EFFECTS OF DISPERSAL AND INSECT HERBIVORY ON SEEDLING RECRUITMENT OF DIPTERYX OLEIFERA BENTH (FABACEAE) A TROPICAL TREE

by

Javier Enrique Ruiz

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Doctoral Committee:

Professor John H. Vandermeer, Chair
Professor Ivette Perfecto
Associate Professor Beverly J. Rathcke
Assistant Curator Iñigo Granzow-de-la-Cerda

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Dedicated to students and farmers everywhere
fighting for social equality

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ABSTRACT

EFFECTS OF DISPERSAL AND INSECT HERBIVORY ON SEEDLING RECRUITMENT OF DIPTERYX OLEIFERA BENTH (FABACEAE) A TROPICAL TREE

by

Javier Enrique Ruiz

Chair: John H. Vandermeer

This dissertation provides evidence for the relative contribution of primary and secondary seed dispersal and insect herbivory on seedling recruitment of *D. oleifera*. I tested the Janzen-Connell and the recruitment limitation hypotheses using seedlings of *Dipteryx oleifera* within a prermanent plot (6.5 ha), located in eastern Nicaragua. Results show that seedling insect herbivory decreases with the distance to the nearest conspecific adult ($r^2 = 0.79$, $p = 0.000$). As a consequence seedling survival was higher away from as oppose to near to a conspecific adult ($r^2 = 0.80$, $p < 0.05$). Bat primary seed dispersal, while removing seeds from the immediate vicinity of adult trees, nevertheless had the effect of congregating seeds below roosting sites usually located far away from adult conspecifics (79% of the cases, n=70 roosts). Bats preferred to roost in two palm species (*Welfia regia* and *Cryosophila warscewiczii*). Rodent secondary seed dispersal from these seed concentrations was required to release seed from seed predators and insect herbivores ($Z = 3.3$, $df = 2$, $p < 0.005$). The presence of unviable seeds at the seed concentrations “masked” the availability of viable seeds, confused rodents and increased secondary seed dispersal. Per each additional unviable seed moved by bats into the seed concentrations, seed predation rate decreased by a factor of ~1.9% ($p < 0.001$), and increased seedling recruitment rate by a factor of ~1.8% ($p < 0.001$). The distribution of

Dipteryx oleifera seedlings is consistent with the Janzen-Connell hypothesis and depends on primary dispersal by bats, secondary dispersal by rodents and the constant threat of insect herbivores acting on the seedlings.

CHAPTER I

ON THE MAINTENANCE OF TROPICAL TREE SPECIES RICHNESS

Understanding tropical rainforest organization. Historically, tropical rain forest research aimed to understand whether the observed pattern of species richness is determined by density dependent biological mechanisms, but recently there has been increasing interest in understanding the role of random processes in tropical rainforest organization. Two hypotheses, the Recruitment limitation hypothesis (Dalling and Hubbell 2002; Hubbell 1999; Hubbell et al. 1999) and the Janzen-Connell hypothesis (Connell 1971; Janzen 1972) represent these two seemingly antagonistic viewpoints. Chapter 2 addresses the reasons why these two bodies of literature have overlapping goals and evidence from field research. Thus, an organic merging of both hypotheses is shown to be possible, and to provide a more inclusive and coherent framework for tropical rainforest field research.

Toward an inclusive and coherent framework for tropical rainforest research. The Recruitment limitation hypothesis proposes that it is random recruitment that determines the spatial pattern and shapes species abundance and richness. Chapter 3 specifically focuses on the evaluation of the spatial distribution of seedling recruitment of the tropical tree species *D. oleifera*. *D. oleifera* , its natural enemies, and roosting palms represent an ideal system to test this hypothesis. The results showed that seedling recruitment is clumped rather than random. These results suggest that it is not random seed dispersal alone that may be largely determining seedling spatial distribution, but rather a mechanism that depends on density. Consequently, Chapter 4 focuses on determining the relative contribution of seed dispersal and seedling predation recruitment and whether seedling survival follows the prediction embodied in the Janzen-Connell hypothesis. The results are consistent with both the density and distance effects of this hypothesis, suggesting that density dependent seed damage and foliar herbivory is what determines the overwhelmingly high seed and seedling mortality closer to the adult tree, where the propagule density is higher. Bat seed dispersal and terrestrial vertebrate seed dispersal contributed to the high seedling survival away from the seed source (the adult tree). Because seedling recruitment would occur away from the nearest conspecific adult tree, tropical tree species' high diversity can be maintained when adult of multiple trees species are located away from each other at low densities.

There is lack concrete evidence about the relative contribution of biological mechanisms on the outcome of the seedling distribution (Howe and Miriti 2000; Howe and Miriti 2004). As a consequence, seedling recruitment distributions have been used to infer whether is it randomness or density dependency biological controls that determine recruitment and forest organization (Russo 2005; Russo and Augspurger 2004; Russo et al. 2006). As a response, Chapter 5 explicitly addresses the question "What is the relative contribution of seed dispersal mechanisms and biological control on seedling survival?" The results regarding the relative contribution of bat seed dispersal and terrestrial seed dispersal on seedling recruitment suggest that secondary seed dispersal by terrestrial vertebrates releases seedlings from negative density effects near the adult tree (*i.e.* pathogens, insect herbiovry, etc.). Chapter 6 focuses on understanding the ecological effect of insect herbirvory on seedling recruitment. The results provide a strong case in favor of the hypothesis that insect herbivory is the main biological interaction determining the Janzen-Connell pattern of seedling distribution for *D. oleifera*.

In Chapter 7, I explore the idea of whether top-down biological control of spider species on insect herbivores could be an important positive indirect effect determining seedling distribution (Van Bael and Brawn 2005; Van Bael et al. 2003) of *D. oleifera*. The results obtained suggest that insect foliar herbivory is inversely related to the presence of eight spider species in seedlings of *D. oleifera*. Spider distribution could contribute to seedling distribution patterns via insect predation and/or insect herbivore harassment.

Chapter 8 focuses on testing the predictions of the two mayor hypotheses in the seedling recruitment dynamics of *D. oleifera*. First, the results provide evidence in favor of the Janzen-Connell hypothesis, as seedling survival increases with the distance to the nearest conspecific adult tree. Second, seedling survival is higher in areas with large numbers of conspecific adult trees as predicted by the recruitment limitation hypothesis.

Chapter 9 presents major conclusions from this research and recommendations for futher fieldwork. In my dissertation research, I aimed to first analyze and synthesize the current literature on the recruitment limitation hypothesis and the Janzen-Connell hypothesis and evaluate the current evidence in favor of either or both hypotheses. The review of the literature and the hypotheses testing approach provided evidence in favor of both hypotheses. I additionally investigated the relative contribution of seed dispersal types on seedling survival pattern and the effect of insect herbivory and spiders' positive indirect effect on the distribution of seedling recruitment. Finally, I conducted a test of the main predictions of each hypothesis. This research provides evidence that was previously lacking in tropical rainforest research, and thus it leads us one step further in the direction of understanding how species richness is maintained in tropical rain forests.

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CHAPTER II

RECONCILING THE JANZEN-CONNELL AND THE RECRUITMENT LIMITATION HYPOTHESES: SEED DISPERSAL, SEEDLING SURVIVORSHIP AND TROPICAL FOREST ORGANIZATION

INTRODUCTION

This chapter explores mechanisms of tree species coexistence proposed by the Janzen-Connell hypothesis (Connell 1971; Janzen 1970) and the recruitment limitation hypothesis (Condit *et al.* 1992; Dalling *et al.* 2002; Hubbell *et al.* 1999). The Janzen-Connell hypothesis (JCH) emphasizes the role of biologically caused negative density-dependence and distance-dependence in tree establishment dynamics. Alternatively, the recruitment limitation hypothesis (RLH) focuses on random dispersal and recruitment into available spots in the forest understory. The JCH and the RLH, as originally formulated, represent two fundamentally antagonistic approaches (negative density-dependency approach vs. random -neutral- approach). The debate between these two views has generally focused on whether tropical rain forest communities are structured by interactions among species (the foundation of the JCH) as opposed to being an accidental collection of individuals at micro-regeneration sites in which competition has little importance (the central argument of the RLH). Studies in tropical rain forests reported evidence in favor of both hypotheses; where little or no distinction between these two approaches was made (Condit et al. 1992; Hubbell and Foster 1986; Wright 2002). It has been recently argued that the considerable overlap and complementarity of these hypotheses' predictions are leading to a re-organization of these central paradigms in tropical rain forest ecology (Howe and Miriti 2000; Howe and Miriti 2004; Nathan and Muller-Landau 2000). This new, more inclusive approach suggests that tropical rain forest organization might be driven by these hypotheses' mechanisms acting -or interacting- at different temporal and spatial scales.

Previous research has laid the groundwork for the potential unification of both approaches into a more inclusive one (Howe and Miriti 2000; Howe and Miriti 2004; Muller-Landau et al. 2002b; Nathan and Muller-Landau 2000). The general argument of this emerging body of tropical rain forest literature proposes that seed dispersal could increase the probability of escape from density and distance-dependent effects as well as increasing the probability of landing in light-gaps or other kind of suitable recruitment sites. Main questions in this new line of research are the degree to which tree species differ in their potential for escape from negative density-dependent effects and whether this escape is related to colonization ability; as well as whether seed and seedling distributions correspond or fail to correspond to each other.

This review examines two specific hypotheses: The relative importance of negative density-dependent effects and random effects on tree species dynamics. In other word, it asks whether density- and distance-dependence effects and recruitment limitation of tropical tree populations are complementary. These hypotheses are evaluated through a literature review covering relevant research on negative density-dependence, species-specific interactions and recruitment limitation. This review is intended to be a step in the direction of a new framework for research on the maintenance of diversity of tropical forests. If these hypotheses are complementary then studies failing to find consistent support for of the JCH may provide support for RLH. This would show that their union provides a stronger theory. Whenever possible, examples cited will come from studies of natural systems or field experiments. Theoretical models are examined to indicate what we might expect to find in those natural systems and experiments. This review indicates several areas in which critical data are lacking are examined.

THE JANZEN-CONNELL HYPOTHESIS

Janzen (1970) and Connell (1971) proposed that high negative density-dependent mortality of seedlings close to conspecific adult trees occur when seeds and seedlings provide cues to species-specific seed predators (Forget 1992), herbivores (Augspurger 1983; Clark and Clark 1985) and pathogens (Augspurger 1983; Augspurger 1984). In this hypothesis there are two main effects driving plant demography. The density dependent effects occur when disproportionately high predation rates affect seedlings at higher densities. The distance dependent effect occurs when predation rates are disproportionately higher closer to a conspecific adult. Thus, seed dispersal away from conspecific adults could lead to escape form natural enemies (Howe and Smallwood 1982). Lowered recruitment near conspecific adults could therefore favor heterospecific seedling establishment, reducing the potential for a species to become locally abundant when reaching adult status. Consequently, tree species are limited from becoming abundant at a local scale and long distances are expected between conspecific adult trees, in which other tree species occupy space in between. This pattern is hypothesized to lead to multiple species coexisting at low densities. While the core of the JCH was originally focused on the expectation that seeds and/or seedlings will exhibit either distance-dependent effects and/or negative density-dependent effects (in relation to the nearest conspecific adult tree), it has subsequently become standard to note that adult tree density is clearly a modifying force (Connell *et al.* 1984; Harms *et al.* 2000; He *et al.* 1997; Schupp 1992; Wenny 1999; Wright 2002).

Under conditions of low adult density, seedlings are less likely to be found by natural enemies than under conditions of high adult density, even though they may be abundant at some focal tree individuals. This low adult density situation could lead to high survival below conspecific adult trees since the expected attractiveness of local cues for seed predators, herbivores and diseases would be relatively low. However, when there is a high density of adult trees, there are likely to be high rates of seed predation and seedling mortality near conspecific adults. Dispersal away from areas of high density of adult trees could release seeds and seedlings from natural enemies. Here, the effects of seed dispersal can also be density-dependent but positive so. That is, when predation rates are high, predator satiation can become important, leading to positive density-dependence. Either seed disperser disperses the seeds by scatter hoarding or leave them for secondary dispersal. Positive density-dependence is not predicted by the JCH, nevertheless it appears to be pervasive in nature. For example, Schupp (1992) evaluating seedling distributions around conspecific focal individuals of *Faramea occidentalis* in Panama, determined that seed survival and seedling establishment was positively correlated with increasing dispersal distance away from adults of *F. occidentalis* but only under a high density of adult trees. This suggests that effective dispersal could occur when either the predator acts as a disperser once satiated or when satiated predators leave seeds for dispersion by other animals; which is more likely to occur at high density of adult trees. Both seedling recruitment and sapling performance did not respond to density

-and distance-dependent effects in subsequent studies of the same population (Condit *et al.* 1992). Condit and colleagues' results suggest that *F. occidentalis* recruits near conspecifics.

Predictions

After examining relevant tropical rain forest literature (Table 2.1), I extracted nine specific predictions: **(1) A negative relationship between predispersal seed predation rate and distance between conspecific adult trees. (2) A positive relationship between predispersal seed predation rate and the density of reproducing trees. (3) A negative relationship between postdispersal seed predation rate and seedling survivorship. (4) A positive relationship between secondary seed dispersal rate and seedling survivorship. (5) A negative relationship between seedling survivorship and seedling density. (6) A positive relationship between seedling survival and distance to the nearest reproductive individual. (7) A positive relationship between distance to a conspecific adult and larger individual size stages. (8) A higher heterospecific seedling survivorship below/near to a nonspecific adult tree. (9) Density-and distance-dependent effects close to parent tree lead to a less clumped distribution of conspecific adult individuals through the forest.** All these predictions have been tested in a wide range of tropical studies with the exception of prediction number eight. The only study addressing establishment of heterospecific seedlings below the crown of adult trees was conducted by Connell & Green (2000). Prediction nine combines the effects with their final spatial distribution; the null hypothesis is supported when random distribution of adult trees is the resulting pattern.

Three literature reviews

Clark & Clark (1984) tested distance-dependent effects on seedling establishment (prediction **6**) in a literature review of 23 papers addressing the JCH (Table 2.1). They predicted that if seedling escape from natural enemies near conspecific adults then over time there would be an increase in the mean distance between conspecific adult trees and survivor seedlings. Clark and Clark found that in 33% of studies, mean distance between adult trees and conspecific seedling increased, but in 77% of the cases seedling mortality was also high farther from the nearest conspecific tree, leading to lower mean establishment distances with respect to the nearest conspecific adult tree. They also categorized the nature of the evidence in each paper as strong or weak –they did not define specifically "strong support" or "weak support"- and concluded that only 12% provided strong evidence for the prediction that mean distance of seedling to adult individual increased over time. This suggests that only a few species escaped from natural enemy effects close to conspecific trees. Since their conclusion comes from short-term evidence (mean time period = 1.3 yrs; 9 studies with ≥1 mo) it has to be considered as preliminary.

Clark & Clark (1984) also tested density -and distance-dependent effects on seed removal, seed predation and seedling survival (predictions **1, 3, 5** and **6**). They counted papers in which the dependent variables (seeds, seedlings) increase or decrease in response to these two effects. They reported that 85% of the papers provided support for both density and distance effects on seed removal, seed predation and seedling survival (this was the paper that coined the term "Janzen-Connell hypothesis"). Forty-nine percent of those studies were considered to provide only weak evidence. Several papers summarized in their review tested either density or distance-dependence of seed removal, seed predation and seedling survival, potentially introducing a bias toward one or the other of those effects. In some studies, the experimental design nonetheless allowed separation of the effects of distance or density on seed or seedling mortality because one or the other effects were controlled, for instance the work of Augspurger (1984). However the design of many other studies limits their analyses to a categorization of what implication distance and/or density effects might have.

Another literature review found little support for the negative density-dependent effects of seed predation. Looking at both tropical and temperate literature, Hyatt *et al.* (2003) tested the predictions of the JCH concerning distance-dependent and negative density-dependent effects of seed predation (predictions **1, 2** and **4**) and seedling establishment (predictions **4, 5** and **6**). They reported a general trend of negative density-dependence for seedling survivorship, but found little evidence for negative density-dependent effects on seed predation, which was generally not different near versus far from conspecific adults. This finding does not support the predictions of Janzen (1970), who proposed that seed predation by density responsive dispersers -- in Janzen's words -- will be higher closer to the parent tree than further away. However, some of their conclusions are flawed because the way seed predation was treated in their analysis, or in the analysis presented in the literature reviewed. They considered that seed removal indicates seed dispersal, whereas seeds can be removed either to be preyed on or to be dispersed. Hyatt *et al.* (2003) failed to consider the dispersal/predation dichotomy, first pointed out by Howe & Smallwood (1982). This confusion seems to be common in the tropical rainforest literature. In many studies removed seeds are assumed to be preyed upon while in other studies seeds removed are considered to be seed dispersed (Vander

Wall *et al.* 2005). Recent research acknowledges this problem (Debout 2004; DeMattia et al. 2004; Forget and Wenny 2005; Pimentel and Tabarelli 2004).

Other methodological considerations also cast doubt on results by Hyatt and colleagues. Most of the studies included in their analysis were from studies of a single species and only a few were from population-level studies. In the focal individual approach, only a small number of adult individuals are selected, in contrast to the population-level approach where a large number are selected. Because most of the studies included are at the focal-individual level, it was not possible for Hyatt and colleagues to directly evaluate negative density effects of the adult trees on seed predation, or dispersal and seedling survival at the population-level and community-level. As these will be discussed below, the existence of a large number of focal-individual level studies limits testing of the predictions at the community-level, which in many ways is the part of the hypothesis that is most interesting. Hyatt *et al*'s conclusions that seed predation could affect distance of seedling establishment from adult tree are based on short-term evidence (mean = 4.1 months/species) and have to be considered as preliminary. In sum, much support was found for the prediction that seedling survivorship increases away from the parent tree (predictions **1, 2** and **4**), however there is confusion in the way in which seed predation was treated (predictions **4, 5, 6**). Their study was also somewhat limited by the low number of tropical rain forest studies included in the analysis.

Third literature review on Janzen-Connell hypothesis

Twenty-five papers explicitly addressing at least one prediction of the JCH in tropical forests were located using Janzen-Connell as key word in Google, seven of which were not included in previous reviews (Clark and Clark 1984; Hyatt et al. 2003). A general description of these papers is presented in Table 2.1. In 84% of the cases there were consistent effects in support of the density and/or distance-dependence predictions of the hypothesis. Supportive effects were defined as seeing the change in the dependent variable measured in each experiment (*i. e.* number of seeds removed/preyed on, seedling growth, mortality, herbivory, etc.). All studies reported at least density or distance effects consistent with the expected pattern. In general, there are few studies with reliable support for the hypothesis as relating to predispersal seed predation (Janzen 1972; Janzen *et al.* 1976; Vandermeer 1974; Wright 1983), positive density-dependent pathogen infection (Augspurger 1983; Augspurger 1984); and negatively-density dependent seedling survival (Schupp and Frost 1989). There was some support for predictions relating to the roles of post-dispersal seed predation (Wright 1983) and post-dispersal seedling fate (Augspurger and Kitajima 1992; Terborgh and Wright 1994).

In general, studies examined were biased in terms of location and time period but not by taxonomic group tested. Published evidence for the JCH is geographically biased. Of all the papers, 67% were conducted in just two countries: Panama and Costa Rica. Furthermore, there was no preference for a particular taxonomic group or species. The mean period of research time was just 2.3 yrs. This could be due to the short-term research grants provided by the National Science Foundation. The longest time period of a research project was 32 years (Connell and Green 2000) and the shortest was just 6 days (Fleming and Heithaus 1981) and one study did not report its duration (Blundell and Peart 1998).

Some predictions of the JCH remain to be empirically explored, and two were often ignored in making predictions. The longest study reported here was conducted by

Connell & Green (2000). They studied seedling establishment dynamics of *Chrysophyllum sp.* for a period of 32 years in a tropical rain forest in Australia. During this period there were six episodes of high seedling recruitment (at intervals of 4 to 10 yrs) and long periods of low seed production and recruitment. Negative density-dependent germination, growth and mortality of seedlings varied markedly over this period, and out of the plants measured during the first year only 2% were still alive after the most recent census. Since seed production can be highly variable within and between years, long-term studies similar to Connell and Green's are necessary to arrive at strong inferences relating to seedling establishment.

Tree species diversity predictions

Some predictions of the JCH remain to be empirically explored, namely there were two often ignored predictions. First, negative density-dependent effects acting on seedlings closer to conspecific parent tree would free-up space for heterospecific seedlings establishment (prediction **8**). The only study that addresses heterospecific seedling establishment near to an adult individual provides partial evidence supporting this prediction (Connell and Green 2000). They compared seedling recruitment of *Chrysophylum sp.* with all other species in relation to distance to a nearest reproductive individual of *Chrysophyllum* between 1965 and 1995. Only 7.4% of conspecific seedlings were beyond two adult crown radii (twice the crown distance) and none were beyond six crown radii. Because *Chrysophyllum* adults were common and widespread, most seedlings of other species were also close to adult *Chrysophyllum*. In contrast to *Chrysophyllum* seedlings, 48% of the seedlings of other species were beyond two crown radii from an adult *Chrysophyllum*, ranging up to 50 radii away. The second often ignored prediction is that negative density-dependent effects closer to a parent tree will reduce the probability of conspecifics establishing close to replacing parent trees. This then would result in large distances between conspecific adults, which would lead to a homogeneous distribution of conspecific adult individuals through the forest (prediction **9**) (Janzen 1970). There was no evidence in Janzen's argument, only a verbal generalization based on observed negative density-dependent seed dispersal in tropical rain forest communities. Janzen's tree spacing prediction was rejected by Hubbell (1979), who reported that juveniles and adult stages of 280 species of a tropical dry forest in Costa Rica were aggregated or clumped rather than evenly spaced, and subsequent studies have confirmed this in other forests.

FUTURE DIRECTIONS

Some areas of the JCH merit further evaluation, for instance the role of life history on seed dispersal and seedling fate. Muller-Landau (2002a) pointed out that in evaluating Janzen-Connell predictions, it is important to include species with different life history traits and representing different dispersal modes. The reasons for this is that the degree to which these species may present density-or distance- dependent effects greatly depends upon dispersal abilities, which also depends on adult individual density, seed production strength and effective dispersal (Muller-Landau *et al.* 2002a). Howe & Miriti (2000; 2004) consider that spatial demography should be explored further. Thus, it is important to ask to what degree conspecific adult tree densities influence establishment dynamics of smaller life stages. Experiments involving short-lived sub-canopy tree species would provide valuable information on seed-to-adult transitions, growth and plant fitness, if the dynamics within this community is similar to that of the long-lived canopy tree community.

THE RECRUITMENT LIMITATION HYPOTHESIS

General description

The second major hypothesis of tropical tree diversity is that of recruitment limitation (RLH), focusing on random processes acting on tree establishment dynamics and demography (Hubbell 2001). The argument of RLH has been narrowed to consider the process of light-gap colonization; because gap-phase colonization has been key in understanding tropical rain forest organization (Brokaw 1985). Hubbell & Foster (1986) suggested that recruitment into light-gaps was a random process where all species have the same chance to win these recruitment spots. They came to this conclusion after observing the large amount of unpredictability as to which tree species surround other trees at Barro Colorado Island (BCI), and asserted that random recruitment largely contributes to this pattern. A variation of this fundamental proposition suggests that dispersal into recruitment spots (for example light-gaps) is at random and that species richness in an area results from an accidental collection of species, and not from the effects of competition (Chesson and Warner 1981; 2001; Hurtt and Pacala 1995; Tilman 1997). Hubbell (1979) introduced the term ‘neutrality’; referring to a random demography (same probability to germinate, migrate, speciate and die) and suggested that tree spatial distribution, species richness and species abundance would be governed by this ecological ‘neutrality’ (Hubbell 2001). If particular individuals of any species do not recruit at a particular light-gap, it is only because sufficient time has not passed for the species to get there. Over the long term, all species will thus have the same probability to reach available light-gaps and species coexistence is achieved.

Empirical scope

The RLH generally focused on one of two possibilities: 1) recruitment occurs at random, or 2) non-random mechanisms determine recruitment. In the first possibility seeds are dispersed at random into recruitment spots in the forest understory, and the result is a sort of accidental species richness, where competitive abilities have little impact on tree species organization (2001; Hurtt and Pacala 1995; Tilman 1994). The second possibility is that dispersal may be guided by non-random process such as masting, light-gap size, or species pool (Clark *et al.* 1999a; Condit *et al.* 1992; Hubbell *et al.* 1999). Empirical tests aim to evaluate the importance of recruitment due to random demography in determining tropical rain forest community organization (Connell and Green 2000; Dalling and Hubbell 2002; Dalling et al. 2002; Hubbell et al. 1999; Nathan and Muller-Landau 2000). Chave (2004) argued that even when the assumption of complete ecological randomness -- neutrality -- is false, because the existence of physiological and life history trade-offs between plant guilds in the degree of dependency on light and light-gap for germination, growth and survival, nonetheless the idea provides a basis for testing neutrality in natural systems. The aspect of the RLH considered here is whether all recruitment limitation is detectable in field experiments. Furhtermore whether tropical rain forest richness could be explained by the RLH.

Predictions

There are 7 relevant studies that explicitly test one or several aspects of the RLH in tropical rainforests (Chave 2004; Dalling and Hubbell 2002; Dalling et al. 2002; Hubbell and Foster 1986; Hubbell et al. 1999; Muller-Landau et al. 2002a; Vandermeer et al. 2000). The evidence in these studies comes from studies at the community-level (Chave 2004; Dalling and Hubbell 2002; Dalling et al. 2002; Hubbell 1999; Hubbell and Foster 1986), or studies of groups of species (about 10 tree species with similar life history trade offs) (Muller-Landau et al. 2002b; Vandermeer 1974). Population-level studies are cited only to indicate what evidence in these studies may lead to new research directions. After a review of the relevant literature the RLH was organized into seven specific predictions: **(1) Tree species recruitment into light-gaps is random. (2) Under dispersal limitation species with inferior competitive traits but with better colonization traits (pioneer tree species) recruit better into light-gaps, thus winning competition by default. (3) There is an individual-to-individual sorting of tree species at the recruitment spot in which competition has little repercussion. (4) Species richness at light-gaps is a function of the light gaps' sizes and their distribution. (5) There is a positive relationship between the species richness of reproductive trees and species richness of saplings at regeneration spots. (6) Seedling/sapling survival should be higher when parent trees are closer to light-gaps. (7) The long term patterns of species abundance rank and species-area functions can be predicted under equal dispersal, survival and mortality of tree species.** Predictions 1-6 have been previously empirically addressed. Prediction 7 has only been analyzed from a theoretical stand point owing to the long-term dynamics of tropical rain forest trees.

Seed dispersal limitation

In field research conducted at BCI, Hubbell *et al.* (1999) evaluated whether species differed in their recruitment potential into light-gaps (prediction **1**). They collected 1.3 x

10^6 seeds in 200 (0.5 m^2) seed traps over 520 weeks of censuses, reporting that only 3 of 314 species reached all seed traps, and most species reached only a small number of monitored traps. Based on these data, they concluded that dispersal limitation is a nearly universal phenomenon. One problem with Hubbell *et al's* method for seed trapping, however, is that large-seeded species will have a very low probability of being dispersed into the seed traps if they rely on secondary dispersal modes (that is dispersal by small mammal or rodent), and recruitment limitation estimates could be biased against these species. It is hard to believe that a big-seeded species, even if it is efficiently dispersed throughout the forest floor, will often be represented in the seed traps. For example, in a field study in the rain forest of Nicaragua, the large seeds of *Dipteryx oleifera*, reached at least 94% of 637 quadrats (10x10 m). However, because a great deal of seed dispersal is due to bats and rodents it would be unlikely to find seeds in many seed traps if we had used this procedure instead (Ruiz *et al. in preparation*). Seed trapping approaches used by Hubbell *et al.* (1999) could be one of the reasons for the observed pattern of dispersal limitation for large seeded species. In contrast, Zagt & Werner (1998) reported a random sorting of pioneer species at light-gaps, where competition to dominate the light-gaps does not depend upon species-to-species competitive abilities, but rather at the individual-to-individual level depending on who reaches a critical size first (prediction **3**).

Seed dispersal limitation and seedling abundance

The only study evaluating both seed dispersal and seedling recruitment in the context of the RLH was conduced at BCI by Dalling *et al.* (2002). They asked whether seed availability of 14 pioneer tree species at light-gaps may reduce their abundance at light-gaps (prediction **2**). Thirty-six light-gaps formed between 1993 and 1995 were used to set up 670 seed traps of the same kind used by Hubbell *et al.* (1999). Seed dispersal was evaluated for 14 major pioneer tree species, and their seedlings < 1 cm (diameter at the base height) were counted using a grid of 1 m^2 at light-gaps. They found a positive relationship between number of seeds in seed traps and number of seedlings in light-gaps. However, some species require larger seed rain (number of seed in the seed traps) than others to attain the number of observed number of seedlings. Wind dispersed species were more abundant at the light-gaps than seed dispersed by rodents. Their results suggest that there are differences in dispersal traits within the pioneer guild. Pioneer tree species dispersed by biotic agents had more variable abundance in light-gaps and generally less seedling abundance. In some case their dispersal was limited to seed traps near reproductive individuals. Distribution of seed traps (Figure 1 in their paper) and small sized light-gaps could favor wind disperse seeds. In a follow-up paper Dalling & Hubbell (2002) reported large differences in mortality rates among these 14 pioneer species in the first two years after seedling emergence, reflecting variation in seed mass and growth rate. Seed rain into light-gaps decreased with seed size. They suggested that an even stronger effect of seed rain on seedling composition of light-gaps would be found with larger sample size and more consistency of the timing of seedling censuses. In general, their results suggest that recruitment success of pioneers is strongly affected by interspecific variation in dispersal, seedling establishment success and seedling growth characteristics. Their results showed evidence against the RLH because there were differences between tree species as to their colonization, germination and growth at the light-gaps.

