

1 RUNNING HEAD: FRAGMENTATION SEEDLING RECRUITMENT FOREST HERB

2

3

4 EFFECTS OF FOREST FRAGMENTATION ON SEEDLING RECRUITMENT

5 OF A TROPICAL HERB: ASSESSING SEED VS SAFE-SITE LIMITATION

6

7 María Uriarte<sup>1\*</sup>, Emilio M. Bruna<sup>2,3</sup>, Paulo Rubim<sup>3</sup>, Marina Anciães<sup>3</sup>, and Inge Jonckheere<sup>4</sup>

8

9 <sup>1</sup> Department of Ecology, Evolution and Environmental Biology, Columbia University, 1200 Amsterdam

10 Ave., New York, NY 10027, USA

11

12 <sup>2</sup> Dept. of Wildlife Ecology & Conservation & Center for Latin American Studies, University of Florida,

13 Gainesville, FL 32611-0430, USA

14

15 <sup>3</sup> Biological Dynamics of Forest Fragments Project, Instituto Nacional de Pesquisas da Amazônia and

16 Smithsonian Tropical Research Institute, Manaus, AM 69011-970 Brazil

17

18 <sup>4</sup> Biosystems Department, Geomatics Group, Katholieke Universiteit Leuven, 3001 Leuven, Belgium

19

20 \*Corresponding author: [mu2126@columbia.edu](mailto:mu2126@columbia.edu)

21

22

## 1 ABSTRACT

2 Studies simultaneously evaluating the importance of safe-site and seed limitation for plant establishment of  
3 are rare, particularly in human-modified landscapes. We used spatially-explicit neighborhood models  
4 together with data from ten 0.5-ha mapped census plots in a fragmented landscape spanning 1000 km<sup>2</sup> to  
5 (1) evaluate the relative importance of seed production, dispersal, and safe-site limitation for the  
6 recruitment of the understory herb *Heliconia acuminata*; and (2) determine how these processes differ  
7 between fragments and continuous forests. Our analyses demonstrated a large degree of variation in seed  
8 production, dispersal, and establishment among and within the ten study plots. Seed production limitation  
9 was strong but only at small spatial scales. Average dispersal distance was less than 4 m leading to severe  
10 dispersal limitation at most sites. Overall, safe-site limitation was the most important constraint on seedling  
11 establishment. Fragmentation led to a more heterogeneous light environment with negative consequences  
12 for seedling establishment but had little effect on seed production or dispersal. These results suggest that  
13 the effects of fragmentation on abiotic processes may be more important than the disruption of biotic  
14 interactions in driving biodiversity loss in tropical forests, at least for some functional groups. These effects  
15 may be common when the matrix surrounding fragments contains enough tree cover to enable movement  
16 of dispersers and pollinators.

17

18 *Key words: Amazonian forest, landscape modification, seedling recruitment.*

19

## 20 INTRODUCTION

21 Seedling recruitment is a critical bottleneck in the population dynamics of many plant species (Horvitz and  
22 Schemske 1994, Wenny 2000). Theoretical and empirical studies also suggest that patterns of seedling  
23 establishment can have major consequences for the structure and composition of plant communities  
24 (Wright 2002, Levine and Murrell 2003). While the importance for seedling establishment of factors ranging

1 from seed predation to gap dynamics are often studied independently (e.g., Clark and Clark 1989), we still  
2 know little regarding the relative importance of individual factors for the establishment of seedlings in most  
3 ecological communities.

4         Successful seedling establishment is generally thought to be limited by either low seed abundance  
5 or a limited number of microsites in which seeds can safely germinate, become established, and grow (i.e.,  
6 seed limitation and safe-site limitation, respectively, reviewed in Turnbull *et al.* 2000). Low seed  
7 abundance can result from either limited production or the limited dispersal of available seeds, while the  
8 factors defining safe-sites can be both biotic (e.g., competitors, seed predators) or abiotic (e.g., light levels,  
9 soil chemistry). If populations are safe-site limited, then increased seed availability will not result in  
10 elevated seedling establishment – additional seeds will simply be arriving in sites where they are unlikely to  
11 germinate or thrive. Although the extent to which seedling recruitment is seed- or safe-site limited remains  
12 controversial (Crawley 1990), there is an increasing awareness that their relative importance varies  
13 spatially and temporally (Turnbull *et al.* 2000). Nevertheless, studies simultaneously assessing their  
14 relative importance remain rare.

15         Deforestation in the tropics is continuing at rates that lack historical precedent (Hansen *et al.*  
16 2008), resulting in the extensive fragmentation of species-rich rain forests (Bierregaard *et al.* 2002). One of  
17 the most common consequences of fragmentation is reduced seedling recruitment, particularly for ‘shade-  
18 tolerant’ or ‘forest understory’ species (e.g., Benitez-Malvido *et al.* 1999, Bruna 2002, Melo *et al.* 2007).  
19 Because fruit production and disperser abundance are often lower in fragments, these reductions in  
20 seedling density have often been attributed to seed limitation (Cardoso da Silva and Tabarelli 2000, Bruna  
21 2002). However, biotic and abiotic changes that follow fragment isolation could also affect the number of  
22 available safe-sites. For instance, in some locations fragmentation may lead to more seed predators  
23 (Burkey 1993), generalist herbivores (Terborgh *et al.* 2001), and intense competition due to an influx of  
24 pioneer tree taxa (Sizer and Tanner 1999). Finally, fragments often have higher air temperatures, reduced

1 relative humidity, and elevated levels of photosynthetically active radiation (Kapos *et al.* 1997, Bruna  
2 unpubl. data), all of which can inhibit germination or result in seed and seedling mortality.

3         Processes influencing seedling recruitment are often spatially heterogeneous, which could have  
4 important consequences for patterns of safe-site and seed limitation. For instance, the density and  
5 distribution of reproductive plants is rarely homogeneous, which can affect pollinator behaviour and  
6 therefore spatial patterns of fruit abundance (e.g., Feinsinger *et al.* 1986, Feinsinger *et al.* 1991). Most  
7 seed dispersal is also highly localized: although some seeds can be dispersed long distances, estimated  
8 mean dispersal distances are frequently less than 20 m (Horvitz and Schemske 1994, Clark *et al.* 1999).  
9 The spatial distribution of seed predators is also influenced by the distribution of seed sources, which alters  
10 post-dispersal patterns of seed abundance (Schnurr *et al.* 2004). Finally, plants are sessile organisms that  
11 engage in competition for resources with nearest neighbors, which are themselves spatially variable in their  
12 size and abundance. Despite an increasing appreciation of how 'neighborhood effects' influence  
13 population and community dynamics, the application of spatially-explicit approaches to elucidate patterns of  
14 safe-site and seed limitation remains virtually unexplored (but see Muller-Landau *et al.* 2002).

15         Safe-site and seed limitation are often evaluated with experimental seed additions and by  
16 manipulating environmental factors presumed to influence seed germination (e.g., litter abundance, Bruna  
17 1999). However, seed addition experiments that comprehensively test for seed limitation can be very labor  
18 intensive, and many environmental factors that influence seedling establishment are difficult to manipulate  
19 in the field (e.g., relative humidity, temperature). An alternative approach that can overcome these  
20 shortcomings is spatially-explicit neighborhood models (reviewed in Muller-Landau *et al.* 2002). These  
21 models use spatially-explicit data on the distribution of potential seed sources, the size and location of  
22 competitors, and other biotic or abiotic variables that may affect seedling establishment to parameterize  
23 spatially-explicit models. The models can then be used to make predict seed dispersal and seedling

1 establishment limitation. They have proven particularly useful for systems in which the processes affecting  
2 establishment operate at scales that make experimental manipulations prohibitive.

3         Here we use spatially-explicit neighbourhood models to elucidate the relative importance of safe-  
4 site and seed limitation for recruitment of the Amazonian understory herb *Heliconia acuminata*, and to test  
5 competing hypotheses explaining differences in seedling abundance between fragments and continuous  
6 forest. Our focal species is the subject of an ongoing demographic study in the Brazilian Amazon (Bruna  
7 2003), and the results of previous work suggest this system is an excellent one with which to investigate  
8 mechanisms of safe-site and seed limitation in tropical understory plants. For instance, the abundance of  
9 seedlings in a demographic plot is positively correlated with how many flowering plants were in it the during  
10 the reproductive season (Bruna 2002), suggesting seed limitation. Evidence for seed limitation also comes  
11 from a two-year seed sowing experiment, in which seed additions to both continuous forest and forest  
12 fragments always resulted in seedling establishment (Bruna 2002). However, seedling establishment was  
13 lower in forest fragments (Bruna 1999, 2002), suggesting changes in fragments influence safe-site  
14 abundance. We address the following two questions:

15         (1) Is seed limitation in *H. acuminata* the result of low seed abundance or limited dispersal, and does  
16         the relative importance of these factors vary between forest fragments and continuous forest sites?

17         (2) Are safe-sites defined by biotic or abiotic conditions in the locations where seeds are dispersed  
18         and seedlings establish, and how does this differ between fragments and continuous forest?

