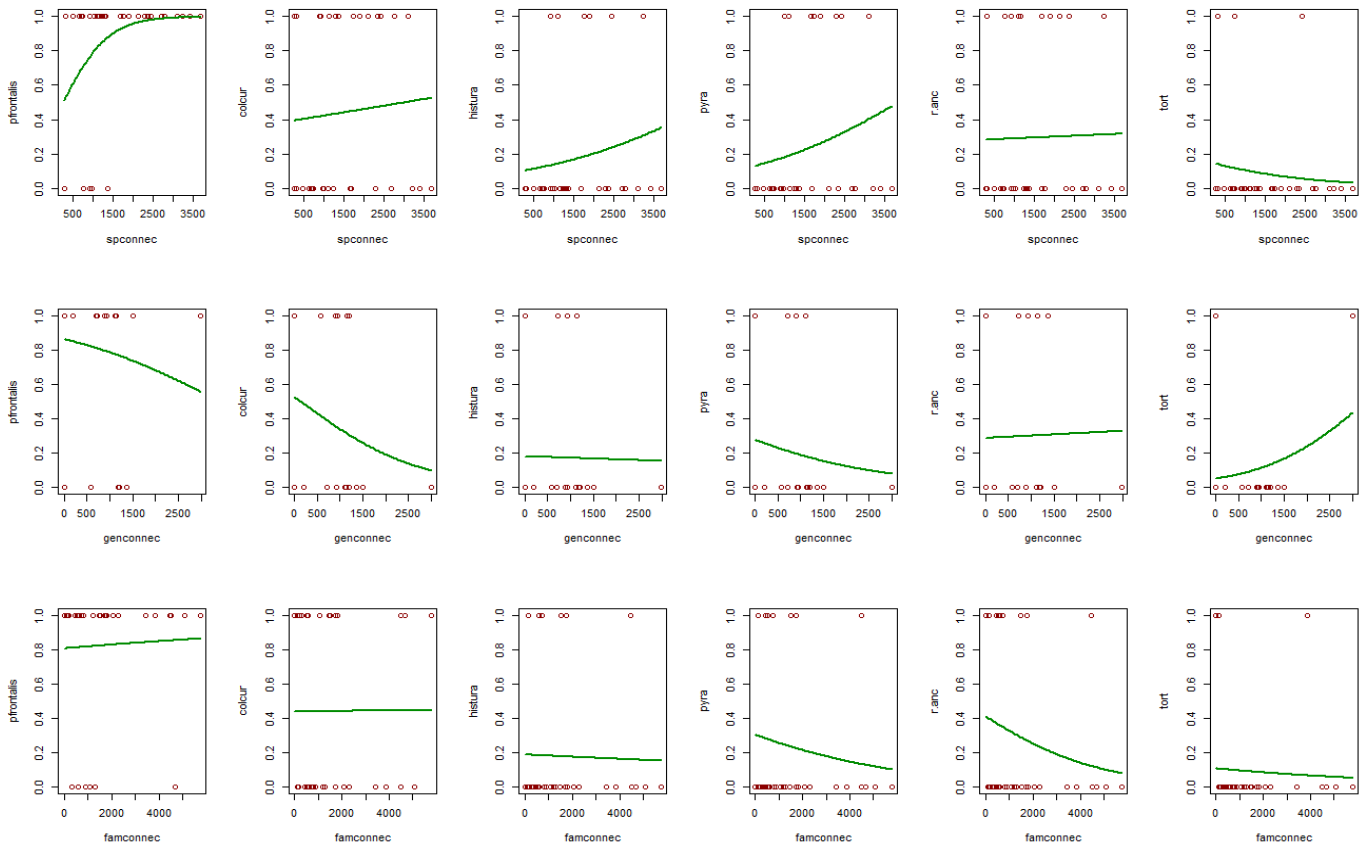
Supplementary Figure 1- Map of Barro Colorado Island with mapped plots used in this study shown.



Supplementary Figure 2- There was a significant positive relationship between log dbh and log fruit crop (Pearson's correlation: r= 0.59, P<0.001).



Supplementary Figure 3- Mean number of fruits collected per week per m^2 over the two year collection period.



Supplementary Figure 4 – The incidence of individual seed predator species (n=6) in relation to connectivity at each species, genus and family level. Row 1= species connectivity, Row2= genus connectivity, Row3= family connectivity. Each column is a different seed predator species. Seed predator species have been ordered according to abundance (left = from most abundant; right = least abundant), and follow the order in Table 2: *Pagiocerus frontalis*, *Heilipus draco*, *Histura* sp., Pyralidae sp., *Riculorampha ancyloides* and Tortricidae sp. Note that none of the relationships are statistically significant (all P>0.05).

Supplementary Table 1 – Connectivity values (at the level of conspecifics) for the focal trees under a range of alpha values.

Tree ID	Species	0.01	0.02	0.04	0.06	0.08	0.1
48123	*N. lineata*	772.9404	1190.661	1374.162	1473.421	1555.641	1625.139
4120	*N. lineata*	1355.802	1665.556	1801.735	1873.213	1932.209	1982.066
4166	*O. oblonga*	1484.346	1495.897	1496	1496	1496	1496
4604	*O. puberula*	2208.098	2315.559	2327.801	2327.996	2328	2328
47004	*O. puberula*	2561.955	2562	2562	2562	2562	2562
20510	*O. oblonga*	755.4973	956.8863	1177.776	1286.253	1346.731	1384.464
20568	*O. oblonga*	796.5819	992.1958	1200.013	1301.334	1358.86	1395.453
20727	*O. oblonga*	768.175	1086.498	1317.781	1377.697	1394.233	1398.988
46787	*O. puberula*	1684.333	2026	2136.785	2148.315	2149.778	2149.971
46796	*N. lineata*	1948.727	2207.114	2352.104	2407	2433.628	2446.863
21345	*B. pendula*	504.7347	728.1696	836.113	851.2628	853.5646	853.9292
21401	*O. oblonga*	776.2929	1041.116	1280.434	1373.741	1413.707	1431.447
21506	*O. whitei*	1139.44	1227.655	1238.824	1238.975	1238.977	1238.977
21632	*O. puberula*	1871.618	2074.55	2104.414	2104.989	2105	2105
22285	*B. pendula*	476.2437	618.5814	713.5001	758.1868	789.6149	813.6075
22288	*B. pendula*	554.4215	683.4989	773.867	814.9263	842.7195	863.6421
22368	*O. whitei*	909.2596	993.2832	1003.999	1004.166	1004.169	1004.169
22404	*B. pendula*	695.8486	845.6929	884.0784	885.9015	885.9946	885.9997
22567	*O. cernua*	218.9194	242.9772	245.8149	245.8471	245.8475	245.8475

47239	*O. puberula*	2064.096	2274.667	2345.722	2353.795	2354.841	2354.979
23625	*N. lineata*	2041.045	2256.963	2379.501	2430.658	2455.925	2468.507
23740	*O. cernua*	226.4228	249.8349	252.5964	252.6278	252.6282	252.6282
24196	*O. whitei*	1060.326	1117.632	1123.094	1123.146	1123.146	1123.146
24911	*N. cissiflora*	0	0	0	0	0	0
49108	*N. lineata*	2136.181	2325.201	2347.681	2347.995	2348	2348
29331	*N. puberula*	0	0	0	0	0	0
48737	*O. whitei*	2712.651	3015.785	3227.061	3295.686	3320.412	3329.802
49205	*O. cernua*	318.8316	551.4567	775.1599	868.7012	913.1236	936.024
48752	*B. pendula*	5216.534	5751.162	5902.798	5953.869	5994.088	6028.114
38188	*O. cernua*	535.5111	766.7865	935.7069	978.9216	990.8503	994.3696
38285	*B. pendula*	3685.778	4823.453	5608.036	5857.472	5960.816	6012.908
38310	*O. whitei*	2971.512	3198.04	3275.719	3287.661	3289.616	3289.937
38314	*B. pendula*	4952.45	5640.735	6060.18	6181.26	6232.021	6259.21
38326	*B. pendula*	4245.28	4673.304	5159.734	5444.059	5617.717	5727.418
38341	*O. whitei*	2447.615	2788.569	3102.072	3227.737	3283.356	3309.459
49579	*O. whitei*	2181.224	2487.889	2828.878	3005.552	3106.709	3169.023
49580	*B. pendula*	5056.882	5392.531	5733.875	5903.451	5995.786	6049.989
38364	*O. whitei*	2659.688	2897.357	3142.244	3256.888	3318.446	3355.588
38370	*O. oblonga*	1885.071	2085.405	2248.181	2309.797	2333.593	2342.885
38391	*O. whitei*	3120.902	3326.977	3485.389	3528.767	3541.001	3544.529

39060	*B. pendula*	4619.613	5253.563	5815.588	6051.789	6165.503	6226.579
39101	*B. pendula*	4256.313	5172.747	5811.255	6027.177	6120.667	6166.997
39116	*O. whitei*	3443.021	3560.016	3599.802	3605.037	3605.845	3605.975
39122	*B. pendula*	4190.486	4967.185	5661.58	5963.051	6108.375	6182.427
39137	*B. pendula*	4271.479	5075.679	5726.821	5983.198	6104.337	6168.744
39155	*B. pendula*	4567.723	5212.711	5760.321	5987.849	6103.617	6172.065
39201	*B. pendula*	4901.87	5319.934	5704.228	5883.178	5974.026	6023.686
39222	*B. pendula*	5004.313	5307.183	5649.085	5850.548	5971.787	6045.977
39554	*O. puberula*	637.7649	795.5003	871.7573	882.3041	883.7651	883.9675
39561	*O. whitei*	3001.894	3224.823	3379.924	3438.476	3466.408	3481.03
39597	*B. pendula*	5257.173	5764.814	5910.887	5961.84	6001.694	6035.341
39634	*O. whitei*	3141.702	3396.706	3454.319	3456.866	3456.993	3457
39704	*B. pendula*	4891.626	5609.975	6102.548	6252.286	6305.196	6325.073
49223	*O. oblonga*	2050.752	2208.006	2229.643	2229.994	2230	2230
39809	*N. lineata*	0	0	0	0	0	0
39843	*O. oblonga*	1594.558	1760.65	1931.141	2028.123	2089.335	2131.393
39891	*O. oblonga*	1302.429	1568.923	1814.179	1957.377	2044.513	2098.09
39901	*O. oblonga*	1260.301	1577.966	1855.609	1997.008	2071.488	2110.801
48791	*O. puberula*	825.8777	855.2143	855.9993	856	856	856
48466	*O. cernua*	425.5257	599.335	791.006	886.0535	938.0645	968.1553
48467	*O. cernua*	162.8525	377.6697	627.7964	760.2605	836.138	881.2516

48481	*O. cernua*	366.7697	562.7069	780.9477	883.8038	935.4922	962.903
48756	*O. puberula*	1216.002	1273.586	1299.817	1303.421	1303.92	1303.989
48814	*O. oblonga*	2144.736	2231.864	2315.2	2359.29	2386.982	2406.559
49182	*B. pendula*	6221.261	6388.103	6426.666	6429.712	6429.975	6429.998
49186	*O. whitei*	3137.74	3299.742	3435.307	3497.176	3527.9	3543.549
49195	*N. cissiflora*	0	0	0	0	0	0
49234	*B. pendula*	5338.505	5793.463	6152.531	6286.493	6347.907	6380.6
49531	*O. oblonga*	2132.404	2243.897	2344.975	2401.432	2434.885	2455.087
35326	*B. pendula*	4223.2	4798.998	5134.512	5244.66	5302.872	5342.951
35328	*B. pendula*	1768.88	3216.98	4050.799	4319.652	4460.345	4558.381
35330	*B. pendula*	3640.185	4410.737	4883.339	5047.599	5133.701	5190.649
48998	*B. pendula*	4333.024	5142.381	5266.362	5268.939	5268.998	5269
35492	*O. whitei*	0	0	0	0	0	0
35876	*B. pendula*	2467.889	3764.854	4687.186	5022.529	5172.943	5245.923
35881	*B. pendula*	3534.731	4341.893	4903.237	5106.164	5212.621	5279.932
49757	*B. pendula*	2349.953	3613.642	4500.839	4819.565	4982.147	5080.877
49760	*N. cissiflora*	0	0	0	0	0	0
35939	*B. pendula*	3792.643	4634.112	5193.819	5361.207	5429.164	5461.739
35948	*B. pendula*	3432.063	4300.603	4909.533	5132.457	5246.816	5315.536
36270	*B. pendula*	4103.457	4989.839	5363.803	5423.06	5436.621	5440.353
49417	*O. oblonga*	0	0	0	0	0	0