Dispersal limitation: pioneer versus shade-tolerant species

Hubbell *et al.* (1999) evaluated whether recruitment success differs between two tropical rain forest tree species guilds – pioneers vs. shade-tolerant tree species (prediction **2**). Out of 229 tree species, 47 were classified as pioneer tree species, 97 were classified as shade-tolerant trees, and 33 species were classified as intermediate between these two categories. They noticed that areas of forest containing light-gaps had more species than non-gap mature forest. However, tree species richness was significantly larger in small light-gaps than in large ones, and only 7% of all saplings were pioneers in the smallest (25 m^2) light-gaps compared with 26% in the largest light-gaps (>400 m^2). Relative and absolute abundance of pioneer tree species did not increase with light-gap size. However, rate of light-gap occupancy by pioneers increased from 2% in the smallest to 49% in the large light-gaps. More light reaching lower forest levels probably accounted for this increase. In contrast to this trend, stem density of shade-tolerant species decreased from 0.86 m^2 in the small light-gaps to 0.66 m^2 in the largest light-gaps. Despite this decline, shade-tolerant species were six times more common than pioneer species in smaller light-gaps. In contrast, in large light-gaps, shade-tolerant species had a gap occupancy rate not significantly different from the pioneer guild. Hubbell *et al.* (1999) concluded that pioneers were potentially wining these spots by default, due to lack of shade-tolerant tree species. Hubbell analyzed species richness versus stem density using species accumulation curves per cumulative number of individual, as suggested by Chazdon *et al.* (1999), and once again came to the conclusion that species richness increase as light-gaps size decreased .

Recruitment by default

Hubbell (1999) noticed a decrease in species richness for a given number of stems in larger light-gaps. Hubbell concluded that this decrease in species richness/stems improves recruitment rates of pioneer tree species as a product of two factors. The first factor, dispersal limitation, matters because there were many more small light-gaps than large ones, and the small light-gaps were more widely distributed throughout the forest floor. Reduction in light-gap number with light-gap size leads to fewer seed sources for large light-gaps, the latter were fewer in number and comprised a smaller fraction of the total forest area due to the much more abundant and widely distributed small light-gaps (predictions **3, 5** and **6**). Hubbell and colleagues concluded that widely spread small light-gaps set the ground for pioneers to take advantage of their better dispersal traits and increase recruitment there, but in large light-gaps pioneers had a light-gap occupancy similar to the shade-tolerant tree species. Dispersal limitation of shade-tolerant tree species was attributed to reduction of mammal disperser populations due to over-hunting in a fragmented habitat. Recent research has shown that decreases in animal-dispersed plant populations have resulted from disperser reduction due to habitat loss, a product of forest fragmentation (How and Lulow, 1997). The second factor considered by Hubbell *et al.* (1999) as contributing to the observed reduction in species richness in large gaps is elevated mortality of shade-tolerant seedlings in light-gaps.

Vandermeer *et al.* (2000) suggested that catastrophic events could lead to dispersal limitation of pioneer and light-gap specialist tree species in large-scale light-gaps. They arrived at this conclusion after the massive destruction by hurricane Joan to the tropical rain forests of southeastern Nicaragua. The area of hurricane damage was estimate to be 300,000 – 500,000 ha (Yih *et al.* 1991). In general, forest regeneration was characterized by resprouting of standing, damaged trunks (Boucher 1990; Granzow-de-la-Cerda *et al.* 1997; Ruiz *et al.* 2001; Vandermeer *et al.* 2001; Vandermeer *et al.* 2000; Yih *et al.* 1991). The analysis for the first 10 years of forest regeneration found higher species accumulation curves versus the cumulative number of individuals in sites with more damage. This indicates that tree diversity is higher in more damaged sites. Dispersal limitation of pioneer and light-gap specialist tree species to the center of the hurricane-struck area could lead to the observation that high tree species richness was maintained following the hurricane. With such a large area damaged, the few pioneers and light-gap specialists that survived the storm did not produced enough recruits to saturate the forest, as they are able to do in tree-fall light-gaps (Hubbell 1999). The percentages of stems that are pioneers in light-gaps at the four hurricanes sites support this idea. In sum, dispersal limitation of pioneer tree species and light-gaps specialist contributed to the observed direct recovery of forest tree species richness after the hurricane. The hurricane forest did not go through the expected early succession stages after severe disturbances (Connell 1978).

FUTURE DIRECTIONS

Areas that merit further examination in the context of the RLH is to consider the JCH. Interspecific competition (Chave et al. 2002; Durret and Levin 1998; Nee and May 1992) and/or attack by natural enemies (Adams 1983; Augspurger 1983; Augspurger 1984; Gilbert *et al.* 2001) could limit tree populations from becoming disproportionately abundant. Mortality due to positive density-dependent attack by natural enemies has not been evaluated – it is not considered part of the fundamental concept of RLH. However effects of natural enemies can keep species from becoming abundant within patches and thus could control multiple species dynamics (Connell and Green 2000). Experimentation using herbivore enclosure cages could be use to test for the role of species-specific interactions controlling plants in the understory (Parker and Clay 2000), similar to the work by Howe (1990). Additionally, in these types of enclosure experiments, there should be enclosures where plant densities are controlled at the light-gaps, with density of a superior competitor tree species decreased. Thus, one could evaluate whether once there is relief from superior competitor tree species, the inferior competitor tree species improves its transition from seedling to large stages. This is a prediction that has not been experimentally tested in RLH empirical research in the tropics (prediction **4**). Long-term field research is necessary to evaluate tropical tree species recruitment limitation, because recruitment limitation can be reduced if seeds are effectively dispersed, or if tree species become more fecund and/or the number of reproductive trees increases. Tree species with different competitive abilities can escape recruitment limitation if they become abundant even when they experience long -and consecutive- periods of low seed production, low dispersal and low establishment.

Empirical tests of specific predictions yield support for random dispersal and seedling establishment for a few tree species. However, methods to assess seed dispersal could introduce bias in favor of small seeded species (prediction **1**). Under dispersal limitation species with inferior competitive traits but with superior colonization traits (pioneer tree species) better recruit into light-gaps, thus winning competition by default (prediction **2**). Long-term research would show whether recruitment by default contributes to community organization. Current field evidence suggests an individual-to-individual sorting of tree species at the recruitment spot where competition has little effect (prediction **3**). Studies at BCI suggest that species richness at recruitment spots (light-gaps) depends on size and distribution of these regeneration spots. Overall, species richness decreases with respect to light-gap size. Small-light gaps can sample large species richness, since small light-gaps are widely distributed and because they make up greater area of the forest floor than large ones (prediction **4**). There is a positive relation between the species richness of reproductive wind-dispersed individuals and species richness of recruits at regeneration spots (prediction **5**). There is some evidence at the population-level suggesting that there is a positive relationship between distance of parent tree to light-gaps and recruitment success (Muller-Landau *et al.* 2002a) (prediction **6**). Predictions concerning to the effects of recruitment limitation on long-term dynamics of population abundance and community richness can only be explored from the theoretical stand point (prediction **7**). Hubbell (2001) showed that, at least theoretically, random dispersal can drive species abundance rank and species areas curve in a predictable way –a measure of population and community organization in long-term simulations. Yet, future improvement in theoretical models should not ignore but rather incorporate the species-specific causes of mortality, fundamental processes in the tropical rain forests as pointed out earlier (Gilbert 2002). Wright (2002) recently argued that in evaluating the effect of species-specific pathogen infection in theoretical simulation it is only necessary to have per-species differential mortality due to pathogen infection, but not necessarily species-specific pathogen infection.

DISCUSION

This section explores ways in which mechanisms proposed by the JCH and RLH might offer a more inclusive approach for understanding tropical rain forest dynamics.

Both propositions are not mutually exclusive considering that seed dispersal 1) leads to escape from negative density-dependent effects associated with conspecific adults and 2) enhances the probability of recruitment intro light-gaps. For instance, Muller-Landau *et al.* (2002b) have reported strong JCH effects on saplings and juveniles of *Trichila tuberculata* due to short-distance dispersal. Muller-Landau and colleagues compared the degree of recruitment limitation of *T. tuberculata* with *Miconia argentea*. *M.argentea* disperses further and produced twice as many seeds as *T. tuberculata*. Although the seed-to-seedling transition for *M.argentea* was very low, it successfully colonized a large number of light-gaps. Their results suggest that dispersal traits are important to escape from negative density-dependent effects, but also show that negative density-dependent effects could prelude recruitment limitation into suitable micro regeneration sites, a result of dispersal limitation. However it is premature to accept the hypothesis that density-dependence and recruitment limitation both control tropical tree species recuitment. Sufficient empirical evidence exists to start to ask questions as to whether recruitment limitation and/or density-dependency drive tree population growth, and community trends, or whether an organic merging of the two ideas is warranted.

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Table 2.1. Evidence from field studies addressing the Janzen-Connell hypothesis. Evidence consistent with hypothesis is labeled as: “+” ; evidence inconsistent with the hypothesis “-”. Weak effects (or unclear effect) both in favor (+) and against (-) are presented. The number between parentheses in 2nd and 3rd column corresponds to the number of species with reported to show effect.

Species (habitat)	Hypothesized pattern of progeny mortality (seed and seedlings)		Main findings and duration of study	Source
	Density-dependence	Distance-dependence		
New taxon, Fam. Bombacaceae, TRF? Costa Rica	+	+	Seedlings survival and mortality due to a predatory insect. Duration = 2 months.	Denslow (1980)
Maximiliana mariposa TRF, Brazil	(+)	+	Clumped distribution of adult trees does not support Janzen-Connell hypothesis, however strong density-dependent effects for seedlings within clumps.	Fragoso et al. (2003)
Andira inermis, TRF (pasture sp.), Costa Rica	+	+	High seed predation; seed germination and establishment were not followed. Duration = 2 months.	Janzen *et al.* (1976)
Caliandra grandiflora, tropical highland forest, Guatemala	+	+	Number of pods with damage decreased in isolated plants. Duration = 2 weeks.	Vandermeer (1974)
Scheelea zonensis, TRF, Panama	+	-	Positive density-dependence seed predation near to adult *Scheelea*. Duration = 2 months.	Wright (1983)
Multiple species, TRF, Costa Rica	+	-	Initial seedling fate in monkey feces after seed dispersed by monkey. High seed removal by secondary seed movements and high seed predation. Duration = 3	Chapman (1989)

			years.	
*Welfia georgi*i, TRF, Costa Rica	+	+	High seedling survival at 10 metes from fruiting *W. georgii* was significantly higher than in each of two other treatments (light-gap, understory away from conspecific adult). Duration = 35 days.	Schupp & Frost (1989)
Euterpe globosa, TRF, Puerto Rico	+	-	High number of seed damaged by *Scolytids* beetle far from fruiting tree. Duration = 3 years.	Janzen (1972)
Tachigalia versicolor, TRF, Panama	-	+	Higher transition probabilities from seed to seedling further from adult tree at low density. Duration = 3 months.	Augspurger & Kitajima (1992)
Multiple species, TRF and temperate forests	(+)*	+*	Meta-analysis of studies published mainly in 90s. Duration of studies considered about 2 yrs. * Seedlings from tropical forests.	Hyatt *et al.* (2003)
Dipteryx panamensis, TRF, Peru and Panama	+	+	Higher rate of seedling survival farther from conspecific adults. Duration = 1 year.	Terborgh & Wright (1994)
Faramea occidentalis, TRF, Panama	+	+	Seeds survival and seedlings establishment were positively correlated with seed dispersal father from parent tree. Duration = 28 weeks.	Schupp (1992)
Bactris acanthocarpa, TRF, Brazil	+	(-)	High seedling mortality near to conspecific adults. Duration =10 months.	Silva & Tabarelli (2001)
Platipodium elegans, TRF, Panama	+	+	High pathogen incidence below conspecific's crown leading to high seedling survivorship away conspecific adult tree. Duration = 55 weeks.	Auguspurger (1983)
Nine species studied, TRF, Panama	+	+	Seed and seedling fate evaluated for nine tree species. Seedling survivorship was larger father from conspecific adults for all species. Pathogen incidence measured. Duration = 1 year.	Auguspurger (1984)
Dipteryx panamensis, TRF, Costa	+	+	Large mean distance of seedlings survivorship to the trunk of the nearest adult.	Clark & Clark (1984)

Rica			Duration = 2 years.	
Dipteryx panamensis, TRF, Costa Rica	+	+	Distance and density effects on seedling survival from nearest cospecific adult trees. Duration = 5 years.	Clark & Clark (1987)
Cecropia peltata tropical dry forest, Costa Rica	+	+	Multiple tree species seed shadow from fruiting tree of *Cecropia peltata* followed the classic Janzen-Connell distribution. Duration = 6 days.	Fleming & Heitnaus (1981)
Muntingia calabura, tropical dry forest, Costa Rica	+	+	Multiple tree species seed shadow from fruiting tree of *Muntingia calabura* followed the classic Janzen-Connell distribution. Duration = 6 days.	Fleming & Heitnaus (1981)
Multiple species study, TRF, Borneo	+	+	Evidence for seedling compensatory mortality trend. Duration = 19 months.	Webb & Peart (1999)
Chrysophyllum sp., TRF, Australia	+	+	Although seedling survival was not affected by density closer to conspecific; sapling mortality increase with sapling density in two censuses. Duration = 32 years.	Connell & Green (2000)
Four species in the genus Shorea tropicarl rain forest, West Kalimantan, Indonseia. TRF	+ (4)	+ (4)	For *Shorea hepeifolia*, *Shorea pinanga*, hebivory damage decrease with distance from conspecific adults. Study's duration unknown.	Blundell & Peart (1998)
Scheelea rosata, tropical rain forest. Costa Rica	+	(-)	High seed mortality closer to the parent tree than farther away. Density dependent effects on seed mortality. Duration = 4 months.	Wilson & Janzen (1972)
Ocotea endresiana, tropical cloud forest, Costa Rica	+	+	Directed dispersal by one of the species of bird to light-gaps enhances seedling survivorship and leads to escape form fungal pathogen. Duration = 1 year.	Wenny & Levey (1998)
Cassia biflora, Sabana, Costa Rica	+	+)	Proportion of pods escaping bruchid infection increased with distance to nearest-neighbour as well as proportion of pods lacking herbivory. Proportion of seed increased with distance to conspecific adult. Duration = 1 month.	Silander (1978)

Nine species, TRF, Panama	+ (9)	- (6) + (3)	Species present at low density: *Cecropia insignis*, *Cordia bicolor*, *Desmopsis panamensis*, *Hasseltia floribunda*, *Miconia argenetea*, *Randia armata*; for these species the density dependence effect is +, and distance dependency effect is - . *Ficus costaricana*, *Ficus yoponensis*, *Laetia procera* were not abundant, for these species density dependence effect is + and distance dependence effect is +. Duration = 26 days.	Wehncke *et al.* (2003)

CHAPTER III

RECRUITMENT LIMITATION OF THE TROPICAL RAINFOREST TREE DIPTERYX OLEIFERA BENTH (FABACEAE) IN SOUTHEASTERN NICARAGUA: ECOLOGICAL EFFECTS OF FOLIAR HERBIVORY

INTRODUCTION

Seed production, seed dispersal and sapling recruitment are critically important processes in population dynamics, precisely because they are almost never completely successful (Clark et al. 1999a; Muller-Landau et al. 2002b). First, the failure of seeds to arrive at all sites limits population growth rates and abundances, a phenomenon referred to as seed limitation. Consequently, seed limitation has important consequences for population and community dynamics and for species diversity at multiple scales (Hurtt and Pacala 1995; Tilman 1994). Seed limitation can arise from limited seed production, or from limited dispersal of available seeds even when seeds are locally abundant. The number of sites that can possibly be reached by seeds depends on the local abundance of adult trees and their fecundity, thus determining the overall [cumulative] seed density (Clark et al. 1998). The variance in seed density depends primarily upon the shapes and sizes of seed shadows, which indicates how seeds are distributed with respect to the nearest conspecific adult (Clark et al. 1998). When adult trees are clumped, as they frequently are (Condit et al. 2000), seed limitation will be further increased (Ribbens et al. 1994). Thus, variance in seed density increases, in which case, often producing recognizable seed aggregations through the forest floor.

An important hypothesis concerning recruitment dynamics is that of Janzen (1970) and Connell (1971). The basic prediction here is that plants' natural enemies (i.e. insect herbivores) will respond in a positive density-dependent manner to their host's abundance near the seed source – the adult tree. Escaping from natural enemies is crucial for plant demographic success (Howe and Smallwood 1982). Thus, seed dispersal and density-dependent effects would be responsible for large mean distances between established seedlings and the nearest conspecific adult tree (Blundell and Peart 1998; Clark et al. 1999b; Condit et al. 1992; Hyatt et al. 2003). Consequently, large numbers of tropical tree species might coexist at low densities and with large mean distances between established individuals (Janzen 1970).

This research focuses on determining the recruitment dynamics of the seed, seedling and sapling stages and to what extent insect herbivory determines the recruitment of *Dipteryx oleifera* in southeastern Nicaragua. The specific hypotheses we are testing are: (1) "Seed distribution hypothesis," if dispersal is random then the per quadrat variance to mean ratio of the distribution of seeds is not statistically different from a Poisson process; (2) "Seedling recruitment hypothesis," the fraction of sub-plots receiving seedling recruitment is less than the fraction with seedling establishment if establishment were random and (3) the Janzen-Connell hypothesis.

METHODS

Study site. The research was conducted in a tropical rain forest site in southeastern Nicaragua (12°05' N., 83°55' W.) from March 2002 to August 2006. The site is known as La Union, and it is accessible by the Caño Negro River. A detailed description of the site was published by Granzow-de-la-Cerda et al (1997).

Species in study. The study species is *Dipteryx oleifera* also known as *D. panamensis*, a neotropical canopy emergent tree common in lowland forests. It can reach a height of about 50 m and diameter at breast height (DBH) of about 1 500 mm (Clark and Clark 1992). Within the research plot there are several individuals with (DBH) greater than 2 000 mm. The wood is very dense, which could allow saplings to withstand damage from falling branches (Clark and Clark 1991).

Methodology. A 6.5 ha plot was established in 2002, and yearly censuses of basic demography of each *D. oleifera* individual have been carried out since that time. This plot is subdivided into 10x10m quadrats. Diameters at the base (DB) of the stem were measured for all seedlings and small saplings. Seedlings are categorized as those individuals with a height ≤ 1m. Height is the vertical distance from the forest floor to the highest green tissue. For the purposes of seedling recruitment estimates, seedlings were classified as either an incoming seedling or an old seedling. Old seedlings are more than one year old and easy to recognize because they have (or there is evidence that they had produced), more than two leaves and their leaflet coloration is darker than those of incoming seedlings (< 1yr old). These phenotypic characters make it easy to detect when seed establishment occurred. For saplings, we measured height, and both diameters at the base (DB) and breast height (DBH). Saplings are individuals with height between 1.01 and 2 m. We also measured stem damage on saplings due to falling branches from the forest canopy, as indicated by scars.

Relative growth rates as a function of the distance to the nearest conspecific adult tree. Relative growth rate defined as $RGR = ln(X(t+1)) - ln(X(t))$, where $ln(X(t))$ is the natural logarithm of the height at time t and $ln(X(t+1))$ is the natural logarithm of the height at time $t + 1$. RGR represents the pace at which the circumference is growing or de-growing. Linear models were fitted in order to determine whether seedling relative growth rate correlates with the distance from the nearest conspecific adult tree.

Seed, seedling and sapling distribution. The variance to mean ratio was computed as a measure of the spatial distribution of seeds, seedlings and saplings. The arithmetic mean = N/J, which corresponds to the total number of individuals per quadrat divided by the total number of quadrats. The variance = $\frac{\sum_{i=1}^{J} i(m-n)^2}{J-1}$, where: i = number of quadrat, n = number of individuals per quadrat, m = arithmetic mean and J = total number of quadrats. Observed values were compared with expected values from a Poisson distribution (Vandermeer and Goldberg 2003; Zar 1999). The Wilcoxon test was used to test for differences between observed numbers of individuals (seed, seedlings or saplings) per unit of area against the expected Poisson distribution expectation of the observed number of individuals per stage per quadrat. A non-parametric test was used because of the scale dependency of variance to mean ratio estimates. For saplings we did not compare the observed distribution with the theoretical Poisson distribution because saplings were generally single individuals per quadrat. From April-March 2002 we counted how many times saplings presented broken stems and compared this kind of damage to sapling relative growth rate from 2002-2003 using a linear regression model.

Recruitment and establishment limitation. The basic recruitment limitation estimates were computed following standard methodology (Muller-Landau et al. 2002b; Nathan and Muller-Landau 2000). In order to assess fundamental seed limitation, we observed the seed rain at each 10x10 m quadrat within the 6.5 ha plot for 2003-2004, 1.27 ha in 2005 and 3.38 ha in 2006. Fundamental seed limitation was computed as the fraction of sites not reached by seeds within the study area. The optimal establishment scenario would be that an adult tree establishes at every site receiving one or more seeds. The fundamental seedling limitation was calculated as the fraction of sites without seedlings. Realized establishment niche is defined as the fraction of sites where seedlings survive one or more years (Muller-Landau et al. 2002b). Seedling establishment occurs when a seedling stays in the forest floor for more than a year (Dalling and Hubbell 2002; Dalling et al. 2002).

Herbivory assessment. In order to determine the effect of insect herbivory on seedling recruitment dynamics a digital photograph of the leaves of each seedling was taken from above the seedling canopy. Leaf area damaged area by herbivory was evaluated from digital photographs taken in early January of 2005 and 2006. Leaflets of *D. oleifera* are remarkably horizontal which allowed us to visually count the percentage of herbivory per leaflet. Leaf area damage per leaflet was summed up and divided by the total number of leaflets in order to assess the percentage of herbivory per seedling. To determine the accuracy of the visual estimation, leaf damage was also measured using a grid on 100 seedlings. There was no significant statistical difference between the methods, allowing us to use the faster method of visual estimation. Regression models were fitted in order to determine whether insect herbivory damage depends on the distance from the nearest conspecific adult tree.

Seedling survival as a function of conspecific density. Seedling survival per measurement period was evaluated using linear models. A first model studied seedling survival (Y_t) a function of seedling density per quadrat in the previous year (S_{t-1}) and sapling number in the previous years (P_{t-1}):

$$Y_t = \mu + \alpha\, S_{t-1} + \beta\, P_{t-1} + \varepsilon \qquad (1)$$

Where μ is the intercept or the average survival of seedlings per quadrat, α is a parameter relating seedling survival for period (t, $t+1$) with the density of seedlings in the previous year (*t*), β accounts for the additional change in seedling survival due to the presence of saplings in each year. The model presented in formula 1 was analyzed using an ANCOVA to test for the significance of each predictor.

RESULTS

Seed and seedling distribution. Seed and seedling distributions are presented in figure 3.1. Results of the yearly variance to mean ratio for seeds from 2003 to 2006 are presented in table 3.1. Results of the yearly variance to mean ratio for seedlings from 2002 to 2006 are also presented in table 3.1. Results of the fundamental seedling limitation and the realized seedling limitation from 2002 to 2006 are presented in table 3.2.

Seedling survival and growth. Seedling survival increased with distance to the nearest conspecific adult trees for all periods 2002-2003, 2003-2004, 2004-2005 and 2005-2006 (Logistic regressions $R^2 = 0.1360$, n = 261 seedlings, p = 0.000; $R^2 = 0.7630$, n = 1 906 seedlings, p = 0.000; $R^2 = 0.858$, n = 1 373 seedlings, p = 0.000 and $R^2 = 0.7270$, n = 2 141 seedlings, p = 0.000, respectively) (Figure 3.2). Seedling survival 2005-2006 was negatively correlated with sapling density per quadrat in 2005 (Table 3.3). Seedling relative growth rates with respect to the distance to the nearest adult *D. oleifera* tree were fitted to a linear function (Figure 3.3). The slopes of the linear functions were very close to zero ($p<0.01$) for each measurement period.

Seedling herbivory. Seedling herbivory decreased linearly with distance to the nearest adult *D. oleifera* in period 2004-2005 ($R^2 = 0.3308$, P = 0.0314, n = 1 226). The distribution of insect herbivory fitted a quadratic function with respect to the adult tree in the period 2005-2006 ($R^2=0.7379$, P=0.0001, n =884) (Figure 3.4).

Sapling growth and distributions. Results of the mean to variance ratio for the sapling stage for each research period (*t*, *t+1*) are presented in table 3.1. Percentage of the transitions from seedling to sapling for each research period (*t*, *t+1*) are presented in table 3.4. Sapling relative growth rate is independent of the distance to the nearest conspecific adult tree for each period from 2002-2006 (F = 0.707, df = 12, p = 0.728; F = 0.495, df = 12, p = 0.896; F = 1.128, df = 12, p = 0.379; F = 0.923, df = 12, p = 0.536, respectively). There was a negative trend between number of stem damages and the relative growth rate of saplings in 2002-2003 ($R^2 = 0.1058$, df = 22, p = 0.1210).

DISCUSSION

Seed stage. Sixty eight percent of the research area received seeds during the most productive year, which is very high considering that *D. oleifera* has large seeds. During two years of high seed production seed limitation was lower (66%). Seed distribution is highly clumped and strongly different from the theoretical Poisson random distribution. This clumped distribution is due, to some extent, to the overwhelming primary seed dispersal by gravity below the crown of the adult tree. Field observations lead us to believe that the biological agent controlling seed mortality below the adult *D. oleifera* trees is the larva of a fly of the genus *Taeniaptera* (Fam. Micopezidae). Seed predation occurs when seeds are germinating and larvae reach the seeds through the small opening (~1 mm) between the valves of the fruit. The observed number of larvae varied from few to hundreds per seed. During 2006 we observed that this type of seed damage seemed to be positive density-dependent, as higher larval seed consumption corresponded with the peak of seed production (around June) below the reproductive adult trees (*unpublished data*). This is the first study reporting larval seed predation on seeds of *D. oleifera* in the literature. However, field studies reporting insect seed predation in tropical rain forest studies are frequent (Bradford and Smith 1977; Wilson and Janzen 1972; Wright 1983). Since terrestrial mammal seed predation could be important we observed that there was no seed dispersal by terrestrial mammals from below the adult trees in 2006 (Ruiz et al. *in preparation*), thus suggesting that terrestrial mammals are not the major force driving seed mortality but the parasit insect.

Seedling recruitment. Seedling limitation was higher in the beginning of the study and less pronounced after two highly productive years (2005 and 2006). Only 12% of the quadrat did not receive seedlings at any given period of time. However, seedling cumulative establishment (≥ 1 yr after germination) only occurred in ~40% of the quadrats during the whole research period (black circles in Figure 3.1). Figure 3.2 also shows that seedling establishment occurred away from adult trees, as expected from the natural enemies control hypothesis (Connell 1971; Howe and Smallwood 1982; Janzen 1970). However, seedling relative growth does not depend on the distance to the nearest conspecific adult tree. These observed clumped distributions of seedlings are consistent throughout the research period and strongly different from a theoretical Poisson distribution (Table 3.1).

Seedling herbivory. Seedling mortality can be due to insect herbivory nearby adult *D. oleifera* trees (Figure 3.4). The overall seedling mortality was two-fold higher when foliar herbivory damage was about 20% during the previous measurement period (Ruiz et al. *submitted*). Seedling distributions in figure 3.1 suggest that the areas with a higher number of seedlings are more ephemeral than those with fewer seedlings, which appeared to be more permanent during the research period. These observations suggest a positive density dependent effect of herbivores within the research plot. Since seedling survival 2005-2006 was negatively correlated with the presence of saplings in 2005. These results suggest that insect herbivores might cue in on saplings and adults, seedling survival is lower nearer to adult conspecific trees and saplings. This evidence is in support of the Janzen-Connell effect on seedling survival distribution of *D. oleifera* (Clark and Clark 1987).

Sapling stage. Sapling recruitment appears to be randomly distributed. Seedling transition to the sapling stage is a rare event, according to our census of the demography of *D. oleifera*. The cumulative percentage of seedling transition to sapling stage was only 0.77%. Sapling survival was very high (> 77 %) over the research period, similar to the annual survival rate of 1-2 cm DBH saplings was 98.0 % for *Trichilia tuberculata*, and 84.4% for *Miconia argentea* in a field study conducted at BCI (Muller-Landau et al *in preparation*). Much of the observed sapling mortality may be due to branches falling from the upper stories as observed earlier (Clark and Clark 1987), and as also suggested in our observational study. Sapling relative growth rate was independent of the distance to the nearest conspecific adult tree. In a field study conducted in a tropical rain forest in Costa Rica, maximum height of seedlings of *D. oleifera* was related with light intensity reaching the forest floor (Clark and Clark 1987).

Life history and recruitment dynamics. In field experiments we noticed that the large seed of *D. oleifera* has sufficient energy necessary for initial seedling growth (~26 cm) (*unpublished data*). Herbivory and other factors resulted in mortality of 60 % of the seedlings and the remaining 40 % reached dormancy. Dormancy is defined as a compensatory point of light where respiration equals photosynthesis. At this stage seedlings are stagnant and vulnerable to damage, which could be the reason why damage to photosynthetic tissue (~20%) from herbivory, is such an important determinant of seedling survival of *D. oleifera* (Ruiz et al. *submitted*). Under these circumstances a small fraction of seedlings would make the transition to saplings, however transition to sapling is a rare event (0.77%) and might also depend on light conditions above the mean light intensity at the forest floor.

Implications for current theories of recruitment and forest organization. Although there is strong evidence for the Janzen-Connell hypothesis at the seed and seedling stages; sapling recruitment seems random which embodies the fundamental prediction of the neutral model (Hubbell 2001). In a sense, these results suggest that both hypotheses can be detected in different life stages. Because the seedling of *D. oleifera* reached ~88% of the research area the chance to recruit in a small light gap (~10x10 m) is larger than if the species were recruitment limited. The research questions that arise are whether light gap formation is randomly distributed and whether these light gaps are largely responsible for the transition from seedlings to saplings.

Our main objectives here were to test hypotheses concerning recruitment dynamics and the effect of biological agents controlling the recruitment of *D. oleifera*. Specifically, we gathered evidence to reject (1) the hypothesis that if seed dispersal is random then the per quadrat variance to mean ratio of the distribution of seeds is not different from a Poisson process. (2) There is some evidence in favor of the “seedling limitation hypothesis” because seedlings were clumped distributed, the fraction of sub-plots receiving seedling recruitment being less than the fraction that would receive seeds if establishment were randomly distributed. Approximately 80% of the research plot received seedling recruitment during the research period. Forty percent of the seedlings survived with 0.77% making the transition to the sapling stage.