19

## 20 METHODS

### 21 Study system and site

22 All field work was conducted at the Biological Dynamics of Forest Fragments Project (BDFFP) located 70  
23 km north of Manaus, Brazil (2°30'S, 60°W). The BDFFP's 1000 km<sup>2</sup> landscape is dominated by non-

1 flooded, high-diversity forests (de Oliveira and Mori 1999). In addition to large expanses of continuous  
2 forest, the BDFFP has several forest fragment reserves ranging in size from 1-100 ha. These fragments  
3 were isolated from 1980-1984 by felling of surrounding forests and, in most cases, burning the downed  
4 trees once they dried. The regenerating vegetation around the fragments is cleared periodically to insure  
5 their continued isolation. The soils in the BDFFP reserves are highly-weathered oxisols, which despite their  
6 relatively high clay content have poor water retention capacity (Laurance *et al.* 1999). Mean annual  
7 temperature is 26° C (range 19-39° C), and average annual rainfall ranges from 1900-2300 mm. There is  
8 a pronounced dry season from June-December. For a description of the field sites and BDFFP history see  
9 Bierregaard *et al.* (2002).

10 *Heliconia acuminata* (Heliconiaceae) is a perennial, understory monocot native to central  
11 Amazonia and the Guyanas (Berry and Kress 1991). In the BDFFP reserves *H. acuminata* flowers and  
12 fruits once per year (January-March). Most reproductive plants have one inflorescence with a total of 20-25  
13 flowers; in our study sites *H. acuminata* is pollinated by two hummingbird species: the long-tailed hermit  
14 *Phaethornis superciliosus* and the straight-billed hermit *Phaethornis bourcieri* (Bruna and Kress 2002).  
15 Each flower produces a maximum of three seeds (mean=1.9 ± 0.02 SE , based on dissection of  $n=873$  ripe  
16 fruits). The seeds germinate 6-7 months later at the onset of the rainy season, and rarely beyond then  
17 (Bruna 2002). These discrete flowering, fruiting, and germination seasons greatly facilitate surveys for  
18 newly established seedlings, and the lack of clonal reproduction (Bruna and Kress 2002) simplifies the  
19 interpretation of seedling emergence patterns.

20 The seeds of all Neotropical *Heliconia* species are exclusively bird-dispersed (Berry and Kress  
21 1991). In our study sites the primary dispersers are white-necked thrush (*Turdus albicollis*), and several  
22 species of manakin (*Pipra erythrocephala*, *P. pipra*, *P. serena*, *Corapipo gutturalis*, *Schiffornis turdinus*, *P.*  
23 *Stouffer*, *pers. comm.*, M. Anciães, *unpubl. data.*). These species can be divided into two broad categories

1 based on their fruit-handling techniques. Thrushes are larger birds that perch to swallow fruits and may  
2 regurgitate seeds locally or defecate them at longer distances. In contrast, manakins are smaller birds that  
3 swallow fruits, immediately move away from the fruiting plant, and regurgitate seeds (Stiles 1979, M.  
4 Anciães, pers. obs.).

5 In January 1998, a series of permanent demographic plots (each 50x100 m) were established in  
6 the BDFFP reserves in which all *H. acuminata* were marked with an aluminum tag and measured. All plots  
7 were subdivided into fifty 10x10 quadrats to facilitate the surveying and mapping of plants (Bruna and  
8 Kress (2002). Since their establishment the plots have been censused annually and all plants have been  
9 mapped. The present study is based on data collected in plots located in continuous forest (N = 6) and 1-  
10 ha fragments (N = 4) during the 2006 and 2007 censuses. In 2006 we mapped all established plants to the  
11 nearest m; we also recorded the number of inflorescences produced by each reproductive plant. In 2007,  
12 we recorded and mapped all new seedlings (Fig. 1).

13

#### 14 MODELING FRAMEWORK

15 We used inverse models parameterized with observational data (reviewed in Muller-Landau *et al.* 2000) to  
16 characterize *H. acuminata* seed production, dispersal, and seedling establishment, and to evaluate the  
17 factors influencing seed and safe-site limitation and how they differ between fragments and continuous  
18 forests. This method assumes that observed spatial variation in seedling abundance is a multiplicative  
19 function of seed production, which is based on the size of potential seed sources, and local dispersal,  
20 which is modeled with a dispersal kernel that adjusts for proximity of the sources to seedling quadrats (e.g.,  
21 Fig. 1). The density of dispersed seeds can then be modified by biotic and abiotic drivers of establishment.

22 To estimate seedling recruitment, we calculated the total number of seedlings in the 2007 census  
23 in each demographic plot's fifty 10x10 m quadrats (Table 1). Although this spatial scale is coarse relative to  
24 the size adult *H. acuminata* plants, the low seedling density (mean no. sdgls/quadrat =  $0.83 \pm 0.85$ ) made it

1 necessary to use this quadrat size to obtain robust parameter estimates for our model (Kobe and  
2 Vriesendorp 2009).

3 The total number of seeds,  $t$ , produced by a reproductive plant during the 2006 flowering season  
4 was estimated as a function of its number of inflorescences ( $m$ ) as follows:

$$5 \quad t = \frac{a * m}{1 + (a/b) * m} \quad \text{[Eqn. 1]}$$

6 where the parameter  $a$  determines the steepness in the increase in seed production with the number of  
7 inflorescences, and  $b$  determines the asymptote of the inflorescence-seed production relationship. Implicit  
8 in this functional form is the assumption that the number of seeds per inflorescence will decrease with the  
9 total number of inflorescences within an individual plant because of pollination limitation or limited resource  
10 availability.

11 *Heliconia acuminata* is bird dispersed and most seeds are dispersed some distance from the  
12 parent plant (M. Anciães, *unpub. manuscript*). For this reason, we chose a lognormal dispersal function,  
13 which is well suited to a variety of dispersal mechanisms including the passage of seeds by animals  
14 (Greene and Johnson 1992). The kernel takes the form:

$$15 \quad f(d) = \frac{1}{\eta} e^{-\frac{1}{2} \left( \frac{\ln(d/X_0)}{X_b} \right)^2} \quad \text{[Eqn. 2]}$$

16 where  $d$  is the observed distance between the flowering plant and the seedling quadrat,  $X_0$  is the distance at  
17 which maximum recruitment occurs (i.e., the mode of the dispersal kernel),  $X_b$  determines the breadth or  
18 spread of the dispersal kernel, and  $\eta$  is a normalization constant equal to the arcwise integration of the  
19 dispersal kernel (Ribbens et al. 1994).

20 Combining local seed production and the dispersal kernel results in a model for the potential  
21 number of seedlings in 10x10 m quadrat  $i$  over the course of a single reproductive season:

$$R_i = \sum_{k=1}^n \frac{a * m_k}{1 + (a/b) * m_k} f(d_{ik}) \quad [\text{Eqn. 3}]$$

2 where  $m_k$  is the number of inflorescences of  $k = 1 \dots n$  plants within the maximal dispersal distance (in  
3 meters) suggested by our model, and  $d_{ik}$  is the distance from quadrat  $i$  to plant  $k$ , and  $f()$  is the appropriate  
4 dispersal kernel.

5 Our previous studies and knowledge of the natural history of this system allow us to exclude  
6 several factors that may define safe-sites in other systems. For instance, predation of *H. acuminata* seeds  
7 in both fragments and continuous forest is very limited (Bruna 1999), as is seedling herbivory by both  
8 vertebrates and invertebrates (Bruna 2002). There is also no evidence that fungal pathogens are a major  
9 source of *H. acuminata* seedling mortality (Bruna, *pers. obs.*), and community-wide rates of seedling  
10 infection in Central Amazonia are extremely low (Benitez-Malvido *et al.* 1999). Finally, under identical  
11 conditions, seeds from fragments are not less likely to germinate than those from continuous forest (Bruna  
12 1999), suggesting the effects of inbreeding on recruitment are limited. Therefore, we emphasize  
13 mechanisms that our previous empirical work suggests are most relevant and that have been shown to  
14 strongly influence seedling recruitment in other herbs: canopy cover (a surrogate for the amount of light  
15 reaching the forest floor) and the density of established conspecific plants (Schleuning *et al.* 2009, Flinn  
16 and Vellend 2005). To this end, we modified the basic inverse model to account for the following factors:

17 (a) *Density dependent interactions with established H. acuminata.* The density of established (>  
18 12 cm ht) *H. acuminata* plants in the 50x100 m demographic plots varies 8-fold (Bruna and Kress 2002),  
19 and there is also substantial variation in density at the 10x10 m scale within census plots (Table 1). There  
20 is therefore the potential for seedlings to be competing with previously-established plants, most of which  
21 are orders of magnitude greater in biomass. We incorporated density dependent interactions in the model  
22 by first calculating the number of established plants in each of the 10x10 m quadrats of each plot, and

1 estimating the effects that this increase in conspecific density would have on seedling recruitment. The total  
 2 number of seedlings  $Sd_i$  potentially recruited in quadrat  $i$  becomes:

$$3 \quad Sd_i = \sum_{k=1}^n \frac{a * m_k}{1 + (a/b) * m_k} f(d_{ik}) + g * P_i \quad \text{[Eqn. 4]}$$

4 where  $P_i$  is the number of adult plants in quadrat  $i$ , and  $g$  is the estimated density dependence parameter.