49482	*B. pendula*	4032.035	4651.693	4979.416	5120.833	5214.492	5281.865
36497	*B. pendula*	4326.754	4848.082	5119.088	5227.048	5298.271	5350.615
36965	*B. pendula*	3287.939	4518.612	5146.456	5281.229	5318.522	5329.805
37078	*B. pendula*	1927.952	3322.299	4069.64	4366.851	4557.761	4694.717
37103	*B. pendula*	3235.746	4155.367	4629.55	4832.409	4966.797	5063.217
49412	*B. pendula*	4424.129	5071.87	5437.845	5518.355	5538.955	5544.638
49379	*N. cissiflora*	7831.67	8004.424	8067.162	8079.121	8083.818	8086.246
4487	*N. cissiflora*	7425.528	7676.969	7864.684	7940.348	7981.018	8006.239
49736	*N. cissiflora*	7455.928	7689.239	7863.175	7935.891	7976.785	8003.038
49741	*B. pendula*	1682.832	1943.262	2008.955	2014.29	2014.91	2014.989
17280	*O. oblonga*	3857.268	5189.456	6007.165	6257.445	6371.384	6435.043
17302	*O. oblonga*	4892.624	5810.705	6309.372	6457.02	6521.867	6557.106
17313	*N. cissiflora*	5220.891	6656.416	7327.875	7500.453	7579.48	7626.537
17314	*N. cissiflora*	3918.21	6019.876	6988.401	7234.093	7345.636	7411.156
49672	*O. puberula*	164.3864	312.6138	438.9729	496.2556	523.1543	535.7944
48936	*O. oblonga*	4623.579	5938.27	6475.672	6543.079	6553.606	6555.514
17368	*N. cissiflora*	4243.376	6315.014	7397.874	7654.156	7738.332	7770.212
19244	*N. cissiflora*	7283.088	7760.583	7984.985	8029.053	8041.957	8046.393
19248	*O. oblonga*	3487.405	4880.545	5782.234	6082.377	6236.458	6332.104
49396	*O. oblonga*	4468.174	5595.461	6219.296	6397.253	6474.128	6514.902
22307	*N. cissiflora*	2124.315	5366.17	7203.514	7565.058	7652.742	7677.704

19291	*B. pendula*	1599.475	1916.099	2074.127	2098.407	2102.819	2103.731
22310	*O. oblonga*	2769.402	4684.4	5894.942	6224.742	6344.263	6394.679
22375	*O. whitei*	0	0	0	0	0	0
22377	*N. cissiflora*	6962.454	7377.672	7702.718	7837.588	7909.472	7952.661
49604	*O. oblonga*	4176.282	5514.077	6212.573	6381.531	6443.507	6473.433
49375	*N. cissiflora*	7622.748	7918.373	8030.372	8053.105	8060.144	8062.569
19713	*B. pendula*	1252.913	1542.162	1781.844	1888.715	1950.231	1989.682
49617	*B. pendula*	352.203	1089	1580.781	1712.936	1757.712	1775.515
49648	*N. cissiflora*	6835.039	7634.967	7909.03	7949.921	7960.888	7964.639
19764	*O. oblonga*	3335.787	4789.933	5755.838	6085.501	6250.934	6348.915
49691	*O. oblonga*	3830.413	5286.573	6168.424	6402.623	6488.437	6525.138
19869	*N. cissiflora*	3358.746	5449.339	6942.263	7425.741	7618.011	7704.56
49679	*O. oblonga*	4360.075	5502.339	6056.886	6222.495	6314.343	6376.284
22329	*O. oblonga*	4430.067	5583.526	6103.551	6252.872	6332.405	6383.376
19924	*N. cissiflora*	7116.355	7457.27	7709.163	7819.262	7884.36	7927.723
49732	*N. cissiflora*	3092.21	4802.797	6045.388	6577.525	6885.563	7087.478
49735	*N. cissiflora*	6844.664	7331.527	7704.221	7847.98	7918.797	7958.772
22350	*N. cissiflora*	2448.326	5036.906	6857.02	7392.109	7591.755	7679.53
23924	*N. cissiflora*	4067.173	6261.989	7450.931	7697.103	7763.661	7785.195
23951	*B. pendula*	243.6739	726.2927	1092.733	1254.753	1356.676	1431.244
29854	*O. puberula*	464.6158	557.7731	640.4404	678.3782	696.2004	704.5754

29855	*O. oblonga*	2491.734	4428.971	5531.156	5879.403	6036.888	6121.342
29896	*N. cissiflora*	2666.256	5382.362	6796.366	7205.126	7390.273	7490.929
48890	*O. oblonga*	3903.313	5181.942	6001.215	6263.944	6388.801	6460.086
48922	*N. cissiflora*	3315.58	5341.873	6864.143	7388.606	7606.163	7705.766
48929	*N. cissiflora*	1970.563	4559.542	6476.859	7075.388	7299.07	7394.038
48930	*N. cissiflora*	3980.805	5726.583	7016.142	7416.787	7566.269	7629.991
48941	*N. cissiflora*	4963.119	6463.963	7377.284	7609.462	7689.905	7725.956
48942	*N. cissiflora*	3750.298	5814.735	7089.031	7415.937	7529.189	7579.761
49374	*B. pendula*	1383.187	1611.999	1784.303	1858.7	1903.685	1935.303
49378	*B. pendula*	1438.757	1739.387	1980.922	2061.185	2090.505	2101.873
49382	*N. cissiflora*	4892.781	6762.149	7456.546	7612.361	7678.741	7712.896
49399	*N. cissiflora*	4529.784	6620.267	7568.707	7733.567	7775.064	7787.914
49602	*O. puberula*	773.9848	809.5472	812.972	812.9998	813	813
49606	*O. oblonga*	5529.396	6229.781	6582.12	6663.572	6693.589	6708.394
49634	*O. oblonga*	4302.487	5458.093	6123.065	6318.91	6413.019	6470.4
49637	*O. oblonga*	5417.032	6084.268	6462.598	6582.583	6641.596	6675.805
49664	*O. oblonga*	4286.842	5349.177	6054.955	6297.599	6421.244	6495.083
49673	*N. cissiflora*	5053.873	6716.227	7546.648	7736.916	7805.336	7835.624
49694	*O. cernua*	0	0	0	0	0	0
49698	*N. cissiflora*	3140.426	5641.947	7224.652	7605.282	7720.431	7761.57
49710	*N. cissiflora*	5506.501	6947.838	7608.201	7766.625	7822.083	7844.153

49712	*O. oblonga*	5643.038	6325.676	6616.727	6673.529	6689.619	6694.961
49722	*N. cissiflora*	4845.301	6357.399	7320.131	7623.657	7748.374	7806.937
49724	*N. cissiflora*	5004.963	6258.567	7148.813	7493.665	7666.981	7763.606
49728	*N. cissiflora*	3931.157	5332.022	6345.533	6770.801	7009.989	7162.091
49730	*N. cissiflora*	3638.027	5144.9	6258.255	6733.745	7002.57	7172.784
49733	*N. cissiflora*	2451.393	4361.557	5748.493	6329.787	6654.12	6857.716
49740	*N. cissiflora*	4607.271	5793.346	6703.421	7104.151	7338.768	7492.942

Supplementary table 2- Model outputs for analyses on the incidence of each of five seed predator species

Species	**Connectivity measure**	**Est.**	**Std. Error**	**z**	**P**
P. frontalis	Species	0.002	0.001	1.840	0.066
	Genus	-0.001	0.001	-0.907	0.364
	Family	0.001	0.001	0.252	0.801
Helipus draco	Species	0.001	0.001	0.436	0.663
	Genus	-0.001	0.001	-1.264	0.206
	Family	0.001	0.001	0.018	0.986
Histura sp.	Species	0.001	0.001	1.018	0.309
	Genus	-0.001	0.001	-0.095	0.924
	Family	-0.001	0.001	-0.156	0.876
Pyralidae sp.	Species	0.001	0.001	1.279	0.200
	Genus	-0.001	0.001	-0.700	0.484
	Family	-0.001	0.001	-0.798	0.425
R. ancyloides	Species	0.001	0.001	0.125	0.901
	Genus	0.001	0.001	0.118	0.906
	Family	-0.001	0.001	-1.221	0.222
Tortricidae sp.	Species	-0.001	0.001	-0.661	0.508
	Genus	0.001	0.001	1.252	0.210
	Family	-0.001	0.001	-0.327	0.743

Chapter 4

Insect herbivory on seedlings of rainforest trees: effects of density and distance of conspecific and heterospecific neighbours

Status: accepted, Ecology & Evolution

Summary

1. Natural enemies of plants such as insect herbivores can contribute to structuring and maintaining plant diversity in tropical forests. Most research in this area has focused on the role of specialised enemies and the extent to which herbivory on individual plant species is density-dependent.

2. Relatively few insect herbivores specialise on a single host plant species. Insect herbivores that feed on more than one plant species may link the regeneration dynamics of their host species through 'apparent competition' or 'apparent mutualism'.

3. We investigated herbivory and survival of seedlings of two tropical tree species (*Cordia alliodora* and *Cordia bicolor*) in the forests of Barro Colorado Island (Panama). We used experiments and observations to assess seedling fate in relation to the presence of conspecifics and heterospecifics across a range of spatial scales.

4. Herbivory significantly increased seedling mortality and was highest at high local densities of *C. alliodora* seedlings. There was also evidence that high local densities of *C. alliodora* increased herbivory on co-occurring *C. bicolor* seedlings.

5. *Synthesis.* The elevated rates of seedling herbivory at high densities of conspecifics documented in our study are consistent with the predictions of the Janzen-Connell hypothesis, which explains how so many plant species can coexist in tropical forests. Our data also highlight the possibility that herbivore-mediated density-dependence, facilitated by herbivores that feed on multiple plant species, can also occur *across* plant species. Enemy-mediated indirect effects of this sort have the potential to structure plant communities.

Introduction

Plants and their insect herbivores account for around half of the macroscopic diversity on earth. The response of herbivores to the distribution of their host plants in the landscape can have profound consequences for plant population dynamics and community structure (Crawley 1989; Maron & Crone 2006).

Insect herbivores are thought to play a key role in plant regeneration dynamics, particularly in highly-diverse tropical forest tree communities (Janzen 1970; Connell 1971). The Janzen-Connell hypothesis predicts that host-specific pests such as insect herbivores will cause seed and seedling mortality to be highest in areas where the density of conspecifics is high, and/or in the vicinity of conspecific adults. This mechanism can facilitate high local plant diversity in tropical forests by limiting population growth of species that are common, and favouring locally rare species (Janzen 1970; Connell 1971).

The Janzen-Connell hypothesis relies on enemies being reasonably host specific (Sedio & Ostling 2013). In terms of their diet breadths, insect herbivores are known to range from specialists to generalists that attack a wide range of species (Novotny et al. 2010). In these instances, host species sharing one or more enemies could experience enemy-mediated interspecific density dependence, increasing or decreasing each other's mortality rates via mechanisms such as 'apparent competition' and 'apparent mutualism' in locations where they co-occur (Holt 1977; Abrams et al. 1998). This could lead to the regeneration dynamics of plant species being linked to heterospecific as well as conspecific plant densities, and to non-specialist enemies shaping distributions of plant species at the community level.