Seedling and sapling growth rates were independent of the distance to the nearest conspecific adult. There was a trend between sapling growth rate and the number of damage to sapling stems from falling branches (Figure 3.5). (3) Insect herbivory decreased linearly with respect to the nearest adult tree in 2005 and showed a non-linear (quadratic) distribution in 2006. These findings are consistent with predictions embodying the natural enemy control hypothesis for seed and seedling, whereas sapling survival seems to be a random process.

In sum, the population of *D. olefiera* is weakly recruitment limitation and establishment is limited at the seed and seedling stages; sapling recruitment and establishment seems randomly distributed. The relative growth rate of seedlings and saplings does not correlate with the distance to the nearest adult tree. However, sapling growth seems to be negatively correlated with damage from falling branches. Insect herbivory was negatively correlated to the distance to the nearest adult *D. oleifera* tree, which suggests that insect herbivores are largely responsible for seedling survival distribution having greather function away from the nearest conspecifc adult tree.

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Table 3.1. Variance to mean ratio for spatial distribution of seeds, seedlings and sapling of *D. oleifera* from 2002 to 2006. Observed seedling distribution were tested against a Poisson distribution using a probability < 0.05. Sample size "N" is number of individuals at the beginning of the research period. Totals of surviving and dead individuals do not add up to "N" because made transitions to upper stages or to lower stages.

Category	Ratio	N	p =	Distribution
Seed 2003	864	1 195	0	Clumped
Seeds 2004	76.91	783	0	Clumped
Seeds 2005	1280	593	0	Clumped
Seeds 2006	1649	2 303	0	Clumped
Seedlings 2002	7.82	299	0.028	Clumped
Seedlings 2003	26.09	1 545	0.002	Clumped
Seedlings 2004	1.73	1 496	0	Clumped
Seedlings 2005	19.37	2 297	0.001	Clumped
Seedling 2006	57.71	2 496	0.001	Clumped
Seedlings establishment 2002-2003	12.06	194	0.028	Clumped
Seedling establishment 2003-2004	3.81	724	0.028	Clumped
Seedling establishment 2004-2005	23.38	825	0.002	Clumped
Seedlings establishment 2005-2006	23.91	1 441	0.005	Clumped
Seedling mortality 2002 -2003	5.96	103	0.028	Clumped
Seedling mortality 2003 -2004	14.66	815	0	Clumped
Seedling mortality 2004-2005	10.23	648	0.005	Clumped
Seedling mortality 2005-2006	28.88	968	0.001	Clumped
Saplings 2002	1.191	105		Slightly clumped
Saplings 2003	0.991	68		Slightly even
Saplings 2004	0.910	74		Slightly even
Saplings 2005	0.938	66		Slightly even
Saplings 2006	0.041	74		Slightly even
Saplings establishment 2002-2003	1.120	55		Slightly clumped

Sapling establishment 2003-2004	1.656	56	Slightly clumped
Sapling establishment 2004-2005	0.833	57	Slightly even
Sapling establishment 2005-2006	0.911	60	Slightly even
Sapling mortality 2002 -2003	0.963	44	Slightly even
Sapling mortality 2003 -2004	1.645	6	Slightly clumped
Sapling mortality 2004-2005	1.042	5	Slightly clumped
Sapling mortality 2005-2006	1.000	3	Random

Table 3.2. The results of the fundamental seed and seedling limitation and seedling establishment limitation for the years 2003 to 2006. Calculations were conducted within a sub plot of 6.5 divided in subplots of 10x10 m.

Year	Analysis	Subplots with seeds	Total subplots	Formulation	Result
2003	Seed limitation	52	127	1 – (52/127)	0.4094
2004	Seed limitation	87	127	1 – (87/127)	0.6850
2005	Seed limitation	59	175	1 – (59/175)	0.6628
2006	Seed limitation	172	338	1 – (172/338)	0.4911
2002	Seedling limitation	117	637	1 – (117/637)	0.8163
2003	Seedling limitation	286	637	1 – (286/637)	0.5510
2004	Seedling limitation	447	637	1 – (447/637)	0.4553
2005	Seedling limitation	436	635	1 – (436/635)	0.3155
2006	Seedling limitation	436	635	1 – (462/635)	0.2747
2002-2003	Seedling establishment limitation	155	338	1 – (155/338)	0.5414
2003-2004	Seedling establishment limitation	154	637	1 – (154/637)	0.7582
2004-2005	Seedling establishment limitation	331	635	1 – (331/635)	0.4804
2005-2006	Seedling establishment limitation	179	637	1 – (179/637)	0.719
2002-2006	Seedling establishment limitation	381	637	1 – (381/637)	0.4019

Table 3.3. Seedling fate, seedling transition to sapling and sapling fate for the period 2002-2006. The overall weighted transition from the seedling to sapling stage is 0.77%. A number of individuals made the transition from the upper stage to saplings ("de-growth"); since these represent a minimal number of individuals these figures are not presented here.

Stage	Period	Survival %	Mortality %	Transition %	Sample n	Total N
Seedling	2002-2003	62.96	37.04			
Seedling	2003-2004	55.81	44.20			
Seedling	2004-2005	51.23	48.80			
Seedling	2005-2006	56.18	43.82			
Sapling	2002-2003	52.38	47.62			
Sapling	2003-2004	82.35	17.65			
Sapling	2004-2005	77.03	22.97			
Sapling	2005-2006	90.91	9.091			
Seedling to sapling	2002-2003			0.67	2	299
Seedling to sapling	2003-2004			3.11	8	257
Seedling to sapling	2004-2005			0.16	1	628
Seedling to sapling	2005-2006			1.11	13	1169

Table 3.4. a) Analysis of Covariance (ANCOVA) for the model presented in formula 1.

Model	Independent variable	D.F.	Sum Sq	*F* value	P
1	Seedling density (Y_{2002})	1	0.0557	0.3352	0.5649
	Saplings density (S_{2002})	2	0.01126	0.33888	0.7141
	Error (ε)	56	9.3038		
2	Seedling density (Y_{2003})	1	0.042	0.2947	0.5876
	Saplings density (S_{2003})	2	0.250	0.8731	0.4187
	Error (ε)	305	43.709		
3	Density seedlings (Y_{2004})	1	0.132	0.9224	0.3374
	Saplings density (S_{2004})	2	0.269	0.9418	0.3908
	Error (ε)	402	57.415		
4	Seedling density (Y_{2005})	1	0.069	0.5958	0.4405
	Saplings density (S_{2005})	1	0.554	4.8146	0.0287 *
	Error (ε)	533	61.368		

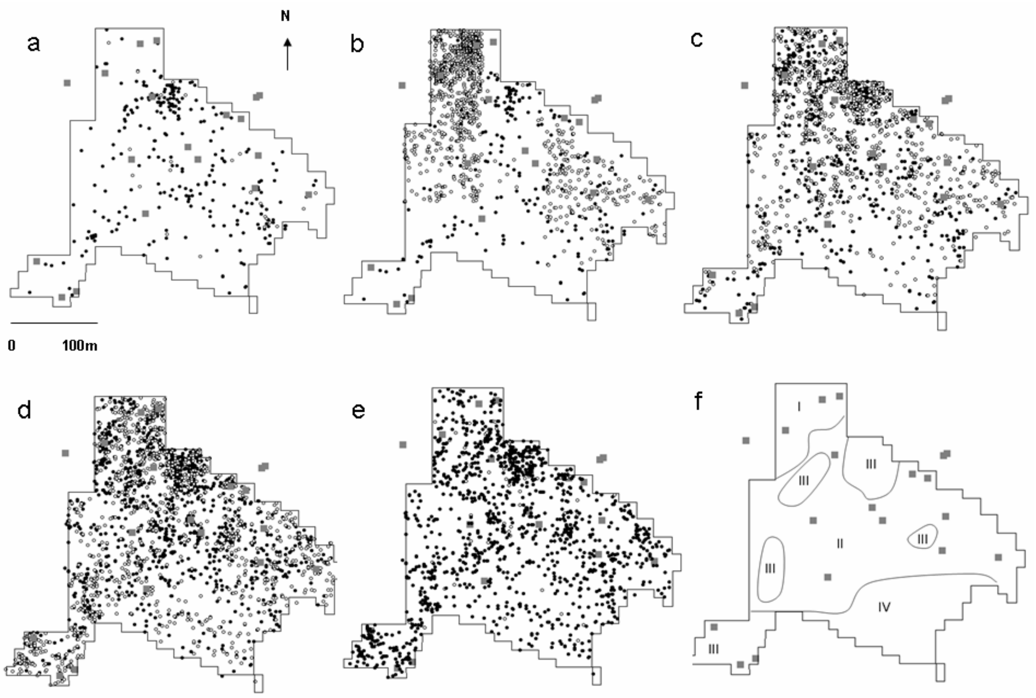
* Statistically signif. (P<0.05).

b) Coefficients for the ANCOVA model see text for details.

Parameter	Estimate	S.E.	*t* value	P
Intercept (μ)	0.37269	0.02638	14.130	< 2e-16*
Seedling density 05 (α)	0.05832	0.06683	0.873	0.3832
Saplings (1) 05 (β_1)	-0.14621	0.04851	-3.014	0.0027 **
Saplings (2) 05 (β_1)	-0.39602	0.33829	-1.171	0.2423

* Statistically signif. (P<0.05), ** Statistically signif. (P<0.01).

Figure 3.1. Observed distribution of seedlings adult trees within a 6.37 ha research plot in Southeast Nicaragua from 2002 to 2006. Gray squares represent adult tree individuals (■); black solid circles represent seedlings older than one year (●) and open circles are incoming seedlings (○). Low caption letters represent consecutive years since the beginning of the research in 2002. I) clumps with high number of seedlings nearby adult trees; II) clumps with low number of seedlings further from large adult trees (northern area); III) Clump with high number of seedlings and IV) with low number of seedlings.


a
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III

Figure 3.2. Weighted mean of seedling survivorship probability of *D. oleifera* versus distance to nearest conspecific adult tree for periods 2002 to 2006. Seedling survival was calculated from 0 to 60 m per each 5 m distance interval with respect to 14 reproductive individuals within a 6.37 ha research plot. Error bars are the standard errors of weighted mean of seedling survival per distance interval between adult individuals located within the research plot.

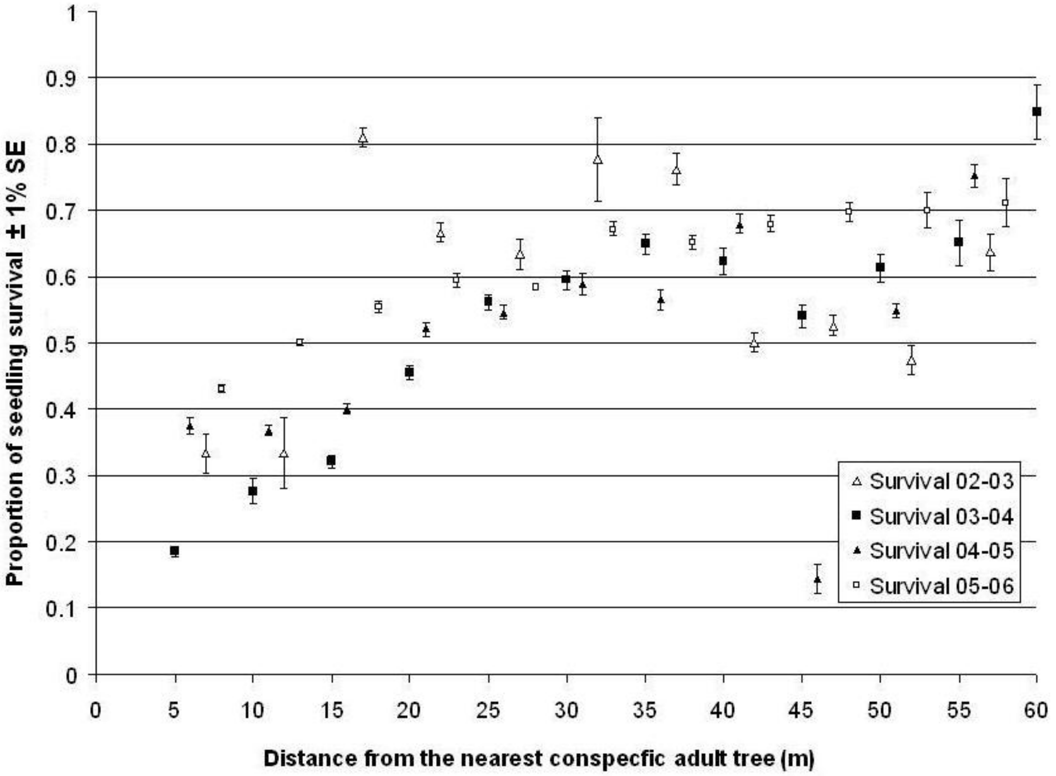


Figure 3.3. Seedling relative growth rates as a function of the distance to the nearest conspecific *D. oleifera* adult tree (m). Lines represent a linear fit and error bars is the 1% standard errors in seedling growth rates between adult tree per distance interval (5 m).

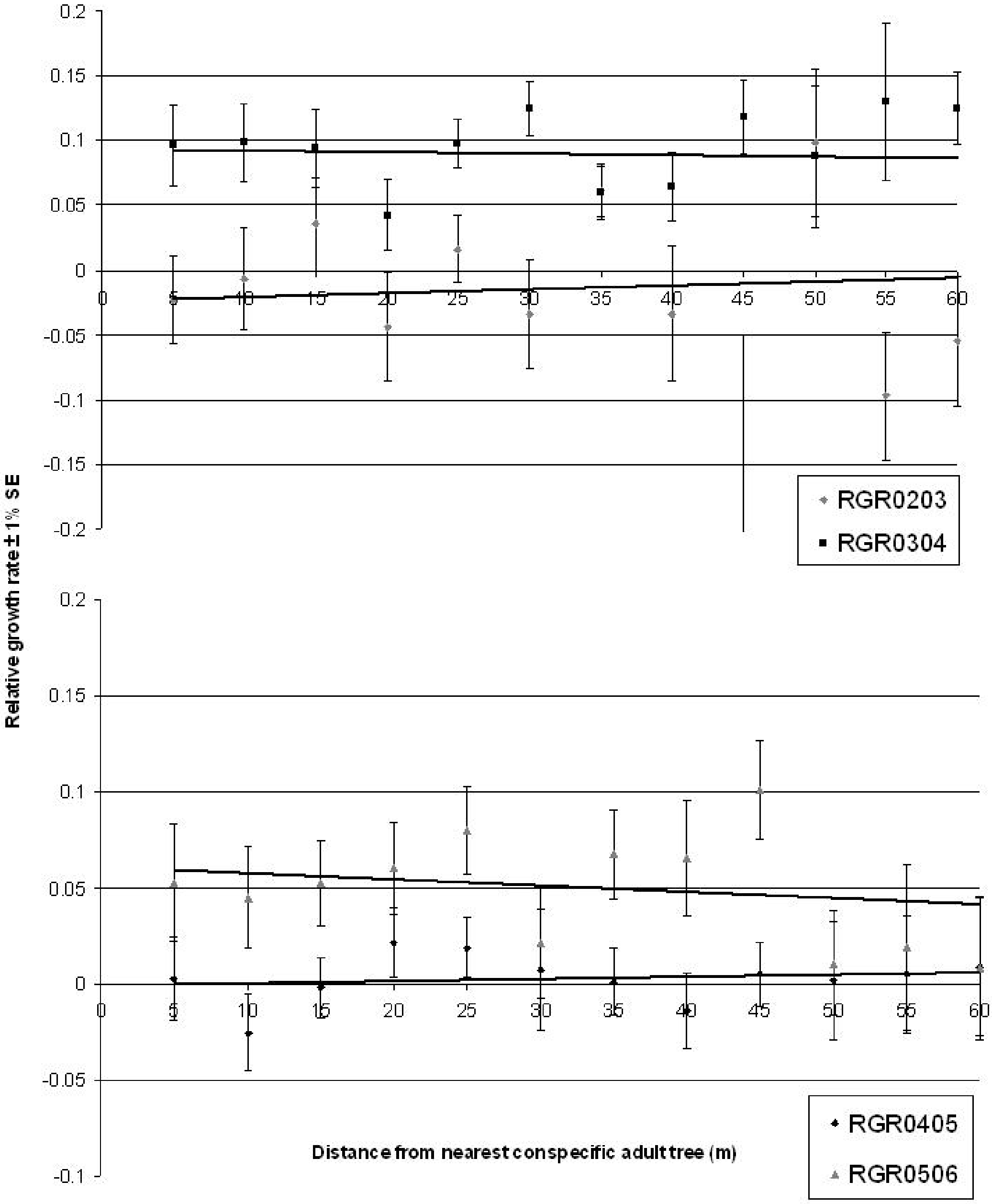


Figure 3.4. Proportion of insect herbivory as a function of the distance to the nearest conspecific *D. oleifera* adult tree (m). Curves represent a statistically significant logistic fit for herbiovry in year 2005 and a statistically significant quadratic fit for herbivory in year 2006. Error bars are the standard errors in insect herbivory between adult individual per distance interval (5 m).

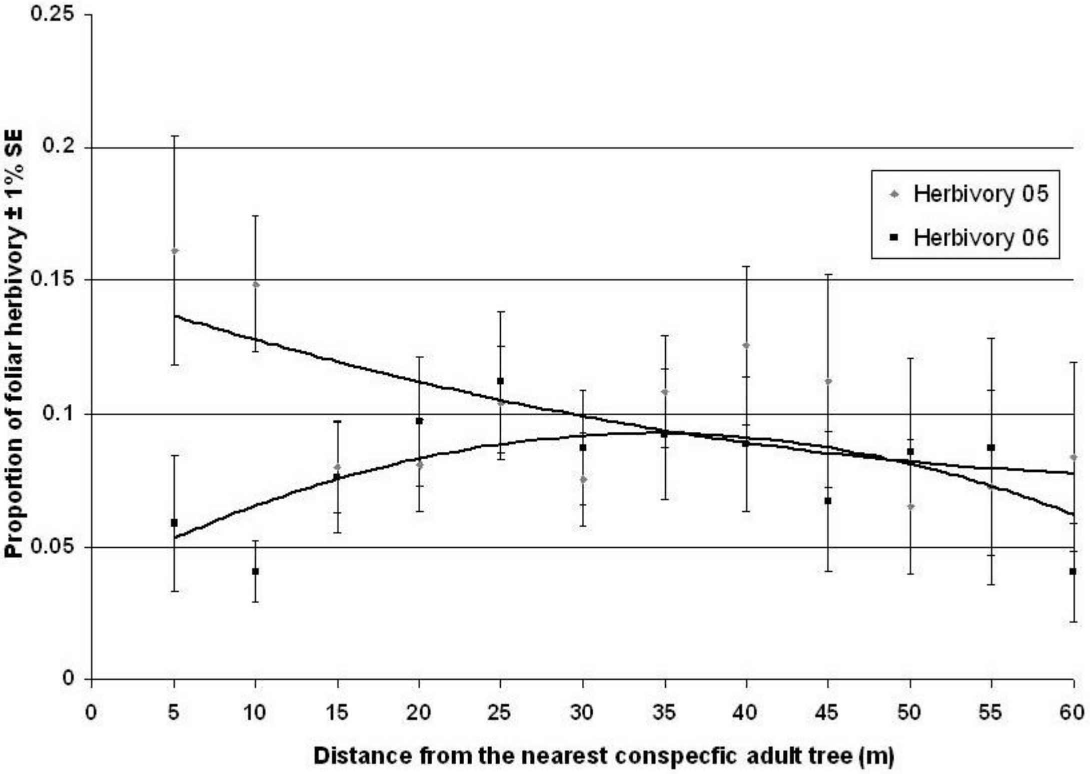
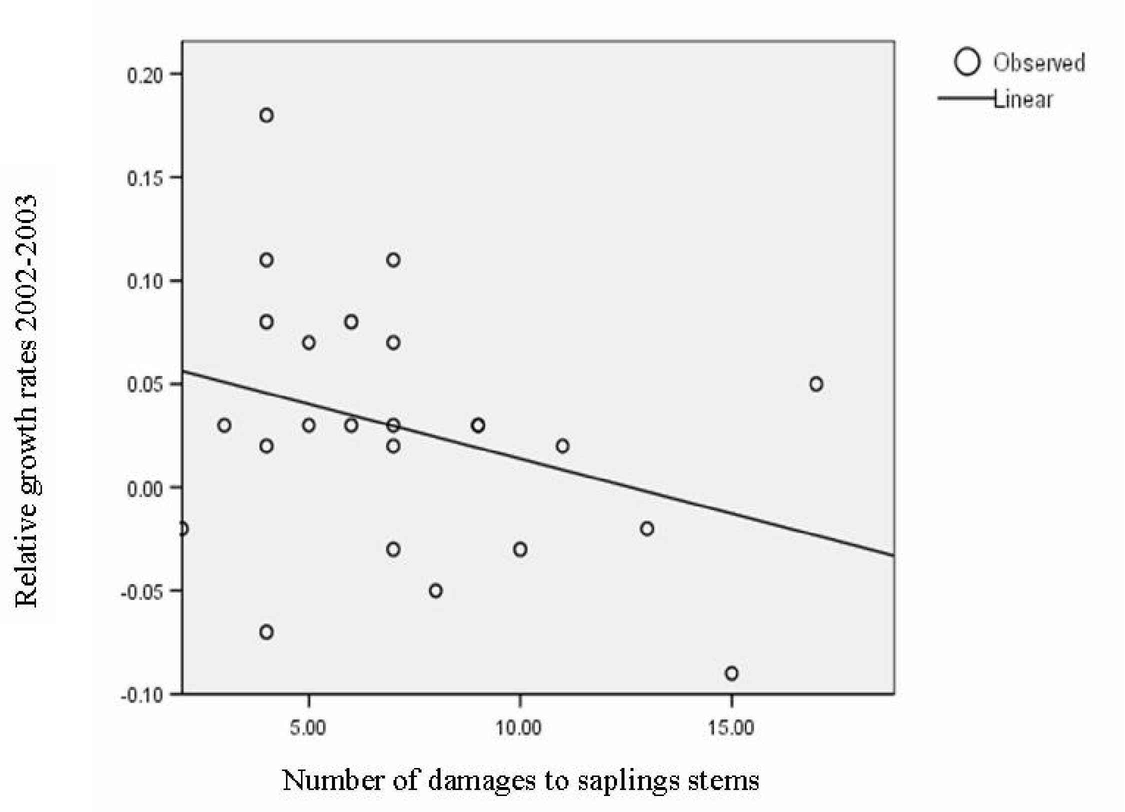


Figure 3.5. Sapling growth rate (2002-2003) as a function of the number of stem damage due to falling branches from the forest canopy.



CHAPTER IV

SEEDLING RECRUITMENT DISTRIBUTION OF *DIPTERYX OLEIFERA* BENTH (FABACEAE) CORRELATES WITH BAT ROOSTING PALMS AND DISTANCE FROM SEED SOURCES

INTRODUCTION

Seedling recruitment away from adult trees is fundamental for species diversity of tropical rain forests (Augspurger 1983; Clark et al. 1999a; Clark et al. 1998; Jansen et al. 2006; Janzen 1970; Schupp 1992). An important hypothesis poses that escape from natural enemies affects the spatial distribution of plant recruitment (Connell 1971; Janzen 1970), this is the Janzen-Connell hypothesis. There are two main effects driving plant diversity in this hypothesis. Density dependent effects occur when disproportionately high predation rates affect seedlings at higher densities. Distance dependent effects occur when predation rates are disproportionately higher closer to the nearest conspecific adult trees. Of course, this is usually also a place where seedlings are at high density.

The hypothesis predicts that escaping natural enemies would result in higher recruitment way from the nearest conspecific adult tree than if there were no effect of natural enemies. As a consequence, high numbers of tree species could coexist at low densities with large distances between them (Condit et al. 1992; Janzen 1970). Although this recruitment pattern has been widely reported in tropical studies (Clark and Clark 1984; Schupp 1990; Schupp 1992; Wyatt and Silman 2004), there is limited empirical evidence regarding the relative contribution of mechanisms involved (Augspurger 1983; Augspurger 1984). More research would lead to better understanding of the relative contribution of primary and secondary seed dispersal to plant recruitment (Howe and Miriti 2000; Howe and Miriti 2004; Howe and Smallwood 1982; Vander Wall et al. 2005) and their relative effect on the strength of the spatial relationship between the mechanisms proposed and the seedling recruitment distribution.

Efforts to understand this phenomenon focus on 1) determining which mechanisms could be involved and 2) devising methods to detect the proposed mechanisms in nature. One important mechanism is seed dispersal because it increases seed movement away from conspecific adult trees. An excellent model species to study the consequences of seed dispersal is the tropical tree species *Dipteryx oleifera*, as we know many details of its dispersal ecology (Clark and Clark 1984; Clark and Clark 1992; Clark and Clark 1991; Clark et al. 1993; Ruiz 2004). This species has an endocarp protected within hard, large pods (6 by 4 cm), which makes thread-tracking approaches suitable to evaluate the ecological effects of dispersal on short-term seedling survival. Throughout the rest of this paper we will refer to the pods as seeds, since seed is the term generally used while referring to propagules in studies exploring the effects of dispersal on recruitment (Vander Wall et al. 2005).

D. oleifera attracts two groups of seed dispersers: 1) bats, which disperse seeds from the adult trees and discard these in seed piles (primary dispersal) (Forget and Milleron 1991) and 2) terrestrial mammals, such as agoutis and squirrels, which could disperse seeds away from below the canopy of adult trees or from seed piled up by bats (secondary dispersal) (Fleming and Heithaus 1981). Terrestrial mammals are classified as either scatter hoarders if they leave seeds scattered individually across the forest floor, or cache-hoarders if they congregate seeds in small seed piles (named caches), on or buried in the forest floor (Jansen et al. 2006). Terrestrial mammals seed dispersal generally occurs when seeds are moved from highly dense areas and then forgotten after being moved to areas of lower densities.

D. oleifera is also associated with at least three palm species of tropical rain forests (*Astrocaryum alatum*, *Welfia regia* and *Cryosophila warscewiczii*), because bats use these palms as roosts while consuming the fleshy part of the propagule and later discard the seeds below. However, there is a fundamental problem with seed dispersal by bats, because after consuming the fleshy parts of the propagules, bats deposit seeds into dense seed piles below the roosting palm. Thus secondary dispersal by terrestrial mammals is fundamental to escaping density dependent mortality at seed piles.

Several important features of the ecology of bat roosting in palms are understood. For example: bat use palms to establish feeding roosts while resting (Fenton and Fleming 1976; Foster and Timm 1976). An issue that has been little explored are the implications that bat roosting has for the seedling recruitment pattern predicted by the Janzen-Connell hypothesis (Fleming and Heithaus 1981), the first goal of this study. The second goal is the evaluation of the density and distance effects on seed fate at experimental seed piles, and the third goal is the quantification of the spatial correlation between the distribution of seedling survival and the distribution of bat feeding roosts.

The working null hypotheses are four 1): Seed fate is independent to the density and distance of experimental seed piles to nearest adult tree of *D. oleifera*. 2) Seedling survival is independent of the distance from natural seed piles created by bats. 3) Seedling survival is independent of the distance from nearest conspecific adult tree. 4) Seedling survival is independent to the distribution of potential roosting palms (*A. alatum*, *W. regia* and *C. warscewiczii*).

METHODS

Study site. The research was conducted in a tropical rain forest site in southeastern Nicaragua from March 2003 to January 2005, at the research site known as La Union (12°05' N., 83°55' W., elevation 10-20 m; mean annual temperature 26°C) (IRENA 1991). The forest is lowland tropical rain forest accessible by the Caño Negro River at which we have worked since 1994. See Yih et al. (1991) or Granzow-de-la-Cerda et al. (1997) for a site description.

Study species. *Dipterix oleifera* is a Neotropical canopy emergent tree common in lowland forests of Central America, Panama and Venezuela. The species is also know as *Dipterix panamensis* (Fournier 2003). It can reach heights of ~50 m and diameters at breast height (DBH) of about 1,500 mm (Clark and Clark 1992). *D. oleifera* is a large seeded species with heavy seeds averaging 25.2 g ± 4.4 SD, n=80, (*unpublished data*). It is dispersed by bats (Forget and Milleron 1991) and terrestrial mammals (*i. e.* squirrels, agoutis, pacas, etc.) (Reid 1997). The wood is very dense and helps saplings to withstand damage inflicted by branches falling from the forest canopy (Clark and Clark 1991).

Astrocaryum alatum. This palm reaches about 7 m in height, with composite leaves used by bats as feeding and resting roosts (Foster and Timm 1976). At La Union site this species is present with ~ 50 individuals per hectare.

Cryosophila warscewiczii. This palm has small to medium-sized palm, reaching about 10 m in height, and with leaflets radiating away from a central point in an umbrella like shape. The life span of leaves of *C. warscewiczii* is approximately 5 years (Freiberg and Freiberg 1999). *Cryosophila* leaves are used by bats as sleeping and feeding roosts, and in order to establish the roost, bats remove tissue from one (or several) leaves at their base, these holes are used by the bats to hang downward as they feed or rest (Figure 4.1). *C. warscewiczii* has 20 to 25 individuals per hectare at La Union site.

Welfia regia. This palm ranges from 5 to 20 m in height. Bats use leaflets to hang downward, and in general remove tissue around the midribs leaflet at which they feed or rest. The number of adult and juveniles individuals of *W. regia* is ~8 individuals per hectare at La Union site.

Bats and terrestrial mammals. Several species of bats were captured around adult *D. oleifera* individuals and/or near natural seed piles. The species of bats trapped over the research period are listed in table 4.1. Terrestrial mammal trapping success has been extremely low in the area, as is frequently observed in studies at lowland rain forests (McClearn et al. 1994). To date, the mammal species captured by the trapping fieldwork at the La Union site are: *Proechimys semispinous*, *Oryzomys spp.*, *Nyctomys sumichirasti*, *Marmosa mexicana*, *Didelphys virginiana* and *Didelphis alventris*.

Mapping. A permanent 6.37 ha plot was established in 2002; the plot is divided up into 10 x 10 m quadrats. Data collected every year: seedlings' xy coordinates, seedling fate and seedling height. The sample size is large, for example from January 2003 to January 2005 there were records for 3,279 seedlings of *D. oleifera*.