5 Using this formulation, negative density dependence is straightforward to interpret. However, positive  
 6 density dependence may be indicative of favorable conditions for establishment or increased pollinator  
 7 visitation (Feinsinger *et al.* 1986, 1991), thereby confounding biotic and abiotic drivers of seedling  
 8 establishment. However, previous research at the site has demonstrated that *H. acuminata* reproductive  
 9 success is independent of local flower density (Bruna *et al.* 2004). This allows us to interpret positive  
 10 density dependence with established plants as a proxy for habitat favorability, an approach often used in  
 11 plant ecology (e.g., Iverson and Prasad 1998). Although a positive or a negative value of  $g$  could mask  
 12 some density dependence of the opposite sign, this approach allows us to identify the predominant effect.

13 (b) *Abiotic Factor – Light levels.* We used hemispherical photography to quantify growing season  
 14 light availability in each of the fifty 10x10 m quadrats in each study plot. Photographs were taken on a  
 15 leveled platform at 1 m from the ground in the center of the quadrat. We used a thresholding algorithm that  
 16 ensures objective and repeatable results (Jonckheere *et al.* 2005), to calculate percent light transmission  
 17 as a measure of available light at each quadrat in each plot. Inspection of the data suggested that seedling  
 18 recruitment increased with light availability up to a point, but fell off abruptly beyond this threshold (Fig. 1a).  
 19 For this reason, the effect of light availability on *H. acuminata* establishment was calculated as a function of  
 20 two estimated parameters:  $L_{thresh}$  determines the light level (transmission,  $TR$ ) beyond which seedling  
 21 establishment stops,  $L$  determines the slope of the relationship between light availability and seedling  
 22 recruitment below the threshold. The effect of light transmission on quadrat  $i$  becomes:

$$f(TR_i) = \begin{cases} \text{if } TR_i < L_{thres}, & f(TR_i) = L * TR_i \\ \text{if } TR_i \geq L_{thres}, & f(TR_i) = 0 \end{cases} \quad \text{[Eqn. 5]}$$

3 Combining equations 4 and 5, the total number of seedlings  $S_i$  expected to establish at quadrat  $i$ :

$$4 \quad S_i = Sd_i f(TR_i) \quad \text{[Eqn. 6]}$$

5

### 6 Model comparison

7 To assess the importance of each individual process on seedling recruitment and how their relative  
 8 importance varied between fragments and continuous forests, we compared alternative candidate models  
 9 using Akaike Information Criterion (Burnham and Anderson 2002). These models described the basic  
 10 mechanisms and potential effects of fragmentation on seed production, seed dispersal, and seedling  
 11 establishment. For instance, to determine the importance of dispersal we compared a model that  
 12 incorporated a dispersal kernel (Eqn. 2) with a null model that assumed that seeds were evenly distributed  
 13 across quadrats. We examined the importance of individual biotic and abiotic factors on recruitment by  
 14 comparing a basic set of models that include all potential combinations of the biotic and abiotic processes  
 15 we considered.

16 To quantify the effects of fragmentation on seed production, dispersal, and establishment, we  
 17 included models that assumed that each of these processes can vary between fragments and continuous  
 18 forests. For instance, fragmentation could affect the effectiveness of pollinators (parameters  $a$  and/or  $b$  in  
 19 Eqn. 1), disperser movement and the shape of the dispersal kernel (parameter  $X_0$  in eqn. 2) or the effect  
 20 that light availability has on establishment (parameter  $L$  or  $L_{thres}$  in Eqn. 5).

21 For all analyses we assumed that the expected density of recruits in a quadrat follows a negative  
 22 binomial distribution, reflecting the high degree of clumping observed in the data (Clark *et al.* 1998). We  
 23 used simulated annealing, a global optimization algorithm, to find the parameter values that maximized the

1 likelihood of observed recruitment densities. We also calculated asymptotic 95% support limits for all the  
 2 parameters. For the goodness of fit calculations, seedling densities were log transformed as log (seedlings  
 3 + 1) to reduce deviation from normality (Zar 1996). A list of estimated parameters is provided in Table 2.

4

#### 5 Assessing seed and safe site limitation

6 To estimate potential seed production limitation, we used data on the number of inflorescence for  
 7 reproductive plants in each plot together with an average of 20 fruits with 3 seeds per fruit produced per  
 8 inflorescence. This is a conservative estimate with respect to tests of seed production limitation because  
 9 this number is the maximum observed seed production and we assume no seed predation (Bruna and  
 10 Kress 2002). Seed production limitation at each study plot was calculated as the proportion of 10x10  
 11 quadrats at which no seeds arrive which was estimated stochastically as a Poisson seed rain with equal  
 12 expectation across all quadrats. The proportion of quadrats ( $n$ ) at which none of the seeds produced in the  
 13 plot ( $s$ ) arrive is:

$$14 \quad \text{Source limitation} = \exp(-s/n) \quad \text{[Eqn. 7]}$$

15 We then used seed production numbers and our estimated dispersal kernels to determine dispersal  
 16 limitation at the quadrat level as the proportion of 10x10 m quadrats in each plot that failed to receive  
 17 seeds. Finally, the difference between dispersal limitation and patterns of established seedlings provided  
 18 an index of safe-site limitation, calculated as  $1 - r/a$ , where  $r$  is the number of quadrats with established  
 19 seedlings and  $a$  is the proportion of quadrats that would receive seeds according to our estimates of seed  
 20 production and dispersal above (see Clark *et al.* 1998). Finally, to assess the effects of fragmentation on  
 21 seedling recruitment processes, we compared the values of all limitation metrics between fragments and  
 22 continuous forests. All analyses were conducted using R statistical software (R Development Team 2008).

23

24 **RESULTS**

1 Our analyses demonstrated both seed and safe-site limitation of *H. acuminata* recruitment at the study site,  
2 with large variation in the magnitude of the processes that determine seed production, dispersal, and  
3 establishment among and within the ten study plots (Table 1 & 3). The most parsimonious model, which  
4 included dispersal, positive density-dependent interactions with established plants, and a positive effect of  
5 light on seedling establishment below a threshold value (Appendix I), produced a moderate fit to the data  
6 ( $R^2= 0.23$ ), a result we expected in light of the complex behavior of animal dispersers in heterogeneous  
7 landscapes (Russo *et al.* 2006). Results highlighted the importance of light and habitat favorability (as  
8 reflected by density of established plants) as the primary factors limiting recruitment of *H. acuminata* in  
9 these forests.

10

#### 11 Seed limitation

12 Assuming that each inflorescence produces 20 fruits with 3 seeds per fruit (Bruna and Kress 2002) and no  
13 dispersal or safe-site limitation, we found virtually no seed production limitation across the study plots at a  
14 scale of 10x10 m (Table 3). However, these numbers change drastically if we evaluate seed production  
15 limitation at a finer spatial grain (Kobe and Vriesendorp 2009). For instance, at 1x1 m scales there would  
16 not be enough seeds to reach 0.01 to 78% of the quadrats at the study plots.

17 AIC values indicated that a model that includes dispersal was a better fit to the data than a null  
18 model that assumes seeds are evenly distributed between quadrats (Appendix I). Moreover, our inverse  
19 modeling results suggest that most seeds are dispersed short distances from reproductive plants, which is  
20 consistent with the potential importance of dispersal limitation (Table 4). Since collection of dispersed  
21 seeds is not feasible in our study system, we could not evaluate the degree of dispersal limitation at the  
22 sites directly. However, we used parameter values from our estimated dispersal kernel together with data  
23 on the location and reproductive effort (# of inflorescences) of reproductive plants to estimate seed  
24 dispersal limitation at the study sites (See Assessing seed and safe-site limitation in Methods section).

1 Results from these simulations show severe dispersal limitation at a 10x10 m scale (Table 3). We also  
2 demonstrated great variation between and within plots in the magnitude of dispersal limitation with the  
3 percent of quadrats that did not receive any seeds ranging from 0% in plot 5750 to 68% in plot PA-CF.

4 We examined the effects of fragmentation on seed limitation using two complementary  
5 approaches. First, we determined whether seed production or seed dispersal limitation differed between  
6 fragments and continuous forests (Table 3). Second, we varied parameters  $a$  and  $b$  in Eqn. 1 and  
7 parameters  $X_0$  in Eqn. 2 to assess whether fragmentation influences either seed production or the mode of  
8 the dispersal kernels, and hence the degree of dispersal limitation. Results from these two approaches  
9 provided consistent answers. First, model comparison showed that the data do not support a difference in  
10 the shape of the relationship between inflorescence number and seed production or in dispersal kernels  
11 between fragments and continuous forests (Appendix II). Second, we found no differences in seed  
12 production limitation (t-statistic = 0.066, p=0.47) or seed dispersal limitation (t-statistic = -0.09, p=0.46)  
13 between 0.5-ha. plots in forest fragments and continuous forests, although both seed production and seed  
14 dispersal limitation were far more variable within and between plots in continuous forests than in fragments  
15 (Table 3).

