

The Ecology of Sharing Mutualists:
Consequences for Plant Performance and Population Dynamics

by

Arietta Elise Fleming-Davies

Department of Biology
Duke University

Date: _____

Approved:

William F. Morris, Supervisor

Mark D. Rausher

William G. Wilson

Justin P. Wright

Robert R. Dunn

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of Philosophy in the Department of
Biology in the Graduate School
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ABSTRACT

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Abstract

Although we often study mutualisms (interactions in which both species benefit) at the level of the individual partners, mutualistic interactions take place in the context of populations and communities. Sharing mutualists with others in a population could result in indirect interactions in the form of mutualist-mediated competition or facilitation. In my dissertation work I asked whether intraspecific competition or facilitation for ants might occur in an extrafloral nectary-bearing (EFN) plant, and what the consequences would be for long-term population dynamics of the plant. My focal species was *Colubrina spinosa* (Rhamnaceae), a neotropical treelet on which I observed 69 ant species at La Selva Biological Station, Costa Rica.

Demonstrating intraspecific competition for mutualists requires that 1) neighbor densities affect mutualist visits to an individual, and 2) change in mutualist visits results in reduced benefit. To determine how mutualist density affects plant benefit, I experimentally manipulated ant abundances on plants over two years and measured growth and survival. To assess competition for mutualists, I excluded ants from conspecific neighbors and followed ant abundance on focal plants. To consider long-term facilitation, in which greater local nectar resources increase local ant abundance, I manipulated nectar resources in a two-year field experiment and estimated ant abundance on *C. spinosa* plants and on baits.

Considering local neighbor density both within a 1m radius and in 5x5 m plots, ant densities on *C. spinosa* plants showed evidence for a small-scale competition effect and a contrasting plot-level facilitation effect. The small-scale competition was sized-based; smaller plants lost ants to larger plants. Ant benefit to plants also depended on plant size. For larger plants, those with greater size-adjusted ant density had higher growth and survival than those with fewer ants than expected for their size.

To determine whether these contrasting competition and facilitation effects could impact population growth or densities, I modeled population dynamics with an integral projection model (IPM). Growth and survival were functions of ant density, which in turn depended on conspecific neighbors, plant size, and mean background ants. Results suggest that larger-scale facilitation of mutualists impacts long-term population growth more than small-scale competition. Population growth rate increased with increasing background ant density, which depended on facilitation at the 5x5m plot scale. In contrast, small-scale competition caused a redistribution of mutualist ants among plants of different sizes, but had very little effect on long-term population growth.

I thus conclude that on the scale of individuals there is evidence of intraspecific competition for ants as well as facilitation in the EFN plant *C. spinosa*, but only facilitation effects lead to appreciable changes in population dynamics. If mutualist-mediated facilitation effects tend to occur over long time scales in other systems as well, facilitation might prove to be more important than competition in other mutualisms.

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Acknowledgements

I thank first of all my advisor Bill Morris for all his good-natured help throughout this project, from discussing ideas to field experiments to analysis and writing. The members of the Morris, Wilson, Wright and Koelle labs (aka the Cogitron group) provided helpful feedback on ideas and manuscripts, as did my committee members Rob Dunn, Mark Rausher, Will Wilson, Jim Clark and Justin Wright.

A great thanks to all those who helped with the substantial amount of fieldwork that went into this dissertation, in particular Gerald Campos Romero, who spent many hours and learned many ants in the course of this project. Also thanks to field assistants Claudia Lizana Moreno, Aaronn Cachon and Enrique Salvicetti, as well as to the many volunteers who provided supplies and help in the field and the lab, including Cynthia Rossi, Mindy McDermott, Adam Calo, Luis Elizondo, Marylin Veiman, Carrol Fleming, Erin Kuprewicz, Carlos Garcia-Robledo, Lydia Gentry, Isabelle Boittin, Kelsey Reider, Evi Paemelaere, and any members of the Q-tip team not otherwise mentioned.