Seed fate assessment. An experiment was conducted in order to evaluate the ecological effects of distance and density on seed fate. In this experiment seeds were labeled with a micro nylon thread produced by Imperial Thread Inc. The tip of the thread was glued to the seed and the bobbin clipped to the forest floor with a plastic toothpick. Previous studies have only evaluated the effect of seed removal, without considering the ultimate fate of removed seeds, for instance Cintra and Horna (1997) but see Witt (2001a) or DeMattia et al. (2004).

The conditions of dispersed, thread-marked seeds were evaluated from January to July 2005. Seeds were classified as preyed on or germinated. Two adult individuals of *D. oleifera* outside the research plot were selected for the experiment. The experimental design is a complete factorial (density x distance) as seeds were experimentally set out in low-density and high-density treatments, below and away from the adult trees (Figure 4.2). The treatment away from the adult tree was set up at 70 meters in order to experimentally evaluate the fate of seed at bat seed piles. For the low-density treatment, two labeled seeds were placed systematically in each of nine spots. These seed spots were 3.33 m apart with one treatment below and the other away from the adult tree ($n_{(below)}$= 9 x 2 and $n_{(below+far)}$ = 18 x 2 for a total of $n_{(low)}$ = 36 seeds). For the high-density treatment, seven seeds were placed in nine locations below and away from the adult trees ($n_{(near)}$ = 7 x 9, and $n_{(below+far)}$ = 63 x 2 for a total of $n_{(high)}$ = 126 seeds). Thus, there were eighteen replications per treatment, with each located below and far each selected adult tree. Kruskal-Wallis tests were used to determine whether seed conditions (preyed on or germinated) depended on density and distance from adult trees. In these analyses we compared the effect of distance and density (independent variables) on seedling fate (dependent variable). This set of experiment includes two distance treatments and two density treatments. The resulting outcomes are two 2x2 comparisons between seed fate and density or distance treatments.

Seedling survival away from the bat roost. In order to determine whether there were effects associated with distance to the nearest natural seed pile on seedling survival for 2004-2005, we mapped the *xy* coordinates for all natural seed piles to the nearest centimeter located within the research plot during February-June 2005. A logistic regression model was fitted to the proportion of seedling survival (dependent variable) as a function of the distance of seedlings to natural seed piles spot (independent variable) using SPSS.

Seedling survival and distance from adult trees. In order to determine the distance effect on seedling fate associated with conspecific adult trees, we mapped all individual seedling and adult trees to the nearest centimeter within the research plot as they were found from 2003 through 2005. Seedlings were classified as individuals with ≤ 100 cm maximum height. Maximum height is the vertical distance from the forest floor to the highest green tissue. An aluminum tag with a unique number was looped around the stems of each seedling as they were found. We recorded whether seedlings were healthy or when leaves were yellowish. Seedlings were considered dead when the aluminum tags were found alone or when seedlings were completely brown and without green tissue. When seedlings were not found for one year they were recorded as "not found", but if they were not found for two years they were considered to have died in the first year they were not found. Each seedling was assumed to be the offspring of its nearest conspecific adult tree.

The weighted means and standard errors were computed for the proportion of seedling survival with respect to distance to nearest conspecific adult tree per each period (t, $t+1$), from 2003 to 2005. The error bars were constructed using the seedling survival per each distance interval (5 m) between 14 adult individual of *D. oleifera* within the research plot. A logistic regression model was fitted to a seedling survival probability as a function of the independent variable distance to nearest conspecific adult tree for each measurement period using SPSS.

Seedling survival and spatial correlation with potential roosting palms. In addition to the mapping of *D. oleifera* we identified and mapped (*xy* coordinates), all adult and juvenile individuals of *W. regia*, *A. alatum* and *C. warsewitzii* within the research plot in January 2005. Adult palm individuals are classified as those with fruit or with evidence of having fruited in past years. Juvenile palms are those with developed stem above the ground but without evidence of fruiting in previous years. Only these three palm species were considered as potential roosting sites. This is based on the fact that these palm species were most frequently used as bat feeding or resting roost during the research and because of their high abundance within the research plot. For the rest of the paper we will refer to these palms as potential roosting palms when evaluating their spatial correlation with seedling recruitment.

In order to test the relationship of the spatial distribution of the roost individuals with the survivorship probability of *D. oleifera* seedlings we used the Partial Mantel test (Manly 1997). We tested for associations of the *xy* coordinates of potential roosting palm individuals by species in 2005 with the *xy* coordinates of seedling survival for 2004-2005. The Partial Mantel test provides a correlation coefficient Z ranging from 0 to 1, where 1 corresponds to a perfect correlation. We used Euclidean distance to compute the distance matrices and to standardize scales between them. Significance for the Z values were tested using a Monte Carlo randomization using 999 permutations. Mantel tests were independently carried out in two areas within the research plot: area with large adults *D. oleifera* or area with smaller adults *D. oleifera* (four 4 adult individuals per each area) using Pop-Tools (Hood 2006). Larger adult adults *D. oleifera* were different to smaller in that their crown were three to four folds larger and small adult *D. oleifera* produced just few tens of seeds.

RESULTS

Seed fate assessment. There were 19 natural seed piles in 2005 with a mean number of 8.7 ± SD 6.30 seeds per natural seed piles within the 6.5 ha plot. Mean distance of natural seed piles to the nearest adult of *D. oleifera* was 43.17 ± 15.56 m (SD). Palms were used as roosting sites in 61% of cases, out of which 17% were *C. warsewitzii* individuals. Non-palm trees were used as feeding roosts in 11% of the cases. In 28% of roosts, we could not identify the host.

Results of the Kruskal-Wallis tests evaluating the effects of density and distance with respect to adult trees are presented in table 4.2. There is a statistically significant trend in seed predation at high density further from the nearest adult tree. There is nearly statistical significant trend in seedling germination further from the nearest adult tree at high density.

Seedling survival versus distance from bat roosts. Seedling survival increased significantly with distance from bat roosts for the period 2004-2005 (Logistic regression, $R^2 = 0.3333$, n = 924 seedlings, p = 0.0008) (Figure 4.3).

Seedling survival versus distance form the adult tree. Seedling survival increased significantly with distance to the nearest conspecific adult trees for both 2003-2004 and 2004-2005 (Logistic regression $R^2= 0.7630$, n = 1,906 seedlings, p = 0.000 and $R^2 = 0.858$, n = 1,373 seedlings, p = 0.000, respectively) (Figure 4.4).

Seedlings survival and distribution of palms. The density of potential rooting palms was very similar between the large-and-small-adult-trees areas (Table 4.3). The results of the Partial Mantel test are presented separately for these two areas of the research plot (Figure 4.5). For smaller rectangle corresponding to area of large adult trees, there was only one, negative trend between seedling survival and any of the palms (*C. warsewitzii,* Z = -0.12016, p = 0.5516; *A. alatum*, Z = -0.2016; p =

0.0731; and *W. regia*, Z = -0.2011; p = 0.33323). For the larger rectangle or area with small adult trees in figure 4.5, the Partial Mantel tests showed significant positive correlations between seedling survival and one of the palms and nearly significant correlation for a second palm (*C. warsewitzii*, Z = 0.1318, p = 0.0841; *A. alatum*, Z = 0.2399; p = 0.0090; *W. regia*, Z = -0.0226; p = 0.418418).

DISCUSSION

Seed fate assessment. The results of the experiment evaluating the density effects on seed fate suggest that seeds escape predation when they are deposited in low density seed piles, either near or far from nearest conspecific adult tree. Seed germination was not different between distance treatments at low density. Seed predation is higher when seeds are located far from adult trees and at high density, than in seeds in piles of low densities thus supporting the idea that terrestrial mammals are more likely to find the larger piles. Seed germination was not quite significantly different between treatments with initial high density of seeds.

Seeding survival and distance from bat roosts. Seedling survival increased with distance to bat roosts. The results showed that seedling mortality is very high at the spot of the natural seed piles. Bat roost were located around 40 meters away from the nearest *D. oleifera* adult individuals; survival probabilities were higher >40 m away from *D. oleifera*. Seed dispersal from natural seed piles could lead, to some extent, to the observed Janzen-Connell seedling survival increase further from adult *D. oleifera* trees. A similar finding was reported in a field study conducted at a premontane moist forest in Costa Rica (Fleming and Heithaus 1981), where frugivorous bats (*i.e. Carollia perspicillata*) deposited seeds of *Cecropia peltata*, *Muntinga calabura* and *Piper spp*. under bat seed piles located ~50 m from food sources. In their study seedling survival was clumped around bat feeding roosts, similar to the findings here. An explanation for the seedling survival pattern of *D. oleifera* found with respect to natural seed piles is that seeds were dispersed by secondary means from these spots, and thus secondary seed dispersal might be largely responsible for the observed seedling shadow. Interestingly, seedling survival was zero within the first two meters from the bat roost.

Seedling survival and distance from adult trees. The observed pattern of seedling survival of *D. oleifera* with distance from adult trees is consistent with the expected seedling recruitment pattern according to the Janzen-Connell hypothesis. Similar results have been reported in previous studies for this species (Clark and Clark 1987; Condit et al. 1992). Our field observations and experiments suggest that this seedling recruitment pattern is determined, to some extent, by the two-step seed dispersal process involving bats and terrestrial mammals. Bats and terrestrial mammals can disperse seeds further from the adult trees thus leading to higher probabilities of seed and seedling survival. In a field study conducted at Barro Colorado Island, Panama, several bat species were observed moving seeds of *D. oleifera* (Bonaccorso et al. 1980). Field observation led us to conclude preliminarily that *Artibeus jamaicensis* is the main bat species dispersing seeds of *D. oleifera* at La Union site in Nicaragua. *A. jamaicensis* were captured near to bat roosting sites, although we did not capture any individual of this bat species carrying a seed of *D. oleifera* in our nets. *A. jamaicensis* is a large sized bat species that can move large seeds like those of *D. oleifera* (Reid 1997). In February 2006 an individual of *A. lituratus* was captured carrying a seed of *Virola sp.* Terrestrial mammals such as *Sciurus granatensis* (squirrels), *Dasyprocta punctata* (agoutis), and *Proechimys* *semispinosus* (spiny rat) prey on or disperse seeds of *D. oleifera* (Bonaccorso et al. 1980). Squirrels were observed during several days near natural seed piles during our fieldwork. In one occasion we were able to see a squirrel moving a seed from a natural seed pile, after which the squirrel buried the seed into the understory.

Seedling survival and correlation with palm distributions. Spatial trends between seedling survival and the distribution of each potential roosting palm species are negative and almost significant or nearly so in area of large conspecific adult trees. It is possible that negative spatial trends between the location of potential roosting palms and the distribution of seedling survival in areas of large adult *D. oleifera* trees might be the result of roost site selection. It has been suggested that bat roosts conceal bats from predators and shelter them from the weather (Timm and Mortimer 1976). The bats might be selecting roosting sites in palms in areas with lower abundances of their natural enemies (*i.e.* owls and boa constrictors), where spatial trends between the location of potential roosting palms and the distribution of seedling survival are positive, significant or nearly so.

Assumptions and errors. The seed experiment probably underestimated the role of terrestrial mammals as secondary dispersers from artificial seed piles. The artificial seed piles were qualitatively distinct from natural seed piles in that their seeds were drier, since seeds used in the experiment were stored during several weeks prior to the experiment being set up. It is probable that terrestrial mammals cue on the strong odor of fresh seeds. Consequently, artificial seed piles might be less likely to be found by these terrestrial mammals, thus leading to a general underestimation of predation. Although we recognize that there are spatial trends between potential bat roosting palms species and seedling survival in areas of small adult individuals of *D. oleifera*, the presence of natural enemies of bats in the crowns or large *D. oleifera* individuals was assumed to enhance this correlation. To our knowledge there has not been a study that evaluated the spatial distribution of predation on bats in tropical rain forests, and thus our explanation is purely speculative; but see Boinski and Timm (1985). Field studies in tropical forest have shown bat preference for palms to establish feeding roosts (Fleming and Heithaus 1981; Foster and Timm 1976), here we present evidence suggesting that bats select for sites with lower densities of adult trees of *D. oleifera* to establish their feeding roosts.

Hypothesis testing. We rejected the null hypothesis that seed mortality and germination is independent of distance from and density of the experimental seed piles. The non-significantly differences in seed survival at experimental low densities with respect to the nearest conspecific adult tree, indicates that seeds at low experimental densities were not more likely to be damaged by seed predators if they were close to an adult tree. On the other hand, seeds at high experimental densities were significantly more damaged further than closer from nearest conspecific adult tree. However, the trend in seed germination was nearly significant further from adult trees at high density. This result suggests that although seed piles were predated at high densities further from the adult tree, a nearly significant number of seed escaped predation under these conditions. For seedlings, the results supported the Janzen-Connell distance hypothesis. A significant fit to the logistic regression allowed us to reject the hypothesis that seedling survival is independent to the distance from seed piles created by bats. The spatial trends in the small-adult-tree-density area led us to reject the hypothesis that seedling survival is independent to the distribution of potential roosting palms *A. alatum* and nearly so for *C. warscewiczii*.

In conclusion, we have presented empirical evidence concerning how seedling recruitment occurs in *D. oleifera*. The long mean distances between surviving seedlings and adult trees supports the distance hypothesis proposed earlier (Connell 1971; Janzen 1970). This seedling survival pattern is critically dependent on the patterns of seed dispersal by bats and terrestrial mammals.

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Table 4.1. Bat species identified within the study area in Southeast Nicaragua. Feeding habits and estimated weight were obtained from Reid (1997).

Species	**Feeding habit**	**Weight (g)**	**Size**
Artibeus intermedius	Frugivorous	40-54	Large
Artibeus jamaicensis	Frugivorous	21-51	Large
Artibeus lituratus	Frugivorous	53-73	Large
Artibeus phaeotis	Frugivorous	9-15	Small
Artibeus watsoni	Frugivorous	9-15	Small
Carolia perspicillata	Frugivorous	21-23	Medium
Chiroderma villosum	Frugivorous	15-18	Medium
Carolia castanea	Frugivorous	11-16	Small
Myotis nigricans	Insectivorous	3-6	Small
Phyllostomus discolor	Insectivorous	26-51	Large
Trachops cirrhosus	Insectivorous	24-36	Medium
Vampyressa nymphaea	Bloodsucking bat	11-16	Small
Glossophaga soricina	Nectivorous	7-12	Small
Hylonycteris underwoodi	Nectivorous	6-12	Small

Table 4.2. Density and distance effects on seed fate of *Dipteryx oleifera*. The values reported are p-values for Kruskal-Wallis test (KW) testing the effect of density and distance on seedling fate. There are four outcomes, first "a" indicates that non-statistics were computed because seed predation was constant at low densities. Second, clockwise, is the value testing the effect of high density on seed predation. Third, is value testing the effect of low density on seed germination. Fourth, is the value testing the effect of high density on seed germination.

		Distance (near versus far)	
		Predated	Germinated
Density	Low	*a*	0.303
	High	**0.014**	**0.087**

Table 4.3. Density of potential roosting palms in two areas within the permanent research plot (individuals/hectares). Area “I” is the area with large adult individuals of *D. oleifera* and area “II” is the area with smaller adult *D. oleifera* individuals.

Area	Palm species	Density/Ha.	Total area
a	*A. alatum*	149	1.5
a	*C. warsewitzii*	39	1.5
a	*W. regia*	21	1.5
b	*A. alatum*	108	2.08
b	*C. warsewitzii*	56	2.08
b	*W. regia*	15	2.08

Figure 4.1. An individual *Artibeus watsoni* roosting in the palm *Criosofila warsewitzii*. Bats were frequently found roosting in palms at La Union site (Photo credit: Delvis Guillén).

Figure 4.2. Seedling survival with respect to nearest bat feeding roost from 2004 to 2005. Seedling survival probability was zero at the location of the natural seed pile and increased meters from these spots. Line represents logistic regression fit (R^2 = 0.3333, n = 924 seedlings, $p < 0.05$).

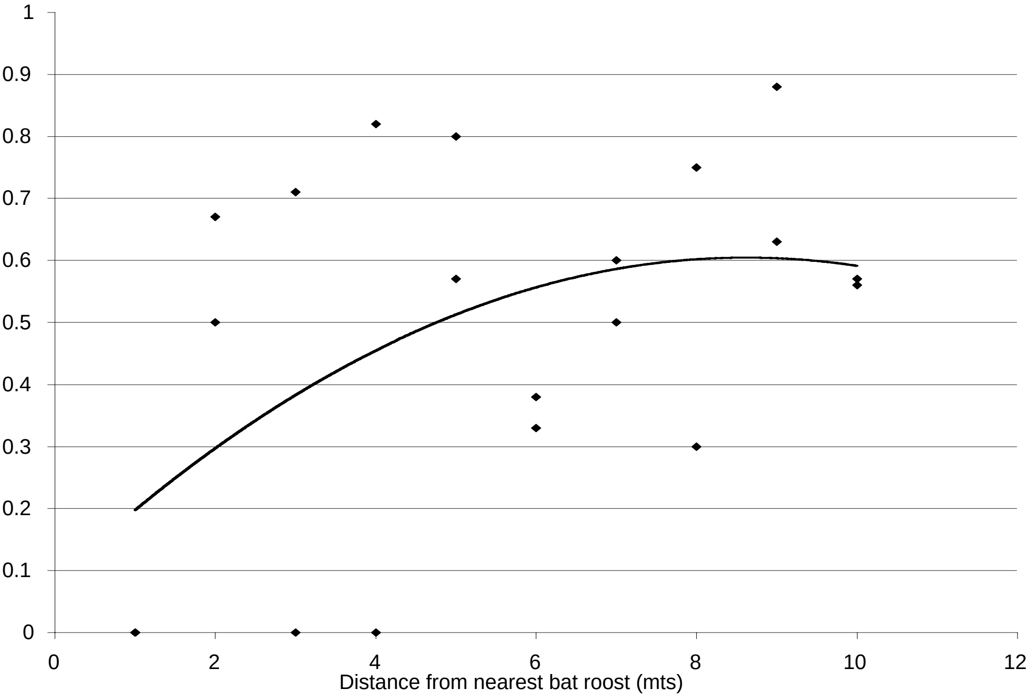


Figure 4.3. Factorial experiment design setup to test for the effect of distance and density on seed fate. Large circle represent two adult *D. oleifera* individuals located outside the research plot. Small squares represent the locations of artificial seed piles; the numbers with arrows represent seed number per treatment and number within bracket represent the distance between distance treatments.

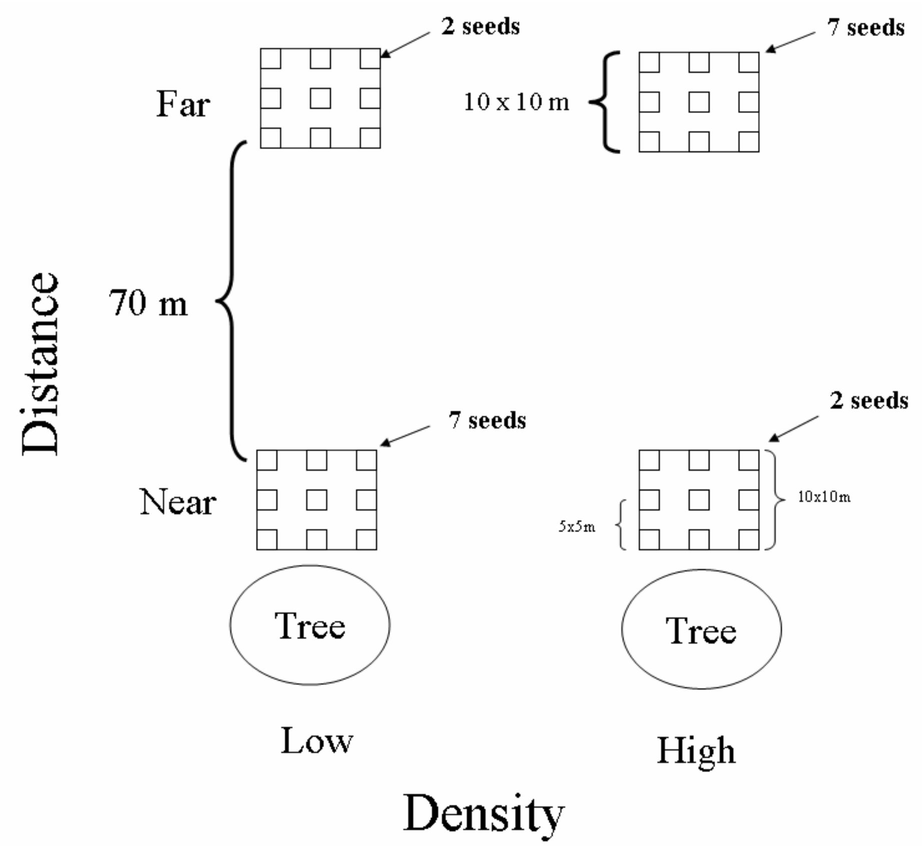


Figure 4.4. Weighted mean of seedling survivorship probability of *D. oleifera* versus distance to nearest conspecific adult tree for periods 2003-2004 and 2004-2005. Total number of seedlings tagged during the measurement period is 3,476. Seedling survival was calculated from 0 to 60 m per each 5 m distance interval with respect to 14 reproductive individuals within a 6.37 ha research plot. Error bars are the standard errors of weighted mean of seedling survival per distance interval between adult individuals located within the research plot. Shaded area is the mean distance ± 95% CI of 19 natural seed piles with respect to nearest adult tree of *D. oleifera*.

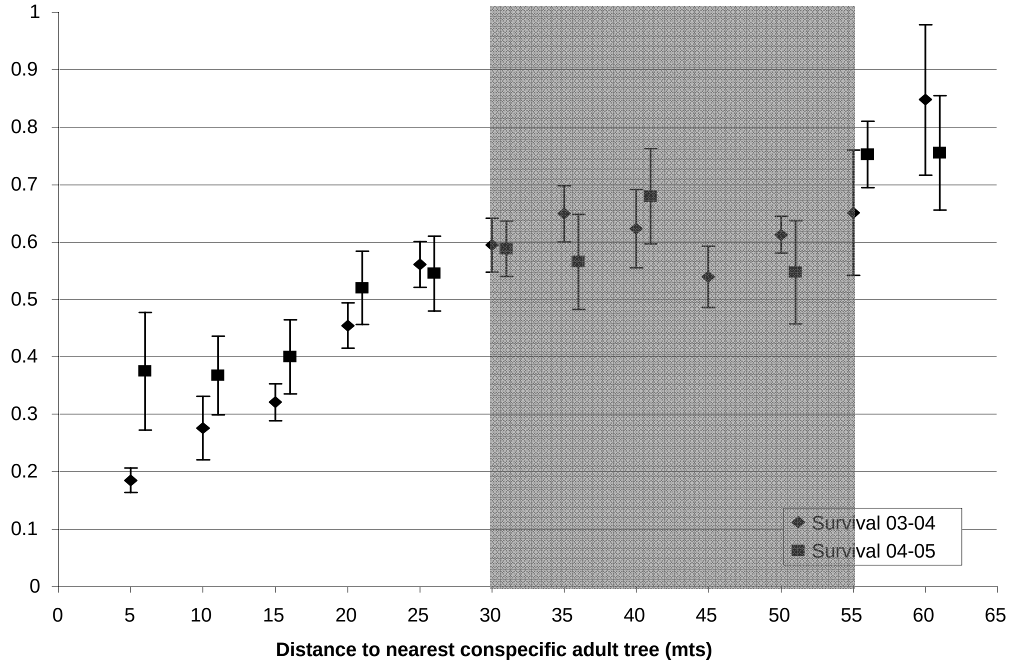
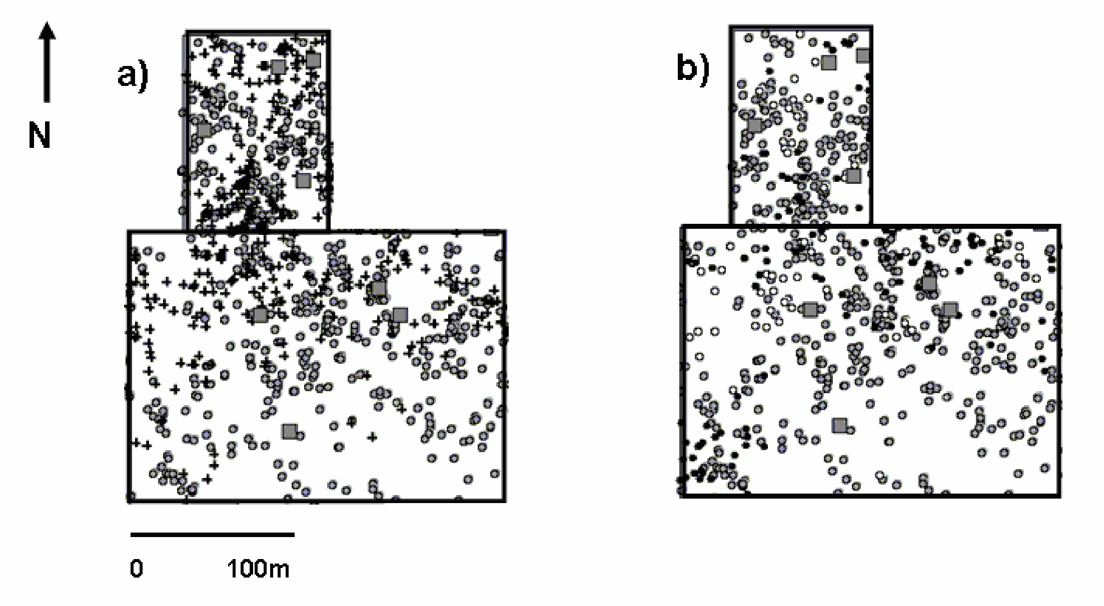


Figure 4.5. Distribution of *D. oleifera* and potential roosting palms within the research plot. Smaller square is referred in the text as the area with large adult individuals of *D. oleifera* and larger square is the area of smaller adult individuals of *D. oleifera*. There are four adult individuals in each area. Solid grey squares represent adult trees. Solid black crosses represent *C. warsewitzii*. Small open circles represent *W. regia* and small black solid circles represent *A. alatum*. Small gray circles represent surviving seedlings during the period 2004-2005.



CHAPTER V

SEED FATE AND SEEDLING ESTABLISHMENT OF DIPTERYX OLEIFERA BENTH (FABACEAE) IN BAT ROOSTS AND CACHES: A TEST OF THE SEED MASKING HYPOTHESIS

INTRODUCCTION

An important ecological consequence of pollination limitation is seed unviability in obligated outcrosser plants (Perry and Starrett 1980). We pose and test the hypothesis that if seed damage decreases with the number of unviable seeds then there will be a "masking effect" for viable seeds. This seed masking effect would determine plant recruitment distribution. We used as a model system *Dipteryx oleifera* and associated mammal seed dispersers to test the "seed-masking hypothesis". Our approach is novel since research on recruitment dynamics mainly focuses on the evaluation of ecological effects on seed and seedling recruitment and little is known about the ecological consequences of pollination limitation on earlier development stages – *i.e.* effect of unviable seeds on the transition from viable seed to seedling. Several hypotheses addressing why trees produce unviable seeds have been studied: 1) that there is pollination limitation (Kelly et al. 2001; Kelly and Kadley 2004), 2) that unviable seeds are the result of environment fluctuations (Kelly 1994; Monks and Kelly 2006), 3) endogamy (intraespecific fecundation) (Fuchs et al. 2002; Quesada et al. 2004) and 4) plant-seed predator interactions (Elliott 1974; Sork 1993). The focus of the present communication is to test for the seed masking effect.

Masking viable seeds among unviable ones could lead to larger recruitment rates because it can lower seed predator search efficiency to a low point where seeds escape consumption by granivores (Kelly 1994; Kelly et al. 2001). For example, squirrels, which spend short periods of time searching for seeds at the forest floor (Reid 1997). Thus when a large number of unviable seeds are produced there can be high probabilities for viable seeds to escape damage due to a lowered squirrel search efficiency. For granivores, there is no nutritional value in unviable seeds, since it is the endosperm that is eaten. For the plant, the cost of producing unviable seeds could be lower than producing viable seeds, because unviable seeds do not have endosperms or the endosperm is undeveloped. However, because the unviable seed only lacks the developed endosperm, plants invest a large amount of energy in producing unviable propagules.

A leading hypothesis proposes that massive seed production can lead to escape seed predation through the process of predator satiation (Curran and Leighton 2000; Kelly 1994). In this hypothesis seed predator abundance increases as a function of high seed density (Nakagawa et al. 2005; Peres and Baider 1997; Rocha and Redaelli 2004; Yen 1983). However, this increase in granivore abundance is then limited by granivore foraging efficiency above a seed density threshold where seeds escape predation. Parallel to this notion is the hypothesis that plants respond to granivore pressures by way of producing large seed crops that could satiate seed predators. This plant antipredator adaptation could lead to higher seed survival rates by alternately starving and satiating seed predators (Herrera et al. 1998; Sork 1993). As a consequence escaping predation during mast years, can result in higher seedling recruitment than would occur during regular seed production years (De Steven and Wright 2002; Kelly 1994). This escape can occur toward the end of the seed production period when rodent seed predators also lose interest in consuming germinating seeds (Forget 1992; Norconk et al. 1998). An alternative idea is embodied in the seed masking hypothesis because it proposes that whenever a larger number of unviable seeds are produced viable seed predation rates would be lower and thus unviable seeds could determine seedling recruitment by the process of seed masking.

We predict that the effect of seed masking on seedling recruitment can be stronger away form the adult tree where seedling survival is high. Field evidence supports the idea that a fraction of diaspores escape damage by seed predator (Wyatt and Silman 2004), insect herbivores (Augspurger 1984) and pathogens (Augspurger 1983; Parker and Clay 2000) away from adult trees where the density of natural enemies is lower (Connell 1971; Howe and Smallwood 1982; Janzen 1970).

We investigated whether production of unviable seeds during a year of high seed production increases germination rates and whether seed masking occurs away form adult trees. Specifically, this research addresses three working null hypotheses: 1) Seed predation rate is independent of the number of unviable seeds. 2) Seed germination and seedling recruitment rate is independent of the number of predated seeds. 3) Seed germination and seedling recruitment rate is independent of the number of unviable seeds. We used naturally occurring bat seed piles and caches of *D. oleifera* located away from the nearest conspecific adult tree to address the research questions and the working hypotheses.