16

### 17 Safe site limitation

18 We considered abiotic and biotic factors that can influence seedling recruitment in *H. acuminata*. The  
19 former included the potential effects of light while the latter accounted for density dependent interactions  
20 with conspecifics. Of these factors, models were most improved by inclusion of positive density  
21 dependence with established plants (Appendix I). A higher number of established plants in the plot,  
22 regardless of their reproductive status, was associated with greater seedling recruitment. The most  
23 parsimonious model also included effects of light. We found a threshold light transmission beyond which

1 seedling establishment fails, as well as a linear positive relationship between light availability and seedling  
2 recruitment below the threshold (Figs. 2 & 3).

3 We also examined whether fragmentation affected the importance of the factors determining safe-  
4 site limitation in our system. Mean light levels were higher in fragments than in continuous forests (t-test on  
5 log transformed light transmission data,  $t = 8.7013$ ,  $df = 478.351$ ,  $p\text{-value} < 2.2e-16$ , Fig. 2b). The most  
6 parsimonious model included differences between continuous forests and fragments in the effects of light  
7 availability on seedling recruitment. Parameter  $L$  in Eqn. 5, which determines the magnitude of the effect of  
8 light on recruitment, increased fivefold from fragments to continuous forests (Fig. 3). We failed to detect  
9 any effects of fragmentation on light threshold levels, or on density dependent interactions with conspecifics  
10 (Appendix II).

11 Given that seedling establishment rates and our calculations of seed dispersal were based on data  
12 collected at the same spatio-temporal scales, we could also calculate safe-site limitation at each of our ten  
13 study plots (Table 3). These calculations demonstrate that seedling establishment limitation is stronger in  
14 fragments than in continuous forests (mean = 0.77 in fragments vs. 0.53 in CF,  $t\text{-statistic} = 5.01$ ,  $p =$   
15  $0.0002$ ), which is consistent with the hypothesis that the seed germination or seedling establishment  
16 environment is less favorable in fragments.

17

## 18 DISCUSSION

19 Studies simultaneously evaluating safe-site and seed limitation of seedling establishment are rare (but see  
20 Flinn 2007, Jacquemyn and Brys 2008), particularly in tropical systems. Furthermore, most studies  
21 investigating recruitment in the tropics have focused on trees (e.g., Muller-Landau *et al.* 2002) despite the  
22 fact that herbs and other understory plants can represent up to 25% of the diversity in tropical forests  
23 (Gentry and Emmons 1987). Our results suggest that the effects of fragmentation on the recruitment of

1 herbaceous species are more complex than previously suggested (Bruna 2003, Cardoso da Silva and  
2 Tabarelli 2000), and that they extend beyond a simple safe-site vs. seed limitation dichotomy.

3

4 Effects of inflorescence abundance and potential dispersal on seed limitation

5 Seed limitation is strong for many plant species (Svenning and Wright 2005, Flinn and Vellend 2005). In our  
6 system the number of inflorescences – and therefore number of potential seeds produced – varied twenty-  
7 fold across the study plots (Table 1). Although seed production limitation at the scale of a 10x10 m quadrat,  
8 that is the percentage of quadrats that did not produce at least one seed, was similar among study areas,  
9 these rates varied from 0.1 to 78% among sites at a scale of 1x1 m, indicating that seed input limitation at  
10 the scale occupied by a reproductive adult plant, can be extremely high (Table 3) (Kobe and Vriesendorp  
11 2009). This result is not surprising since forests in Central Amazonia have amongst the lowest recorded  
12 levels of plant fertility in the tropics (Gentry and Emmons 1987).

13         Although previous work suggested there was a large influx of seeds from continuous forest into  
14 nearby forest fragments (Bruna 2003), our results suggest this is not the case – the average estimated  
15 dispersal distance for *H. acuminata* was less than 4 meters (Table 4, Fig. 1), resulting in strong dispersal  
16 limitation across and within sites (range 2-68%). Previous efforts to describe the dispersal kernel of  
17 vertebrate dispersed seeds have focused primarily on measuring movement patterns and seed passage  
18 rates of frugivores (e.g., Westcott and Graham 2000), and there has been some criticism in the literature of  
19 seed dispersal kernels derived from seedling quadrat or seed trap data (e.g., Holbrook and Loiselle 2007).  
20 However, the short mean dispersal distances we estimated for *H. acuminata* are consistent with those of  
21 other understory herbs (Svenning and Skov 2002, Cain et al. 1998) and the foraging behavior of *H.*  
22 *acuminata*'s dispersers. Many frugivores cache food, have nest sites to which they return after foraging  
23 (e.g., Russo *et al.* 2006), or swallow and digest seeds for relatively long periods (e.g., Westcott and  
24 Graham 2000). Manakins, however, rarely swallow seeds but rather regurgitate them after a few minutes

1 while perching on nearby trees (Stiles 1979). Radio telemetry data and foraging experiments conducted at  
2 the study site further support our conclusion that short distance dispersal is prevalent in *H. acuminata*  
3 (Uriarte, Anciães, and Bruna unpublished data). This pattern has also been observed for other *Heliconia*  
4 species, possibly resulting from their large seed sizes relative to bird gut size (Stiles 1979, Schleuning *et al.*  
5 2009). We are therefore confident that the estimated dispersal kernels accurately reflect dispersal limitation  
6 for *H. acuminata*.

### 8 Effects of fragmentation on seed production and dispersal limitation

9 Our results showed that the relationship between inflorescence number and seed production (Eqn.  
10 1) did not vary between fragments and continuous forest. This suggests that pollination rates were  
11 relatively unaffected by fragmentation, perhaps because hummingbirds appear to readily move through the  
12 secondary growth surrounding fragments (Antongiovanni and Metzger 2005, Stouffer *et al.* 2006).  
13 However, the effects of fragmentation on seed production are likely to differ with mating systems and  
14 pollinator behavior (Aizen and Feinsinger 1994). By altering plant resources (e.g., water, light, nutrients),  
15 fragmentation may also affect maternal resource limitation which could lead to differential seed production  
16 between fragments and continuous forests without any pollination decline (e.g., Aizen and Feinsinger  
17 1994). Although we did not explicitly test for effects of the abiotic environment on seed production, model  
18 comparison failed to support consistent differences in seed production between fragments and continuous  
19 forests, and previous observations suggest per-capita seed production is similar in these two habitat types  
20 (Bruna and Kress 2002). However, seed production was far more variable within and between plots in  
21 continuous forests than in fragments with very high seed production in some of the continuous forest plots  
22 (Table 1). These large populations probably indicate habitat suitability (e.g., edaphic control) at some but  
23 not all of the continuous forest sites.

1           The magnitudes of seed dispersal limitation were comparable for fragments and continuous forests  
2 (Table 3). Moreover, model comparison failed to support the notion that disperser behavior lead to different  
3 dispersal distances for seeds in fragments vs. continuous forests (Appendix II). We therefore find little  
4 support for the hypothesized increases in dispersal limitation following fragmentation, which contrasts  
5 sharply with work in other systems documenting limited immigration of seeds into forest fragments  
6 (Cordeiro *et al.* 2009, Flinn and Vellend 2005). Previous research at the study site has demonstrated that,  
7 despite having lower population densities in forest fragments (Stouffer *et al.* 2006), *H. acuminata*'s avian  
8 frugivores readily move among fragments (M. Anciães, unpubl. data). However, species differ considerably  
9 in their response to fragmentation and the resulting landscape matrix (VanHoutan *et al.* 2007). The extent  
10 to which seed dispersers, and hence plant seed shadows, are affected by fragmentation depends on  
11 dispersers' range sizes, fidelity to natal territory, and physical and behavioral limits (Sodhi *et al.* 2004).

12

### 13 Effects of abiotic and biotic factors on safe-site limitation

14 In contrast to the limited effect of fragmentation on dispersal limitation, our models suggest major effects of  
15 fragmentation on the availability of safe sites -- safe-site limitation, that is, the percent of quadrats that  
16 failed to receive at least one seed, varied from 45 to 82% across study sites. In contrast to previous studies  
17 demonstrating the importance of fragmentation on disperser behavior and plant colonization (see review in  
18 Flinn and Vellend 2005), biotic factors -- specifically negative density dependent interactions among  
19 seedlings and established plants -- appeared relatively unimportant. Rather, abiotic factors seem to be the  
20 most important control on seedling establishment.

21           Two abiotic factors appear to exert major and positive influences on seedling establishment. Light  
22 availability influenced seedling establishment at two levels. Seedlings failed to establish above threshold  
23 values of canopy opening, implying limitation by high light (Fig 2). Elevated levels of photosynthetically  
24 active radiation are associated with higher air temperatures and reduced relative humidity (Kapos *et al.*

1 1997, Bruna unpubl. data), increasing risk of seed and seedling desiccation or mortality. Below the  
2 estimated threshold value, however, seedling establishment increased with light levels (Fig. 3). Slight  
3 increases in light availability also led to greater seedling growth and survival in an experimental study with  
4 another Amazonian *Heliconia* species (Schleuning *et al.* 2009), and similar results have been observed for  
5 tropical tree seedlings (Montgomery and Chazdon 2002).