The importance of enemy-mediated indirect interactions in ecological communities has been demonstrated in many systems (e.g. Muller & Godfray 1999; Tompkins et al. 2000; Morris et al. 2004). Despite this, the role of these types of interactions and their relative importance

compared to effects produced by specialist enemies have received little attention in tropical plant communities (but see Garzon-Lopez et al. 2015).

Numerous studies in tropical forests have investigated patterns of herbivory and/or plant survival in relation to either conspecific plant densities (reviewed in Comita et al. 2014) or the composition of the surrounding plant community (Weiblen et al. 2006). In most cases the identity and diet breadth of the herbivores remains unknown, making it difficult to assess the likely mechanisms behind observed patterns. Moreover, the extent to which observed patterns of herbivory suppress plant performance is typically unknown (but see Bagchi et al. 2014), making it challenging to infer the likely ecological impact of the herbivores.

We used experiments and field surveys to investigate patterns of herbivory on seedlings of two closely-related tropical tree species, *Cordia alliodora* (Ruiz & Pav. Oken) and *Cordia bicolor* (A.D.C.) (Boraginaceae). During preliminary studies (field observations and feeding trials in the laboratory), the tortoise beetle *Ischnocodia annulus* (Fabricus 1781) (Coleoptera: Chrysomelidae) was identified as an abundant insect herbivore shared by *Cordia alliodora* and *Cordia bicolor.*

We investigated patterns of herbivory in relation to host plant density, and assessed the potential for recruitment dynamics of the two *Cordia* species to be coupled via the foraging behaviour of the shared enemy. More specifically, our studies were designed to address the following questions: (1a) Do *Cordia* seedlings experience intra- and/or interspecific density-dependent seedling herbivory; and if so, (1b) at what spatial scale do any density effects occur? (2a) Is herbivory of *Cordia* seedlings higher closer to conspecific and congeneric adults than elsewhere in the landscape; and (2b) if so, at what distance do herbivory levels decline? (3) Does herbivory affect *Cordia* seedling survival?

Our results exploring the effects of conspecific distance- and density-dependent herbivory are relevant in the context of the Janzen-Connell hypothesis. Measuring the effects of congeneric distance- and density- dependent herbivory allows us to assess whether enemy-mediated indirect interactions between *Cordia* species occur at our study site.

Materials and methods

Study Site

Barro Colorado Island (BCI) is a 15.6 km² island located in Gatun Lake, Panama, (Lat: 9.1543, Long:-79.846) that was isolated from the surrounding mainland in 1914 when the Rio Chagres was dammed to form part of the Panama Canal. The island is covered with tropical semi-deciduous forest. The northeast half experienced widespread clearing during the late 1800s, whilst the other half has been little disturbed for up to 1500 years. BCI's climate is seasonal, with a dry season from December to April/May and an average yearly rainfall of 2612mm (Leigh 1999).

Most investigations presented in this study were carried out in four mapped forest plots (one ten hectare, one twenty-five hectare and two six hectare; Supplementary Fig. 1) that were established in the mid-1990s. In these plots every free-standing woody stem larger than 20cm diameter at breast height (dbh) has been tagged, measured (dbh) and identified to species level. These plots were last censused in 2013 and 2014.

Study System

The genus *Cordia* (Boraginaceae) consists of trees, shrubs, lianas, vines and erect herbs. Three species occur on BCI and overlap in their reproductive phenology, with the majority of seeds germinating in May and June. *Cordia alliodora* is a tall pioneer tree species with wind-dispersed seeds that germinate to form dense seedling carpets in the vicinity of reproducing

trees. *Cordia bicolor* is typically slightly smaller than *C. alliodora* and produces single-seeded fruits (ca 1.5cm in length) that are dispersed by mammals and birds. *Cordia lasiocalyx* is a small tree with fruit that resemble those of *C. bicolor* (Croat 1978). Since its dbh rarely exceeds 20cm it does not appear in the plot data sets, and seeds and seedlings of *C. lasiocalyx* appear to be considerably less common on BCI than those of *C. alliodora* and *C. bicolor*. For these reasons, we omitted *C. lasiocalyx* from our studies.

At our field site, newly-germinated seedlings of all three species are attacked by the target beetle *Ischnocodia annulus* (Chrysomelidae) (Fig. 1). Data from a long-term Malaise trap study on BCI show that the activity of *I. annulus* peaks during May and June, when *Cordia* seedlings germinate (D.M. Windsor, pers. comm.). Whilst we are confident that *I. annulus* causes the majority if not all of the herbivory to young *Cordia* seedlings, we cannot completely exclude the possibility that other insect herbivores also feed on *Cordia* seedlings. As our study is focused on enemy- mediated density- dependence and enemy-mediated indirect interactions, this will not change the interpretation of our results.



Figure 1- Feeding marks of *Ischnocodia annulus* on newly germinated *Cordia alliodora* seedlings.

While pathogens have been shown to cause substantial mortality in young seedlings of other tropical tree species (e.g. Augspurger 1984; Bell et al. 2006) the number of seedling with signs of pathogen attack was negligible in our studies (only 0.02% of 4120 individuals

showing signs of pathogen attack throughout our studies). We are therefore confident that insect herbivores are the most important enemies of young *Cordia* seedlings in our study.

Data Collection

To address our study questions we used a combination of experimental work and observational surveys on natural *Cordia* seedlings conducted across three field seasons (2013, 2015 and 2016).

For experiments, newly- germinated seedlings were collected beneath reproducing *C. alliodora* trees near the laboratory clearing in the north-eastern part of BCI. Seedlings were placed individually in pots filled with sterilised soil and allowed to acclimatise in a shade house for a minimum of two days before being taken to the field (still in their pots). All seedlings were taken to the field at the cotyledon stage. Experimental seedlings were free from insect herbivores and showed no evidence of pathogen attack when experiments were initiated. Specific study methods to address each question are described below.

Do *Cordia* seedlings experience density-dependent seedling herbivory; and if so, at what spatial scale do conspecific and congeneric density effects occur?

Study 1

To investigate how *C. alliodora* seedling herbivory was affected by the density of conspecific seedlings at a small spatial scale, and by the distribution of adults of *C. alliodora* and *C. bicolor* at a large spatial scale, experimental seedlings were introduced to the 25 ha mapped plot (Supplementary Fig. 1). The 25 ha mapped plot was divided into 625 20×20m subplots (Supplementary Fig. 2) using an existing grid of PVC markers. To minimise problems with edge effects, we excluded all subplots within 40m of the mapped plot edge. In each of the remaining 441 subplots, seedlings were placed at nine locations, guided by the existing PVC

markers: one in each of the corners, one halfway along each edge of the subplot, and one in the centre (Supplementary Fig. 2). At one of these nine locations, selected at random, we placed a cluster of eight potted seedlings. A single potted seedling was placed at the remaining eight locations. Thus there were 16 seedlings in total per subplot, eight in a 'high' density and eight in a 'low' density treatment.

The experiment was established between 17th May and 3rd June 2016 with seedlings placed in 120 subplots. In total, 1869 seedlings were placed at 1089 locations (PVC poles). It was not possible to place seedlings at all nine locations in 20 subplots because of steep terrain, lianas or dense undergrowth. All introduced seedlings were monitored weekly for the first month, then every ten days. In total we completed seven censuses, with the last census taking place on 5th August 2016. At each census, seedlings were scored for presence or absence of herbivory. To quantify the abundance of adult *Cordia* trees in the study area, every mapped adult of *C. alliodora* and *C. bicolor* in the 25 hectare plot (including those close to plot edges) was visited and its reproductive status recorded during 15th and 31st May 2016. Adults were scored as reproductive if flowers, fruits or recently-established seedlings were observed.

Study 2

To assess levels of seedling herbivory in relation to conspecific and congeneric abundances, 27 20×20m subplots located in three of the mapped forest plots (Supplementary Fig. 1) were surveyed. The 27 survey plots were selected to represent varying densities of *Cordia* adult individuals: no *Cordia*, one *C. alliodora*, one *C. bicolor*, multiple *C. alliodora*, multiple *C. bicolor*, low density (two-three individuals) mixed species and high density (>four individuals) mixed species. Within each of these subplots, where access was possible, nine 0.5m² quadrats were set up, one at each corner of the subplot, one at the mid-point of each subplot edge, and one in the centre. In each quadrat, every newly- germinated (i.e. current year) *C. alliodora* and *C. bicolor* seedling was counted and scored for presence or absence of

herbivory. A total of 255 quadrats were surveyed, 105 of which had *Cordia* seedlings in them. Censuses took place from 25th May 2015 and 21st June 2015.

Is herbivory of seedlings higher closer to conspecific and congeneric adults than elsewhere in the landscape; and if so, at what distance do herbivory levels decline?

Study 3

To assess whether seedlings of *C. alliodora* suffer elevated insect attack rates close to conspecifics and/or congeneric adult trees, and to explore the spatial extent of such effects, we introduced potted seedlings to 18 sites in the mapped forest plots and subsequently scored them for herbivory.

Six adults of each of *C. alliodora*, *C. bicolor* and *Guapira standleyana* (Woodson) were selected from the four mapped plots. *Guapira standleyana* (Nyctaginaceae) was selected as a heterofamilial species to provide a non-*Cordia* comparison. This species is similar in size and abundance to the studied *Cordia* species, but is not closely related to them and is not a host of *I. annulus*. The 18 sites were chosen as the adult trees were reproductive (i.e. seeds and/or seedlings present in their vicinity) and were separated from the nearest *Cordia* adult by a distance of at least 30m. At each tree, a linear transect of 26 potted *C. alliodora* seedlings (hereafter referred to as 'focal' seedlings) was established, oriented in a random direction, with single plants spaced at 1m intervals from 0m to 25m. This distance was chosen to reflect the typical extent of natural *C. alliodora* seedling carpets observed in preliminary surveys (mean 18m, maximum 23m). If there was another *C. alliodora* tree within 30 m of a focal tree, we selected an alternative transect direction. All natural seedlings in a 0.5m² quadrat surrounding each focal seedling were counted. Focal seedlings were scored for presence or absence of herbivory every two or three days from 24th June 2015 until 15th July 2015, when

herbivory had levelled off. This allowed us to obtain an accurate herbivory score for each focal seedling, even in cases where a focal seedling later died.

Study 4

To assess whether seedlings of *C. alliodora* and *C. bicolor* experience elevated herbivory close to reproducing *C. alliodora* adults, potted *C. alliodora and C. bicolor* seedlings were introduced (still in their pots) to sites close to each of four *C. alliodora* trees. Seedlings of both species were obtained from seeds collected on BCI and germinated in a shade house. At each tree, 11 *C. alliodora* and 11 *C. bicolor* seedlings were positioned randomly within an area of approximately 2 × 2 m within the seedling carpet of the adult tree. The precise location was selected haphazardly from sites with suitable terrain. An additional 11 *C. alliodora* and 11 *C. bicolor* seedlings were placed within a similar-sized area further away from each adult tree, outside the seedling carpet, but within five meters of the periphery of the seedling carpet. We refer to seedlings in the two distance categories as 'near' and 'far' seedlings, respectively. Seedlings were transferred to the field between 5th and 29th July 2013 and revisited to score herbivory (presence/absence) and survival (alive/dead) approximately every two day until 28th August 2013, when herbivory had levelled off.

Does herbivory affect Cordia seedling survival?

Survival of *C. alliodora* and *C. bicolor* seedlings from Study 4 (above) following herbivory was recorded. To determine how herbivory co-varies with *C. alliodora* seedling survival, two *C. alliodora* seedling carpets were selected on 1st June 2015. Within each seedling carpet, 20 intact seedlings and 20 seedlings with herbivory were haphazardly selected, numbered, and their fate (alive or dead) followed for six weeks.

Statistical Analysis

Study 1- Data were analysed using GLMs with quasibinomially distributed errors (to account for underdispersion). To test for spatial autocorrelation in herbivory, focal seedling herbivory (a binary response) was modelled as a function of the proportion of seedlings with herbivory within 10 different radii (from 0 to 100m). Additionally, the proportion of seedlings with herbivory within a subplot was modelled as a function of seedling treatment (high or low density), summed basal area of *C. alliodora* adults, and summed basal area of *C. bicolor* adults within the neighbourhood. Subplot was used as a random effect. After trying several different neighbourhood distances, 50m from the central point of each subplot was selected as the most appropriate by comparing model AIC values, and was thus used in the final model.