I thank the administrative staff at La Selva Biological Station, in particular Deedra McClearn, Bernal Matarrita, Danilo Brenes, Cynthia Rossi, and all the Taller staff. Jack Longino, Ronald Vargas, and all the teachers and students of the Ant Course 2008 made it possible for me to identify the many ant species I observed in this work. In addition, despite studying only one focal plant species, I received quite a bit of help

identifying EFN plant species, in particular *Inga* spp., from Orlando Vargas and Nelson Zamora. Thanks to Javier Guevarra of MINAE for his extremely efficient help with Costa Rican research permits. The Duke Biology Department administrative staff was also very helpful with logistics.

Field work was conducted with permission from the Costa Rican Ministry of Energy and the Environment (MINAE permits 146-2007-SINAC; 061-2008-SINAC; 198-2008-SINAC; 054-2009-SINAC). This work was supported by the Organization for Tropical Studies, the Duke Center for Latin American and Caribbean Studies, Sigma Xi, a National Science Foundation Graduate Research Fellowship, and a National Science Foundation Dissertation Improvement grant to A. Fleming-Davies.

1. Introduction

Mutualisms are interactions between two species or guilds of species in which both sides benefit. In the early literature of interspecific interactions, mutualisms were largely ignored relative to competition and predation, as mutualisms were thought to be destabilizing and therefore rarer than antagonistic interactions (e.g. May 1973). However, since then mutualisms have received considerable attention in both experimental and theoretical work (Boucher et al. 1982, Bronstein 1994). Mutualisms are ubiquitous in nature, and can help to stabilize competitive interactions and promote species coexistence (Lee and Inouye 2010).

Although we often study mutualisms as isolated interactions between the partner species, mutualisms take place in the context of populations and communities. For populations above some minimum density, most individuals will share mutualists with others in the population. The shared mutualists may be from either a single partner species or of a guild of potential partners. Sharing a common mutualist among individuals within a population, or even among different species, could cause indirect interactions between those individuals or species. For example, if the resources or services provided by the common mutualist are a limiting factor for the partner species, then those partners might compete for mutualists (Addicott 1985). Facilitation might also occur, in which the presence of several partners increases the number of mutualists visiting an area (short-term facilitation) or the population of the shared mutualists (long-

term facilitation) (Waser and Real 1979, Rathcke 1983). Therefore being near other conspecific or heterospecific individuals may be advantageous, if their presence increases the numbers of the shared mutualist partner. Characteristics of the partners that share mutualists could influence whether facilitation or competition occurs. For example, larger individuals might draw mutualists away from smaller individuals, but several small individuals might collectively attract more mutualists. Many pollination and seed dispersal mutualisms show density-dependent effects of sharing mutualists, with facilitation of neighboring plants at low plant densities, but competition at high densities (Moore and Willson 1982, Manasse and Howe 1983, Rathcke 1983, Feldman 2008). Potentially both facilitation and competition could occur in the same system, perhaps over different temporal or spatial scales, or at different population densities (Addicott 1985).

Mutualist-mediated competition could have important consequences for population dynamics, by introducing a new source of negative density dependence (Addicott 1985, Cushman and Addicott 1989). In contrast, intraspecific facilitation via mutualists may introduce a source of positive density dependence, in which mutualist visits increase with population density, thus causing population growth rate to increase with population density as well (Groom 1998, Hackney and McGraw 2001). If both facilitation and competition occur in the same system, one or the other might have greater long-term demographic effects. Whether facilitation or competition effects

dominate in long-term population dynamics may depend on external factors such as the availability of other resources (Stanton 2003), or on the temporal scales at which the effects occur.

In my dissertation research, I addressed how indirect interactions may arise from sharing mutualists in a generalized mutualism between plants that produce extrafloral nectaries (EFNs) and the ants that visit them. Using experimental manipulations of an EFN plant, I looked for evidence of mutualist-mediated competition or facilitation, considering different temporal and spatial scales. I also assessed how characteristics such as size or the identity of the partner species might influence benefit or the outcome of sharing mutualists. In my work I addressed the following main research questions: **1)** Do EFN plants compete for mutualist ants, or is there a facilitative effect, in which being near other EFN plants is beneficial? **2)** At what spatial and temporal scales do facilitation or competition effects occur? **3)** Are the effects of sharing mutualists size-dependent? How does benefit depend on plant size? **4)** What are the long-term demographic effects of sharing mutualists for an EFN plant?