6 The second abiotic factor, habitat favorability, as reflected in the strong positive effects of  
7 established plant density on seedling establishment, may reflect underlying soil quality. Understory herbs  
8 tend to have specific microhabitat requirements (Whigham 2004), and in a site near ours Costa *et al.*  
9 (2005) found strong links between soil structure and herb distribution and abundance. Furthermore, fine-  
10 scale spatial variation in soil properties and soil chemistry have previously been shown to influence the  
11 probability of seed germination (reviewed in Baskin and Baskin 1998). Studies of tree dynamics at some  
12 sites in the BFDDP have uncovered links between tree biomass, soil texture and nutrient properties  
13 (Laurance *et al.* 1999), however little is known of how these properties influence seed germination.  
14 Experiments manipulating variables that have been shown to be key for germination of plants in other  
15 tropical systems (e.g., leaf litter, water availability, herbivory) would help in elucidating the importance of  
16 these factors of plant recruitment.

17 Safe site limitation, specifically, the effect of light on seedling establishment, appears to be the  
18 critical driver of differences in population demography of *H. acuminata* between fragments and continuous  
19 forests (Fig. 3). To our knowledge no other studies have compared spatial heterogeneity in light availability  
20 in fragments and continuous forest, nor how this heterogeneity influences seedling recruitment. One of the  
21 most common consequences of fragmentation is reduced seedling establishment, particularly for forest  
22 understory species like *H. acuminata* (e.g., Benitez-Malvido *et al.* 1999, Bruna 2002). After isolation,  
23 increased light levels often lead to seed dessication or high rates of seedling mortality for shade tolerant  
24 species (Bruna *et al.* 2002). These effects could be manifest either through different thresholds for

1 establishment or different responses to ambient light levels in fragments and forest. Our results suggest  
2 that plants in continuous forests respond more strongly to slight increases in light levels (Fig. 3). Understory  
3 light levels were greater and more spatially heterogeneous in fragments than in continuous forests, possibly  
4 creating an environment in which higher photosynthetic activity is associated with greater  
5 evapotranspiration and lower net carbon assimilation (Kitajima 1994). Minimizing water losses rather than  
6 maximizing carbon gain may be a more sensible strategy for shade tolerant species in stressful  
7 environments (Walters and Reich 2000).

8         Two important caveats to our conclusions bear discussion. First, we examined the importance of  
9 seed vs safe-site limitation during one season, uncovering high spatial variation in the importance of these  
10 two factors within quadrats in a 0.5 plot and between plots. A number of studies suggest that temporal  
11 variation in the factors that drive recruitment is likely to have important effects on plant population dynamics  
12 (Schupp 1990, Connell and Green 2000, Ibañez *et al.* 2007), so that the relative importance of safe-site  
13 and seed limitation will vary spatially and temporally (Turnbull *et al.* 2000). For instance, the abundance of  
14 disperser, pollinator, and predator populations can all vary from year to year (Pascarella 1998). In addition,  
15 inter-annual differences in climate (e.g., rainfall) may also lead to variation in seed production (Wright 2005)  
16 and seedling establishment (Ibañez *et al.* 2007), both of which can in turn affect frugivore populations  
17 (Wright *et al.* 2009). Given the importance of light in limiting seedling establishment for *Heliconia acuminata*  
18 – a result we believe is potentially indicative of dessication – dry years may drive inter-annual variation in  
19 seedling establishment for this species. Understanding how this variation interacts with the spatial  
20 processes that determine plant recruitment, particularly in the context of human habitat modification, is a  
21 critical component of biodiversity conservation. The methods presented in this paper can easily incorporate  
22 inter-annual variation in seed production (Eqn. 1) or in the processes that drive safe site limitation (Eqns. 4-  
23 6) by estimating separate parameters for each year for which data are available, which we believe is a  
24 fruitful avenue for future research. Second, remnant forest patches are rarely protected from hunting, fire,

1 or other forms of human disturbance like those at the BDFFP are, and all of these factors could exacerbate  
2 the factors leading to low plant recruitment in fragments and biodiversity loss (Galetti et al. 2009) Our study  
3 provides much needed empirical data on how the strength of both pre-dispersal and post-dispersal factors  
4 influences recruitment in an Amazonian understory plant, as well as how the pattern of limitation varies  
5 spatially. However, we believe it is essential to conduct similar studies in a diversity of fragmented  
6 landscapes – as well as well as with a broader diversity of plant functional groups – to better predict the  
7 consequences of fragmentation on plant recruitment (Flinn and Vellend 2005). To date dispersal  
8 mechanisms have received the most attention, with studies suggesting less dispersal limitation for wind and  
9 vertebrate dispersed species (Flinn and Vellend 2005, Muscarella and Fleming 2009, Cordeiro et al. 2009).  
10 Furthermore, most of the attention in tropical systems has focused on tree species (e.g., Norden et al.  
11 2009), despite the fact that understory plants represent up to 25% of the plant diversity in tropical forests  
12 (Gentry and Emmons 1987). Future research should move beyond these systems to explore the  
13 effectiveness of key traits associated with plant performance and functional strategies in predicting the  
14 relative importance of dispersal and safe-site limitation (Grime 1977, Reich et al. 1997, Westoby 1998,  
15 Westoby et al. 2002, Wright et al. 2004). For instance, shade-tolerant species are predominantly dispersed  
16 by animals, and exhibit a contrasting set of functional traits from the pioneers, such as large seed size, low  
17 fecundity, longer life-span, high density wood, low specific leaf area, and low leaf N content (Wright et al.  
18 2004). These characteristics may lead to greater dispersal limitation and a greater availability of safe sites  
19 relative to pioneers. An understanding of the importance of life-history traits on seed and safe-site  
20 limitation, coupled with adult plant performance data can be used to evaluate population viability and to  
21 identify the demographic transitions most critical to population establishment and growth (Bruna 2003).

22

23 **ACKNOWLEDGEMENTS**

1 We thank Liza Comita, Kerry Woods, Kathryn Flinn, and an anonymous reviewer for helpful comments on  
2 the manuscript. We would also like to thank the technicians and students who helped conduct the  
3 censuses and are grateful for the logistical support of the BDFFP and its staff. Financial support was  
4 provided by the US National Science Foundation (award DEB-0614339 to MU and DEB-0614149 and INT  
5 98-06351 to EB) and the Institute for Food and Agricultural Sciences, Center for Latin American Studies,  
6 and College of Agricultural and Life Sciences at the University of Florida. This is publication number -- -- --  
7 in the BDFFP Technical Series.

8

## 9 LITERATURE CITED

- 10 Aizen, M.A. and P. Feinsinger. 1994. Forest fragmentation, pollination, and plant reproduction in a chaco  
11 dry forest, Argentina. *Ecology* 75: 330-351.
- 12 Antongiovanni, M. and J. P. Metzger. 2005. Influence of matrix habitats on the occurrence of insectivorous  
13 bird species in Amazonian forest fragments. *Biological Conservation* 122, 441-451.
- 14 Baskin, C. C. and J. M. Baskin. 1998. Seeds: ecology, biogeography, and evolution of dormancy and  
15 germination. Academic Press, New York.
- 16 Benitez-Malvido, J., G. Garcia-Guzman and I.D. Kossmann-Ferraz. 1999. Leaf-fungal incidence and  
17 herbivory on tree seedlings in tropical rainforest fragments: an experimental study. *Biological*  
18 *Conservation* 91: 143-150.
- 19 Berry, F. and W. J Kress. 1991. *Heliconia: an identification guide*. Smithsonian Institution Press,  
20 Washington D.C., USA.
- 21 Bierregaard, R. O., C. Gascon, T. E. Lovejoy, and R. Mesquita. 2002. *Lessons from Amazonia: the ecology*  
22 *and conservation of a fragmented forest*. pp. 478. Yale University Press, New Haven.
- 23 Bruna, E. M. 1999. Seed germination in rainforest fragments. *Nature* 402: 139.

- 1 Bruna, E. M. 2002. Effects of forest fragmentation on *Heliconia acuminata* seedling recruitment in central  
2 Amazonia. *Oecologia*: 132, 235-243.
- 3 Bruna, E. M. 2003. Are plants in rain forest fragments recruitment limited? Tests with an Amazonian herb.  
4 *Ecology* 84: 932-947.
- 5 Bruna, E. M. and W. J. Kress. 2002. Habitat fragmentation and the demographic structure of an  
6 Amazonian understory herb *Heliconia acuminata*. *Conservation Biology* 16: 1256-1266.
- 7 Bruna, E. M., W. J. Kress, O. F. da Silva, and F. Marques. 2004. *Heliconia acuminata* reproductive  
8 success is independent of local flowering plant density. *Acta Amazonica* 34: 467-471.
- 9 Burkey, T. V. 1993. Edge effects in seed and egg predation at two neotropical rainforest sites. *Biological*  
10 *Conservation* 66:139-143.
- 11 Burnham, K. P. and D. R. Anderson. 1998. Model selection and inference: A practical information-theoretic  
12 approach. Springer-Verlag, New York, NY, USA.
- 13 Cain, M.L., H. Damman, and A. Muir. 1998. Seed dispersal and the Holocene migration of woodland herbs.  
14 *Ecological Monographs* 68: 325-347.
- 15 Cardoso da Silva, J. M. and M. Tabarelli. 2000. Tree species impoverishment and the future flora of the  
16 Atlantic forest of northeast Brazil. *Nature* 404: 72-74.
- 17 Clark, D. B. and D. A. Clark. 1989. The role of physical damage in the seedling mortality regime of a  
18 neotropical rain forest. *Oikos* 55: 225-230.
- 19 Clark, J. S., M. Silman, R. Kern, E. Macklin, E., and J. HilleRisLambers. 1999. Seed dispersal near and far:  
20 Patterns across temperate and tropical forests. *Ecology* 80: 1475-1494.
- 21 Clark, J.S., E. Macklin, and L. Wood, 1998. Stages and spatial scales of recruitment limitation in southern  
22 Appalachian forests. *Ecological Monographs* 68: 213-235.
- 23 Connell, J.H and P.T. Green. 2000.. Seedling dynamics over thirty-two years in a tropical rain forest  
24 tree. *Ecology* 81: 568-584.