Study 2- Numbers of *C. bicolor* seedlings in our observational plots were too low to test for intra- or inter-specific density effects on *C. bicolor* herbivory rates. Data on *C. alliodora* seedling herbivory were analysed using a GLMM with binomially distributed errors. Seedling herbivory (proportion of seedlings with signs of herbivory per 0.5m² quadrat) was modelled as a function of local density of *C. alliodora* seedlings (in 0.5m² subplot), local density of *C. bicolor* seedlings (in 0.5m² subplot), density of *C. alliodora* adults in plot (20m × 20m), density of *C. bicolor* adults in plot (20m × 20m), summed basal area of *C. alliodora* adults in plot (20m × 20m) and summed basal area of *C. bicolor* adults in plot (20m × 20m) (all fixed effects). Plot number was included as a random effect to account for non-independence of seedlings within the same plot. To account for overdispersion in the data, an observation-level random factor was included in the model. Here, each data point receives a unique level of a random effect that models the extra variation present.

Study 3- Data were analysed using Generalised Linear Mixed Effects Models (GLMMs) with binomially distributed errors. Seedling herbivory at the final census on each focal seedling (a

binary response) was modelled as a function of the distance (m) of the seedling from adult tree, local seedling density (the number of natural *Cordia* seedlings in the 0.5m² quadrat surrounding the focal seedling), adult tree species (*C. alliodora*, *C. bicolor* or *G. standleyana*) and the interaction between adult tree species and local seedling density (all fixed effects). Adult tree identity was included as a random effect to account for non-independence of seedlings within the same transects.

Study 4- Data were analysed using Generalised Linear Models (GLMs) with quasibinomially distributed errors (to account for overdispersion). Seedling herbivory and seedling survival at the final census (binary response) were both modelled as a function of seedling location (inside/outside seedling carpet), focal seedling species (*C. alliodora* or *C. bicolor*), number of days the seedling was in the field and the interaction between distance from adult tree and focal seedling species. Adult tree identity was included as a fixed effect rather than a random effect, as there were only four levels.

Study 5 - Data were analysed with a GLM with binomially distributed errors. Seedling survival (a binary response) was modelled as a function of initial seedling state (intact/herbivory).

In all analyses the maximal models (including all fixed effects) were simplified by sequentially dropping terms until arriving at a minimum adequate model following the procedures recommended by Crawley (2007). Maximal and minimal adequate models for all analyses are shown in Supplementary Table 1. All analyses were run in RStudio, R version 3.3.1 (2016-06-21) - "Bug in Your Hair" using the packages lme4 (Bates et al., 2015) and multcomp (Hothorn et al., 2008) for statistical analyses, and ggplot2 (Wickham, 2009), for figures.

Results

Do Cordia *seedlings experience density-dependent seedling herbivory; and if so, at what spatial scale do conspecific and congeneric density effects occur?*

In Study 1, the proportion of *C. alliodora* seedlings experiencing herbivory was significantly lower in the low density (one seedling treatment) than in the high density (eight seedlings) (β= -0.4389, Std. Error = 0.2071, t= -2.119, P= 0.0354; Fig. 2). The likelihood of focal *Cordia* seedlings being attacked by herbivores was not affected significantly by the summed basal area of either conspecific or congeneric adults in the neighbourhood (conspecifics: Deviance= -0.0203, F= 0.0516, P= 0.8205; congenerics: Deviance= 0.3177, F= 0.8125, P=0.3685). Study 1 revealed no evidence of spatial autocorrelation in herbivory of *C. alliodora* seedlings across the twenty-five hectare plot (see Supplementary Table 2). Whilst some distances came out as significant in some analyses, this was not consistent over time periods (see Supplementary Table 2).

In Study 2, a total of 788 *C. alliodora* seedlings were present in the surveyed plots, of which 388 (49.2%) had signs of herbivory. Only eight *C. bicolor* seedlings were observed, which is too few to analyse. There were no significant effects of basal area of either *C. alliodora* or *C. bicolor* adults on the likelihood of seedling herbivory (*C. alliodora*: χ^2=1.5632, df= 1, P= 0.2112; *C. bicolor*: χ^2= 0.0159, df=1, P= 0.8996). In Study 2, there was a trend for *C .alliodora* seedlings surrounded by high numbers of conspecific seedlings in the immediate ($0.5m^2$) neighbourhood to be more likely to experience herbivory (β = 0.034, SE = 0.018, z = 1.917, P = 0.055).

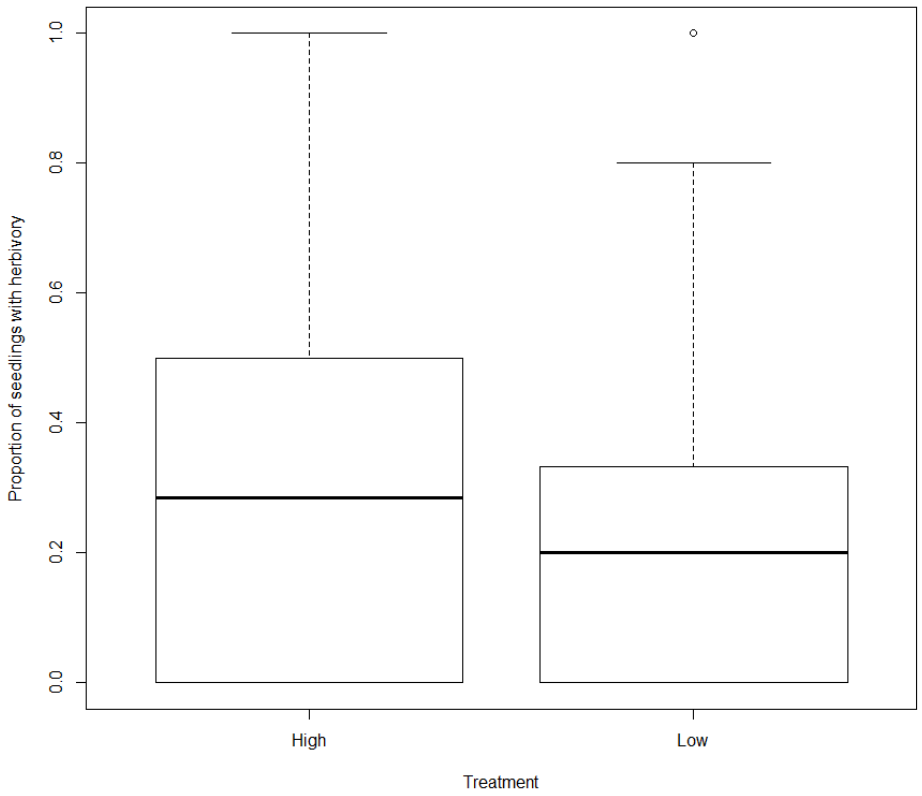


Figure 2- Proportion of seedlings with herbivory in high (eight seedling) and low (one seedling) density treatments.

Is herbivory of seedlings higher closer to conspecific and congeneric adults than elsewhere in the landscape; and if so, at what distance do herbivory levels decline?

In Study 3, of the 468 focal seedlings, 42% had experienced herbivory by the end of the experiment: 58%, 41%, and 27% under *C. alliodora, C. bicolor* and *G. standleyana*, respectively. Neither the distance from an adult tree nor local seedling density significantly affected the likelihood of herbivory on focal *Cordia* seedlings (Distance: χ^2= 2.7822, df= 1, P= 0.09; Density: χ^2= 0.41, df= 1, P= 0.5243). The only fixed effect in the final model was adult tree species: the likelihood of potted *C. alliodora* seedlings being attacked differed significantly between adult tree species (χ^2=10.37, df= 2, P=0.006). More specifically, *C. alliodora* seedlings were less likely to experience herbivory close to hetero-familial adults

(i.e. *G. standleyana*) than under conspecific adults (β= -1.474, SE= 0.404, z= -3.648, P<0.001). There was also a trend for *C. alliodora* seedlings to experience less herbivory close to congeneric *C. bicolor* adults than under conspecific adults (β = -0.756, SE = 0.395, z = -1.915, P=0.055) and for seedlings close to *G. standleyana* to experience less herbivory than those close to *C. bicolor* adults (β= -0.719, SE= 0.399, z= -1.800, P= 0.072) (Fig. 3).

In Study 4, 59% of the 176 focal seedlings experienced herbivory (*C. alliodora* near 74%, *C. alliodora* far 32%, *C. bicolor* near 74%, *C. bicolor* far 56%). All terms were retained in the final model. The significant interaction between distance and species (χ^2= 39.234, df= 1, P <0.001) indicates that the magnitude of the distance effects on seedling herbivory differs between the two species. Seedlings of both *C. alliodora* and *C. bicolor* placed close to *C. alliodora* adults experienced higher herbivory than those placed further away (Fig. 4). This distance effect was significant for both species (*C. alliodora*: β=1.891, SE= 0.126, z=14.970, P<0.001, *C. bicolor*: β= 0.797, SE=0.123, z= 6.506, P <0.001), but was more pronounced for *C. alliodora*. Seedlings of *C. bicolor* experienced significantly higher levels of herbivory than those of *C. alliodora* outside seedling carpets (β=1.086, SE=0.119, z=9.144, P<0.001; Fig. 4) but not within seedling carpets (β=0.008, SE=0.129, z=-0.064, P=1.000; Fig. 4). The likelihood of being attacked increased significantly with time (deviance =21.71, df=1, P<0.001) and also differed among sites (deviance =51.024, df=1, P=<0.001).

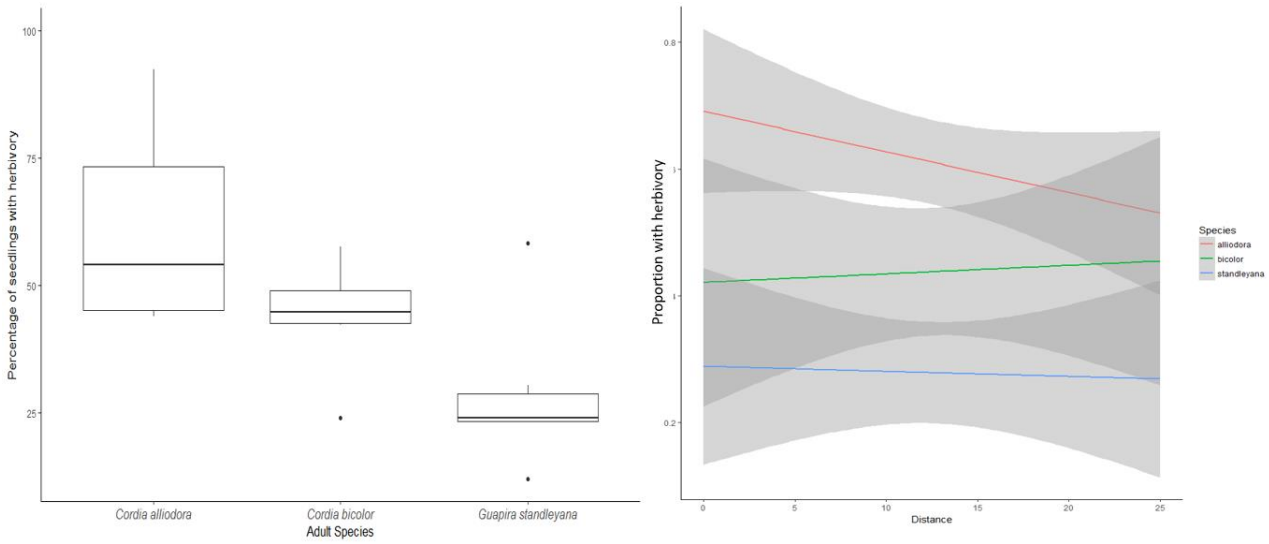


Figure 3- (Left) The percentage of *Cordia alliodora* seedlings placed close to conspecific, congeneric (*C. bicolor*) and heterofamilial (*G. standleyana*) attacked by *Ischnocodia annulus*. Whiskers represent lowest and highest values (outliers excepted), horizontal lines of the boxes refer to q25, median and q75 (bottom to top), and black dots represent outliers (Right) Proportion of seedlings with herbivory at distances (0-25m) from conspecific, congeneric and heterofamilial trees, note all trends are non-significant.