1.1 Evidence of competition and facilitation for mutualists

Competition for mutualists has been found in ant-aphid interactions (Cushman and Addicott 1989, Breton and Addicott 1992, Fischer et al. 2001), pollination systems (Free 1968, Pleasants 1980, Kephart 1983, Rathcke 1983), seed dispersal mutualisms (Davidson and Morton 1981), and obligate ant-plant interactions (Davidson et al. 1989,

Young et al. 1997, Palmer et al. 2000). Most work has focused on interspecific competition (but see Moore and Willson 1982, Manasse and Howe 1983, Morales 2000, Moeller 2004). Additionally, most previous work has provided only indirect evidence of competition for mutualists (Addicott 1985).

Previous evidence of facilitation for mutualists comes mainly from pollination biology (but see Holbrook and Schmitt 2004). Co-flowering plants may increase total pollinator visits to an area, thus creating a benefit to being near other flowering individuals (Lavery 1992, Moeller 2004). Long-term facilitation may occur when sequentially flowering plant species benefit one another by maintaining pollinator populations (Waser and Real 1979, Rathcke 1983). Pollination systems are not representative of most other mutualisms, in that sharing mutualists with conspecifics is frequently necessary in order for plants to benefit from the interaction. In EFN plant-ant protection mutualisms, however, there exists a wider range of possible outcomes, including the possibility that mutualist visits are highest when no conspecific neighbors are present. Therefore findings from EFN plant-ant interactions might be more readily generalized to intraspecific facilitation in other non-pollination mutualisms.

1.2 Extrafloral nectary bearing (EFN) plant-ant mutualisms

Ants often engage in herbivore-defense mutualisms with plants, in which the plant provides a food source for the ants, and the ants help to defend the plant from

natural enemies (Janzen 1966). Some plants produce nectar at extrafloral nectaries (EFNs) on leaves or stems to attract predators (or parasitoids), including ants (Bentley 1977). Ant-EFN plant interactions are generally facultative mutualisms (i.e. populations can persist in the absence of the mutualist partner). EFN plants typically receive a smaller magnitude of benefit from their guild of ant visitors compared to myrmecophytes, plants which provide domiciles and food bodies and are typically obligately dependent on one or few specialized ant species (reviewed in Rosumek et al. 2009). However, much evidence supports that generalized plant-protection interactions are true mutualisms (i.e., both plant and ant partners benefit). Herbivory increases when ants are excluded (e.g. Bentley 1977, Costa et al. 1992, but see O'Dowd and Catchpole 1983), often resulting in lower seed set (Horvitz and Schemske 1984, Oliveira et al. 1999). Generalist ants can derive a significant portion of their resources from EFN plants (Davidson 1997, Ness et al. 2009), and plant-derived resources, including extrafloral nectar and homopteran honeydew, may play a key role in structuring ant communities in tropical forests (Tobin 1991, Blüthgen 2000). Selection on extrafloral nectary characteristics in the presence of ants has been directly measured in several EFN species (Rudgers 2004, Rutter and Rausher 2004).

Most EFN mutualisms are very diffuse interactions, with many species of ants that visit many species of EFN plants. The magnitude of benefit to EFN plants has been shown to depend on the identity of the ant species present (Horvitz and Schemske 1984,

Ness 2003, Miller 2007, Agarwal and Rastogi 2008). Plants with EFNs are found in over 90 families (Koptur 1992) and contribute up to one third of plant species in tropical forests (reviewed in Blüthgen and Reifenthat 2003). Interactions among EFN plants may be especially important in disturbed high-light habitats such as forest edges, secondary forests, and treefall gaps where EFN species are particularly abundant (Bentley 1976, Schupp and Feener 1991, Fiala and Linsenmair 1995). Unlike myrmecophytes, most EFN plants do not produce specialized structures to house ant colonies, and most ants do not nest on the plant. This suggests that individual plants are likely to share the same mutualist ants, and that competition or facilitation effects could occur over relatively short time scales, since plant benefit is affected by daily foraging decisions by ant visitors. There is evidence that EFN plants compete with homopterans for ant attendance (Buckley 1983, Sakata and Hashimoto 2000), and that ants compete with other arthropods for EFNs (Heil et al. 2004). Competition or facilitation for ants among EFN plants has not previously been studied.