- 1 Cordeiro, N.J., H. J. Ndangalasi, J. P. McEntee, and H. Howe. 2009. Disperser limitation and  
2 recruitment of an endemic African tree in a fragmented landscape. *Ecology* 90: 1030-1041.
- 3 Costa, F.R.C., W.E. Magnusson, and R.C. Luizao. 2005. Mesoscale distribution patterns of Amazonian  
4 understorey herbs in relation to topography, soil and watersheds. *Journal of Ecology* 93:863-878.
- 5 Crawley, M. J. 1990. The population dynamics of plants. *Philosophical Transactions of the Royal Society of*  
6 *London B Biological Sciences* 330: 125-140.
- 7 Hansen, M. C., S. V. Stehman, P. V. Potapov, T. Loveland, J. R. G. Townshend, R. DeFries, K. W. Pittman,  
8 B. Arunarwati, F. Stolle, M. K. Steininger, M. Carroll and C. DiMiceli 2008. Humid tropical forest  
9 clearing from 2000 to 2005 quantified using multi-temporal and multi-resolution remotely sensed  
10 data. *Proceedings of the National Academy of Sciences* 105: 9439-9444.
- 11 de Oliveira, A. A. and S. A. Mori, S. A. 1999. A central Amazonian terra firme forest. I. High tree species  
12 richness on poor soils. *Biodiversity and Conservation* 8: 1219-1244.
- 13 De Steven, D. and S.J. Wright. 2002. Consequences of variable reproduction for seedling recruitment in  
14 three neotropical tree species. *Ecology* 83: 2315-2327.
- 15 Feinsinger, P., K. G. Murray, S. Kinsman, and W. H. Busby. 1986. Floral neighborhood and pollination  
16 success in four hummingbird-pollinated cloud forest plant species. *Ecology*, 67, 449-464.
- 17 Feinsinger, P., H. Tiebout III and B. E. Young, B. E. 1991. Do tropical bird-pollinated plants exhibit density-  
18 dependent interactions? Field experiments. *Ecology* 72: 1953-1963.
- 19 Flinn, K.M. 2007. Microsite-limited recruitment controls fern colonization of post-agricultural forests.  
20 *Ecology* 88: 3103-3114.
- 21 Flinn, K.M., and M. Vellend. 2005. Recovery of forest plant communities in post-agricultural landscapes.  
22 *Frontiers in Ecology and the Environment* 3: 243-250.

- 1 Galetti, M., HC Giacomini, R. S. Bueno, C. S.S. Bernardo, R.M. Marques, R. S. Bovendorp, C. E. Steffler,  
2 P. Rubim, S. K. Gobbo, C. I. Donatti. 2009. Priority areas for the conservation of Atlantic forest  
3 large mammals *Biological Conservation* 142: 1229-1241
- 4 Gentry, A. H. and L. H. Emmons. 1987. Geographical variation in fertility, phenology and composition of  
5 the understory of neotropical forests. *Biotropica*, 19, 216-217.
- 6 Greene, D.F., C. D. Canham, K. D. Coates, and P. LePage. 2004. An evaluation of alternative dispersal  
7 functions for trees. *Journal of Ecology* 92: 758-766.
- 8 Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to  
9 ecological and evolutionary theory. *American Naturalist* 111: 1169-1194.
- 10 Holbrook, K. M. and B. A. Loiselle. 2007. Using toucan-generated seed shadows to estimate seed dispersal  
11 in Amazonia Ecuador. Pp. 300-321 in A. J. Dennis, E. W. Schupp, R. Green, and D. W. Westcott,  
12 editors, *Seed Dispersal: Theory and its Applications in a Changing World*. CABI Publishing,  
13 Wallingford, Oxfordshire, UK.
- 14 Horvitz, C. C. and D.W. Schemske. 1994. Effects of dispersers, gaps, and predators on dormancy and  
15 seedling emergence in a tropical herb. *Ecology* 75: 1949-1958.
- 16 Ibáñez I, J.S. Clark, S. LaDeau, and J. Hille Ris-Lambers. 2007. Exploiting temporal variability to  
17 understand tree recruitment response to climate change. *Ecological Monographs* 77: 163–177
- 18 Iverson, L.R. and A.M. Prasad. 1998. Predicting abundance of 80 tree species following climate change in  
19 the eastern United States. *Ecological Monographs* 68: 465-485.
- 20 Jacquemyn, H. and R. Brys. 2008. Effects of stand age on the demography of a temperate forest herb in  
21 post-agricultural forests. *Ecology* 89: 3480-3489.
- 22 Jonckheere, I., K. Nackaerts, B. Muys, and P. Coppin. 2005. Assessment of automatic gap fraction  
23 estimation of forests from digital hemispherical photography. *Agricultural and Forest Meteorology*,  
24 132, 96-114.

- 1 Kapos, V., E. Wandelli, J. L. Camargo, and G. Ganade. 1997. Edge-related changes in environment and  
2 plant responses due to forest fragmentation in Central Amazonia. *Tropical forest remnants:  
3 Ecology, management, and conservation of fragmented communities* eds W. F. Laurance & R. O.  
4 Bierregaard, Jr., pp. 33-44. University of Chicago Press, Chicago, IL, USA.
- 5 Kitajima K. 1994. Relative importance of photosynthetic traits and allocation patterns as correlates of  
6 seedling shade tolerance of 13 tropical trees. *Oecologia* 98:419–28
- 7 Kobe, R.K. and C.F. Vriesendorp. 2009. Size of sampling unit strongly influences detection of seedling  
8 limitation in a wet tropical forest. *Ecology Letters* 12: 220-228.
- 9 Laurance, W. F., P.M. Fearnside, S. Laurance, P. Delamonica, T.E. Lovejoy, J. M. Rankin-de Merona, J. Q.  
10 Chambers, J. Q. and C. Gascon. 1999. Relationship between soils and Amazon forest biomass: A  
11 landscape-scale study. *Forest Ecology and Management* 118: 127-138.
- 12 Levine, J. M. and D. J. Murrell. 2003. The community-level consequences of seed dispersal patterns.  
13 *Annual Review of Ecology Evolution and Systematics* 34: 549-574.
- 14 Melo, F. P. L., D. Lemire, and M. Tabarelli. 2007. Extirpation of large-seeded seedlings from the edge of a  
15 large Brazilian Atlantic forest fragment. *Ecoscience* 14: 124-129.
- 16 Montgomery, R.A. and R.L. Chazdon. 2002. Light gradient partitioning by tropical tree seedlings in the  
17 absence of canopy gaps. *Oecologia* 131: 165-174.
- 18 Muller-Landau, H. C., S. J. Wright, O. Calderón, S. P. Hubbell, and R. B. Foster. 2002. Assessing  
19 recruitment limitation: concepts, methods and examples for tropical forest trees. Pages 35-53 in  
20 *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation*. J. Levey, W. R. Silva and M.  
21 Galetti, editors. Oxfordshire, UK: CAB International.
- 22 Muscarella, R. and T. H. Fleming. 2007. The Role of Frugivorous Bats in Tropical Forest Succession.  
23 *Biological Reviews* 82: 573-590.