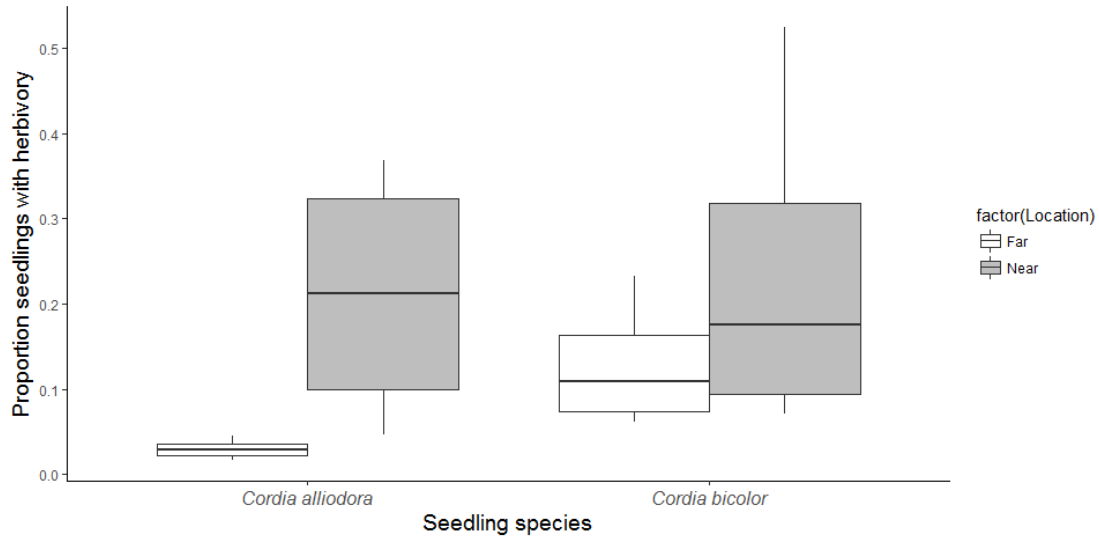


Figure 4- Proportion of seedlings with herbivory by *I. annulus* at different distances (near = inside seedling carpet, far= outside seedling carpet) from adult *C. alliodora* trees. Whiskers represent lowest and highest values), the horizontal lines of the boxes show q25, median and q75 (bottom to top), and black dots represent outliers.

Does herbivory affect Cordia seedling survival?

In Study 5, seedlings of *C. alliodora* were significantly less likely to survive to the end of the experiment if they experienced herbivory (7% survival) than those that did not (40% survival, $\beta = -2.107$, SE =0.682, z = -3.091, P = 0.002; Supplementary Fig. 3). Similarly, in Study 4 seedlings with herbivory were more likely to experience mortality than those without ($\beta = 4.555$, SE = 0.265, z =17.170, P <0.001). The proportions of seedlings that survived throughout the experiment with or without herbivory were 42% and 99%, respectively. The effect of herbivory on seedling survival did not differ significantly between species.

Discussion

Our studies of patterns of herbivory on seedlings of two tropical tree species revealed that herbivory is common and widespread in young (<two months) *Cordia* seedlings. Across data sets, 25-59% of the surveyed seedlings showed signs of herbivory. Seedlings subject to herbivory were less likely to survive than unattacked seedlings, suggesting that patterns of herbivory may translate into patterns of seedling recruitment. The results from our analyses revealed intra-specific density-dependence in one species (*C. alliodora*) and the potential for enemy-mediated interspecific interactions between the two studied *Cordia* species.

Do* Cordia *seedlings experience density-dependent seedling herbivory?

Consistent with predictions of the Janzen-Connell hypothesis (Janzen 1970; Connell 1971) and results from numerous empirical studies (Comita et al. 2014), we found evidence for small scale density-dependent herbivory in *C. alliodora* seedlings. In contrast with many other studies (but see Bell et al. 2006; Bagchi et al. 2014), we have also identified the likely mechanism driving these patterns: herbivory by insects, particularly the beetle *Ischnocodia annulus*.

The documented small-scale density dependence in herbivory suggests a behavioural response by herbivores, which may be more likely to locate clusters of seedlings than more isolated individuals and to feed on multiple plants in such a cluster. Aggregative responses of this sort by herbivores can stabilise consumer-resource interactions (Hassell & May 1973, 1974; Hassell & Pacala 1990). If responses of this sort are frequent in herbivore-plant interactions (e.g. Alvarez-Loayza & Terborgh 2011), this could favour plant species coexistence under the Janzen-Connell mechanism.

Although local seedling density (in the surrounding 0.5m²) did not affect herbivory rates on focal seedlings in Study 4, herbivory was higher under conspecific adults (where there is a higher density of conspecific seedlings) than under congeneric or heterofamilial adults. This suggests that the density of conspecific seedlings does affect herbivory rates, but at a slightly larger scale than we tested.

At what spatial scale do conspecific and congeneric density effects occur?

We did not find evidence of density-dependent herbivory at larger scales (20 × 20m) in our studies. In both Study 1 and Study 2, herbivory did not increase with the summed basal area of adult trees (either *C. alliodora* or *C. bicolor*) in the wider neighbourhood. These results are consistent with those of Zhu et al. (2015) who carried out a comprehensive study of density dependence for 50 North American tree species. They found that effects of conspecific density were pervasive, but the strength varied across life-stages, with the impact of conspecific seedlings being stronger than that of conspecifics in other size classes. Similarly, a meta-analysis of experimental studies of conspecific density-dependence in seedlings found that Janzen-Connell effects were restricted to relatively small spatial scales (Comita et al. 2014). One possible explanation for the lack of density effects at larger spatial scales is that it

is masked or counter-acted by the heterogeneous effects of environmental suitability (Chen et al. 2010, Johnson et al. 2017).

Is herbivory of seedlings higher closer to conspecific and congeneric adults than elsewhere in the landscape; and if so, at what distance do herbivory levels decline?

Previous studies of tropical trees suggest that distance-responsive natural enemies can play a key role in seedling recruitment (Adler & Muller-Landau 2005; Swamy & Terborgh 2010). Herbivory on both *Cordia* seedling species was significantly higher close to reproductive *Cordia alliodora* individuals than further away (outside seedling carpets) (Study 4). Since herbivory was associated with increased seedling mortality, this suggests that the recruitment of *Cordia alliodora* could be suppressed around adult trees, as envisaged by the Janzen-Connell hypothesis (Janzen 1970; Connell 1971). Furthermore, this mechanism could also lead to suppressed recruitment of *C. bicolor*, thus linking the regeneration dynamics of these two species.

Our transect experiment (Study 3) also revealed a trend for higher levels of herbivory close to adults of a congeneric species. Higher herbivory of *C. alliodora* seedlings close to adults of *C. alliodora* than close to adults of *C. bicolor* could result if the dense seedling carpets associated with *C. alliodora* (but not *C. bicolor*) are particularly attractive to enemies. Alternatively, herbivores may have a feeding preference for *C. alliodora* seedlings regardless of abundance, causing them to aggregate near the adult trees. Working in Peru, Alvarez-Loayza & Terborgh (2011) found that mortality rates of conspecific seedlings within seedling carpets were much higher than those of heterospecific seedlings, but their study does not allow a comparison of the survival of these seedlings outside seedling carpets.

All transects of Study 3 (including heterofamilial *G. standleyana* transects) experienced herbivory, suggesting that *Cordia* enemies can readily disperse and locate seedlings over

distances of 30m (the minimum isolation distance from other *Cordia* adult trees). More extensive and systematic exposure of experimental seedlings, combined with widespread trapping of beetles would be needed to assess how enemy pressure varies across the landscape in relation to *Cordia* distances and densities.

Does herbivory affect Cordia seedling survival?

Herbivory was strongly associated with subsequent seedling mortality for both *Cordia* species. While herbivory can be a symptom of plant stress (White 1984), the direction of causality in this case is likely to be top-down, with herbivores reducing plant fitness, perhaps by reducing the leaf surface area available for photosynthesis in the shaded forest understorey. Previous studies of tropical tree seedlings have documented similar results (Eichhorn et al. 2010; Norghauer & Newbery 2014).

Herbivory thus appears to be an important source of mortality during the early months of seedling establishment. However, it is worth noting that, independent of herbivory, survivorship will inevitably be low for young seedlings of *C. alliodora*, which is a light-demanding species with seedlings that are unable to persist for extended periods in the shaded understorey. Thus, if seedlings germinate and remain in shaded conditions, mortality will be inevitable within a few months or years unless a light gap opens nearby. Nonetheless, short-term dynamics within seedling carpets could still determine the distribution of survivors in the longer term, providing the template on which later causes of morality act (Harms et al. 2000). Further longer-term study is needed to investigate the full implications of seedling mortality for *Cordia* population dynamics.

Community-level consequences

In the shaded understorey of tropical forests seedling densities are usually low, and competition between seedlings for light and other limiting resources is weak (Svenning et al.

2008; Paine et al. 2008). This opens up the opportunity for alternative mechanisms, such as interactions with natural enemies, to play a role in maintaining and structuring plant diversity (Wright 2002). In the case of *C. alliodora*, density-dependent herbivory is likely to cause density-dependent mortality. If other co-occurring plant species show similar effects, this could promote species coexistence and diversity (Bagchi et al. 2010; Chen et al. 2010).

Our results also highlight potential for enemy-mediated indirect interactions between *Cordia* species. We documented higher herbivory on *C. alliodora* seedlings close to adults of *C. bicolor* than close to non-*Cordia* species, and found elevated rates of herbivory of *C. bicolor* inside *C. alliodora* seedling carpets. Although our pattern-based study does not reveal the longer-term consequences of shared herbivory in the system, our observations suggest that enemy-mediated indirect interactions with potential consequences for species recruitment might operate in this system. Positive aggregative responses by a consumer to multiple resource species can lead to short-term apparent competition (Holt & Kotler 1987; Holt & Bonsall 2017): a negative relationship between the population growth rates of the resource species. Shared natural enemies can alter the balance of competitive dominance between resource species (Hanley & Sykes 2009), potentially promoting coexistence, for example by favouring species that are poor competitors but which can cope with high enemy pressure (Kuang & Chesson 2009).

Working at the same field site, Garzon-Lopez et al. (2015) found that seed mortality caused by mammalian and insect seed predators in their three study species (all trees) was better explained by densities of both conspecifics and heterospecifics, rather than conspecific densities alone. If such effects are widespread in tropical forests they could act as an important structuring force. Since herbivores and other plant enemies are often specialised at the level of genus or family (e.g. Gilbert & Webb 2007; Novotny et al. 2012), we would expect plant species to be linked to the density of (and distance to) closely related plant

species, potentially leading to phylogenetic overdispersion of plant communities and ultimately increasing 'phylodiversity' (Webb et al. 2006). Uncovering the full role of indirect interactions will require additional experiments for a wider range of species, quantifying both the functional response of natural enemies to co-occurring species, and the longer-term consequences for plant population dynamics and diversity.

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Author Contributions

HD, SG, OL and MB conceived and designed the study. HD carried out data collection for all studies except study 2. CF carried out field work for Study 2 and undertook initial surveys of the study system. HD analysed the data, interpreted the results and drafted the manuscript with input from all authors.