METHODS

Study site. The research was conducted in a tropical rainforest site in southeastern Nicaragua from January 2006 to August 2006 (12°05' N, 83°55' W), mean annual temperature 26°C (IRENA 1991). The forest is a lowland tropical rainforest known as La Cooperativa Unión, accessible by Caño Negro River where we have worked since 1994 (Granzow-de-la-Cerda et al. 1997; Ruiz et al. 2001; Vandermeer et al. 2000).

Mapping. The study was conducted within a 6.37 ha plot (10x10 m quadrats) established in 2002. During 2006 we mapped and followed the fate of 946 seeds present at two seed pile types as they appeared in a 3.2 ha subplot within the 6.37 ha plot. The sample size is large in comparison to other studies of the relative contribution of dispersal (Cintra 1994; Cintra and Horna 1997; DeMattia et al. 2004), or distance and density effect studies (Augspurger 1984; Clark and Clark 1984; Hyatt et al. 2003; Schupp 1992).

Study species. *D. oleifera* is a Neotropical canopy emergent tree common in lowland forests of Central America, Panama, and Venezuela. It can reach a height of ~50 m and diameter at breast height of about 1,500 mm (Clark and Clark 1992). This is a large seeded species ~ 6x4 cm; dispersed by bats (*i.e. Artibeus jamaicensis*, *Artibeus literaturs*, etc.) (Bonaccorso et al. 1980) and non-flying mammals (*i.e.* squirrels, agoutis, pacas, etc.) (Cintra and Horna 1997; Forget 1992; Forget 1993; Forget and Milleron 1991). The wood is very dense and allows saplings to withstand damage by branches falling from the forest canopy (Clark and Clark 1991).

Bats and rodents. Several species of bats were captured near to and far from adult individuals of *D. oleifera*, and near to bat roosts. The species of bats trapped over the research period, are displayed in table 5.1. Non-flying mammal trapping success has been extremely low, as is frequently observed in lowland evergreen rainforest studies (McClearn et al. 1994). To date, the species captured by the trapping fieldwork at the La Cooperativa Unión site are: *Proechimys semispinous*, *Oryzomys spp.*, *Nyctomys sumichirasti*, *Marmosa mexicana*, *Didelphis virginiana*, and *Didelfhis alventris*.

Field observations led us to preliminarily conclude that *Artibeus jamaicensis* is the main bat species dispersing seeds of *D. oleifera* to bat roost at the study site in Nicaragua. *A. jamaicensis* is a large sized bat species that can move large seeds like those of *D. oleifera* (Reid 1997). *A. jamaicensis* individuals were captured near to bat roosts, although none were captured carrying a seed of *D. oleifera* in our nets. In February 2006 an individual of *A. lituratus* was captured carrying a seed of *Virola sp.*

Seed fate assessment, germination, and seedling recruitment. In order to evaluate seed fate we mapped and threaded seeds as they appeared within a 3.2 ha subplot from January to July 2006. We super-glued a micro precision nylon thread to all seeds found within this area. The thread consists of a micro fiber attached to the seed and the bobbin was clipped to the forest understory with a toothpick. Each bobbin was numbered with a unique number such that seed identity was recorded during the research. Previous studies have only evaluated the effect of seed removal (Cintra 1994; Cintra and Horna 1997; Vander Wall et al. 2005), as pointed out in a review of the literature on seed dispersal (Vander Wall et al. 2005). The seed threading approach allowed us to follow seed movements and ultimately to determine seed fate (DeMattia et al. 2004; Forget and Wenny 2005; Witt 2001b). We used the threading procedure developed earlier and innovated this method to account for the fate of thousands of seeds. When seeds were found eaten we did not thread these, although we did noted their position. Seed conditions were measured at least once each month for a period of eight months. Propagule conditions were classified as: predated, unviable (when threaded but without or with undeveloped endosperm), germinated, and recruited (1 yr after germination).

Bat roosts and caches of *D. oleifera* were systematically located as we conducted the seed censuses during 2006. We noted whenever the seeds were congregated into bat roosts as opposed to caches. In general, bat roosts were located below bat roosting palms (Ruiz *et al.*, *in preparation*). Seeds at bat roosts were with either bat feces or with marks of manipulation by bats. For example, pods were partially covered with the fleshy membrane that bats eat. Cached seeds had signs that they had spent a longer time in the forest floor (*i.e.* seeds had bare pods, without vestiges of fleshy membrane around them; pod coloration was darker).

Early during the seed production season we noticed that a considerable proportion of seeds were unviable (smaller and lighter seeds). We used pocketknives to split the seeds' pods and determined whether seeds were unviable about one month after the germination time. Unviable seeds did not have endosperm, or the endosperm or the pods were undeveloped, and never germinated. Unviable seeds were distinct from viable seeds in their physical characteristics (Figure 5.1), which allowed us to precisely distinguish between these two seed conditions in the field. Also, unviable seeds were smaller, whiter, and with softer pods than viable seeds. When seeds were moved and opened by rodents, we were able to distinguish between viable and unviable seeds by their coloration and hardness (density) of the pods.

Several statistical analyses were used to test the seed masking hypothesis. Means and standard errors of number of seed fates (predated or germinated) were calculated in order to compare differences between bat roost and caches. Mean and standard errors of the distance to the nearest conspecific adult tree were computed in order to carry out comparisons between bat roosts and caches. Data corresponding to the number of seeds and seedlings were analyzed using generalized linear models (Faraway, 2005). In specific rate models, which have the following general form:

$$\ln(Y) = \ln(Z) + \alpha X + \varepsilon \qquad (1)$$

Where Y indicates the number of positive outcomes out of Z trials, α is a vector of parameters for the X vector of predictors and ε is i.i.d. normal error, which is obtained once the iterative process for fitting a Poisson Generalized Linear Model (GLM) is done. The process of fitting Negative Binomial GLMs, includes the additional correction for over dispersion in the dataset (Faraway, 2005). Specifically, we studied the rate of predated seeds as a function of unviable seeds and type of seed pile (bat roosts or caches) using a Poisson GLM. Data corresponding to the number of germinated seeds and seedling recruits were analyzed using Negative Binomial GLM, since data was overdispersed (Faraway, 2005). The rate of seed germination was studied in a model as a function of unviable seeds, and in another model as a function of predated seeds, in both considering the type of seed pile. The rate of seedling recruitment was studied using a Negative Binomial GLM as a function of unviable seeds, and in another model as a function of predated seeds, in both considering the type of seed pile as before.

RESULTS

Mean number of seeds at the bat roosts and caches. There were 638 seeds in a census of 60 bat roosts and 308 seeds in a census of 32 caches. This sample corresponds to 27.92% of the seed production. There was no difference in the mean number of seed fates between bat roosts and caches (Figure 5.2). Out of the total number of seeds at the bat roosts none was moved from bat roosts and further congregated into caches or vice versa. Sixty-six percent of seeds at these seed piles were predated.

Distance to nearest conspecific adult. The mean distance from nearest conspecific adult was 41.90±2.43 m for caches and 40.94±1.48 m for bat roosts. Mean secondary seed dispersal is ~ 1.5 m from where the seed was first found (Figure 5.3a).

Seed fate at bat roosts and caches. Results of the Poisson generalized linear model are presented in table 5.2. For each additional seed deposited the rate of seed predation decreased to 94% of what it was before its addition. Results of the Negative Binomial GLM show that there was no significant difference in the rate of seed germination as a function of unviable seeds. However, for each predated seed in the pile the rate of change in seed germination decreased to 96% of what it was before its addition. Per each additional unviable seed the rate of seedling recruitment is increased to106%. Per each additional damaged seed the rate of seedling recruitment is increased to 106% of what it was before the additional seed was damaged.

Seedling fate. Seedling survival increased with distance to the nearest *D. oleifera* adult (quadratic regression: $r^2 = 0.713$, n=578, p = 0.004) from April 2006 to April 2007 (Figure 5.3b).

DISCUSSION

Results of this study support the seed masking hypothesis at the two seed pile types. The overall effect of unviable seeds on seed predation was the reduction of seed predation. For each unviable seed deposited into the seed piles the rate of seed predation is decreased and predated seeds diminished the rate of seed germination but increased seedling recruitment. Then, increasing the number of unviable seeds decreases the rate of predation, which concomitantly increases the rate of germination and seedling recruitment. There was no difference in the mean number of unviable or viable seeds between bat roosts and caches. Both seed pile types were located around forty meters from the nearest conspecific adult tree into an area of high secondary seed movement and high seedling recruitment rates (Figure 5.3). Thus suggesting a positive trend between seed masking and seedling recruitment distribution away from the conspecific adult tree.

Unviable seeds reduced seed predation and as a consequence can increase seedling recruitment. The negative effect of unviable seeds on seed predation in seed pile types is consistent with the pattern expected from the seed masking hypothesis. Unviable seeds were produced earlier presumably during flowering. Pollination biology of *D. oleifera* suggests that outcross pollination limitation could be an important mechanism involved in the production of unviable seeds. In a field study, outcrossing occurred in a large proportion of the flowers of *D. olefiera* during low reproductive years because pollination competition forced bees to pollinate among flowering individual adult trees (Perry and Starrett 1980).

There was a negative effect of seed predation on germination. This means that there is a significant strong pressure of granivores on seedling recruitment. In field studies conducted in a tropical rain forest, the rodents *Sciurus granatensis* (squirrels), *Dasyprocta punctata* (agoutis), and *Proechimys semispinosus* (spiny rats) predated or dispersed seeds of *D. oleifera* (Bonaccorso et al. 1980). Our field trapping assessment shows that squirrels (*Sciurus richmondi*), and spiny rats (*Proechimys semispinous*), are the principal agents of secondary seed movements. Squirrels were observed during several days near bat roosts. In one occasion we were able to see a squirrel moving a seed from a bat roost and then the squirrel buried the seed into the understory. *Proechimys semispinous*, a potentially important seed disperser, was captured during mammal trapping assessment. An alternative explanation for escaping seed predation is that of alternation between high and low seed production periods, which can reduce seed predator community and thus the overall rates of seed predation at the plant community level (Curran and Leighton 2000; Ruscoe et al. 2005; Schnurr et al. 2002). The results presented here support the seed masking idea since the number of unviable seeds increased propagule survival by way of reducing seed predator search efficiency at the seed piles. Unviable seeds were produced abundantly, which may have had reduced granivore consumption to 94% of what it was before the addition of an unviable seed. Because of this reduction in seed predation a number of viable seeds escaped damage.

There were no statistically significant effects of the number of unviable seeds on the rate of seed germination or the rate of seedling recruitment. This effect is lost because of the lack of statistical power from the approximations used to fit Negative Binomial GLM (Faraway, 2005). The lack of statistical power is related to the low sample size of individuals germinating and later recruiting. The explanation to the pattern of seedling survival is that bats congregated viable and unviable seeds away from the nearest conspecific adult tree, where only 4.55% of the individuals at the seed piles survived one year after germination. Seeds escaped rodent predation when removed from seed piles (Witt 2001a), forgotten (Forget 1990), or mistakenly taken as unviable seeds by the seed predators. This provides evidence in favor of the observed seedling survival recruitment pattern (Figure 5.3b), typically reported in lowland tropical rain forests (Condit et al. 1992; Ruiz 2004). Since there is a strong positive correlation between seedling survival and the distance to the adult trees, this pattern suggests that seed masking can contribute to the high seedling survival observed away from the adult tree (Figure 5.3b).

There is a positive trend between the number of predated seeds and the rate of seedling recruitment (Figure 5.4). Seed predation at the seed piles can reduce negative density dependent effects on seedlings (i.e. herbivory, fungi infection, etc.) at the seed pile's spot or further away when seeds were moved but not predated. This effect is non-significant due to the low sample size of seedlings, however, negative density dependent effects have been reported at the focal tree individual level (Antonovics and Levins 1980; Augspurger and Kitajima 1992; Augspurger 1983; Augspurger 1984), or the tree community level (Condit et al. 1992), in previous fieldwork. The decrease in viable seed and seedling density close to the seed piles could reduce the risk of infection by pathogens or damaged by insect herbivores.

An overwhelming majority of hermaphroditic species was found to be self-incompatible. In a hand pollination experiment, 24 out of 28 (80%) species of lowland tropical rainforest were self-incompatible (Bawa et al. 1985). These results suggest that seed masking due to cross-pollination limitation could be an important mechanism involved in the process of seedling recruitment. Because, if seed masking in obligated outcrosser tree species increases seedling survival further from the adult tree species, then it could support the assertion that multiple tree species could coexist with long distances between adult individuals (Janzen 1970). More research is needed in order to evaluate the relative temporal and spatial components of the seed masking hypothesis at the community level.

The results presented here addressed the null hypotheses: 1) Seed predation rate is independent of the number of unviable seeds. The result showed that the rate of seed predation decreased with the number of unviable seeds. 2) Seed germination and seedling recruitment rate is independent of the number of predated seeds. Seed predation significantly reduced seed germination, although there was a positive trend between number of predated seeds and seedling recruitment rate. 3) Seed germination and seedling recruitment rate is independent of the number of unviable seeds. Seed germination rate did not depend on the number of unviable seeds or seed pile types. However, since unviable seeds decreased seed predation then there is evidence to suggest a seed masking effect. We conclude that masking increases seedling recruitment away from conspecific adult trees in *D. oleifera*.

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Table 5.1. Bat species identified within the study area in Southeast Nicaragua. Feeding habits and estimated weight were obtained from Reid (1997).

Species	**Feeding habit**	**Weight (g)**
Artibeus intermedius	Frugivorous	40-54
Artibeus jamaicensis	Frugivorous	21-51
Artibeus lituratus	Frugivorous	53-73
Artibeus phaeotis	Frugivorous	9-15
Artibeus watsoni	Frugivorous	9-15
Carolia perspicillata	Frugivorous	21-23
Chiroderma villosum	Frugivorous	15-18
Carolia castanea	Frugivorous	11-16
Myotis nigricans	Insectivorous	3-6
Phyllostomus discolor	Insectivorous	26-51
Trachops cirrhosus	Insectivorous	24-36
Vampyressa nymphaea	Bloodsucking bat	11-16
Glossophaga soricina	Nectivorous	7-12
Hylonycteris underwoodi	Nectivorous	6-12

Table 5.2. Proportion of seed and seedling fate of *D. oleifera* in response to number of unviable seeds and seed predation at bat seed piles and caches. Letters represent the direction and type of effect of unviable seeds on predation (UP); unviable seeds on germination (UG); unviable seeds on seedling recruitment (UR); seed predation on germination (PG); seed predation on seedling recruitment (PR), seed predation on seedling recruitment (PR); germination on seedling recruitment (GR). BC is the baseline of comparison for Poisson generalized linear models (GLMs) and Negative binomial (GLMs). The sign “-o” represents negative effects and “->” represents positive effects. C= Cache, B = Bat roost, NU = No. Unviable seeds, NP = No. Predated seeds, NG = No. Germinated seeds. Rate corresponds to the effect of either (U, P or G) on P or G or R (second column). Dep. Var. = dependent variable.

Model	Direction and type of effect	Predictor	Dep. Var.	Rate of Dep. Var. (95% CI)	Wald Z
	BC	C		1	-
a.	U -o P	B	P	1.00 (0.85; 1.18)	0.008
		NU	P	0.94 (0.91; 0.97)	-3.486*
b.	U - -o G	B	G	0.92 (0.57; 1.50)	-0.106
		NU	G	0.99 (0.90; 1.11)	-0.34
c.	P –o G	B	G	0.91 (0.59; 1.42)	-0.382
		NP	G	0.96 (0.94; 0.99)	-2.740*
d.	U - -o R	B	R	0.76 (-1.10; 3.22)	0.9076
		NU	R	1.06 (-2.22; 3.75)	-0.6909
e.	P -> R	B	R	0.76 (-1.10; 3.22)	0.9076
		NU	R	1.06 (-2.22; 3.75)	-0.6909
f.	G -> R	B	R	0.92 (-0.90; 3.22)	-0.2651
		NG	R	1.15 (-1.85; 3.69)	5.8688*

*(P<0.005).

a. (Goodness of fit (GF): chi^2=85.55, df=85, P>0.46)

b. (Dispersion parameter (DP) [±S.E.] = 1.88 ± 0.64; GF: chi^2=92.82, df=84, P>0.24).

c. (DP [±S.E.] = 2.41 ± 0.90; GF: chi^2=91.92, df=84, P>0.26).

d. (DP [±S.E.] = 1.48 ± 1.03; GF: chi^2=21.862, df=84, P=1.790e-05).

e. (DP [±S.E.] = 1.34 ± 0.852; GF: chi^2=21.447, df=84, P=2.203e-05).

f. (DP [±S.E.] = 3358± 45839; GF: chi^2=39.322, df=84, P=2.893e-09)

Figure 5.1. a) Seed with undeveloped endosperm (unviable seed) and b) seed with developed endosperm (viable seeds) (Photos: Javier Ruiz).

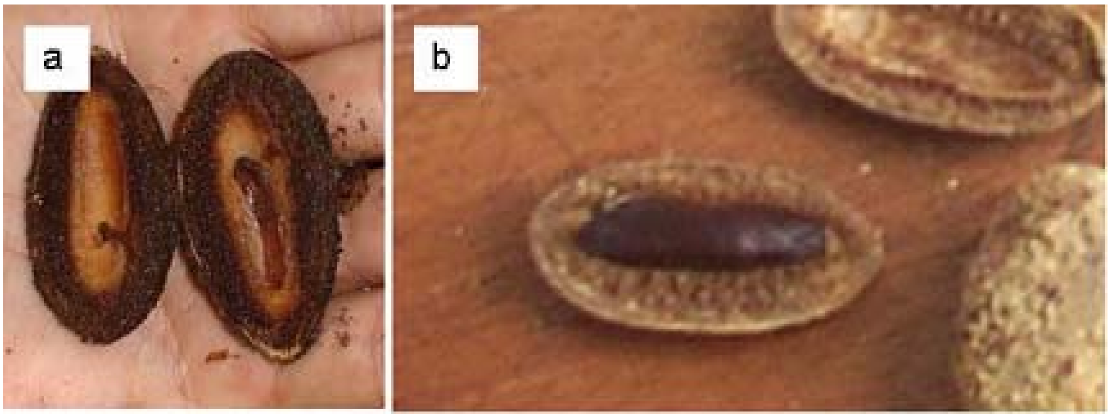


Figure 5.2. Mean and standard error of the number of seeds present at bat roosts and caches.

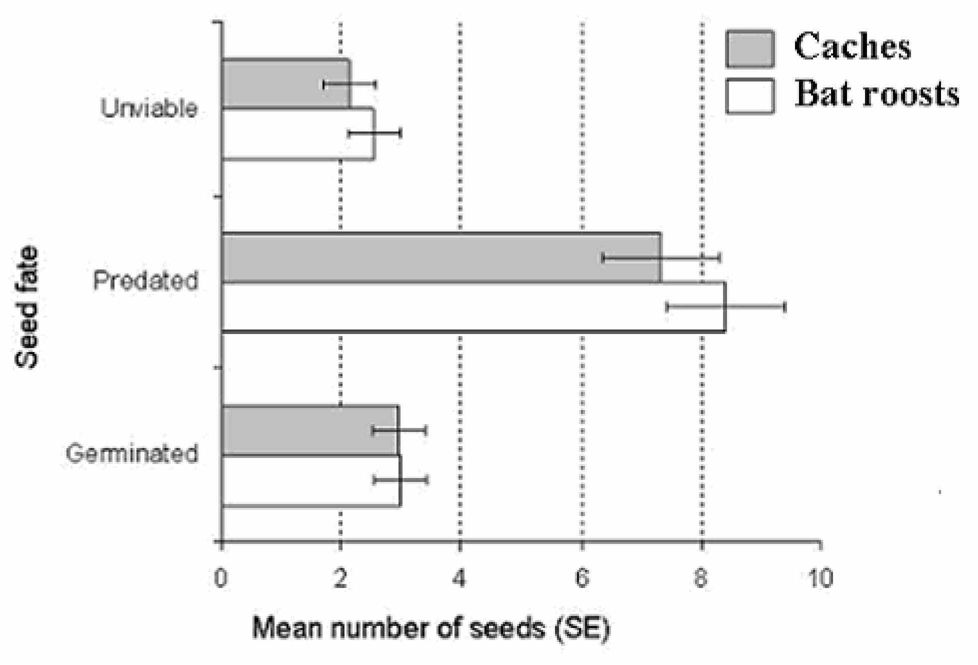


Figure 5.3. a) Mean distance of bat roosts and caches with respect to adult trees (± 96% SE). b) Secondary seed movement (meters) with respect to the distance to nearest conspecific adult. X-axis represents the distance to the nearest conspecific adult tree. Box within broken lines indicate distance where secondary seed movements are frequent. Inserted chart represent the mean secondary seed movements (meters) as a function of the distance to the nearest conspecific adult.

a,b)

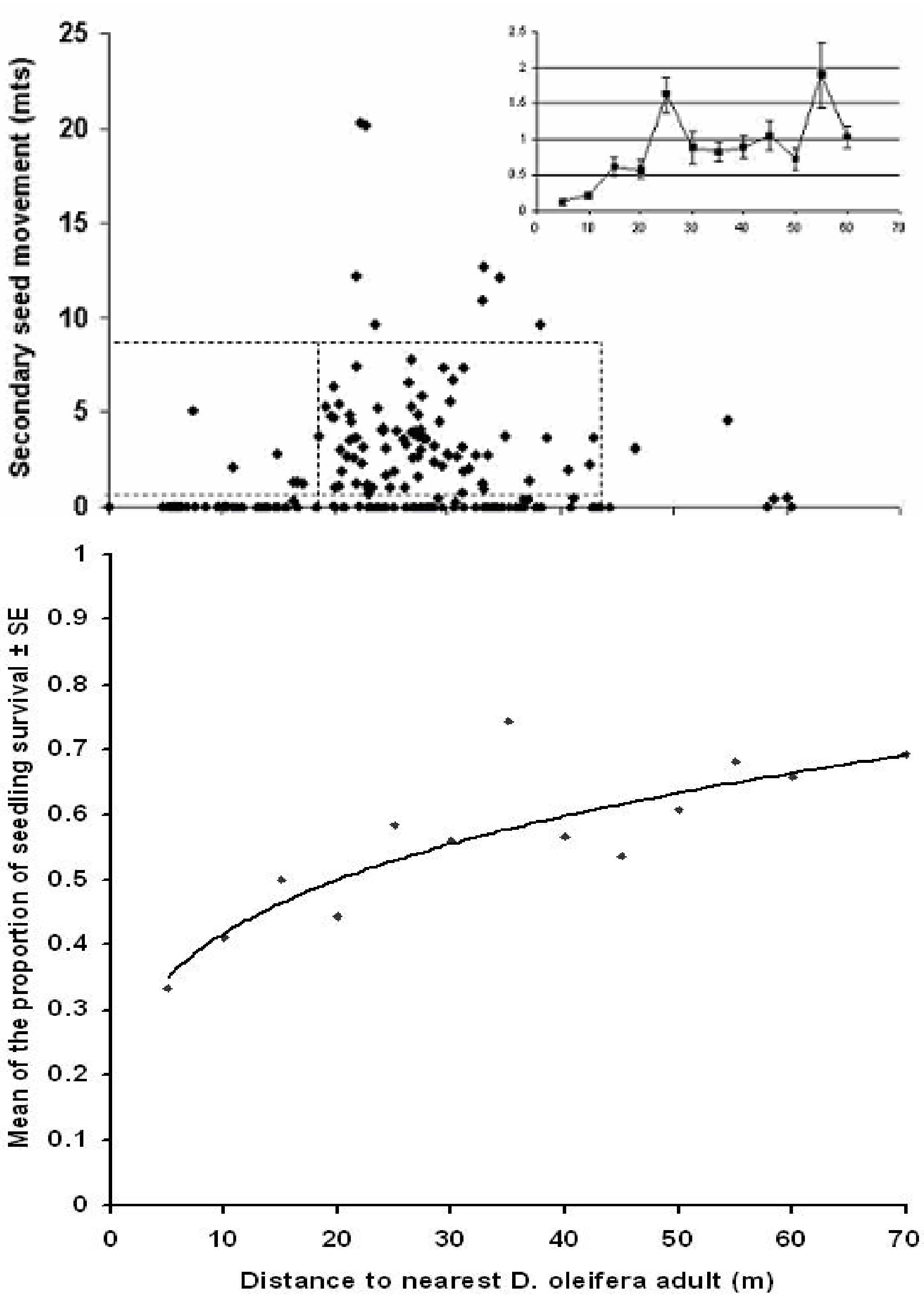
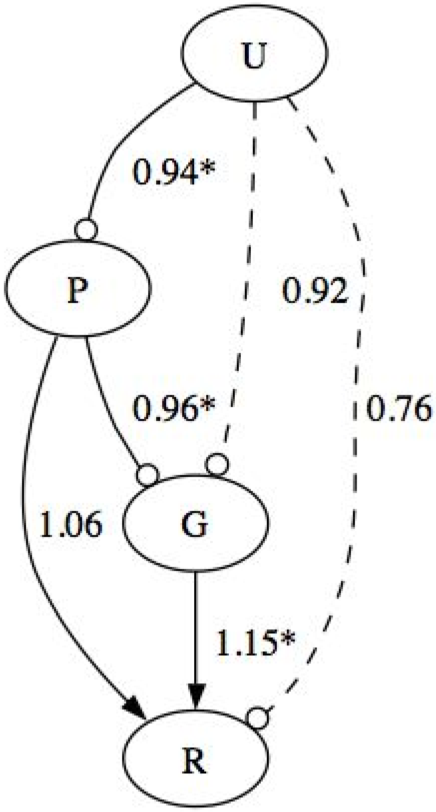


Figure 5.4. Pathways diagram of the seed fate of *D. oleifera*. Lines with open circles represent negative direct effects and arrow represent positive indirect effect.



CHAPTER VI

SPIDER PRESENCE CORRELATES WITH LOW HERBIVORY, LOW SEEDLING MORTALITY AND SEEDLING GROWTH IN THE TROPICAL RAIN FOREST TREE DIPTERYX OLEIFERA BENTH (FABACEAE)

INTRODUCTION

Reduced herbivory can lead to higher recruitment rates in tropical rain forest tree species. It is generally regarded that foliar herbivory reduces plant net productivity (Clark et al. 1993; Coley 1983; Coley 1990; Coley et al. 1985); foliar herbivory accounts for about 10 percent of the leaf damage in tropical tree species (Dirzo 1987). This damage can negatively effect seedlings and adult trees demography (Coley 1983; Coley 1990; Dirzo 1987), and can be severe enough to cause seedling mortality (Sullivan 2003). The vast majority of herbivory is by Lepidopteran larvae (Coley and Barone 1996; Coley P. D. and A. 1996; Coley 1990; Dirzo 1987). However, herbivory damage may be reduced by upper trophic levels (Gruner 2004; Hairston et al. 1960; Levins 1974; Moreau et al. 2006) and plant survival may be determinated by top-down effects or indirect positive interactions between predators and plants. For instance, arboreal ants (Floren et al. 2002) and birds (Van Bael et al. 2003) are important predators of arthropod herbivores. Spiders can also be important if their presence correlates with lower herbivory and seedling survival. Field research provides little evidence of the ecological effect of spiders on arthropod herbivore populations (Basset 2001).

Low seedling recruitment in tropical rain forests suggests that strong pressures from plant natural enemies (Augspurger and Kitajima 1992; Augspurger 1983; Augspurger 1984; Clark and Clark 1984; Hyatt et al. 2003). For instance, less than 3 percent of recruit survival over three decades was reported in a tropical forest in Australia (Connell and Green 2000). Because reduced herbivory can increase plant net productivity, it could result in high survival and higher recruitment rates in tropical rain forests. This study test (1) whether spider presence correlates with lower herbivory, (2) whether lower herbivory levels correlate with survival and (3) the growth of seedlings of *Dipteryx oleifera*, a shade-tolerant tropical rain forest tree species. The study focuses deliberately on the seedling stage because it is when herbivory effects on survival can be strongest.

METHODS

Study site and species. Fieldwork was conducted in the La Union site located in Southeastern Nicaragua (12°05' N., 83°55' W., elevation 10-20 m); mean annual temperature 26°C (IRENA 1991) from January 2005 to January 2006. This is within the lowland tropical rain forest at where we have been conducting research since 1989 (Vandermeer et al. 2000), and it is accessible by Caño Negro River where we have worked since 1994. A detailed description of the site was published earlier (Granzow-de-la-Cerda et al. 1997; Yih et al. 1991).

D. oleifera, previously known as *Dipteryx panamensis* (Fournier 2003), is a neotropical canopy emergent tree common in lowland forests of Central America, Panama and Venezuela. Adultscan reach heights of ~50 m and diameter at the breast height (DBH) of about 1,500 mm (Clark and Clark 1992). The wood is very dense and allows saplings to withstand damage inflicted by branches falling from the forest canopy (Clark and Clark 1991).

Mapping. A permanent plot of 6.37 ha was established in 2002; this plot consists of 10x10 m quadrats. Yearly censuses of basic demography of each individual of *D. oleifera* have been assessed since the beginning of the study. The sample size is large, for example from 2005 to 2006 there were records for 1,983 seedlings of *D. oleifera*. Seedlings are considered to be individuals with maximum height ≤ 100 cm. Maximum height is the vertical distance from the forest floor to the highest green tissue. An aluminum tag with a unique number was looped around the stem of each seedling as these were found. We recorded whether seedlings were healthy or when leaves were yellowish. Seedlings were considered dead when the aluminum tags were found alone or when seedlings were completely brown and without green tissue.

Herbivory assessment. For each seedling, a digital photograph of all the leaves and leaflets were made. Leaf area infected by herbivory was evaluated from digital photographs taken in early January 2005 and early January 2006. Digital photographs were taken from above the seedling canopy. Leaflets of *D. oleifera* are remarkably horizontal which allowed us visually estimate the percentage of herbivory per leaflet from digital photographs. Leaf area damage per leaflet was summed up and divided by the total number of leaflets in order to assess the percentage of herbivory per seedling. In order to determine the accuracy of the estimated herbivory damage, 100 seedlings were randomly sampled, 50 per each year. For these 100 seedlings a new estimation of leaf area lost was conducted using a grid. Because there were not significant differences between the methods, the visual estimation is reported here.