- 1 Pascarella, J.B. 1998. Hurricane disturbance, plant-animal interactions, and the reproductive success of a  
2 tropical shrub. *Biotropica* 30: 416-424.
- 3 R Development Team. 2008. A language and environment for statistical computing. Vienna, Austria.
- 4 Reich, P. B., M. B. Walters and D. S. Ellsworth. 1997. From tropics to tundra: Global convergence in plant  
5 functioning. *Proc. Natl. Acad. Sci. USA* 94: 13730-13734.
- 6 Russo, S.E., S. Portnoy, and C.K. Augspurger. 2006 Incorporating animal behavior into seed dispersal  
7 models: Implications for seed shadows. *Ecology* 87: 3160-3174.
- 8 Schnurr, J. L., C. D. Canham, R. S. Ostfeld, and J. Inouye. 2004. Neighborhood analyses of small-mammal  
9 dynamics: Impacts on seed predation and seedling establishment. *Ecology* 85: 741-755.
- 10 Schupp, E. W. 1990. Annual variation in seedfall, postdispersal predation, and recruitment in a neotropical  
11 tree. *Ecology* 71: 504-515.
- 12 Schleuning, M., V. Huaman, and D. Matthies. 2009. Experimental Assessment of Factors Limiting Seedling  
13 Recruitment of an Amazonian Understory Herb. *Biotropica* 41: 57-65.
- 14 Sizer, N. and E. V. J. Tanner. 1999. Responses of woody plant seedlings to edge formation in a lowland  
15 tropical rainforest, Amazonia. *Biological Conservation* 91: 135-142.
- 16 Sodhi, N.S., L.H. Liow, and F.A. Bazzaz. 2004. Avian extinctions from tropical and subtropical forests.  
17 *Annual Review of Ecology Evolution and Systematics* 35: 323-345.
- 18 Stiles, F.G. 1979. Annual cycle in co-adapted community of hummingbird and flowers in a very humid  
19 tropical forest of Costa Rica. *Revista De Biología Tropical* 27: 75-101.
- 20 Stouffer, P.C., R. O. Bierregaard, C. Strong, and T.E. Lovejoy. 2006. Long-term landscape change and bird  
21 abundance in Amazonian rainforest fragments. *Conservation Biology* 20: 1212-1223.
- 22 Stouffer, P.C. and R.O. Bierregaard. 1995. Effects of forest fragmentation on understory hummingbirds in  
23 Amazonian Brazil. *Conservation Biology* 9: 1085-1094.

- 1 Svenning, J.C. and S.J. Wright. 2005. Seed limitation in a Panamanian forest. *Journal of Ecology* 93: 853-  
2 862.
- 3 Svenning, J.C. and F. Skov. 2002. Mesoscale distribution of understorey plants in temperate forest: the  
4 importance of environment and dispersal. *Plant Ecology* 160:169-185.
- 5 Terborgh, J., L. Lopez, V.P. Nunez, M. Rao, G. Shahabuddin, G. Orihuela, M. Riveros, R. Ascanio, G. H.  
6 Adler, T. D. Lambert, and L. Balbas. 2001. Ecological meltdown in predator-free forest fragments.  
7 *Science* 294: 1923-1926.
- 8 Turnbull, L. A., M. J. Crawley, and M. Rees. 2000. Are plant populations seed-limited? A review of seed  
9 sowing experiments. *Oikos*, 88, 225-238.
- 10 Van Houtan, K.S., S. L. Pimm, J. L. Halley, R. O. Bierregaard, and T.E. Lovejoy. 2007. Dispersal of  
11 Amazonian birds in continuous and fragmented forest. *Ecology Letters* 103: 219-229.
- 12 Walters M. B. and P. B. Reich PB. 1996. Are shade tolerance, survival, and growth linked? Low light and  
13 nitrogen effects on hardwood seedlings. *Ecology* 77:841-853.
- 14 Wenny, D. G. 2000. Seed dispersal, seed predation, and seedling recruitment of a neotropical montane  
15 tree. *Ecological Monographs* 70: 331-351.
- 16 Westcott, D. A. and D. L. Graham. 2000. Patterns of movement and seed dispersal of a tropical frugivore.  
17 *Oecologia* 122: 249-257.
- 18 Westoby, M., D. S. Falster, A. T. Moles, P. A. Veski and I. J. Wright. 2002. Plant ecological strategies:  
19 Some leading dimensions of variation between species. *Annual Review of Ecology and*  
20 *Systematics* 33: 125-159.
- 21 Westoby, M. & I. J. Wright. 2006. Land-plant ecology on the basis of functional traits. *Trends in Ecology &*  
22 *Evolution* 21: 261-268.
- 23 Whigham, D.E. 2004. Ecology of woodland herbs in temperate deciduous forests. *Annual Review of*  
24 *Ecology Evolution and Systematics*, 35: 583-621.

- 1 Wright, I. J., P. B. Reich, M. Westoby, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428:  
2 821-827.
- 3 Wright, S. J. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence.  
4 *Oecologia*, 130, 1-14.
- 5 Wright, S. J., C. Carrasco, O. Calderon, and S. Pato. 1999. The El Niño Southern Oscillation variable fruit  
6 production, and famine in a tropical forest. *Ecology* 80: 1632-1647.
- 7 Wright, S.J. 2005. The El Niño Southern Oscillation influences tree performance in tropical rainforests. In:  
8 Eldredge Bermingham; Christopher W. Dick; Craig Moritz (Ed.), *Tropical rainforests: Past, present,*  
9 *and future*: 295-310. Chicago and London: University of Chicago Press.
- 10 Zar, J. H. 1999. *Biostatistical analysis*. Prentice Hall, Upper Saddle River, N.J
- 11

1 Table 1. Number of reproductive parents in 2006, seedlings in 2007 in the 10 study plots, and percent of  
 2 10x10 quadrats within each plot with no seedlings. Mean and standard deviation for Gap Light Index (GLI)  
 3 were calculated from hemispheric photos.

4

5	<u>Plot name</u>	<u>Size</u>	<u># infl. in 2006</u>	<u># Sdlgs 2007</u>	<u>%quads. w/o sdlgs</u>	<u>Mean GLI</u>
6	2107	1-ha	10	8	42	0.088 (0.034)
7	2108	1-ha	30	11	40	0.086 (0.056)
8	5751	1-ha	27	9	41	0.091 (0.026)
9	5753	1-ha	6	6	46	0.141 (0.117)
10	5750	CF	56	44	29	0.049 (0.021)
11	5756	CF	35	34	31	0.031 (0.004)
12	Cabo Frio	CF	5	18	36	0.096 (0.032)
13	Dimona	CF	2	8	43	0.031 (0.013)
14	Florestal	CF	37	41	27	0.071 (0.0211)
15	Porto Alegre	CF	3	8	43	0.130 (0.057)

16

1 Table 2. Definition of estimated model parameters.

2

3	Parameter	Description
4	$a$	Linear effect of number of inflorescences on seed production
5	$b$	Asymptote of no. inflorescence-seed production relationship
6	$X_0$	Mode of the dispersal kernel
7	$X_b$	Variance of the dispersal kernel
8	$g$	Effect of adult density on recruitment
9	$L$	Effect of light transmission on seedling establishment
10	$L_{thresh}$	Maximum light transmission threshold for seedling establishment

11

1 Table 3. Estimated limitation in seed production and dispersal and seedling establishment in the study plots. Total seed production was calculated by  
 2 assuming that each inflorescence produces 20 flowers each with 3 seeds (Bruna and Kress 2002) and all seeds are distributed evenly among  
 3 quadrats. Dispersal limitation was calculated by simulating dispersal of produced seeds using estimated parameters for Eqn. 2 and calculating the  
 4 percent of quadrats that received no seeds. Seedling establishment limitation was calculated as the proportion of quadrats to which seeds are  
 5 estimated to disperse but where seedlings fail to establish. See *Assessing seed- and safe site limitations, Methods*, for more details.

6

SITE	SIZE	NO. INFL	EST. SEED PROD.	PROD. LIM.	DISP. LIM.	ESTAB. LIM.
2107	1ha	10	600	<0.000001	0.42	0.72
2108	1ha	30	1800	<0.000001	0.30	0.71
5751	1ha	27	1620	<0.000001	0.02	0.82
5753	1ha	6	360	0.00075	0.54	0.83
5750	CF	56	3360	<0.000001	0.00	0.58
5756	CF	5	300	0.0067	0.02	0.61
Cabo Frio	CF	2	120	0.09	0.52	0.42
Dimona	CF	2	120	0.09	0.64	0.61
Florestal	CF	37	2220	<0.000001	0.16	0.45

PA	CF	3	180	<0.000001	0.68	0.56
----	----	---	-----	-----------	------	------

Table 4. Maximum likelihood parameter estimates and support intervals for the most parsimonious model.

Parameters	MLE	Lower S.I.	Upper S.I.
<i>a</i>	0.67	0.41	1.00
<i>b</i>	4.60	1.00	10.00
<i>L<sub>fragment</sub></i>	4.10	3.36	11.62
<i>L<sub>CF</sub></i>	21.48	20.19	45.04
<i>Conspecific Dens. Dep.(g)</i>	0.03	0.03	0.04
<i>X<sub>0</sub></i>	3.64	2.22	4.18
<i>X<sub>b</sub></i>	0.82	0.66	0.86
<i>L<sub>thresh</sub></i>	0.33	0.28	0.40

## FIGURES

Figure 1. Spatial distribution of flowering parents (filled gray circles) and seedling counts in 10x10 quadrats (open circles) at the Florestal plot. Size is proportional to the number of inflorescences and seedling counts respectively.

Figure 2. (a) Light availability (Gap Light Index, GLI; higher values are associated with greater light availability) and seedling densities per quadrat in our study plots. (b) Distribution of light in 10 x 10 m quadrats in 1-ha (n = 200) and continuous forest (n = 300) (CF) plots

Figure 3. Relationship between light availability (GLI) and seedling establishment. We assumed a linear increase in seedling establishment with light up to a threshold level beyond which establishment did not occur. See Table 3 for parameter values.