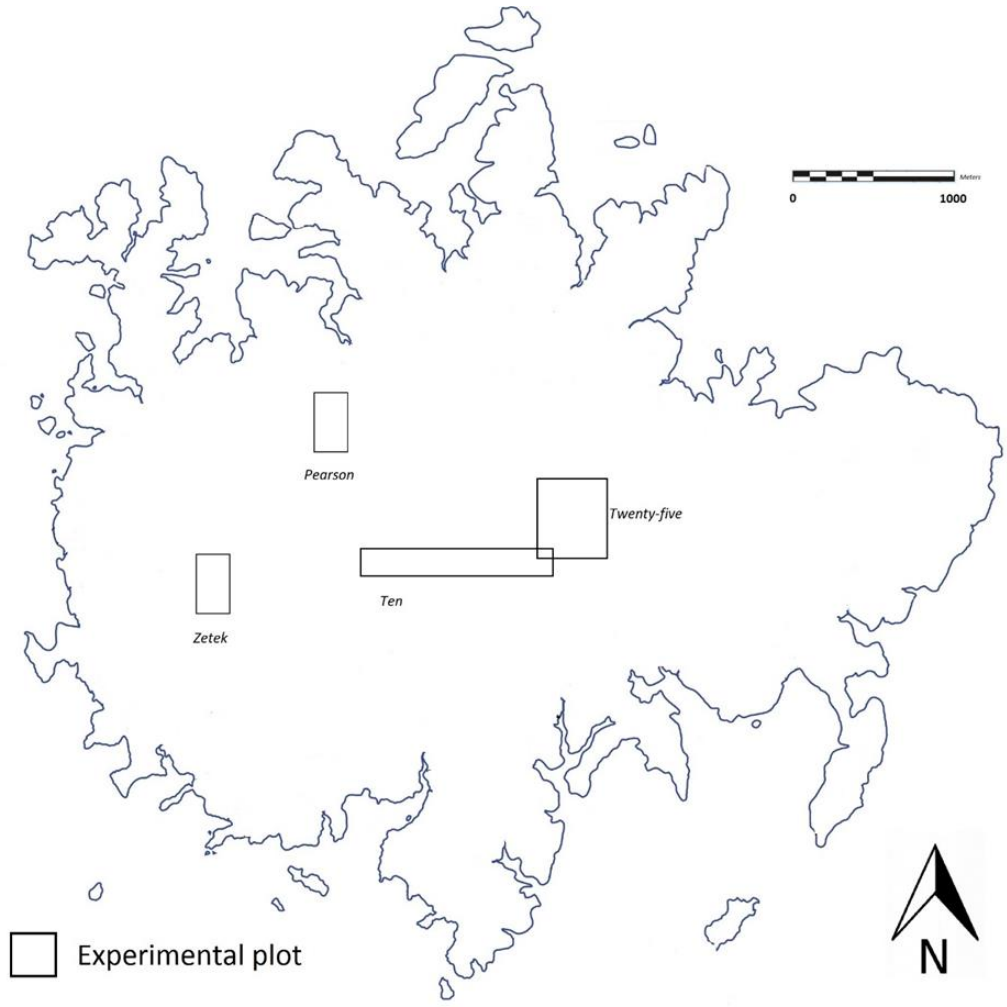
Data Accessibility

Final data sets for all studies will be made available on Dryad (doi to be added).

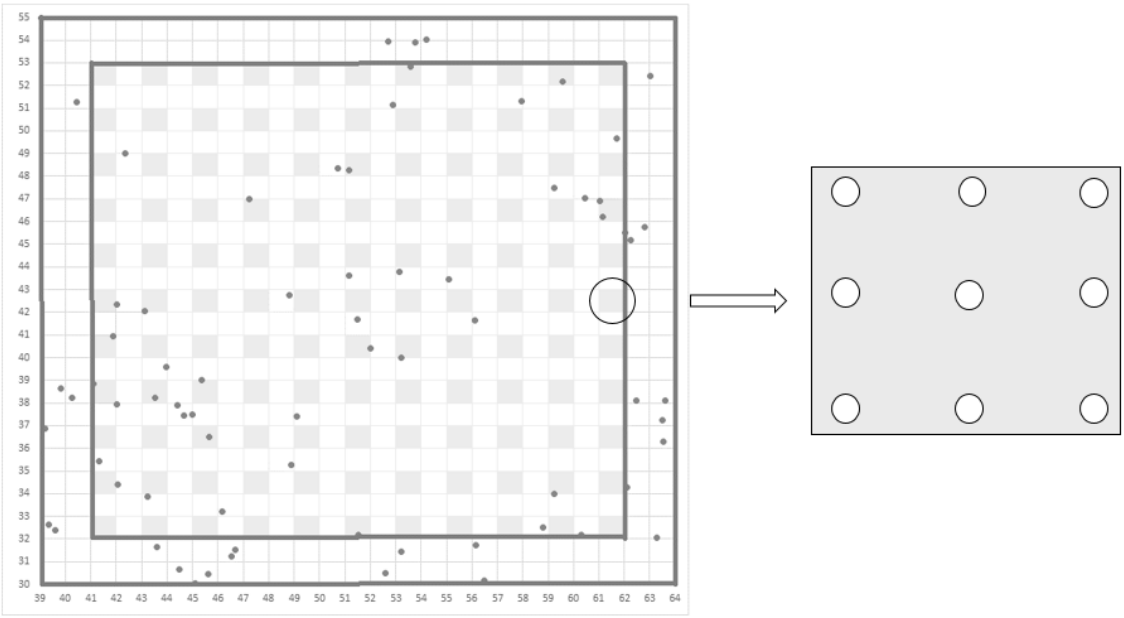
Supplementary Information

Chapter 4

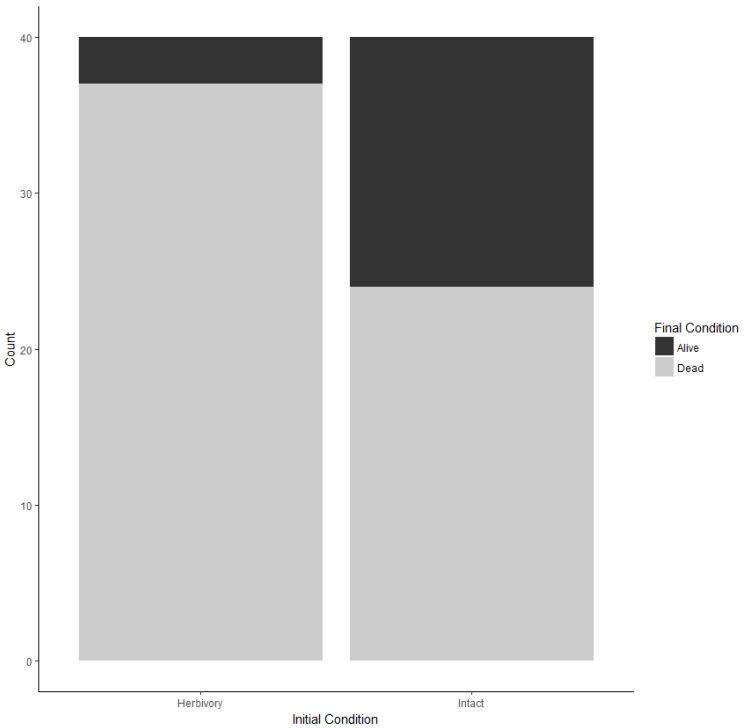
Supplementary Material Chapter 4



Supplementary Figure 1 - Map of Barro Colorado Island showing the location of experimental plots.



Supplementary Figure 2- The 20m × 20m grid system of the twenty-five hectare plot including the 40m buffer zone along the plot edges. Grey dots show the location of adult *Cordia* trees. Shaded squares indicate subplots where potted seedlings were introduced to nine locations according to the design on the right.



Supplementary Figure 3- Final fate (alive or dead) of seedlings with either herbivory or no herbivory at the end of a six week census period.

Supplementary Table 1 - Structure of the statistical models used, indicating the type of model used and the response and explanatory variables for both maximal and minimum models.

Study	**Model Type**	**Error Structure**	**Response variable**	**Random effects**	**Fixed effects (maximal model)**	**Fixed effects (minimum adequate model)**
Study 1	GLMM	Quasibinomial (to account for underdispersion)	Seedling herbivory (proportion per subplot)	Plot number	Focal seedling treatment (high/low density) + summed basal area of *C. alliodora* adults in neighbourhood+ summed basal area of *C. bicolor* adults in neighbourhood.	Focal seedling treatment.
Study 2	GLMM	Binomial	Seedling herbivory (proportion per subplot)	Plot number + to account for overdispersion in the data, an observation level random factor	Local density of *C. alliodora* seedlings (in 0.5m² subplot)+ local density of *C. bicolor* seedlings (in 0.5m² subplot) + summed basal area of *C. alliodora* adults (20m x 20m) and summed basal area of *C. bicolor* adults (in 20 x 20m plot).	Local density of *C. alliodora* seedlings (in 0.5m² subplot).

				was included in the model.		
Study 3	GLMM	Binomial	Herbivory (binary response)	Adult tree ID	Seedling distance (m) from adult tree +local seedling density + adult tree species (*C. alliodora*, *C. bicolor* or *G. standleyana*) + adult tree species*local seedling density	Adult tree species
Study 4	GLM	Quasibinomial (to account for overdispersion)	Seedling herbivory (binary response)	NA	Location from adult tree (inside/outside seedling carpet) + focal seedling species (*C. alliodora* or *C. bicolor*)+ number of days focal seedling in field+ distance from adult tree* focal seedling species+ adult tree ID.	All terms, including the interaction term.
Study 4	GLM	Binomial	Seedling survival (binary response)	NA	Herbivory by *I. annulus* (yes/no).	Herbivory by *I. annulus* (yes/no).
Study 5	GLM	Binomial	Seedling survival (binary).	NA	Initial seedling state (intact/herbivory).	Initial seedling state (intact/herbivory)

Supplementary table 2- Model outputs for spatial autocorrelation analysis

Model	Distance	Random Effects		Fixed Effects			
		Variance	Std. deviation	Estimate	Std. Error	Z value	P value
Census Two	Intercept	20.85	4.566	-6.121	1.802	-3.397	0.001
Census Two	10-20m	20.85	4.566	1.728	1.458	1.186	0.236
Census Two	20-30m	20.85	4.566	-7.395	4.203	-1.76	0.078
Census Two	30-40m	20.85	4.566	-5.339	6.4	-0.834	0.404
Census Two	40-50m	20.85	4.566	1.212	5.021	0.241	0.809
Census Two	50-60m	20.85	4.566	-22.5	10.921	-2.06	0.039
Census Two	60-70m	20.85	4.566	4.372	4.955	0.882	0.378
Census Two	70-80m	20.85	4.566	2.309	5.867	0.394	0.694
Census Two	80-90m	20.85	4.566	-3.369	9.054	-0.372	0.71
Census Two	90-100m	20.85	4.566	-48.23	20.011	-2.41	0.016
Census Three	Intercept	0.5829	0.7635	-3.14	0.562	-5.586	2.32E-08
Census Three	10-20m	0.5829	0.7635	1.192	0.566	2.108	0.035
Census Three	20-30m	0.5829	0.7635	1.639	1.193	1.374	0.169

Census Three	30-40m	0.5829	0.7635	3.356	1.191	2.819	0.005
Census Three	40-50m	0.5829	0.7635	-3.295	2.401	-1.372	0.17
Census Three	50-60m	0.5829	0.7635	1.914	2.059	0.93	0.353
Census Three	60-70m	0.5829	0.7635	3.325	2.041	1.629	0.103
Census Three	70-80m	0.5829	0.7635	-1.507	2.694	-0.559	0.576
Census Three	80-90m	0.5829	0.7635	-0.348	3.226	-0.108	0.914
Census Three	90-100m	0.5829	0.7635	-4.357	2.904	-1.501	0.133
Census Four	Intercept	0.3697	0.6081	-2.3	0.552	-4.161	3.17E-05
Census Four	10-20m	0.3697	0.6081	0.279	0.482	0.058	0.954
Census Four	20-30m	0.3697	0.6081	0.724	1.169	0.62	0.535
Census Four	30-40m	0.3697	0.6081	0.841	0.943	0.892	0.373
Census Four	40-50m	0.3697	0.6081	1.418	1.402	1.012	0.312
Census Four	50-60m	0.3697	0.6081	0.826	1.492	0.553	0.58
Census Four	60-70m	0.3697	0.6081	2.523	1.511	1.67	0.095
Census Four	70-80m	0.3697	0.6081	-1.842	1.888	-0.976	0.329
Census Four	80-90m	0.3697	0.6081	0.014	2.129	0.006	0.995
Census Four	90-100m	0.3697	0.6081	-2.01	1.781	-1.128	0.259