Spider census. Spiders we sampled all species of spiders present in seedlings of *D. olefiera*, including different morphs of each species, within the research area in

2004. Samples were deposited at the Natural History Museum of the University of Central America in Managua, Nicaragua, and samples were taken for identification by an expert at Escuela de la Frontera Sur (ECOSUR) in Mexico (Guillermo Ibarra-Núñez). In early January 2005 and early January 2006, we recorded the presence or absence of spiders in all seedlings present within the research area. Researcher effect on the subject of study has been considered in studies of herbivory, with result suggesting a reduction of herbivory visitation rate due to researcher manipulation (Cahill et al. 2004; Schnitzer et al. 2002). Here, the field research team comprised the first author and two experienced undergraduate students. We approached each seedling very carefully, trying to make the least amount of disturbance possible while examining each seedling for spiders. We used two categories for spider location in each seedling of *D. oleifera*: 1) Present, when spiders were spotted on a seedling or when abandoned spider webs were found within or below leaflets and 2) absent, without a spider or without evidence of spiders.

Herbivory and relation to seedling demography. The effect of herbivory on seedling survival was measured in 1,280 seedlings in 2005 and 703 seedlings in 2006 in a total of 34,800 leaflets. One-way ANOVA (Model I) was used to compare the initial herbivory area in 2005 (dependent continuous variable) between surviving and dead seedlings in 2006 (independent variable with two categorical levels).

The growth of 514 surviving seedlings, whose height was measured during the two years of the observational study, was evaluated using linear models. A first model studied seedling height in the second year (Y_t) a function of the height in the first year (Y_{t-1}), the presence/absence of spiders in both years (S_t, S_{t-1}) and the percentage of herbivory in both years (H_t, H_{t-1}):

$$Y_t = \mu + \alpha\, Y_{t-1} + \beta_1 S_t + \beta_2 S_{t-1} + \gamma_1 H_t + \gamma_2 H_{t-1} + \varepsilon \qquad (1)$$

Where μ is the intercept which gives an idea of the average growth per seedling, α is a parameter relating the current height with the height from the previous year, β_i accounts for the additional growth due to the presence of spiders in each year, γ_i measures the effect of herbivory on seedling growth, and ε is the error which is assumed to be identical and independently distributed. The model presented in formula 1 was analyzed through an analysis of covariance (ANCOVA) in order to test for the significance of each predictor, and based on this result the model was simplified by a process of backward elimination (Formula 2).

$$Y_t = \mu + \alpha\, Y_{t-1} + \beta_1 S_t + \beta_2 S_{t-1} + \gamma_1 H_t + \varepsilon \qquad (2)$$

RESULTS

Spider census, herbivory and seedling demography. Four families of spiders were found in *D. oleifera*: 1) *Teudis sp.*, Family Anyphaenida*e*. 2) *Episinus sp.*, and *Episinus cognatus*, Family Theridiidae. 3) *Mangora sp.*, Family Araneidae and 4) Family Ctenidae.

On average seedling with lower initial herbivory in 2005 (<20%) survived in 2006 ($F_{1,1278}$ = 7.5696, df = 2, p < 0.001) (Figure 6.1). Seedling survival was ~40% from 2005 to 2006. The ANCOVA, table 6.1, of the model presented in formula 1 revealed no significant effect for herbivory in the year 2005 (H_{t-1}) and a marginally significant effect for the presence of spiders in 2006 (S_t). The model presented in formula 2 was also a significant regression (R^2=0.72, $F_{4,509}$ = 333.5, P<0.05). In model 2 herbivory in the year 2005 (H_{t-1}) is dropped, and all coefficients are statistically significant, see table 6.2. The results of this model show that seedling growth is not related to size, since the coefficient relating the size in 2006 (Y_t) to the size in 2005 (Y_{t-1}) is close to one, and occurs with an almost constant value for all the seedlings, approximately 8 cm, this value being increased by approximately 1.5 cm for each year that spiders were present in the seedlings, and decreasing 7.5 mm in height for each percent unit of herbivory. Figure 6.2 shows seedling height in 2006 as a function of its value in 2005, with coded symbols for the presence of spiders in panel A and for different intensities of herbivory in panel B.

DISCUSSION

The results support the hypotheses that low levels of herbivory correlate with higher seedling survival, higher levels of herbivory are inversely correlated with seedling growth and that spider presence correlates with seedling growth. Because lower levels of herbivory correlate with seedling survival of *D. oleifera* it is subject to alternative interpretations. The first alternative explanation is that plant species responds to herbivory damage in relation to life history (Coley and Barone 1996). For example, in an experiment conducted in two tropical rain forests in Southeastern Nicaragua, different levels of artificial herbivory were applied to seedlings of *V. ferruginea*, a light demanding tree species. Results showed that these seedlings were able to resprout even when artificial hervivory levels were >70% (Ruiz 1999). Results contrast with the observation for *D. oleifera* because a loss of 20% of initial leaf area is severe enough to cause seedling mortality. These contrasting results could be explained on the basis of their seedling establishment ecology. For example, *V.*

ferruginea has very small seeds (1-2 mm long) with limited resources, thus survival after germination has to depend on its ability to accumulate resources very quickly, a trait that could also make *V. ferruginea* recover faster after an initial artificial herbivory of 70-100%. In addition, adult trees of *V. ferruginea* are also known for their ability to accumulate biomass very quickly (Boucher 1997). On the other hand, *D. oleifera* has large seeds (4-6 cm long) and an endosperm with sufficient energy for germination and initial seedling recruitment (~1 mo after germination) as was observed during germination trials (*unpublished data*). Therefore, its recruitment might not depend on the initial accumulation of biomass but on maintaining seedling biomass. The second alterantive explanation is related to the compensatory point of respiration; low light levels are the norm in tropical rain forests, for example, the mature forest at La Selva is heavily shaded, with very low light levels of 1-2% at the forest understory (Clark and Clark 1985). In comparison, the forest understory at our site is also shaded, with mean light level ~9% (*unpublished data*). Under these conditions mature forest tree species such *D. oleifera* are more likely to stand low light condition by staying at the compensation point of light where, respiration equals photosynthesis. This suggests why a small initial leaf area lost (~20%) could be large enough to drive seedlings below their compensation point of light and mortality. As an example of a third alternative explanation, in a study conducted in the rain forest of La Selva, seedling longevity of *D. olefiera*, defined as days after first measurement until seedling mortality, was inversely correlated with leaf area lost to herbivory. Only 20% of the initial number of seedlings survived when mean leaf area lost was ~24%. Seedling survival was positively correlated with the number of leaves 7 to 24 months post germination (Clark and Clark 1985), thus supporting the idea that on average biomass accumulation might not be fast enough to counteract leaf area losses.

These results also support the finding for the *D. oleifera* population in Nicaragua, which suggests that lower levels of herbivory could lead to high seedling mortality. In figure 6.2 a clear pattern is that, in general, seedlings with spiders in any year tend to be above the isogrowth line, with no special effect of having spiders in both years when compared to having spiders in just one year. In regard to herbivory, it is clear that seedling experiencing high herbivory (above 50%) tend to be below the isogrowth line implying a negative effect of herbivory on growth.

While spider presence correlates with lower herbivory and defend seedling form herbiovres, different spider species can reduce leaf area. For example in a study of the plant *Styrax benzoin* in Malaysia, 19% of seedlings had spiders whose presence caused a reduction in 2-43% of leaf area (Kiew 1982). Because several spider species roll up leaves where they nest, spider presence can also correlate with lower leaf area of *D. oleifera* (Clark and Clark 1985). Spider families reported here are web spiders, and these kinds of spiders are unlikely to be leaf-rollers, since these construct webs between leaflets, between midribs or below leaflets.

Web spiders were found in seedlings of *D. oleifera* which might not be predator of Lepidopteran larvae. Spiders in the familiy Salticidae are more likely to be predators of Lepidopteran larvae, because these do patrol leaves and hunt small insects (*personal observation*). However, the presence of web could decrease visitation rates of adult stages of arthropod herbivores, thus potentially explaining why there is a negative correlation between spider presence and lower herbivory. Despite this fact, the mechanisms leading to reduction in herbivory via spider presence remain to be investigated.

Most studies suggest that control of arthropod herbivores via predation can be an important mechanism in regulating the intensity of herbivory. For example, ants and birds prey upon arthropod herbivores, which resulted in a reduction of herbivory in two studies conducted in tropical rain forests. The study conducted at the lowland rain forest of Kinabalu National Park, Malyasia, showed that the proportion of the Lepidopteran larvae decreased with the proportion of arboreal ants (Floren et al. 2002). Lepidopteran larvae were more abundant in undisturbed forests were arboreal ants were rare but not in disturbed forests where arboreal ants were abundant. Larvae "offering" experiments lead to the conclusion that arboreal ant predation is the mechanism controlling on Lepidopteran larvae abundance. In the second study, the role of birds as predators of herbivore insects was studied on three tree species, *Anacardium excelsum*, *Cecropia longipes* and *Cecropia peltata*, at Barro Colorado Island, Republic of Panama (Van Bael et al. 2003). Results showed that the density of arthropod herbivores was lowered in wild tree branches due to bird predation. In addition, the average leaf area was significantly lower in caged branches than in branches with access for bird visitations, however this interaction was not significant at the understory.

Although lower herbivory correlated with seedling growth this result was not consistent throughout the study. For instance, the comparison between herbivory damage in 2005 and seedling height in 2005 was not statistically significant. However, the backward elimination allowed us to study the relationship between herbivory and spider presence with seedling growth and survival. A general pattern is that seedling growth is directly related with the presence of spiders. Because, these results were nearly significant toward the end of the research, which lead us to think that, if spiders effect changes over time, then conducting these censuses more frequently may lead to a better understanding of the effect of herbivory on seedling survival. In a field study, seedling longevity was positively correlated with accumulation of lower herbivory (Clark and Clark 1985). Here we estimated insect herbivory in a way, which only allow us to investigate whether high initial herbivory significantly correlates with further seedling mortality. The results show that seedlings with lower herbivory (<20%) in the previous year on average survived to the next year, although several seedlings resisted damage up to 50%.

An aspect that remains to be investigated is whether spider predation on arthropod herbivores is a mechanism that significantly leads to lower herbivory and higher seedling growth and survival. The study here addresses the research question at individual seedlings, however there is little evidence about the long-term effects of herbivory on tree population recruitment and growth. In sum, the results presented here show that higher herbivory correlates with higher seedling mortality and lower spider presence is significantly correlated with lower herbivory levels and seedling growth of *D. oleifera*.

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Table 6.1. Analysis of Covariance (ANCOVA) for the model presented in (1), see text for details.

Variable	D.F.	Sum Sq	*F* value	P
Height 05 (Y_{t-1})	1	35472	1098.72	< 2.2e-16*
Presence of spiders 06 (S_t)	1	166	5.14	0.02377*
Presence of spiders 05 (S_{t-1})	1	328	10.157	0.00153*
Herbivory 06 (H_t)	1	1214	37.5917	1.81e-09*
Herbivory 05 (H_{t-1})	1	15	0.4677	0.494377
Error (ε)	485	15658		

* Statistically significant ($P<0.05$)

Table 6.2. Coefficients for the model presented in (2), see text for details.

Parameter	Estimate	S.E.	*t* value	P
Intercept (μ)	8.127	0.90389	8.991	< 2e-16*
Height 05 (α)	0.832	0.02509	33.171	< 2e-16*
Presence of spiders 06 (β_1)	1.444	0.61784	2.337	0.01983*
Presence of spiders 05 (β_2)	1.683	0.51823	3.247	0.00125*
Herbivory 06 (γ)	-0.075	0.01185	-6.328	5.65e-10*

* Statistically significant ($P<0.05$)

Figure 6.1. Seedling fate in 2006 as a function of herbivory in 2005.

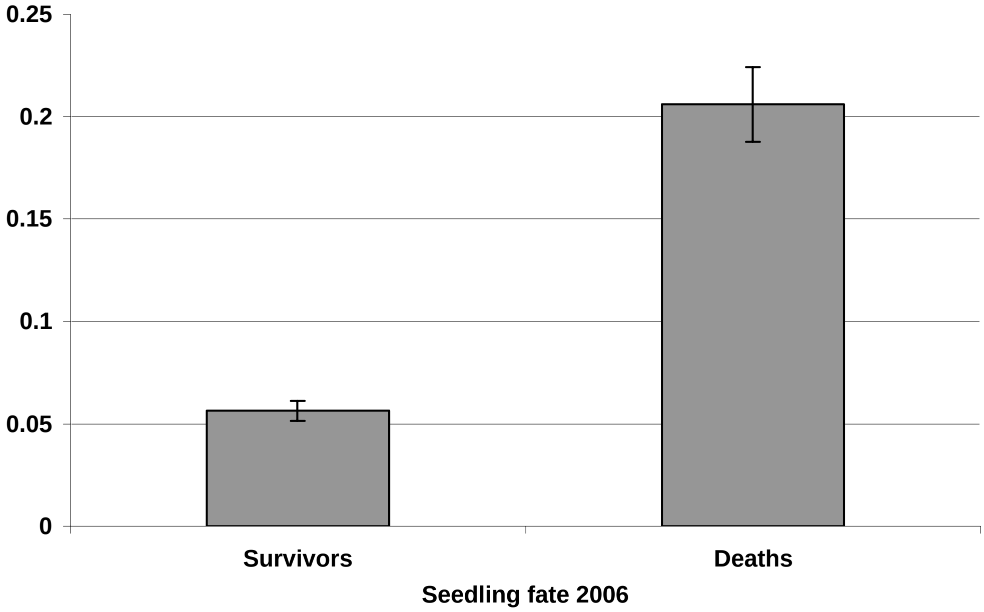
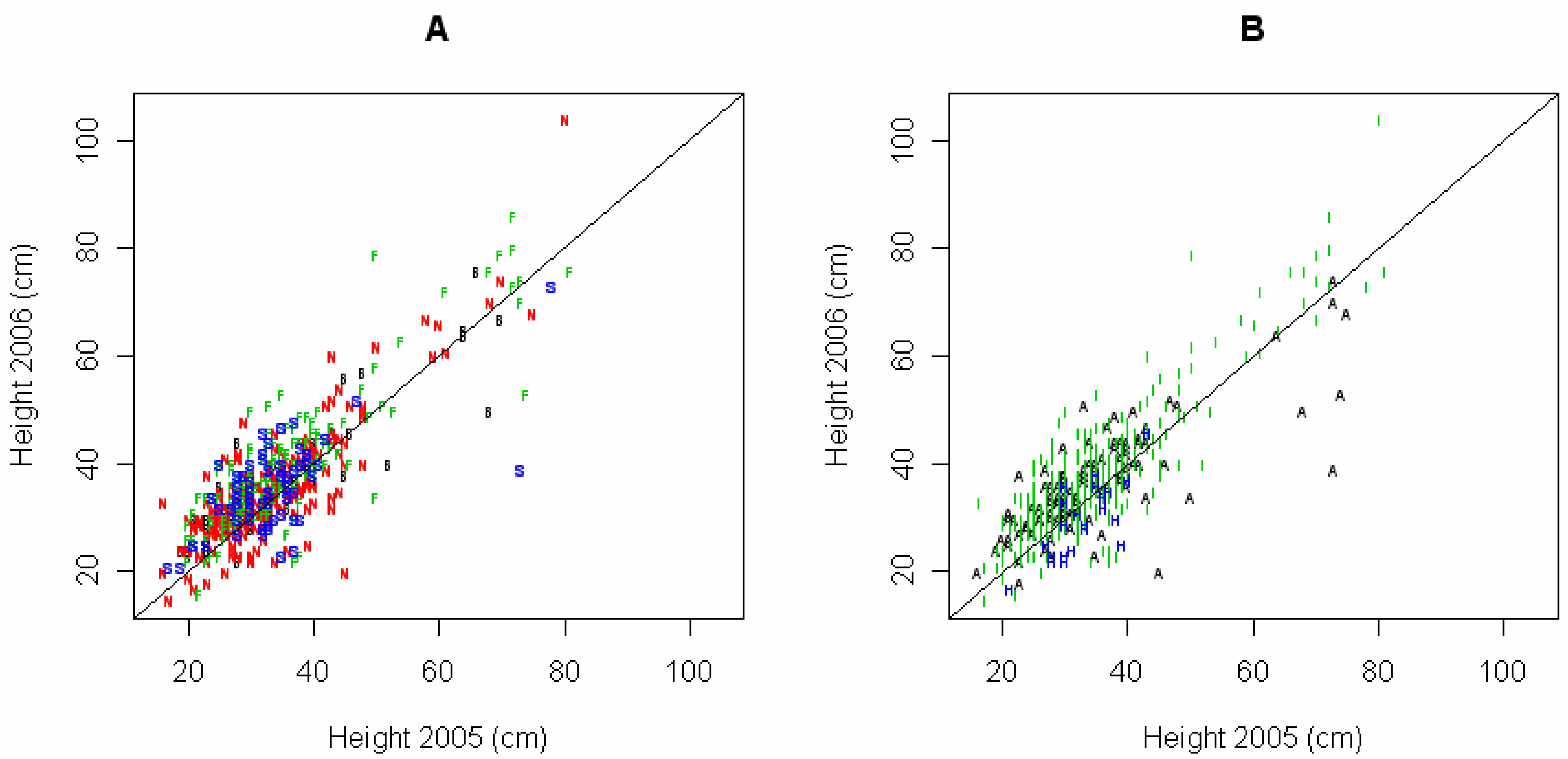


Figure 6.2. Seedling height in 2006 as function of its value in 2005. In Panel A seedlings are coded according to the presence of spiders: B (black) stands for seedlings that presented spiders in both years, N (red) for seedlings without spiders in both years, F (green) for seedlings with spiders in 2005, S (blue) for seedlings with spiders in 2006. In Panel B seedlings without herbivory are presented as A (black), with an intermediate herbivory < 50 %, as I (green) and with high herbivory > 50 % as H (blue).


A
B
Height 2006 (cm)
20
40
60
80
100
Height 2005 (cm)
20
40
60
80
100

CHAPTER VII

ECOLOGICAL CONSEQUENCES OF PRIMARY AND SECONDARY SEED DISPERSAL ON SEED PREDATION, INSECT HERBIVORY AND SEEDLING DEMOGRAPHY OF DIPTERYX OLEIFERA BENTH (FABACEAE)

INTRODUCTION

Seed dispersal away from seed sources can be fundamental for plant demography (Howe and Miriti 2004). Because higher density of conspecific seeds and seedlings nearby adult trees correlates with disproportionately higher damage by granivores (Howe and Smallwood 1982), arthropod herbivores (Clark and Clark 1985) or pathogens (Augspurger 1983) than away where both seedlings and their natural enemies are at lower densities. This idea is know as the Janzen-Connell hypothesis (Connell 1971; Janzen 1970), which also considers the distance effect, which would occurs when seed are moved away from the high density conditions where seeds and seedlings escape damage. Although it is fundamentally important to understand the effect of density and distance on survival, an aspect that have had received minimal investigation is that of the relative contribution of primary and secondary seed dispersal on plant demography in the context of this hypothesis – the first focus of this study. Primary seed dispersal occurs when seeds fall below the adult tree by gravity, or are dispersed away by birds or flying mammals. Secondary dispersal occurs when seeds are moved from the understory after primary seed dispersal.

Resent research showed that there is minimal evidence about the relative contribution of dispersal mechanisms on seed and seedling population demography dynamics (Howe and Miriti 2000; 2004). When thinking about tropical systems, the reasons for this shortcoming have been due, to a large extent, to missunderstandings about what is and what is not seed dispersal (Vander Wall et al. 2005). However, whenever secondary seed dispersal was effectively measured, the overall ecological consequences on plant demography have been reported as positive (DeMattia et al. 2004; Forget 1990; Russo 2005). For example, vertebrate secondary seed dispersal of *Vouacapoua americana* in experimental caches (Forget 1990) and, for of *Virola calophylla* were seeds scattered across the forest floor leaded to higher seedling survival than below the adult conspecific trees or below spider monkey sleeping sites (Russo 2005). In addition, it could be the case that upper trophic interactions could also contribute to the positive effect of secondary seed dispersal on seedling establishment via a reduction in insect herbivory - the second focus of this paper.

In specific, we seek to test the general hypothesis: 1) if secondary seed dispersal increases the survival probability of *D. oleifera*. This increase in survival would result from seed dispersal leading to lower seed and seedling mortality away from the nearest conspecific adult, where herbivory would be lower. This general hypothesis will be evaluated trough three specific hypotheses.

- if the proportion of seed survival and seedling survival correlates with secondary seed dispersal events then seedling growth would be higher.
- if seedlings escape insect herbivory then higher seedling survival correlates with distance to nearest adult tree.
- if lower proportions of insect herbivory correlates with spider presence then spider effects on seedlings is positive.

METHODS

Study site. The research was conducted in a tropical rainforest in southeastern Nicaragua from January 2006 to April 2007, at the research site know as La Union (12°05' N, 83°55' W, elevation 10-20 above sea level, mean annual temperature = 26°C, mean annual precipitation = 5000 mm) (IRENA 1991). The forest is lowland tropical rainforest accessible by Caño Negro River, where we have worked since 1994. For a description of the site see Granzow-de-la-Cerda et al. (1997) or Yih (1989).

D. oleifera system. This is an excellent system to study the consequences of primary and secondary seed dispersal on plant recruitment since we know many details of the dispersal ecology of *D. oleifera* (Clark and Clark 1984; Clark and Clark 1992; Clark and Clark 1987; Clark and Clark 1991; Clark et al. 1993; Forget 1992; Forget and Milleron 1991; Ruiz 2004). *Dipteryx oleifera* is a Neotropical canopy emergent tree common in lowlands, also know as *Dipteryx panamensis*. It can reach heights of ~50 m and diameter at breast height (DBH) of about 1,500 mm (Clark and Clark 1992). *D. oleifera* is a large seeded species with heavy fruits averaging 25.2 g ± 4.4 SD, n = 80, (*unpublished data*). This species has an endocarp protected within hard and large pods, which makes threading approaches suitable for evaluating seed dispersal, seed predation and seedling recruitment. For the rest of this paper we will refer to the pods as "seeds," since "seed" is the term generally used while referring to diaspores in studies exploring dispersal and recruitment dynamics of tropical tree species. *D. oleifera* is primarily dispersed by bats (*i. e. Artibeus jamaicensis*, *Artibeus literaturs*, etc.) and secondarily dispersed by non-flying mammals (*i.e.* squirrels, agoutis, pacas, etc.) (Reid 1997). Primary dispersal occurs when seeds fall from the crown of the tree to the forest floor or when bat move the propagule to bat feeding places. Bats would be definied as primary dispersers because they pick the fruits off the tree and not off the ground. Secondary seed dispersal occurs when seeds are move from the forest floor by terrestrial vertebrates. Terrestrial vertebrates scatter seeds or congregate seeds in small seed piles named “caches” or bury seeds into the forest floor (Forget and Milleron 1991; Jansen et al. 2006).

Secondary seed dispersal occurs when some seeds are moved and forgotten in the understory and left unretrieved. Previous field research had shown that seeds of *D. oleifera* escaped consumption away from the nearest conspecific adult and seedling survival and seedling growth increased with distance to nearest conspecific adult tree (Guillén 2006) (Figure 7.1a). In another field study conducted in Nicaragua, insect herbivory decreased with distance to the nearest adult *D. oleifera*. The presence of spiders in seedlings is correlated with seedling survival (Martínez 2006); spiders might harass or predate insect herbivores in complex ways (Figure 7.1b), similarly to the observation made earlier in an agroecosystem in Mexico (Vandermeer et al. 2002).

Bats and terrestrial vertebrates. Several species of bats were captured around adult *D. oleifera* individuals and/or near wild bat roosts. The species of bats trapped over the research period are listed in Table 7.1. Terrestrial mammal trapping success has been extremely low in the area, as is frequently observed in studies at lowland rain forests (McClearn et al. 1994). To date, the species captured by the trapping fieldwork at the La Union site are: *Proechimys semispinous*, *Oryzomys spp.*, *Nyctomys sumichirasti*, *Marmosa mexicana*, *Didelpiys virginiana* and *Didelphis alventris*.

Seed and seedling mapping. The study was conducted within a 6.37 ha plot established in 2002. During 2006 we mapped and followed the fate of 2,814 seeds as they appeared within a 3.2 ha subplot. The seed sample size is large for studies of the relative contribution of seed dispersal (Cintra 1994; Cintra and Horna 1997; DeMattia et al. 2004), and the sample size for seedlings is comparable to studies of distance and density effects (Augspurger 1984; Clark and Clark 1984; Hyatt et al. 2003; Schupp 1992).

Seed fate assessment. In order to evaluate seed fate with respect to the nearest conspecific adult *D. oleifera* tree, we threaded and mapped to the nearest centimeter all seeds as they appeared within a 3.2 ha subplot from January 2006 to July 2006. A micro precision nylon thread produced by Imperial Thread Inc. (Northbook, Illinois), was super-glued to all seeds found within this area. This nylon bobbin thread consists of a microfiber ending in a bobbin, which was clipped to the forest understory with a toothpick. Each bobbin was numbered with a unique number. Previous studies have only evaluated the effect of seed removal (Cintra 1994; Cintra and Horna 1997; Vander Wall et al. 2005) but see DeMattia et al. (2004) or Forget and Wenny (2005). When seeds were found eaten, we did not thread these, although we did coordinate their positions. Seed conditions were measured at least once each month during eight months. Seed conditions were classified as: predated, intact or germinating.

Seed fate with respect to seed piles. Seeds of *D. oleifera* at bat seed piles and caches were systematically located during the censuses in 2006. In general, bat seed piles were generally located below bat-roosting palms (Ruiz *et al*., *in preparation*). Seeds at bat seed piles were found with either bat feces or marks of manipulation by bats. For example, pods were partially covered with the fleshy membrane that bats eat. Cached seeds had been longer on the forest floor (*i.e.,* seeds had bare pods, without vestiges of the exocarp, and pod coloration was darker).

Seed survival hazard functions. Seed survival was analyzed using a Cox proportional hazard models (CPHM) (Cox and Oakes 1984), following the programming in R 2.5.1 developed by Fox (2002). The first model tests the effects of the covariates seed dispersal mode (β), final distance to the nearest conspecific adult tree (∂) and seed hoarding (cache and bat seed piles) (μ) on the proportional hazard regression:

$$h_{(t)} = h_{0(t)}e^{\beta M+\partial D+\mu H} \qquad (1)$$

where h_0 is the baseline mortality hazard computed from the adjusted hazard function; M corresponds to primary and secondary seed dispersal (0 and 1 respectively); and D is a metric covariate, corresponding to the maximum dispersal distance from the nearest conspecitic adult tree (meters). The variable H codes for primary seed dispersal and when seeds were moved into bat seed piles and caches (0 or 1 respectively). Time measured as census period is t, and β, ∂, μ are the covariates of the coefficients computed above.

The model was simplified by a backward process because seed hoarding, H, does not have a statistically significant effect on the estimated seed hazard function:

$$h_{(t)} = h_{0(t)}e^{\beta M+\partial D} \qquad (2)$$

Seedling demography. In order to determine whether seedling fate depends on the distance to the nearest conspecific adult *D. oleifera* tree, we followed germination from June 2006 to April 2007. We mapped the *xy* coordinates to each seedling within the plot. For seedlings that germinated from secondarily dispersed seeds, we mapped and coded their dispersal modes and their *xy* positions. Seedlings are considered to be individuals with maximum height ≤ 100 cm. Maximum height is the vertical distance from the forest floor to the highest green tissue. An aluminum tag with a unique number was looped around the stem of each seedling as these were found. We recorded height, number of leaves and leaflets for all seedlings recruiting in 2006.

Relative growth rate was calculated to evaluate the proportion of the change in height. This is the basic relative growth rate (RGR) calculated by the formula $RGR = ln(X(t+1)) - ln(X(t))$, where $ln(X(t))$ is the natural logarithm of the height at time t and $ln(X(t+1))$ is the natural logarithm of the height at time $t + 1$. RGR represents the velocity at which the circumference is growing or shrinking. Linear models were fitted in order to determine whether seedling relative growth rate correlates with the distance from the nearest conspecific adult tree. A note was taken to describe whether seedlings were healthy or when leaves were yellowish or brown. Seedlings were considered dead when the aluminum tags were found alone or when seedlings were completely brown and without green tissue. When seedlings were not found for one month, they were recorded as “not found,” but if they were not found the next month they were considered to have died the previous month after they were not found. Each seedling was considered to be the offspring of its nearest conspecific adult tree.

Seedling herbivory assessment. In order to determine the effect of insect herbivory on seedling recruitment dynamics a digital photograph of the leaves of each seedling was taken from above the seedling canopy. Leaf area damaged by herbivory was evaluated from digital photographs taken in early January of 2005 and 2006. Leaflets of *D. oleifera* are remarkably horizontal which allowed us to visually count the percentage of herbivory per leaflet. Leaf area damage per leaflet was summed up and divided by the total number of leaflets in order to assess the percentage of herbivory per seedling. To determine the accuracy of the visual estimation, leaf damage was also measured using a grid on 100 seedlings. There was no significant statistical difference between the methods, allowing us to use the faster method of visual estimation. The insect herbivore damage to leaflets consisted largely of holes in the interior as well as at the edges of the leaflets.

Spider census. In order to evaluate the effect of spiders on insect herbivory of seedling, we sampled all spider species present on *D. olefiera* seedlings, including different morphs per each species, within the research area in 2006 and 2007. Samples were deposited at the Natural History Museum of the University of Central America in Managua, Nicaragua, and also were taken for identification by an expert at Escuela de la Frontera Sur (ECOSUR) in Mexico (Guillermo Ibarra Núñez). During each census the presence or absence of spiders was recorded on all seedlings within the research area. We used two categories for spider location in each seedling of *D. oleifera*: 1) present, when spiders were spotted on a seedling or when abandoned spider webs were found within or below leaflets and 2) absent, without a spider or without evidence of spider webs.