1.3 Outline of the dissertation

In the following chapters I present my dissertation research on how indirect interactions might arise from sharing mutualists among EFN plants, and the resulting consequences for plant populations. In my first research chapter (Chapter 2) I investigate how plant benefits from mutualist ants vary with mutualist density, plant size, and ant species, in the neotropical understory tree *Colubrina spinosa* (Rhamnaceae).

I manipulated ants on *C. spinosa* juveniles in a two-year field experiment, using ant exclusion and nectar addition treatments in order to induce an extended range of ant densities. Benefit from ants depended on plant size, with no effect of ants on performance of very small plants. For larger plants, those with greater size-adjusted ant density had higher growth and survival than those with fewer ants than expected for their size. Growth and survival benefits varied with the presence of different ant taxa.

In the second main chapter (Chapter 3) I present evidence that conspecific neighbors affect ant visits to *C. spinosa* individuals. To assess competition for mutualists, I excluded ants from conspecific neighbors and followed ant abundance on focal plants. To consider long-term facilitation, I manipulated nectar resources in a two year field experiment in which I added artificial nectar or excluded ants from EFN plants, and estimated local ant abundance both on *C. spinosa* plants and on protein and sugar baits in 5x5 m plots . Considering local neighbor density at both the 1m radius and 5x5 m plot level, ant densities on *C. spinosa* plants show evidence for a small-scale competition effect and a contrasting facilitation effect at the level of 5x5 m plots. Results from the short-term neighbor manipulation showed evidence of size-based competition for ants, in which smaller plants lose ants to larger plants. Ant abundance estimates from baiting also supported a plot-level facilitation effect over two years, with highest ant abundance observed in nectar-added plots, but only during periods of high rainfall. Pooling all species, ants preferred protein baits to sugar baits in the added-nectar plots, more so

than in control or reduced-EFN plots. This could introduce another source of facilitation, if a stronger preference for protein leads to more aggressive predation of herbivores in areas with high nectar resources.

Considering results from the first two chapters together, I found evidence that neighboring conspecifics affect ant density on a focal EFN plant, and that the resulting changes in ant density can affect growth and survival probability. Thus *C. spinosa* competes intraspecifically with close neighbors for the benefits provided by ants. At slightly larger temporal and spatial scales, there is evidence of facilitation, in which plants growing at higher densities will receive more ant visits and therefore experience higher growth and survival. In my last chapter (Chapter 4) I used an integral projection model to ask whether the effects of ant-mediated competition or facilitation might impact population dynamics of *C. spinosa*.

The integral projection model (IPM) presented in the final research chapter (Chapter 4) modeled plant sizes and neighbor densities as continuous distributions. Growth and survival are functions of ant density, which in turn is determined by local conspecific neighbor density, plant size, overall mean ant density, and intraspecific competition strength. To look at the effects of mean ant levels and competition for ants on population dynamics, I varied mean ant density and intraspecific competition strength in different runs of the model.

Results from the model suggest that plot-scale facilitation of mutualists impacts long-term population growth more than small-scale competition. The stable population growth rate (λ) increased with increasing ant density, with growth rates crossing $\lambda=1$ (indicating a population that is neither growing nor declining) at intermediate ant densities. Facilitation at the plot level raised the mean ant density, thus increasing population growth rates. In contrast, intraspecific competition caused a redistribution of ant densities among plants of different sizes, but this had little effect on long-term population growth. There was little difference in asymptotic population growth rate or population size distribution among models that set competition to either zero, experimental levels, or 10 times experimental levels. The stronger effect of facilitation than competition on population growth could be due to the two processes occurring at different temporal scales, with facilitation effects seen over longer time periods than competition effects. If mutualist-mediated facilitation effects tend to occur over long time scales in other systems as well, facilitation might prove to be more important than competition in other mutualisms.

2. Sources of variation in a multispecies facultative plant-protection mutualism: ant density, ant species, and plant size influence plant benefit

2.1 Introduction

Mutualisms, interactions between two species in which both benefit, are common in nature. Probably the majority of mutualisms are facultative interactions involving multiple partner species in both mutualist guilds (Bronstein 1994, Stanton 2003). However, a disproportionate amount of work on the benefits of mutualism has focused on obligate mutualisms involving pairs of species. In obligate pairwise mutualisms, the range of possible outcomes is narrow: either the two species interact sufficiently frequently that both receive enough benefits to allow them to persist, or they do not. In contrast, the outcomes of multispecies facultative mutualisms are likely to be more varied. This greater variance is in part trivial: by definition, one outcome – persistence in the absence of the other guild – is not possible for obligate mutualism. But there are less trivial reasons why facultative multispecies mutualisms may show greater variation in outcome.