Census Five	Intercept	0.1447	0.3803	-1.574	0.401	-3.929	8.54E-05
Census Five	10-20m	0.1447	0.3803	0.054	0.421	0.129	0.898
Census Five	20-30m	0.1447	0.3803	0.685	0.741	0.937	0.349
Census Five	30-40m	0.1447	0.3803	1.42	0.783	1.814	0.07
Census Five	40-50m	0.1447	0.3803	0.607	1.076	0.565	0.572
Census Five	50-60m	0.1447	0.3803	0.263	1.115	0.235	0.814
Census Five	60-70m	0.1447	0.3803	0.346	1.218	0.284	0.776
Census Five	70-80m	0.1447	0.3803	-0.95	1.309	-0.726	0.468
Census Five	80-90m	0.1447	0.3803	-1.945	1.66	-1.172	0.241
Census Five	90-100m	0.1447	0.3803	0.146	1.357	0.107	0.914
Census Six	Intercept	0	0	-1.319	0.38	-3.472	0.001
Census Six	10-20m	0	0	0.43	0.397	1.084	0.279
Census Six	20-30m	0	0	1.907	0.616	3.095	0.002
Census Six	30-40m	0	0	0.17	0.798	0.214	0.831
Census Six	40-50m	0	0	-1.803	1.1	-1.64	0.101
Census Six	50-60m	0	0	-0.45	1.005	-0.448	0.654
Census Six	60-70m	0	0	1.278	1.095	1.167	0.243

Census Six	70-80m	0	0	0.032	1.23	0.026	0.979
Census Six	80-90m	0	0	0.726	1.439	0.504	0.614
Census Six	90-100m	0	0	-2.343	1.415	-1.656	0.098
Census Seven	Intercept	0.2068	0.4548	0.48	0.639	0.752	0.452
Census Seven	10-20m	0.2068	0.4548	0.214	0.338	0.634	0.526
Census Seven	20-30m	0.2068	0.4548	-1.073	0.606	-1.771	0.077
Census Seven	30-40m	0.2068	0.4548	0.142	0.6	0.236	0.814
Census Seven	40-50m	0.2068	0.4548	1.103	0.813	1.356	0.175
Census Seven	50-60m	0.2068	0.4548	0.013	0.869	0.015	0.988
Census Seven	60-70m	0.2068	0.4548	-0.52	0.938	-0.555	0.579
Census Seven	70-80m	0.2068	0.4548	-1.411	1.088	-1.297	0.195
Census Seven	80-90m	0.2068	0.4548	0.938	1.328	0.706	0.48
Census Seven	90-100m	0.2068	0.4548	-1.019	1.102	-0.924	0.355

Supplementary Table 3 - Model outputs for the statistical analyses.

Model	Final model	Response	Effects	Estimate	Std. Error	Z value
Study 1	Herbivory~	Herbivory	Intercept (high	-0.80	0.15	-5.18
	treatment		density)	-0.43	0.21	-2.08
			Low density			
Study 2	Herbivory~	Herbivory	Intercept	-0.57	0.41	-1.39
	conspecific seedling		Number conspecific	0.03	0.02	1.91
	density		seedlings			
Study 3	Herbivory~ adult	Herbivory	Intercept (*Cordia*	0.50	0.28	1.77
	tree species		*alliodora)*	-7.56	0.39	-1.92
			Cordia bicolor	-1.47	0.40	-3.65
			Guapira standleyana			
Study 4	Herbivory~	Herbivory	Intercept (Species:	-3.41	0.48	-7.04
			C. alliodora,	1.09	0.12	9.14
			Distance: far, Site1)	1.89	0.13	14.97

			Species (*C. bicolor)*	0.55	0.12	4.53
			Distance (near)	0.38	0.12	3.15
			Site 2	0.86	0.13	6.89
			Site 3	0.04	0.01	4.64
			Site 4	-1.09	0.18	-6.24
			Number days			
			C. bicolor x near			
Study 4	Survival~ herbivory	Survival	Intercept	-4.23	0.26	-16.28
			Herbivory	4.55	0.27	17.18
Study 5	Survival~ herbivory	Survival	Intercept	-4.23	0.26	-16.28
			Herbivory	4.55	0.27	17.18

Chapter 5

General Discussion

Despite decades of research, the question of what maintains and structures tropical forest plant diversity remains topical (Fricke et al. 2014; Stump & Chesson 2015; Murphy et al. 2017; Stump & Comita 2018). Numerous recent studies document evidence for conspecific negative density-dependence in patterns of plant mortality in line with the Janzen-Connell hypothesis (synthesised in Comita et al. 2014). However, surprisingly little work has been done to assess patterns of density-dependence *across* species.

With the knowledge that many plant enemies have diet breadths that span more than just one species (Gilbert & Webb 2007; Novotny et al. 2010), investigating the effects of enemies with diets spanning several plant species is timely. This thesis aimed to uncover the role of non-specialist insect enemies in structuring tropical tree communities with a special focus on insects attacking seeds and young seedlings. Below, I discuss each of the main findings.

Main findings

1. **Insects are a major cause of mortality in the early life stages of tropical trees**

The work presented in this thesis shows that insect herbivores and seed predators can cause a substantial amount of damage to their hosts during the early stages of tropical tree regeneration, making them important natural enemies to consider in these systems. For example, on individual Lauraceae trees, up to 96% of seeds were attacked by pre-dispersal seed predators (chapters 2 and 3), and the majority of *Cordia alliodora* seedlings close to *Cordia* trees were attacked by insect herbivores within weeks of germination (Chapter 4). The early stages in the plant life cycle are important demographic bottlenecks, as a large percentage of mortality happens at the seed and seedling stages (Fenner & Thompson 2005; Leck et al. 2008). In the context of tropical forests, processes operating at the early life stages can be important for patterns of diversity at later stages (Green et al. 2014) and

diversity-enhancing processes are known to operate at the seed-to-seedling transition stage (Harms et al. 2000; Bagchi et al. 2014). This makes it particularly important to investigate what the main causes of mortality at these stages are, and whether the main drivers of mortality are density-dependent. Nevertheless, many studies investigating the Janzen-Connell hypothesis evaluate the density-dependence of seedling survival without attempting to uncover the mechanism responsible for any patterns observed (but see Bagchi et al., 2010, 2014). Where the causes of mortality have been considered, particular attention has been given to the effects of pathogens on these early life stages (Bell et al. 2006; Mangan et al. 2010; Bagchi et al. 2010b). Although we did not specifically quantify pathogen mortality within our systems, our observations suggest that the effects of pathogens in our two study systems appears to be minimal at the studied life stages. By quantifying losses to particular groups of enemies, and understanding how different enemy groups interact, we can assess how each may contribute to structuring plant communities (Fricke et al. 2014; Tiansawat et al. 2017).

2. Insects respond to spatial variation in resource densities

Within both study systems, we found evidence of insects responding to spatial variation in resource densities across several spatial scales. In the Lauraceae system we saw density-dependence (the direction of which varied between years) both at a local (individual tree fruit crop) and population level (the wider Lauraceae neighbourhood), and the insect seed predators responded to both conspecific and heterospecific seed densities.

In the *Cordia* system, conspecific density-dependence was recorded for *C. alliodora* at small spatial scales, but not at larger scales. Schupp (1992) showed that the direction of density-dependence can switch depending on the spatial scale at which it is being studied. Hence, studying density effects, whether within or across species, across multiple spatial

scales is important. Testing density effects at large scales can be challenging. Mapped plots (such as the ForestGeo plot network, of which the 50ha plot BCI is part) are an excellent resource to achieve this.

3. There is the potential for indirect interactions to occur between tree species in tropical forests

The studies presented in this thesis suggest that there is potential for indirect interactions between host plant species mediated via their shared insect enemies. For Lauraceae, we detected strong potential for indirect interactions between five species using a quantitative food web approach (Chapter 2) and found that the patterns of seed predator attack and premature fruit abscission rates were affected not only by the densities of conspecifics, but also of congenerics in the neighbourhood (Chapter 3). Within the *Cordia* system, we recorded higher levels of seedling attack for *C. bicolor* close to *C. alliodora* adults than further away, and also detected a trend for *C. alliodora* seedlings to experience greater herbivory rates close to congeneric adults than elsewhere in the landscape. It is difficult to predict how widespread these types of interactions may be in tropical forests. One potential reason why little attention has been given to enemy-mediated indirect interactions in tropical forest plant communities could be the scarce information on patterns of plant-enemy associations. As many natural enemies of plants are known to attack more than one host species (Gilbert & Webb 2007; Novotny et al. 2010), the potential for indirect interactions could be high (Holt & Bonsall 2017), but identifying and quantifying the interactions is more difficult. Some studies (e.g. Gilbert & Webb 2007) have suggested that phylogenies may help in predicting which plant species are most likely to share enemies. Closely-related plant species are thought to be more likely to share natural enemies as they will often have similar morphology and chemistry. Studies have indeed shown phylogenetic signals in the host ranges of herbivorous insects (Novotny et al. 2002;

Novotny & Basset 2005) and pathogens (Gilbert & Webb 2007) providing promising insights into this area. Other studies suggest that species traits such as seed size may be good indicators of which plant species may be most likely to share enemies (Beckman & Muller-Landau 2011) since host species with similar morphological trait values are likely to be attractive to the same enemies. Compiling trait data for the multitude of species in tropical forests is challenging, but information is rapidly accumulating in various trait databases (Wright et al. 2013). The quantitative food web approach presented in Chapter 2 of this thesis has not previously been applied in the context of tropical forest plants and their enemies, but could be a useful tool in identifying enemy diet breadths and the potential for enemy-mediated indirect interactions although it may be more difficult to apply to enemy groups such as seedling herbivores and pathogens than to the internally feeding seed predators targeted in our study, due to the difficulty in quantifying interaction rates for these types of enemies.

Given the scarcity of studies on the topic, it is too early to tell whether or not the results obtained from the studies presented in this thesis are representative of the wider plant community. Further work will be needed to tell if enemy-mediated indirect interactions are widespread within tropical plant communities, and whether they should be taken into account alongside the more commonly studied conspecific density effects outlined in the Janzen-Connell hypothesis (Janzen 1970; Connell 1971).

4. Indirect interactions may be asymmetric, and there can be temporal variation in the strength and direction of indirect interactions

The results presented in this thesis suggest that indirect interactions in our study systems may be asymmetric (with one species experiencing a negative effect whilst the other experiences a weak or neutral effect). In the Lauraceae system, species that produced the

greatest volume of fruits (*B. pendula* in 2015 and *O. puberula* in 2016) seemed to have a strong effect upon species producing fewer offspring, possibly by acting as a source of enemies to the rest of the community (Chapter 2). Similar data is not available for the *Cordia* system so further information is required to assess whether the less abundant *Cordia* species has a similar strength effect on the more abundant species.

Results from these studies are in line with results obtained from work conducted in other systems: indirect interactions are often asymmetric in nature, particularly those involving plants and a shared herbivore (Chaneton & Bonsall 2000). For shared predation to promote coexistence, the enemy must attack their main host (the most abundant species) more than other hosts (Chesson & Kuang 2008; Stump & Chesson 2015). If the shared enemy does not differentiate between hosts, it may exclude the host that is less able to cope with a high enemy load from the local community (Bonsall & Hassell 1997).

Although difficult to ascertain whether it is a true pattern from just two years' worth of data, we also found temporal variation in enemy attack. In Lauraceae, the percentage of fruits attacked by enemies differed between the two years (Chapter 2), with lower enemy attack rates in years of high seed abundance, in line with results from other studies (Xiao et al. 2017). The direction of density effects of conspecifics and congenerics on seed predator attack and fruit abscission rates also differed between the two years (Chapter 3). These results may be confounded by the strong El Niño event of 2015, but if it is a true reflection of the dynamics of these systems, it could have important implications for enemies in the Lauraceae system.