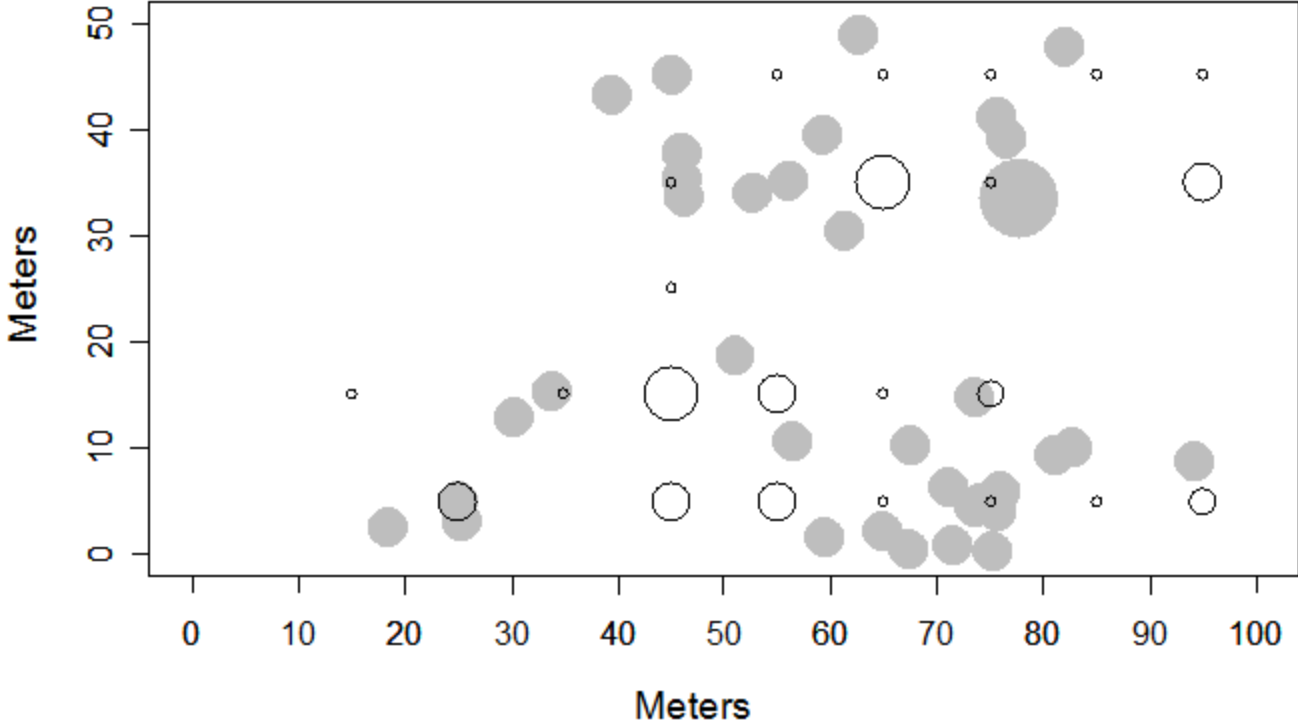


Figure 1

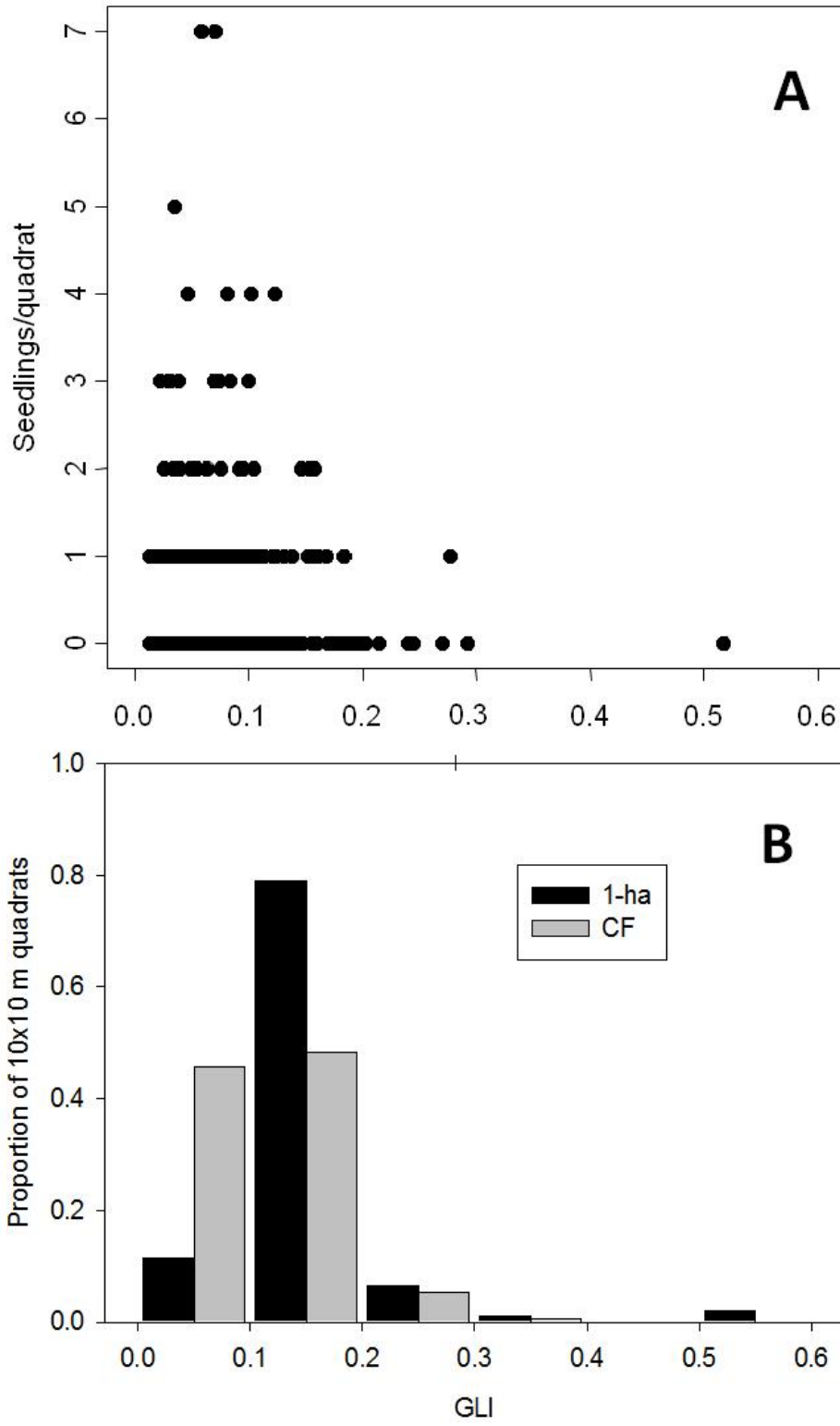


Figure 2

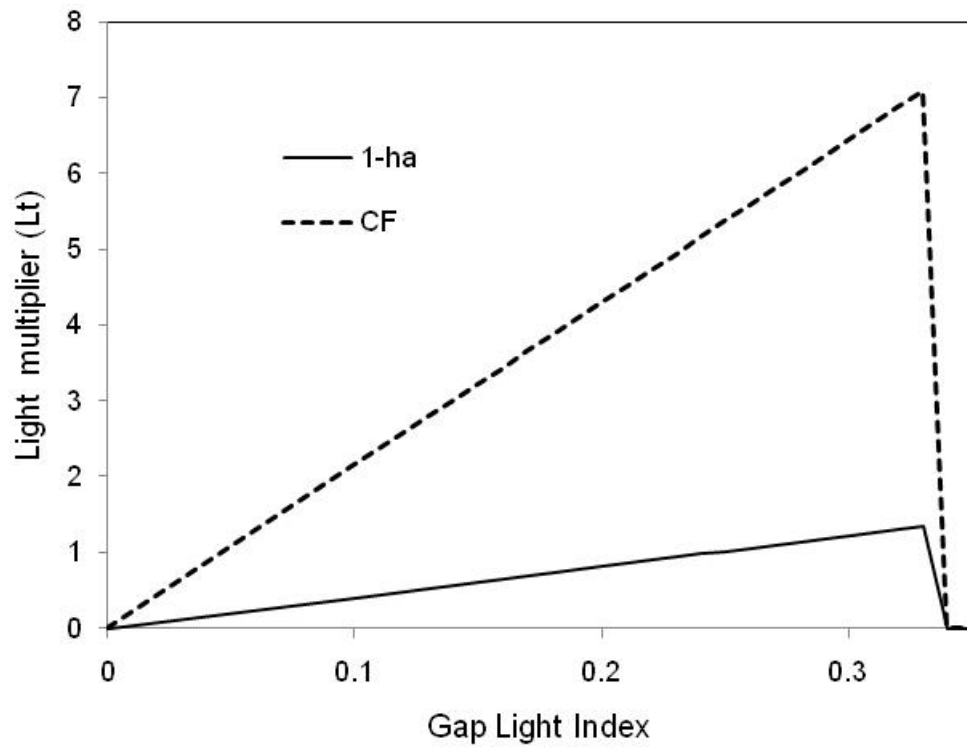


Figure 3