First, with multiple species in each guild, the densities of potential partners are likely to vary greatly in space and time, both because species within each guild are likely to interact with each other (e.g., by competition) and because mutualists may be switching between potential partners. Second, the amount of benefit is likely to vary with the identity of the mutualist partner species more so than in obligate interactions,

because there is less incentive to benefit a facultative partner (as fitness of one partner is not necessarily as closely tied to that of the other), so there is more opportunity for exploiters (species that obtain benefits from a partner but provide no benefits in return). Thus to assess how important a role multispecies facultative mutualism plays in the population and community dynamics of the species involved, we must understand the sources of variation in the benefits each species receives.

Variation in mutualist species identity and density can have important ecological and evolutionary consequences. If mutualist density varies over space and time, or benefit varies with partner identity, this might lead to less consistent selection to maintain mutualistic traits such as production of food rewards (Gomulkiewicz et al. 2003), and may also provide an upper limit to benefit that prevents runaway population growth (Holland et al. 2002). Limiting mutualist benefit is also a necessary condition for competition for mutualists, in which the individuals of the same or different species may compete for mutualist resources or services (Addicott 1985). These two sources of variation have been well-explored in pollination and seed dispersal systems (reviewed in Stanton 2003), but less so in protection mutualisms, such as the interactions between plants that produce nectar at extrafloral nectaries on leaves or stems (EFN plants) and the ants (or other predators or parasitoids) that visit them. Most EFN mutualisms are multispecies facultative interactions, with many species of ants that visit many species of EFN plants. In this study I manipulated ant abundances on a facultatively mutualistic

EFN plant in order to ask how mutualist ant density and species identity vary among plants and how this variation affects the benefit received by the plant.

EFN plants generally receive a smaller magnitude of benefit from their guild of ant visitors than myrmecophytes, plants which provide domiciles and food bodies and are typically obligately dependent on one or a small number of specialized ant species (reviewed in Rosumek et al. 2009). However, much evidence supports that generalized plant-protection interactions are true mutualisms (i.e., both plant and ant partners benefit). Herbivory increases when ants are excluded (e.g. Bentley 1977, Costa et al. 1992, but see O'Dowd and Catchpole 1983), often resulting in lower seed set (Horvitz and Schemske 1984, Oliveira et al. 1999). Generalist ants can derive a significant portion of their resources from EFN plants (Davidson 1997, Ness et al. 2009).

Ant abundance on a given species of EFN plant is likely to vary over time and among individuals in a population, as it depends on local conditions such as the community of ant species present in an area, as well as the presence of conspecific and heterospecific EFN plants with which an individual plant may compete for ant attendance. The different ant species that visit a given plant species may vary in the quality of protective services they provide to the plant, perhaps more so than in myrmecophytic systems because ants may have less incentive to protect plants that provide them with only a nectar resource. The magnitude of benefit to plants has been shown to depend on the ant species present in obligate ant-plants (Bruna et al. 2004,

Raine et al. 2004, Frederickson 2005), and in several EFN-bearing species (Horvitz and Schemske 1984, Ness 2003, Miller 2007, Agarwal and Rastogi 2008), although usually only two ant species were compared at a time. Mutualisms involving many ant species, whether with myrmecophytes, EFN plants, or ant-dispersed plants, have many possible combinations of ant visitors and often numerous rare visitors. This makes it difficult to consider effects of each species separately. This is particularly true for EFN plants; a single individual may be visited by several different ant species over a short time interval or even simultaneously. One way to measure differences in benefit to plants among ant species without considering each species separately is to group species using a characteristic that might predict benefit, such as individual body size. Larger ant body size has been correlated with increased benefits to the plant in other ant-plant mutualisms (Ness et al. 2004), and larger ant species associated with more EFN plant species in a desert mutualistic network (Chamberlain and Holland 2009). It is likely that larger ants prey on different herbivores than smaller ants do, and this could lead to size-based differences in benefit, although not necessarily in the direction of increased benefit with larger ants.