Future directions

The work presented in this thesis is of a purely empirical nature, and only begins to document patterns produced by non-specialist insect enemies. Other approaches should be considered (see postscript). Further experimental studies, theoretical work and a good predictive framework for the breadth of enemy host ranges could add substantially to our understanding of the role of non-specialist enemies in tropical forest plant communities.

Empirical studies

Although the observational data collected in this thesis suggest that insects are important enemies in the early life stages of tropical trees, it would be important to quantify the effect of insect enemies on seeds and seedlings experimentally. Carefully designed experiments removing either host plant species or the natural enemies should be undertaken. This would allow the strength and direction of indirect interactions to be uncovered. Removal experiments have been undertaken in host-parasitoid systems in the tropics (Morris et al. 2004) and in non-tropical plant-herbivore systems (reviewed in Chaneton & Bonsall 2000), but have yet to be undertaken in systems involving tropical plant species sharing insect enemies.

One difficulty with studying long-lived organisms such as trees is detecting long-term apparent competition (Holt 1977) (i.e. apparent competition where the population of natural enemy responds demographically to prey densities). We are only able to speculate on long-term patterns in the study systems investigated in this thesis. Trapping of predators over multiple years would be necessary to assess how they respond to fluctuations in prey densities and to predict how this may affect the system in the long-term.

Theoretical work

Whilst theoretical work has shown that shared enemies have the potential to shape the communities of which their hosts are members (Holt & Lawton 1993; Chesson & Kuang 2008) using results from experiments to help uncover numerical and functional responses (see postscript of this thesis) of enemies could help improve these models and aid in predicting the strength and direction of interactions over a range of resource densities (Abrams et al. 1998). Comparing results from experiments to model predictions would also allow us to see if what we expect to find is what plays out in real systems

Assessing patterns of host use

Due to the overwhelming number of species present in tropical forests, it is a major challenge to work out which plant species are experiencing attack exclusively by specialists, which by generalists and which by both. Building a framework to help predict where they may be occurring will aid in understanding how each group affects plant community structure and diversity. A combination of use of phylogeny or morphological traits as previously suggested and detailed study of trophic interactions (e.g. Novotny & Basset 2005; Novotny et al.2010), or with a quantitative food web approach where possible.

In addition, when assessing the potential for enemy-mediated indirect interactions based on observed patterns of host associations (as done for the Lauraceae enemies in Chapter 2), it would be useful to test for host-associated genetic differentiation in the enemy population (study 2 postscript). This may be important in these types of studies, as if cryptic host races associated with individual plant species (Mccoy et al. 2001; Blair et al. 2005) occur, seemingly non-specialist (based on morphological identifications) enemy species may in fact act like highly host-specific enemies.

Conclusions and applied significance

Overall, this thesis shows that enemies that attack more than one host plant species have the potential to affect the reproductive outputs and link the regeneration dynamics of their host species via enemy-mediated indirect interactions. This could have demonstrable consequences for the distribution and diversity of plant species within tropical forest systems.

Understanding how insect enemies of plants respond to the presence of multiple host plant species can be useful in an applied context. Whilst the applied implications of studying enemy-mediated indirect interactions have been discussed in the literature (Chailleux et al. 2014), the potential importance of non-specialist enemies is often not considered. Some of the ideas discussed in this thesis could be of relevance to tropical agroforestry, where economically valuable species may share pests with non-valuable species. Less valuable species could be used as a form of biocontrol, acting as 'trap crops' to lure pests away from the main crop. In addition, understanding the concepts of enemy-mediated indirect interactions could be important in the context of anthropogenic activities such as selective logging. For example, where economically valuable timber species are removed from the forest, this could have unexpected consequences on the dynamics of other species with which the logged tree species chares enemies.

Postscript

In field based ecology, studies sometimes fail despite careful planning. Three studies that were initiated during the course of this thesis could not be completed due to a variety of reasons. Below, I outline the aims and methods of these studies, and give a brief explanation of why they were not included in the data chapters of the thesis.

Study 1: The effects of shared enemies at the seed-to-seedling transition stage

Background and aims

Understanding the processes causing mortality at individual life stages (such as the pre-dispersal seed mortality caused by insects in the Lauraceae system) can reveal important patterns, but whether these patterns are also found at other life stages (which would further highlight their importance) cannot be ascertained from looking at only one life stage. Since suppressed recruitment under high resource abundances could serve as a mechanism allowing species to coexist (as proposed by the Janzen-Connell hypothesis), understanding how the abundance of conspecifics and alternative host species in the neighbourhood affects recruitment is important.

This study aimed to understand whether survival of Lauraceae at the seed-seedling transition stage is affected by densities of conspecific and/or congenerics and confamilials in the surrounding landscape.

Methods

As a common and reliable fruiter, *Beilschmedia pendula* was selected as the focal species for this study. Four seed traps were set up under each of 16 trees (we had aimed for a minimum of 20 reproductive adults of this species but this was not possible). 3 x 1m² seedling plots were established next to each trap, resulting in a total of 192 plots. In each

plot, emerging *B. pendula* seedlings were counted and tagged with bird bands. Plots were be censused weekly/ bi-weekly, then monthly thereafter. At each census old and new recruits were be recorded. Data on the distribution of Lauraceae in the mapped plots were to be used to assess neighbourhood identity. Analogous to the work presented in Chapter 3, we aimed to calculate whether successful establishment of seedlings was related to local seedling densities (i.e. seedling densities in the $1m^2$ plots) and to the connectivity of the focal tree to other Lauraceae individuals within the mapped forest plots.

Reason for incompletion

Over the course of the study fewer than 100 seedlings were recorded across the network of plots, too few to undertake a meaningful analysis. If more time had been available it may have been possible to establish a wider network of seedling plots to increase sample numbers.

Study 2: Population genetics study of *Pagiocerus frontalis*

Background and aims

The investigations that form Chapter 2 of this thesis suggest that there is the potential for indirect interactions between species in the Lauraceae family mediated by their shared pre-dispersal insect seed predators. The scolytid beetle, *Pagiocerus frontalis,* formed the vast majority (nearly 90%) of these seed predating insects. I hypothesised that this beetle has the potential for mediating indirect interactions between some of its host species by suppressing the reproductive success of trees in parts of the forest where there are many fruiting Lauraceae trees.

This prediction was built upon the assumption that *P. frontalis* individuals associated with different species form a homogeneous population. One challenge when predicting whether

insect herbivore species are likely to cause inter-specific density or distance effects on their hosts is the cryptic host race formation observed in some systems (Mccoy et al. 2001; Blair et al. 2005). Whilst an insect herbivore species may appear to be associated with multiple host plant species, there is a possibility that individuals encountered on different plant species may in fact form separate, reproductively isolated, host races with little or no interbreeding. If this is true, these seemingly non-specialist enemies may in fact act like host species-specific enemies, with little potential for mediating indirect interactions between their host species. The possibility of host-associated population differentiation has typically not been tested for in previous studies of indirect interactions mediated by shared enemies, despite its potential to drastically change predictions of how these enemies may shape their host communities.

The main objective of the proposed study was to test whether *P. frontalis* forms a genetically panmictic population or whether individuals reared from different Lauraceae species form separate genetically distinct population clusters.

The molecular data was intended not only to form a part of one of my DPhil thesis (Chapter 2), but would also allow me to prepare a separate publication on factors influencing the genetic population structure of tropical insect herbivores. Although herbivorous insects in tropical forests form an important part of global biodiversity, few studies have investigated what factors contribute to structuring insect populations in diverse tropical forest ecosystems.

Methods

Pagiocerus frontalis samples had been collected during the peak of Lauraceae fruit production (February- August) in 2015 and 2016 as part of my data collection for a quantitative plant-seed predator food web (Chapter 2).

To assess genetic differentiation between insect individuals collected from different tree species and from sites differing in their spatial isolation from each other, I used the data base of available samples to select up to ten *P. frontalis* individuals reared from each of ten individual trees per Lauraceae species (for a total of 700 samples), making sure that spatial location and host plant species were not confounded. In total, I was able to select 124 samples that met these criteria, with only one individual for some host plant species. This was considerably fewer than I had hoped for (Table 1).

Table 1- Samples of *P. frontalis* available to use in population genetics study

Host plant species	**Number of tree individuals available for analysis**	**Number of *P. frontalis* individuals available for analysis**
B. pendula	8	38
O. oblonga	6	25
O. puberula	5	50
O. cernua	0	0
O. whitei	2	3
N. cissiflora	3	4
N. lineata	2	4

For each *P. frontalis* individual I sequenced the mitochondrial cytochrome c oxidase subunit 1 (COI) region. Based on analysis of these gene sequences, I was planning to test whether individuals reared from the same Lauraceae species are more genetically similar

than individuals reared from different host species, and if so, whether the phylogenetic distance and trait similarity of different pairs of Lauraceae species explains the genetic distance observed between insects associated with them. I was also planning to test whether the genetic dissimilarity of individuals increases with increasing distance between collection sites (i.e. isolation by distance analysis).

Reasons for incompletion

DNA extraction and sequencing was attempted twice. In the first trial I used primers 3 and 10 (to enable us to sequence two regions of DNA sequence known to be suitable for population genetic analysis), but we were unable to extract sufficient amounts of DNA for sequencing. I tried a second batch using primers 3 and 4, and was able to get higher quantities of DNA with this. Nevertheless, only six samples produced sequence reads that were useable. The potential reasons for the low sequencing success rate were discussed with a member of the genetics department who supervised the lab work. It seemed likely that the hard cuticle of the beetle made it difficult to extract sufficient quantities of DNA from the beetles (M. Herbert, pers. comm.).

Following the unexpected relocation of the Zoology department, the laboratory work was delayed by several months and some equipment that may have helped in sample preparation was no longer available for use. Due to the low number of samples available, and after two failed attempts, I decided not to proceed any further with the project.

Study 3: Functional response of *Ischnocodia. annulus* to varying densities of host species

Background and aims

Knowing that a plant enemy attacks more than one host species can give some insights into how it may affect the dynamics of its host species. However, to truly uncover its effects, it is necessary to understand how it responds to varying densities of its host species. This can help in predicting the strength and direction of any potential indirect interactions over population fluctuations of the host plants species. The aim of this study was to uncover the functional response of the beetle *Ischnocodia annulus* to *Cordia alliodora* and *Cordia bicolor* seedlings in the field.

Methods

The Zetek (6ha) plot on Barro Colorado Island was used for this experiment. Every *Cordia* adult tree in the plot was visited and its reproductive status recorded. *Cordia bicolor* seedling carpets were located and seedling densities assessed using $1m^2$ quadrats located within these seedling carpets. The densities were categorised as:

Low – 1-5 seedlings, Medium- 6-10 seedlings, High- 11+ seedlings

Cordia alliodora seedlings were collected from beneath trees in the laboratory clearing and potted in sterilised soil and allowed to acclimatise in a shade house for two days before being taken into the forest. The experiment was then set up in blocks. Each block consisted of five low density *C. bicolor* quadrats, five medium density *C. bicolor* quadrats and five high density *C. bicolor* quadrats. These were in random locations in the Zetek plot.

Within each *C. bicolor* density treatment (Low/Medium/High), one replicate of each of five *C. alliodora* densities treatments was placed. These *C. alliodora* densities were 0, 1, 10, 30 and 70 seedlings (for a total 15 experimental sites per block). In total, we had

sufficient *C. alliodora* seedlings for 6 blocks. These were revisited weekly and both the *C. alliodora* and *C. bicolor* seedlings were scored for herbivory (binary response: yes/no).

Reasons for incompletion

During the second week of the experiment, the majority of the *C. alliodora* seedlings were destroyed in storms on the island. We continued to monitor the remaining seedlings for a third week, however, the number of seedlings was too low to conduct a meaningful analysis. Due to the phenology of the species, and the considerable time it had taken to establish the experiment in the first place, it was considered unfeasible to restart the experiment.

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