Herbivory and relation to seedling demography. The growth of 98 surviving seedlings, whose height, leaves, leaflets, spider presence or absence, and herbivory were measured during April 2006, November and April 2007 was evaluated using linear models. A first model studied seedling growth in the third measurement period (Y_{t+1}) as a function of the height in the first period (Y_{t0}) or second period (Y_t), the presence/absence of spiders during these three periods (S_{t0}, S_t, S_{t+1}), the percentage of herbivory (H_{t0}, H_t, H_{t+1}), the number of leaves (L_{t0}, L_t, L_{t+1}), the number of leaflets (Le_{t0}, Le_t, Le_{t+1}), seed dispersal modes (M_{t0}, M_t, M_{t+1}) and primary versus secondary seed dispersal mode (D_{t0}, D_t, D_{t+1}):

$$Y_t = \mu + \beta_1 S_{t0} + \beta_2 S_t + \beta_3 S_{t+1} + \gamma_1 H_{t0} + \gamma_2 H_t + \gamma_3 H_{t+1} + \delta_1 L_{t0} + \delta_2 L_t + \delta_3 L_{t+1} + \zeta_1 Le_{t0} + \zeta_2 Le_t + \zeta_3 Le_{t+1} + \eta_1 M_{t0} + \eta_2 M_t + \eta_3 M_{t+1} + \theta D_{t0 \rightarrow t+1} + \varepsilon \qquad (3)$$

where μ is the intercept, which gives an idea of the average relative growth rate, β_i accounts for the additional growth due to the presence of spiders each time, γ_i measures the effect of herbivory on seedling growth, δ_i measures the effect of leaves/height on seedling growth, ζ_i measures the effect of leaflets/height on seedling growth and ε is the error, which is assumed to be identical and independently distributed with variance σ^2_ε, $\varepsilon_t \sim N(0, \sigma^2_\varepsilon)$. The model presented in formula 3 was analyzed through an analysis of covariance (ANCOVA) in order to test for the significance of each predictor. Based on the results, the model was simplified by a process of backward elimination:

$$Y_t = \mu + \gamma_1 H_{t+1} + \theta\, D_{t0\rightarrow t+1} + \varepsilon \qquad (4)$$

RESULTS

Seed fate. A total of 848 out of 2,814 seeds (30.13%) were dispersed below the adult *D. oleifera* tree by gravity. Primary dispersal mean distance to nearest *D. oleifera* adult individual was 23.77 ± 0.47m (SE) and secondary seed dispersal distance to the nearest *D. oleifera* is was 24.40 ± 0.14m (SE). The mean distance of seed piles from nearest adult *D. oleifera* was 41.90 ± 2.43m (SE) for caches and 40.94 ± 1.48m (SE) for bat seed piles. There were 575 seeds in a census of 60 bat seed piles and 277 seeds in a census of 32 caches, corresponding to 30.20% of the seed production. There was no statistical difference in the mean number of seeds between bat seed piles and caches (Figure 7.2).

Secondary seed dispersal was frequent away from the nearest adult *D. oleifera* tree (Figure 7.3a). A proportional hazard model was fitted to the data of 2,814 seeds (Formula 2), the results suggest that for each meter away from the nearest adult tree of

D. oleifera the probability of seed damage, 78%, decreased by 1.16%. Secondary seed dispersal decreases the probability of seed damage by 23.94% (Table 7.2).

Seed pile dynamics and seedling fate. Seedling survival increased with distance to the nearest *D. oleifera* adult (quadratic regression: $r^2 = 0.713$, $p = 0.004$) from April 2006 to April 2007 (Figure 7.3b). Seedling fate was no different between seed piles types ($X^2 = 0.61$, df = 1, $p = 0.4348$); 17 out of 43 seedlings dispersed from caches survived and 25 out of 90 seeds dispersed from bat seed piles survived one year after germination. There was a statistically significant difference in the number of surviving seedlings after primary seed dispersal versus seedlings surviving after secondary dispersal ($X^2 = 12.62$, df = 1, $p = 0.0004$) one year after germination. Only 27 out of 111 seedlings survived after secondary dispersal and 64 out of 136 seedlings survived when dispersed by primary means alone.

Spiders present on seedlings of D. oleifera. Four families of spiders were found in *D. oleifera*: 1) *Teudis sp*., Family Anyphaenidae. 2) *Episinus sp*., and *Episinus cognatus*, Family Theridiidae. 3) *Mangora sp.*, Family Araneidae and 4) Family Ctenidae. The numbers of spiders present in census of all seedling seedlings of *D. oleifera* were, 27 spiders in April 2006, 7 spiders in November 2006 and 22 spiders in April 2007. Twenty six percent (67/252) of the total number seedlings presented at least one spider during the research period.

Seedling fate and relation to herbivory, seed dispersal and spider presence. Insect herbivory damage followed a negative trend with respect to the nearest adult *D. oleifera* individual ($r^2 = 0.29$, $p = 0.059$; linear regression) (Figure 7.4). Seedling mortality was correlated with insect herbivory levels > 25% of seedling leaf area (Ruiz et al. *submitted*). Seedlings that emerged after secondary seed dispersal events experienced no statistically significant difference in insect herbivory damage than these seedlings that were dispersed primarily by bats on year after germination (F = 0.153, p = 0.697, df = 1, 98).

The results of the linear model in table 7.3a suggest that seedling growth from November 2006 (Y_t) to April 2007 (Y_{t+1}) is on average 0.8983 cms, this value decreased by approximately 0.8303 due to foliar herbivory in November 2006. Seedling growth decreased 0.0032 as a function of the distance to the nearest conspecific adult tree. Table 7.3b present the estimations for statistical coefficients. Spider presence correlated negatively with insect herbivory one year after germination (F= 4.2808, df=1,118, p = 0.0407).

DISCUSSION

Seed fate. The results presented here are in concordance with the distance and density predictions of the Janzen-Connell hypothesis. For the distance effect, we observed that ~30% of the seed production, based on the proportion of all seeds that you found on the ground, was dispersed below the adult tree by gravity. Remarkably, terrestrial mammals did not move seeds from below the adult tree (Figure 7.3a), perhaps avoiding their natural enemies present near adult trees of *D. oleifera* (*i.e.* boa constrictors and owls). Field observations suggest that predation on bats (Boinski and Timm 1985) and small terrestrial vertebrates (Becker et al. 1985) can be higher nearby feeding sites. Since seeds remained at high density below adult trees, this condition might have led to higher rates of seed damage by insects (*personal observation*). Insect larvae of the family Taeniaptera developed in the decomposing exocarp and destroyed the endosperm during the first stage of germination. Because, about 40% of the seeds were moved away the nearest conspecific adult (around 20-

40m), the effect of this fly seems to be localized below the adult tree (Figure 7.3a). A large number of seeds were scattered throughout the research plot by bats. Bats might be scattering seeds during flights or depositing seeds at low densities below temporal roosting sites distributed randomly, thus bat seed scattering could be an important on seedling survival. In addition, there is compound bat seed dispersal and secondary seed dispersal which decreased the risk of seed predation at the forest floor (Table 7.2), because bat seed scattering and secondary seed dispersal can be reducing insect herbivory. Although, 30% of the seeds moved away from the adult trees were congregated into bat seed piles or caches. Seedlings that germinated from seed piles after secondary seed dispersal presented no statistically significant difference in damage by insect herbivory than seedlings that germinated after bat seed dispersal.

Effects of seed dispersal and insect herbivory on seedling fate. Seedling survival increased away from the nearest *D. oleifera* adult (Figure 7.3b), where insect herbivory is lower (Figure 7.4). The negative trend between seedling herbivory and distance to the nearest conspecific tree suggest that the combined effect of primary and secondary seed dispersal released seedlings from insect herbivory away from the conspecific adult, thus providing evidence in favor of the distance effect (Howe and Smallwood 1982). Insect herbivory was not lower between seedlings germinating after secondary seed dispersal and seedlings germinated after bat primary seed dispersal. Also seedlings can escape insect herbivory after secondary seed movements away from seed piles types. Seedling survival increased with respect to the distance to seed piles – bat seed piles and terrestrial vertebrate seed piles (Ruiz and Boucher *submitted*).

Effects of insect herbivory on seedling growth. The effect of insect herbivory damage on seedling growth accumulated over time. During November of 2007 seedling growth was independent of insect herbivory. An alternative explanation for this pattern is that seedlings could escape insect herbivory when there is a flush in leaf production but insect herbivores abundance is low (Coley and Barone 1996; Coley 1990). Second, there is a negative trend between seedling herbivory and seedling growth (April 2007). During this period even when insect herbivory was lower (<25% of the leaf damage area), this damage led to a reduction in seedling growth rate. Food scarcity may have led to higher insect herbivory during April of 2007 in *D. oleifera*, simply because seedlings of *D. oleifera* seem to have had more foliage than other species in the forest floor.

Seed dispersal and its relation to seedling growth and spider presence. There was no difference in seedling growth rates between seedlings that were dispersed by primary means versus those that were secondarily dispersed. There is a slightly negative significant correlation between seedling growth and distance to the nearest conspecific adult tree (Table 7.3a,b). Although, it is generally considered that higher insect herbivory near adults may reduce the growth of seedlings below the nearest conspecific trees (Coley and Barone 1996). Furthermore, the results show that seedling growth rate is independent to spider presence. Field observations suggest that spiders were rare during the research, thus without their natural enemies, insect herbivores may have had considerable increased foliar herbivory damage. There is not enough evidence to support the hypothesis that if spiders preyed on insect herbivores then there would be a positive indirect effect of spiders on seedling growth (Figure 7.1b).

Experimentation involving insect herbivores can help us to understand the relative strength of spider predation on insect herbivores and foliar herbivory, similar to the work by Oedekoven and Joern (2000) and Philpott et al. (2004). Field experiments involving spider additions and insect herbivores would be necessary to understand the effect of spiders on insect herbivory and seedling fate, if any. For example, in a study conducted in a tropical rain forest in Panama, the effect of bird predation on arthropod herbivores was controlled using bird exclosures placed in branches at the forest canopy. Arthropod herbivore abundance increased in these exclosures, which acted as enemy-free spaces for arthropod herbivores. The effect of herbivores was several times larger within the treatment than outside exclosures, thus providing evidence in favor of the hypothesis that birds defend plants from arthropod herbivores (Van Bael et al. 2003). However, these results were not observed in the forest understory treatment (Van Bael and Brawn 2005). For the population of *D. oleifera* in study, exclosure with spider addition can be placed above seedlings in order to determine the effect of spider presence on insect herbivores, foliar herbivore and seedling fate and growth.

Assumptions and errors. We assumed that bats scattered seeds across the forest floor and below their roosting sites. However, our field observations only allow us to detect bat dispersal when seeds are transported to bat roosting palms. We correlated the presence of spiders with the proportion of leaves consumed by insect herbivores. Although, we assumed that spiders predate upon insect herbivores, little is known about the interaction between spiders and insect herbivory in the *D. oleifera* system. Since we did not observe insect herbivory during the day, we think that insect herbivory and associated spider activity occurs during the night. Another aspect that merits more investigation is a potential temporal effect of spiders on seedling fate. Foliar herbivory estimation only accounts for a static evaluation of foliar insect damage. An accurate estimation of insect herbivory have to include the measurement of herbivory rate. A small ring around a leaflet midrib can be placed in order to estimate rates of insect foliar herbivory over time, for example see: Eichhorn et al. (2006). However, an advantage of the stand herbivory estimation is that it allows us a faster estimation.

Hypothesis testing. The results presented here will be used to address three specific working hypotheses. First, if the proportion of seed survival and seedling survival correlates with secondary seed dispersal events then seedling growth would be higher. Since the probability of seed and seedling damage decreases with respect to the distance to the nearest adult of *D. oleifera* and secondary seed dispersal decreased the probability of seed damage. Seedling survival and growth rates were higher after bat seed scattering and secondary seed dispersal one year after germination. These results suggest a compound effect between bat seed scattering and terrestrial vertebrate secondary seed dispersal can be shaping the seedling survival function with respect to the nearest conspecific adult. We accept the notion that that primary (bats) and secondary seed dispersal are important mechanisms for escaping natural enemies located closer to the *D. oleifera* adult.

Second, if seedlings escape insect herbivory then higher seedling survival correlates with lower herbivory away the nearest adult tree. Seedling herbivory decreased with distance to the adult tree, mainly because after bat seed dispersal and vertebrate seed dispersal, seedlings were present at lower densities away from these adult trees. Because there was no difference in the level of insect herbivory on seedlings that were scattered by bats with those that were also secondary dispersed, this result suggest that secondary seed dispersal reduced density effects near the seed piles. Also the compound effect of bat seed dispersal into seed piles and secondary seed dispersal from these seed piles contributed significantly to escaping natural enemies. We provide evidence in favor of the hypothesis that seedling herbivory is lower when seedlings are away from the nearest conspecific adult, where seedling survival is higher. Third hypothesis, if lower proportions of insect herbivory correlates with spider presence then spider effects on seedlings is positive. There is a trend suggesting that spider presence correlates with lower insect herbivory. However these results are not statistically significant, which seems to be temporally constrained by sample size.

Implications for current debate on tropical rain forest organization. The results presented here provide evidence in favor of the seed and seedling distribution predicted by the Janzen-Connell hypothesis. The mechanism largely responsible for the distribution of seedlings is insect herbivory, which was higher close to the nearest conspecific adult where seedling density is high. Bat seed dispersal and vertebrate seed dispersal are fundamental to escape insect herbivory associated with high seedling density. The negative trend between spider presence and seedling growth rate suggest that spider might have a positive indirect effect on seedling growth and survival. We conclude that seed dispersal, herbivory and upper trophic interactions are fundamental mechanisms determining the distribution of seedling in tropical rainforest. Future field research should include the important role of upper trophic levels on shaping the distribution of plant distribution. Recently, Hubbell (2001) null model of tropical rainforest organization provide valuable insight about how dispersal can be a driving force in the organization of tropical rain forest. Result on the relative contribution of species interactions poses the challenge to develop mechanistic models aiming to understand tropical rain forest organization.

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Table 7.1. Bat species identified within the study area in Southeast Nicaragua. Feeding habits and estimated weight were obtained from Reid (1997).

Species	Feeding habit	Weight (g)	Size
Artibeus intermedius	Frugivorous	40-54	Large
Artibeus jamaicensis	Frugivorous	21-51	Large
Artibeus lituratus	Frugivorous	53-73	Large
Artibeus phaeotis	Frugivorous	9-15	Small
Artibeus watsoni	Frugivorous	9-15	Small
Carollia perspicillata	Frugivorous	21-23	Medium
Carollia castanea	Frugivorous	11-16	Small
Chiroderma villosum	Frugivorous	15-18	Medium
Vampyressa nymphaea	Fruigivorous	11-16	Small
Myotis nigricans	Insectivorous	3-6	Small
Phyllostomus discolor	Insectivorous	26-51	Large
Trachops cirrhosus	Insectivorous	24-36	Medium
Glossophaga soricina	Nectivorous	7-12	Small
Hylonycteris underwoodi	Nectivorous	6-12	Small

Table 7.2. Results of the Cox proportional hazard model for seeds of *D. oleifera*.

Covariates	Coef .95 CI	exp(coef)	Z	P
Distance to adult nearest	-0.0117	0.988 (0.985-0.992)	-6.33	2.4e-10*
Secondary disersal	-0.2736	0.0823 (0.647-0.894)	-3.33	8.8e-04*

*(P<0.005). Partial Likelihood test= 50.2, df = 2, p = 1.26e-11.

Table 7.3. a) Result of the ANCOVA model evaluating the effect of secondary seed dispersal, hebivory damage and spider presence on seedling growth rate and b) the coefficient estimates for the model.

a)

Var. Independent	Estimate	Std. Error	t-value	Pr(>|t|)	Sig.
Intersept (RGR)	0.898282	0.084035	10.689	3.09e-15	***
H_N06	-0.245367	0.170621	-1.438	0.15588	
H_A07	-0.830267	0.070574	-11.764	< 2e-16	***
Lef_N06/Alt_N06	-0.093672	0.118795	-0.789	0.43366	
(Le_N06/Alt_N06)	-0.189976	1.040730	-0.183	0.85581	
Distance to adult	-0.003211	0.001157	-2.775	0.00744	**
as.factor(S_N06)	0.096511	0.139215	0.693	0.49097	
as.factor(S_A07)	-0.003666	0.073276	-0.050	0.96027	
as.factor(Dmod)	0.006024	0.082929	0.073	0.94235	

Sig. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1. Multiple $R^2 = 0.7438$, Adjusted $R^2 = 0.7079$ (F = 20.69 on 8 and 57 DF, p = 2.797e-14).

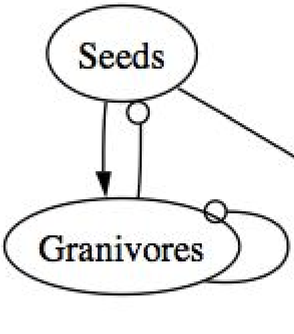
b)

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.824044	0.035475	23.229	< 2e-16	***
H_N06	-0.186588	0.134524	-1.387	0.17040	
H_A07	-0.811940	0.067082	-12.104	< 2e-16	***
Distance	-0.003041	0.001014	-2.999	0.00390	**

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1. Multiple $R^2 = 0.7353$, Adjusted $R^2 = 0.7225$ (F = 57.4 on 3 and 62 DF, p < 2.2e-16).

Figure 7.1. Diagrammatic representation of the *D. oleifera* study system. Each ellipse connects one or several ellipses that it affects, the arrows represent a positive effect, small open circle at the end of the lines represents a negative effect. a) Escaping seed predation (granivory) occurs at high seed density, indicated by the arrow connecting granivores and seedlings. Inversely, seed predation has a negative effect on seeds, represented by an open circle at the end of the connection between granivores and seeds. b) Insect herbivores have a negative effect on seedlings and seedlings have a positive effect on insect herbivores, indicated by the corresponding symbols. The interaction between spider species or between granivores is a negative one, represented as and open circle connecting the spider or granivores ellipse with itself. Seedlings are more likely to advance to juvenile stage when these escape biological control.

a)


Seeds
Granivores
Seedlings
Juveniles

b)

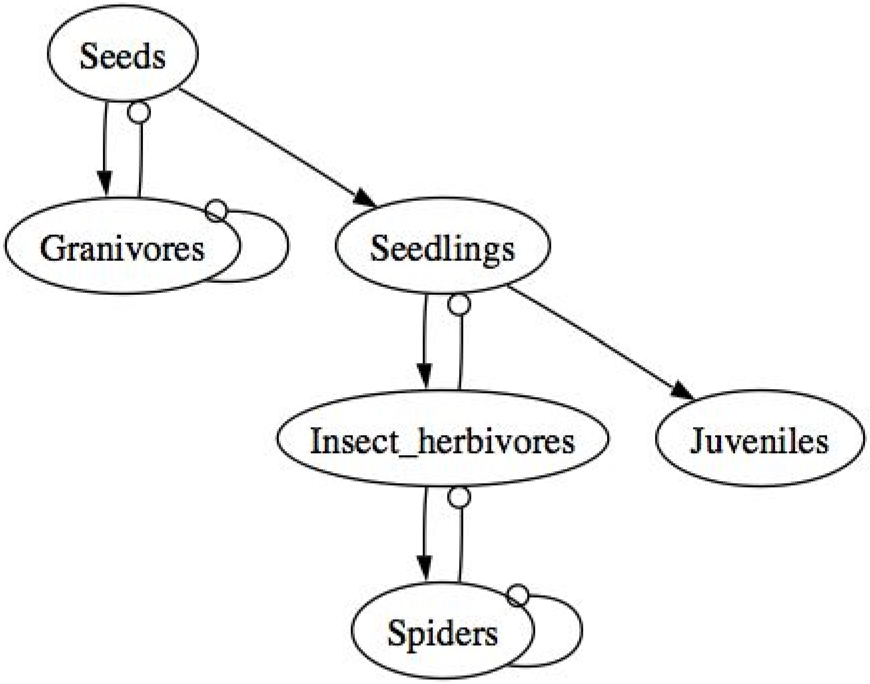

Seeds
Granivores
Seedlings
Insect_herbivores
Juveniles
Spiders

Figure 7.2. Mean number of seeds per bat roost and cache. Error bars are 1% standard error of the mean per seed congregation (bat roost or cache). Shaded column represents cashes.

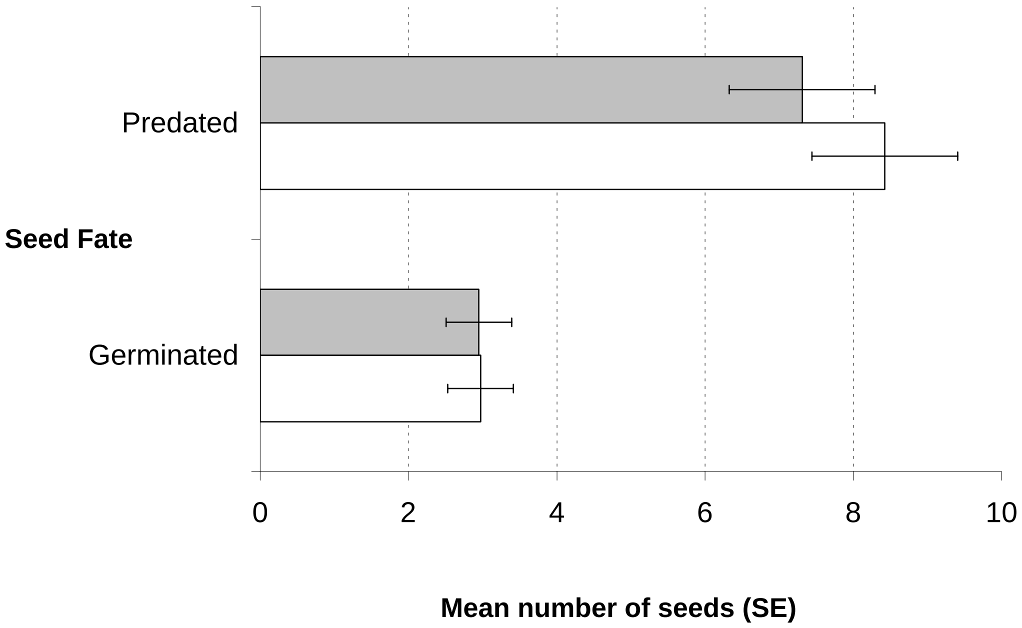


Figure 7.3. a) Secondary seed movement (m) as a function of the distance to nearest adult tree. X-axis represents primary seed dispersal or distance to nearest conspecific adult tree and Y-axis represent seed secondary movement (m). Box within broken lines indicates distance interval where secondary seed movement is frequent. Inserted chart represent the mean secondary seed movements (m) as a function of the distance to nearest adult tree (0-70 m). b) Mean seedling survival as a function of the nearest conspecific adult *D. oleifera* tree.

a,b)

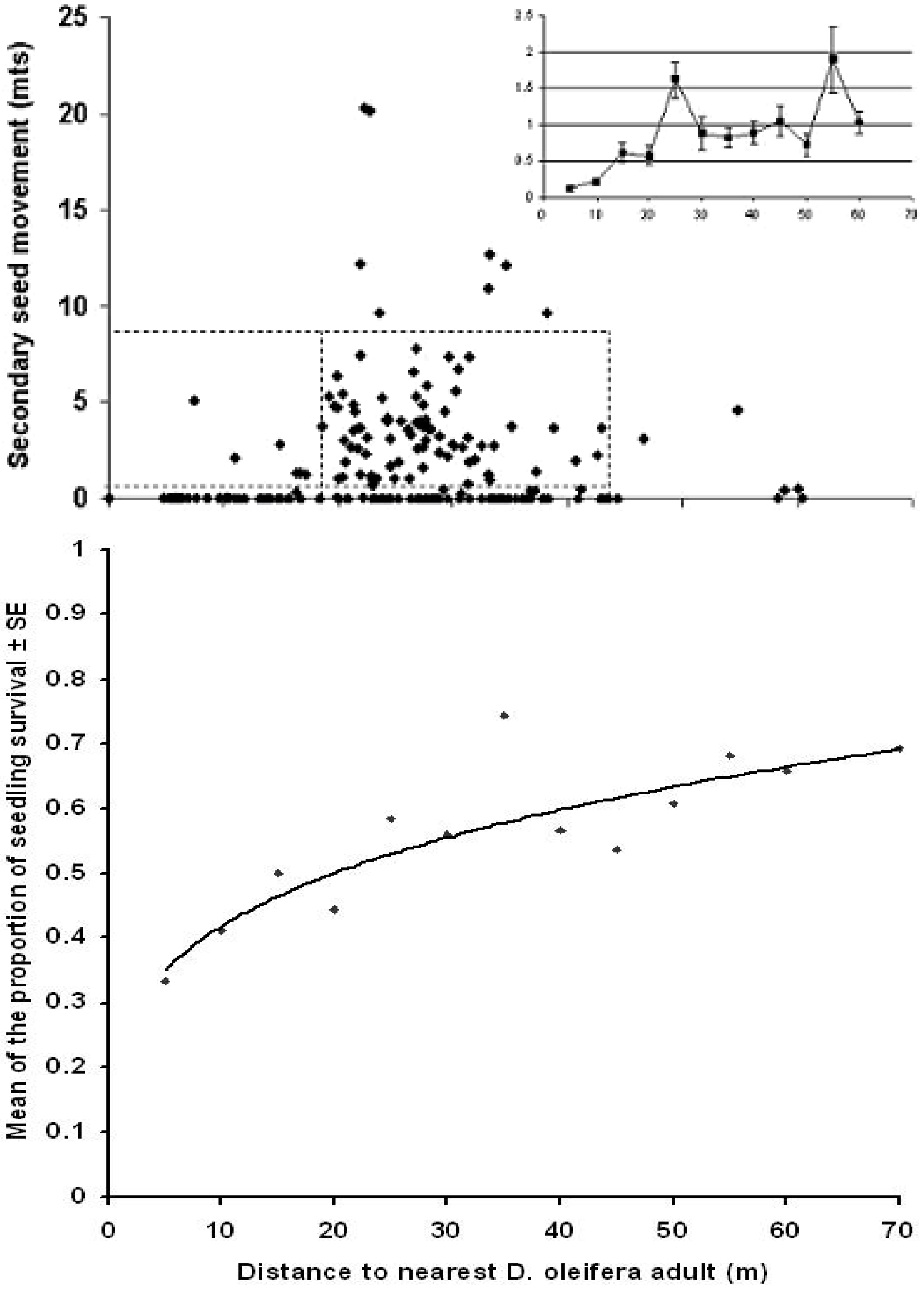
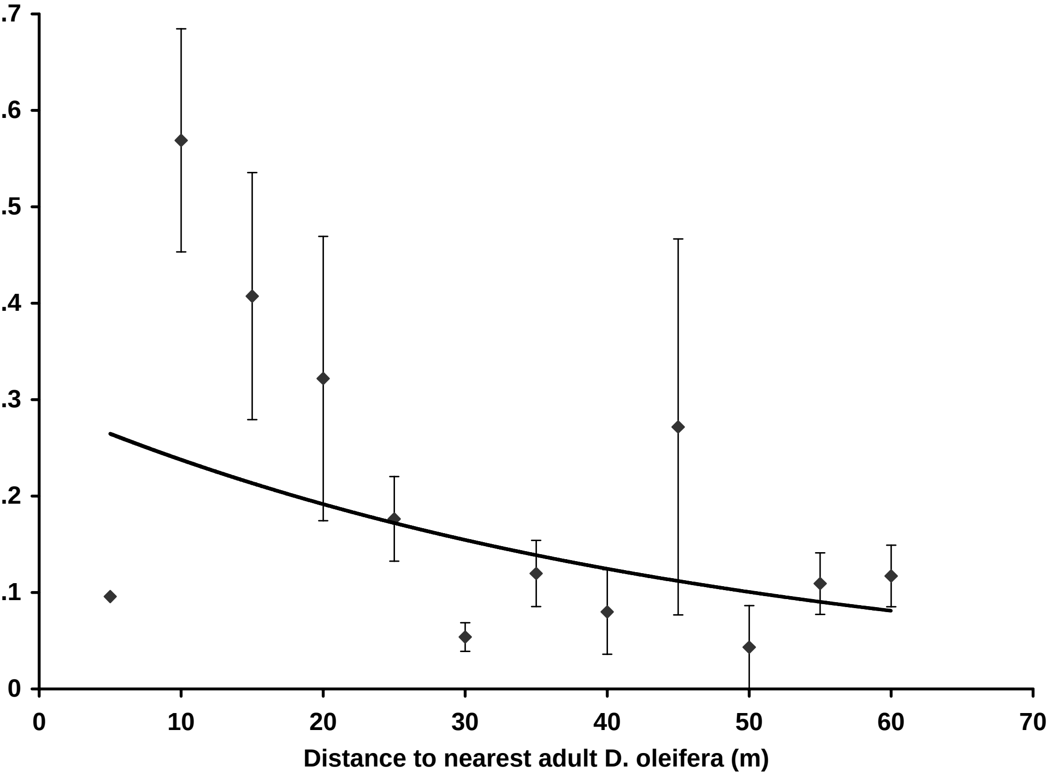

Secondary seed movement (mts)
25
20
15
10
5
0
2.5
2
1.5
1
0.5
0
0
10
20
30
40
50
60
70
Mean of the proportion of seedling survival ± SE
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
Distance to nearest D. oleifera adult (m)

Figure 7.4. Mean insect herbivory proportion as a function of the nearest conspecific adult *D. oleifera* tree. Error bars are the 1% standard errors computed per each 5m.



CHAPTER VIII

A COMPARISON BETWEEN THE JANZEN-CONNELL AND RECRUITMENT LIMITATION HYPOTHESES USING DIPTERYX OLEIFERA BENTH (FABACEAE) SYSTEM IN A TROPICAL RAINFOREST IN NICARAGUA

INTRODUCTION

It is well-known that tropical rainforests are extremely diverse. A significant goal in community ecology is to explain the mechanisms maintaining large numbers of co-occurring species at small spatial scales. For example, in a single hectare of Amazonian forest there can be individuals with diameter at breast height (DBH) of 10 cm or greater from more than 280 different hardwood tree species (Oliveira and Mori 1999; Valencia et al. 1994). Similar richness can be found in a forest in Southeast Nicaragua, where about 240 hardwood tree species have been reported in an area of 0.9 ha across four sites (Vandermeer et al. 2000). An obvious question arises from this pattern: how can so many tree species coexist in tropical forests? For more than half a century this question has been the focus of a great deal of research, both empirical (Chesson 2000; Connell 1971; Connell 1978; Hubbell 1979; Hubbell and Foster 1986; Hubbell et al. 1999; Janzen 1970; Wills and Condit 1999) and theoretical (Chave 2004; Chave et al. 2002; Hubbell 2001; Hurtt and Pacala 1995; Nathan and Casagarandi 2004; Tilman 1994). This research largely focuses on determining and understanding what mechanisms could be responsible for high tree species richness and devising methods to detect the proposed mechanisms in nature.