In species that participate in a mutualistic interaction throughout the lifetime of an individual, the nature of benefit might change with age or life stage. This could influence long-term population dynamics if different size classes are affected differently by mutualist presence and contribute differentially to population growth. For example,

if the smallest juveniles benefit most from mutualist presence, but contribute little to population growth, this could minimize the impact of the mutualism on population dynamics of that species, even if individuals do receive benefits. This size- or stage-based variation might be important in systems such as herbivore-defense mutualisms involving trees, which are long-lived and may engage in the mutualism over their entire lifetime. Plant ontogeny affects the benefit received by myrmecophytes (Del Val and Dirzo 2003, Izzo and Vasconcelos 2005, Trager and Bruna 2006). Variation in benefit with plant size has not been well-studied in EFN plants, and the broader range of potential partner ants suggests visitor species and benefit are more likely to change over the lifetime of an individual.

The diffuse nature of EFN plant-ant mutualisms makes this an ideal system to ask questions about how benefit to plants varies with mutualist abundance, partner identity, and plant size in a multispecies facultative interaction. I manipulated ant abundance on *Colubrina spinosa* (Rhamnaceae) plants by excluding ants from some plants and attracting ants to others with artificial extrafloral nectar, in order to ask the following questions: 1. Do ants benefit *C. spinosa*, as measured by plant growth and survival? 2. How does benefit scale with ant abundance and plant size? 3. What is the mechanism of benefit to plants, *i.e.* does the presence of mutualists reduce leaf herbivory damage? 4. Does benefit depend on which ant species are present, or on characteristics of those species such as body mass?

2.2 Methods

2.2.1 The study system

I conducted all research presented here at La Selva Biological Reserve, Heredia Province, Costa Rica, in secondary or disturbed tropical rainforest areas containing high densities of EFN plants. The focal species, *Colubrina spinosa* (Rhamnaceae), is a tropical treelet that has extrafloral nectaries located in pairs at the base of the leaf blade. I have observed 69 species of ants from 22 genera visiting the nectaries of *C. spinosa* at La Selva (Appendix A, Table A-1). The most common ant visitor was *Ectatomma ruidum*, accounting for approximately 19% of all ant observations (n=251 observations with ants present on *C. spinosa* plants in control plots). The five most common ant species make up about 47% of all visits (instances of at least one individual observed on a plant) and 63% of all individual ants observed, and the five most common genera account for 75% of visits and 78% of individual ants (Appendix A, Table A-2). Although some of the ant species observed (e.g. *Crematogaster* spp., *Solenopsis picea*, some *Pheidole* spp.) nest in stems or leaves on the plant, *C. spinosa* produces no specialized domatia in which ants can nest, and most ants visit plants only to forage. The most common herbivore is the caterpillar *Cyclomia disparilis* (Geometridae), a generalist species that has been found primarily on *C. spinosa* at La Selva (Dyer and Gentry 2002). *C. disparilis* accounted for about 31.4% of all individual herbivores observed.

2.2.2 Field experiment

In order to induce an increased range of variation in ant density, independent of local environment and among-plant differences in nectar production, I manipulated ant abundance on *C. spinosa* juveniles with three treatments: ant exclusion, artificial nectar supplementation, and control. I applied treatments to 20 plants in each of five 5x5m plots per treatment, for a total of 100 plants per treatment. Plots were blocked among five similar sites within La Selva, with treatments assigned randomly to the three plots in each site. Treated *C. spinosa* individuals were chosen to represent a range of sizes, from 1-year-old juveniles to juveniles almost of reproductive size (range: 1 to 47 mm stem diameter). Within each size class, individuals were chosen randomly to be manipulated. I marked and followed all other unmanipulated *C. spinosa* juveniles in a plot (n=732 plants across all plots in addition to treated plants) in the same way as manipulated individuals. Adult trees were not used due to the difficulty in effectively manipulating ants on very large individuals.