In response to the question of tropical tree species richness in tropical rainforest there are two hypotheses that have been the most widely debated. These hypotheses are the Janzen-Connell hypothesis (JCH) and the Recruitment Limitation hypothesis (RLH). Although both hypotheses fall within the "community dispersal assemblage paradigm", these are fundamentally different with regard to the way in which dispersal operates. RLH is part of the Hubbell's Neutral Theory, as stated in the Recruitment Limitation hypothesis, it is the dispersal limitation of propagules that drives recruitment dynamics and ultimately shapes local species richness and abundance. For example, in a hypothetical forest containing a single tree species, the chance that a propagule reaches a suitable regeneration site (namely, light-gaps, or any other regeneration site) is a function of the local density of adult trees (Hubbell 2001). At low density of adults in a tree population and under stochastic light-gap formation, the chances of colonization are lower. This is because there is not enough recruitment leading to an effective replacement of a low density patch of adult trees, unlike the high recruitment expected under a higher density of adult trees (Figure 8.1a).

The Janzen-Connell hypothesis contrasts with this idea because it proposes that host-specific predators and herbivores can organize the spatial distribution of tree species, thus maintaining high tree diversity in tropical rainforests (Figure 8.1b) (Janzen 1970; Connell 1971). The basic proposition is that natural enemies tend to congregate more often where seeds and seedlings congregate, specifically, near conspecific adults. Consequently, pathogen incidence could lead to recruitment of conspecific seedlings away from adult trees were seed and seedling damage is lower. This would result in large distances between adult trees, potentially explaining how tropical rainforests maintain high tree species diversities at low densities. Due to its appealing elegance and simplicity, this hypothesis has found its way into general ecological knowledge with a large body of field data and/or experimentation to support it. A large number of field studies addressing the hypothesis need to be reexamined due to recurrent flaws (Ruiz 2004). As noticed in a recent literature review, in some studies seed removal events are assumed to be either seed dispersal or seed predation (Vander Wall et al. 2005) and many studies present inconclusive or confusing evidence (Clark and Clark 1984; Hyatt et al. 2003).

An organic synthesis of both hypotheses could provide a stronger framework for tropical rainforest research (Figure 8.2). First, under the RLH it is the local density of adult individuals which determines the recruitment dynamics because the probability that a seed arrives at a particular spot is mainly determined by the availability of seed sources - the density of the adult trees (Figure 8.2b). In the JCH, seed and seedling survival at a particular spot is mainly determined by the distance from the adult tree. In both cases, local interactions between dispersers such as bats, terrestrial vertebrates, and herbivores, are involved in the seedling dispersal and recruitment process. However, in the RLH the overwhelming determining factor is the regional scarcity of seed sources, while in JCH the overwhelming determining factor is the proximity of seeds and seedlings to conspecific adult trees and the behavior of seed and seedling dispersers and consumers. The present study focuses on determining to what extend these two hypotheses explain the distribution of seed and seedling survival in a lowland tropical rainforest, using as a model system *Dipteryx oleifera* and its associated seed dispersers and natural enemies.

METHODOLOGY

Study sites: The research was conducted in a tropical rainforest in Southeastern Nicaragua from February 2002 to April 2007, at the research site known as La Union (12°05' N., 83°55' W., elevation 10-20 m; mean annual temperature 26°C, mean annual precipitation = 5000 mm) (IRENA 1991). The tropical rain forest is accessible by the Caño Negro River, and is well studied (Granzow de la Cerda et al. 1997; Yih et al. 1991)

Study species: *Dipterix oleifera* is a Neotropical canopy emergent tree common in lowland forests of Central America, Panama and Venezuela. The species is also know as *Dipteryx panamensis* (Fournier 2003). It can reach heights of ~50 m and a diameter at breast height (DBH) of about 1,500 mm (Clark and Clark 1992). *D. oleifera* is a large seeded species with heavy seeds averaging 25.2 g ± 4.4 SD, n=80, (*unpublished data*). It is dispersed by bats (Forget and Milleron 1991) and terrestrial vertebrates (*i.e*. squirrels, agoutis, pacas, etc.) (Reid 1997). The wood is very dense, which allows saplings to withstand damage inflicted by branches falling from the forest canopy (Clark and Clark 1991).

Bats and terrestrial mammals: Fifteen species of bats were captured around adult *D. oleifera* individuals and/or near natural seed piles. The species of bats trapped over the research period are listed in Table 1. Terrestrial mammal trapping success has been extremely low in the area, as is frequently observed in studies at lowland rain forests (McClearn et al. 1994). To date, the terrestrial mammal species captured by the trapping fieldwork at the La Union site are the following: *Proechimys semispinous*, *Oryzomys spp.*, *Nyctomys sumichirasti*, *Marmosa mexicana*, *Didelphys virginiana* and *Didelphis alventris*.

For *D. oleifera*, bats facilitate primary seed dispersal and terrestrial vertebrates facilitate secondary seed dispersal. In the process of primary seed dispersal, bats have been observed to swoop down upon *D. oleifera* and quickly grab the fruits, most likely as means to escape predation upon natural enemies (owls and boas). As a consequence, bats move propagules long distances, on average ~40 m from the nearest conspecific adult of *D. oleifera* (Ruiz et al. submitted). In general, bats prefer to roost in a select few species of palm trees (*Welfia regia*, *Astrocaryum alatum* and *Criosofila warsewitzii*) (Ruiz et al. submitted). Roosting palms can provide protection to bats against rain and predators from above the canopy. However, the inhabitation of a bat roost leads to the accumulation of discarded seeds below the roost site. Terrestrial vertebrates may then consume or disperse seeds from located in these sites. Thus, terrestrial vertebrates act either as seed consumers or seed dispersers. In our field observations we noticed that seed removal by terrestrial vertebrates from below adult trees was very low. This lower seed movement below fruiting trees may be due to the fact that terrestrial seed dispersers avoid visiting sites where natural enemies of small vertebrates may be waiting to prey on them. Instead, terrestrial vertebrate may prefer to move seeds from bat seed piles located away from the fruiting trees. Terrestrial mammals can either scatter seeds from bat seed piles across the forest, or they may congregate the seeds into caches.

Method to evaluate seed demography: Field observations were conducted in a permanent plot ~6.5 ha established in 2002 at the Union site. In order to evaluate seed fate we mapped and threaded seeds as they appeared within a 3.2 ha subplot from January to July 2006. We super-glued a micro precision nylon thread to all seeds found within this area. The very fine thread was attached to the seed and the bobbin was clipped to the forest floor with a toothpick (Figure 8.3). Each bobbin was numbered with a unique number such that seed identity was recorded during the research. Previous studies have only evaluated the effect of seed removal (Cintra 1994; Cintra and Horna 1997; Vander Wall et al. 2005), as pointed out in a review of the literature on seed dispersal (Vander Wall et al. 2005). The seed threading approach allowed us to follow seed movements and ultimately to determine seed fate (DeMattia et al. 2004; Forget and Wenny 2005; Witt 2001). Seeds found eaten and unthreaded were not included in the analyses, although we did note their position. Seed conditions were measured at least once each month for a period of eight months. Seed conditions were classified into four groups: predated, unviable (when threaded but without or with undeveloped endosperm), germinated, and recruited (1 yr after germination).

Method to evaluate seedling demography: Seedlings were located and mapped each year from 2002 to 2006 at the La Union site. An aluminum tag with a unique number was looped around the stem of each seedling in order to evaluate survival and mortality. Seedlings were considered dead if the seedlings were missing, or if they were dry and without green tissue. Each seedling was assumed to be the progeny of the nearest conspecific adult tree.

The coordinates (x, y) of each adult tree of *D. oleifera* were recorded and later used to select areas with a high, medium or low number of adult individuals of *D. oleifera* within the research plot (Figure 8.4).

Hypotheses testing: Since the hypothesized probability of seed and seedling arrival to a location in the forest floor is different for each hypothesis, it is possible to examine evidence for these two hypotheses using multiple linear regression. The general formulation of this model is as follows:

$$y = a + bx + cz + e_{ij}$$

Where the objective is to determine whether the variation in the dependent variable (*y*) is correlated with (*x*) or (*z*). The value *a* represents the mean; *b* is the coefficient relating the number of adult individuals and the mean; *c* is the coefficient relating the distance to the nearest conspecific adult tree and the averaged mean and e_{ij} is the error, which is assumed to be identical and independently distributed with variance σ^2_ε, $\varepsilon_t \sim N(0, \sigma^2_\varepsilon)$.

RESULTS

Seed fate: Seed fate correlated positively with the distance to the nearest conspecific adult tree (for damaged seeds) and with the number of adult trees (for germinated and unviable seeds) in 2006 at Union site (Figure 8.5, Table 8.2).

Seedling survival patterns: Seedling survival of *D. oleifera* correlated positively with both distance to and number of conspecific adult tree between 2002 and 2006 at La Union site (Figure 8.7, Table 8.2).

DISCUSION

Seed fate: Unviable seed distribution correlated positively with increasing distance to the nearest conspecific adult and the number of adult trees. Because reproductive trees were located in the vicinity of one another, the results suggest that bats moved the unviable seeds long distances from the seed sources. Since bats consume the exocarp, it would be energetically more efficient to move unviable seeds, since these are lighter than the more heavy viable seeds. However, the seed dispersal functions suggest that bats did not discriminate between viable seeds and unviable seeds. This may be due to the fact that the nutritive reward that bats obtained from consuming the exocarp of either seed type is similar, eliminating the need to discriminate between them. Overall, viable seeds were dispersed away from the distance to the nearest conspecific adult tree, where seed predation was lower (Figure 8.5b). Seed germination correlated positively with both, distance to and density of conspecific adult trees, thus suggesting that survival is higher away from the vicinity of a high density of conspecific adult trees. These are results that provide evidence in favor of both the JCH and RLH at the seedling stage.

Seed germination distribution and seedling recruitment patterns provided evidence in favor of both hypotheses. There is evidence in favor of the RLH during 2002-2003, because seedlings were at very low density at the beginning of the study. Seedling survival during this period was not a function of the distance to the nearest conspecific adult tree but the density of adult conspecific. Between 2003 and 2004, the seedling recruitment distribution was more indicative of possible Janzen-Connell effects, because seedling survival increased with the distance to the nearest adult *D. oleifera*. In the periods 2004-2005 and 2005-2006 the seedling survival distribution was consistent with both hypotheses. This may be due to the fact that during the period 2004-2006 there was high seed production across both years and as a consequence, seeds were dispersed to more than 90% of the research plot. High seed production could lead to predator satiation and longer seed movement due to bat and terrestrial vertebrates. This assumption is supported by the observed seedling distribution, which reached almost all subplots (10x10m) within the forest floor. In the 2005-2006 period, seedling mortality due to insect herbivory was higher closer to the nearest conspecific adult tree (Ruiz et al. submitted). These results also suggest a negative effect of proximity to adult trees on seedling survival.

Seedling recruitment dynamics and tropical rainforest organization: The results of the seed and seedlings recruitment of *D. oleifera* provide evidence in favor of both hypotheses (JCH and RLH) in a tropical rainforests of Southeastern Nicaragua. Recently, Muller-Landau *et al.* (2004), proposed that a merging of these two hypotheses would provide us with a stronger framework in the process of understanding the nature of what determines the maintainance of the immense tree biodiversity in tropical rainforests. Result from this study aim to contribute to the organic merging between these two major hypotheses in the process of developing a stronger framework for understanding tropical rain forest communities.

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Table 8.1. Bat species identified within the study area in Southeast Nicaragua. Feeding habits and estimated weight were obtained from Reid (1997).

Species	**Feeding habit**	**Weight (g)**
Artibeus intermedius	Frugivorous	40-54
Artibeus jamaicensis	Frugivorous	21-51
Artibeus lituratus	Frugivorous	53-73
Artibeus phaeotis	Frugivorous	9-15
Artibeus watsoni	Frugivorous	9-15
Carolia perspicillata	Frugivorous	21-23
Chiroderma villosum	Frugivorous	15-18
Carolia castanea	Frugivorous	11-16
Myotis nigricans	Insectivorous	3-6
Phyllostomus discolor	Insectivorous	26-51
Trachops cirrhosus	Insectivorous	24-36
Vampyressa nymphaea	Bloodsucking bat	11-16
Glossophaga soricina	Nectivorous	7-12
Hylonycteris underwoodi	Nectivorous	6-12

Table 8.2. Result of the multivariate regressions for seed and seedling fate in two tropical rain forest of Southestern Nicaragua. Each row presents the result of a multivariate model. First column correspond to dependent variables, "SDU07" = damaged seeds, "SIU07" = viable seed 2007, SGU07 = germinated seeds at Union site respectively in 2007. PU = seedling survival a union site for the periods 2002-2003, 2003-2004, 2004-2005, PB02-03 = seedlings at Bodega site for the periods2002-2003, 2003-2004, 2004-2005 and 2005-2006. Beta coefficients (ß) for the variable distance (Dist), and for the variable number of individuals (Num), and the regression "R" coefficient. Bold values represent significant statistical probabilities ($p<0.05$).

Var. Dep.	Dist (ß)	Num (ß)	Dist (p)	Num (p)	R
SDU07	0.0023	-0.0007	**0.0123**	0.2014	0.2270
SIU07	-0.0020	0.0010	**0.0013**	**0.0068**	0.4940
SGU07	-0.0003	-0.0003	**0.0123**	**0.0270**	0.4380
PU02-03	0.0007	-0.0499	0.1813	**0.0068**	0.3232
PU03-04	0.0014	0.0106	**0.0000**	0.2962	0.3917
PU04-05	0.0012	-0.0169	**0.0000**	**0.0313**	0.3943
PU05-06	0.0014	-0.0169	**0.0000**	**0.0004**	0.5034
PB02-03	8.86E-005	-0.0050	0.6300	**0.0000**	0.3420
PB03-04	-1.1E-005	0.8220	0.9550	**0.0000**	0.3530
PB04-05	3.86E-005	-.0060	0.8690	**0.0000**	0.2940

Figure 8.1. Empirical expectations of the Recruitment Limitation hypothesis and the Janzen-Connell Hypothesis.

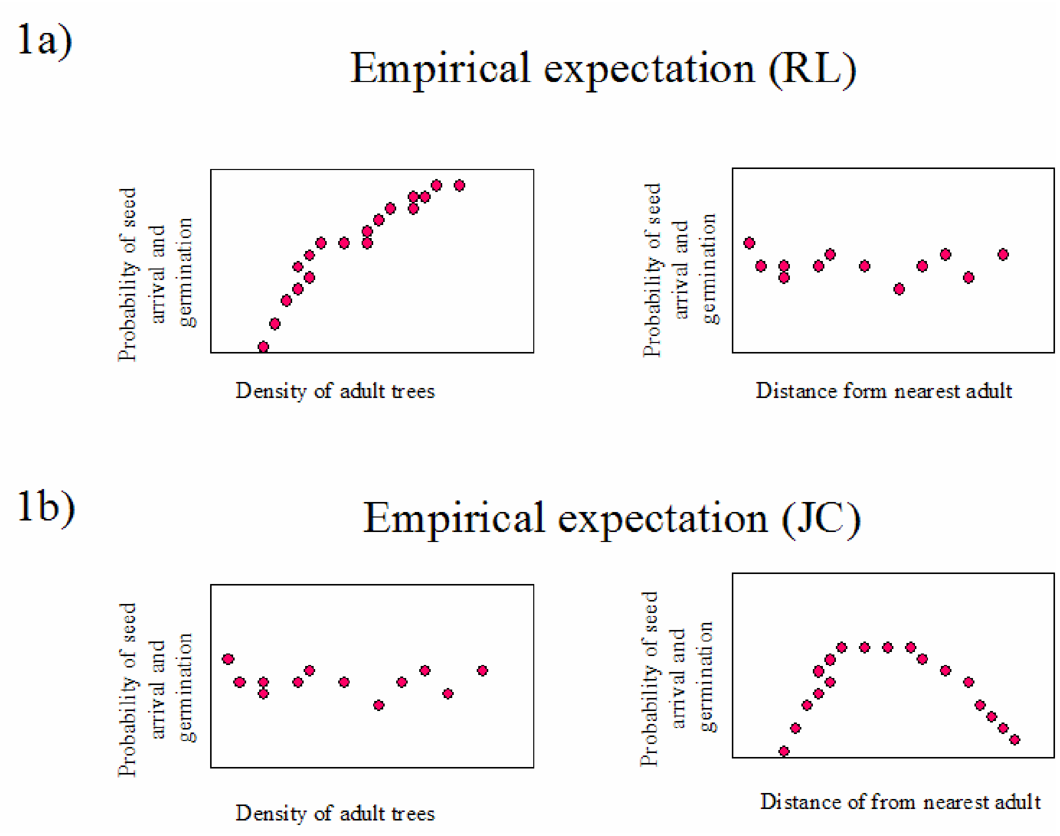


Figure 8.2. Schemas representing the Recruitment Limitation hypothesis (a) and the Janzen-Connell hypothesis for large seeded tropical trees species (b).

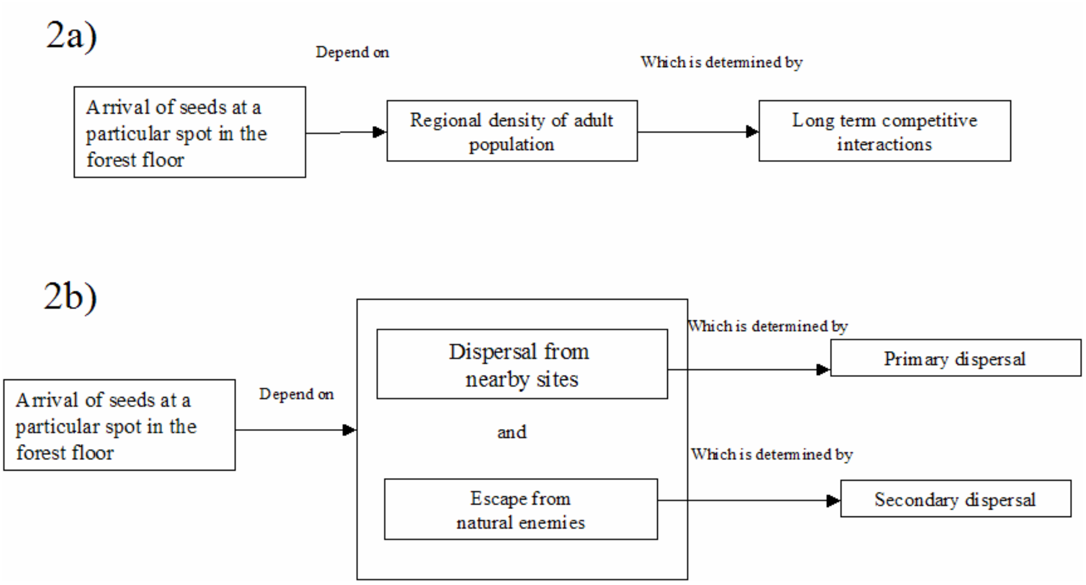


Figure 8.3. Threading method to follow seed fate. This method consists in attaching a nylon thread to each seed (Photo: Javier Ruiz).

Figure 8.4. Map of the research plot at Union site. Gray squares are represent the location of adult *D. oleifera*. Circles colors represent three distinct areas within the research plot. Gray circles represent isolated adult *D. oleifera*, yellow cirles are small adult individuals and green circles represent large adult *D. oleifera* individuals.

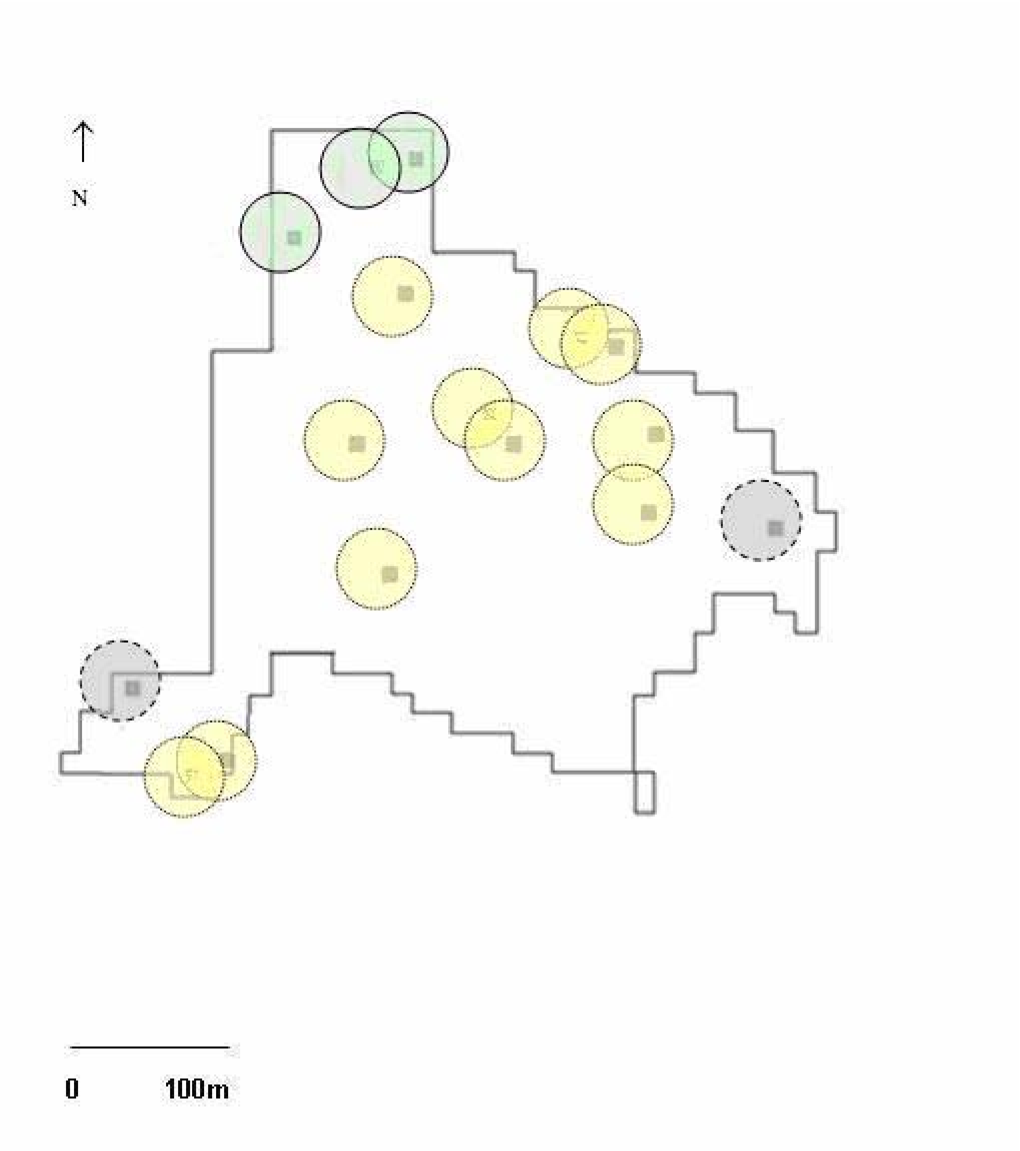


Figure 8.5. Weighted mean proportion of seed fates of *D. oleifera* (*y)* as a function of distance to nearest tree (*x)* and number of adult trees at three areas varying in number of adult tree at La Union site. Data were gathered from threading approach conducted within a 3 ha plot. Percentage of seed at each seed fate category was compared independently by way of using multivariate regression models. Sample size is = 3,529 threaded seeds during the seed production season of 2006.

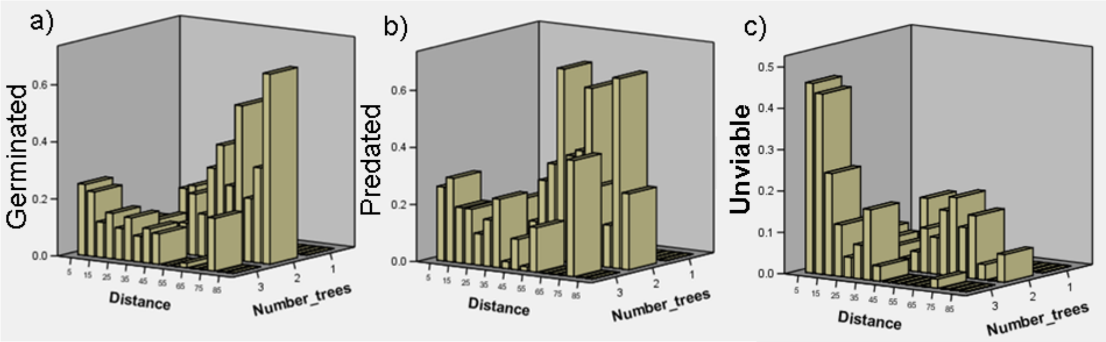
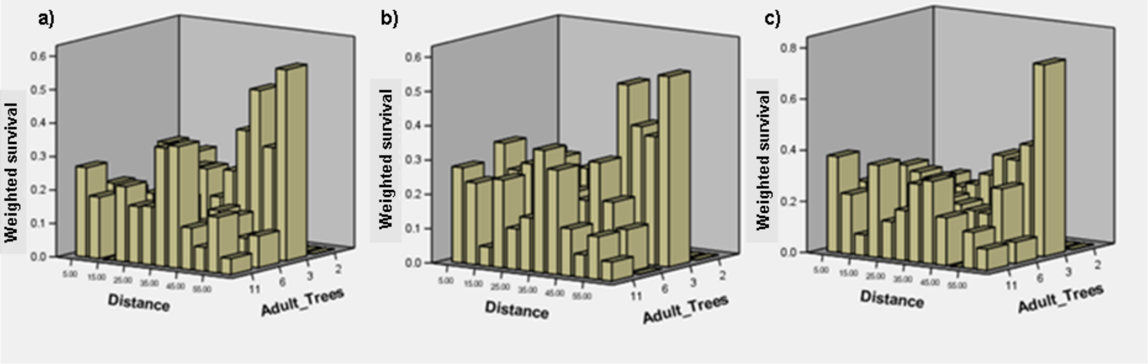


Figure 8.6. Weighted mean of the percentage of seedling survival of *D. oleifera* (*y)*, as function of distance to nearest tree (*x)* and number of adult trees *(z)* in four areas varying in adult individuals numbers at La Bodega site. Data were gathered at a 6.5 ha plots in the tropical rainforest of Southeast Nicaragua. a) Correspond to period 2002-2003, b) period 2003-2004 and c) 2004-2005. During the research period we followed the fate of 1,532 seedlings of this species



CHAPTER IX

CONCLUSIONS

Summary of Major Findings

- Seedling survival distributions of *D. oleifera* are clumped and strongly related to the distance to the nearest conspecific adult tree in an undistributed forest in Southeastern Nicaragua. These seedling survival distributions are strongly dependent on seed dispersal by bats and terrestrial vertebrates. Primary seed dispersal by bats congregates seeds below bat roosting palms, and secondary seed movements by terrestrial rodents further dispersed seeds away from these highly dense seed piles. Seedling survival probability is higher away form the adult tree and strongly correlated with the processes of primary and secondary seed dispersal.
- Because bats also scattered seeds throughout the forest floor, bats' primary seed dispersal is fundamental to seedling survival away from adult trees.
- Secondary seed dispersal is on average a short distance for *D. oleifera* (~1.5 m). This result comes from the observations of the seed fate of a large number of seeds monitored using a seed threading approach.
- There are spatial correlation between seedling survival and the location of potential roosting palms located away form the nearest large adult tree of *D. oleifera* (~35 m). Secondary seed dispersal released seeds from negative density dependent effects associated with the location of the seed piles.
- A seed-masking effect existed from inviable seeds, increasing the survival of viable seeds during a year of high seed production. This masking effect was evaluated in two seed pile types, bat seed piles and caches. Because of the

presence of inviable seeds, seed predator search efficiency was lowered such that the addition of inviable seeds to the seed piles significantly decreased seed predation, increased seed germination rates and consequently increased seedling recruitment further away from the nearest conspecific adult tree.

- Terrestrial vertebrates did not consume seeds below the adult trees, perhaps in order to avoid their natural enemies present (*i.e.* owls and boas). Instead, seed damage is predominantly due to the larval stage of a fly species (family Taeneaptera).
- Insect herbivory decreased with the distance to the nearest conspecific adult tree. These results imply that insect herbivory is the mechanism controlling seedling mortality closer to the nearest conspecific adult tree, where insect herbivory and seedling mortality are higher than they are farther away from the adult tree.
- The presence of eight spider species in seedlings of *D. oleifera* correlated significantly with lower insect herbivory and correlated positively with seedling growth. Spiders might be harassing or predating insect herbivores, suggesting a positive indirect effect of spiders on seedling survival and growth. Spiders were more common away from the nearest conspecific adult tree, thus suggesting that spider species also contribute to the observed Janzen-Connell seedling distribution.
- Tropical tree species richness could be maintained when adult trees are located away from each others at low densities.

Suggestions for Future Research

- The statistical models used to assess comparisons and correlations only incorporated linear parameters. However, some relationships between variables were not strictly linear. This poses the challenge of implementing and/or developing statistical methods to better address non-linearity.
- Second order spatial statistics can be used to evaluate cross correlations between the location of trees and seedling recruitment and seedling mortality. The implementation of such pair-wise estimations poses the challenge of writing efficient computer scripts in order to minimize computational power required to run multiple-to-multiple pair-wise estimations.
- The Ripley cluster coefficients would make it possible to statistically estimate the location and size of clumps and thus to determine the direction and magnitude of spatial and temporal effects of natural enemies of seedlings of *D. oleifera* and their associated spiders species.
- Developing cellular automata models would allow one to examine which dispersal and predation regimens might generate theoretical or unstable equilibria in the population of *D. oleifera* at various spatiotemporal scales. This would pose the challenge of developing and testing theoretical scenarios that resemble the empirical observations of the relative contributions of seed dispersal types, insect herbivory and spider presence on seedling survival of *D. oleifera*.