In the ant exclusion treatment, I applied Tanglefoot (The Tanglefoot Company, Grand Rapids, MI), a sticky resin that ants cannot cross, on a 2.5 cm strip of tape around the base of stems. Surrounding plants that touched focals were clipped to prevent ant access. I applied tape without Tanglefoot to the bases of nectar supplement and control plants to control for any effects of the tape. For nectar supplementation plants, I attached 50 mL centrifuge tubes containing sugar solution to the stem using plastic-coated wire. I

used a 15% sucrose solution, a common sugar and concentration for EFN plants that has been used in previous artificial nectar manipulations (e.g. Bluthgen and Fiedler 2004). Small holes at the base of tubes with a 3 cm piece of thread provided access to the sugar water (after Lanza 1988). Active removal of the sugar solution by ants was necessary; liquid did not flow from the tubes passively. The 50 mL tubes were placed so that the base of the tube was at the same height as the lowest leaf-bearing branch to approximate the height of the lowest naturally occurring EFNs, and tube height was measured as a covariate. For very small *C. spinosa* individuals that could not support the weight of the tube (n=22 plants), I attached tubes to a 30 cm x 8 mm metal stake placed in contact with the plant. Control plants were marked and had tape placed at the base of their stems, but were otherwise unmanipulated.

I maintained treatments for two years, from September 2007 to August 2009. They were renewed approximately every four to six weeks, replacing the Tanglefoot on exclusion plants and replacing sugar water tubes on nectar supplement plants. For the first three months of the experiment, ants were censused on manipulated plants at the time of treatment renewal; treatments still significantly changed ant abundance on plants after four to six weeks.

To measure plant growth, I measured stem diameter and height prior to treatment application and every six months thereafter. Plant survival was also recorded at the time of growth measurements. I estimated overall herbivory damage prior to

treatments (August 2007) and again after 2 years of treatments (August 2009) by visually estimating the percentage of leaf damaged or missing on five different leaves on each of two haphazardly chosen branches. I also estimated herbivory damage to new leaves and new leaf production on two marked branches per plant, at the times of ant censuses (nine times over two years of manipulations). New leaves were marked with small pieces of plastic-coated wire.

I censused ants and potential herbivores on all plants one month prior to applying treatments, and then nine times over the two years of manipulations. Potential herbivores were identified to order. Individual ants were collected in 96% ethanol for identification (using online keys in Longino 2009). To determine mean dry biomass per individual of different ant species, I weighed groups of 10 collected individuals (Scientech SA80 analytical balance). For genera with species of similar body sizes (compared by Webers length, Longino 2009), only one species was weighed, and its mass used as an estimate for all similarly-sized species of that genus.

2.2.3 Data analysis

All statistical analyses were performed in R (version 2.9.2, R Development Core Team 2009). The ant manipulations described above induced a continuous range of ant abundance on *C. spinosa* juveniles, from no ants present on most ant-excluded plants to much higher than control levels on some plants with supplemented nectar (treatment coefficient: ant exclusion= -0.93, nectar supplement= 2.24, $LR=1135$, $df=2$, $P<0.001$, zero-

inflated negative binomial model of total ants over 1 year; Appendix B, Figure B-1).

The best measure of potential protection benefit to plants is likely to be the density of ants (number of ants per unit plant size) rather than the absolute number of ants. For example, a single ant would be able to forage over a much larger percentage of all leaf area of a seedling than of an adult tree. However, it is not clear a priori what measure of plant size should be used to compute ant density. Ants forage on the surfaces of stems and leaves, so ants per total plant surface area might be a good measure. Or if ants benefit plants by deterring leaf-chewing herbivores, ants per total leaf area might better represent potential protection. Total surface area and leaf area are difficult to measure, but they are likely to scale allometrically with stem diameter (Price and Enquist 2006), which I used to measure plant size. Therefore I used ants per unit stem diameter when calculating ant density, as described below.

As plants change in size over ontogeny, the average density of ant visitors is also likely to change. Therefore, to see if the benefits plants received depended on ant density, accounting for changes in average ant density with plant size, I computed the density of ants observed on each plant relative to the density of ants it would be expected to have given its size, which I refer to as “residual ant density.” I computed it by the following procedure. First, I computed the expected density of ants given plant size by regressing ant density (total number of ants of all species divided by stem diameter) against stem diameter, using plants in control plots (year 1: n=255 plants, year

2: n=257; 9 ant surveys per plant over 2 years). Only control plants were used, as those represented the natural range of ant abundances expected on unmanipulated *C. spinosa* individuals. Due to the high number of plants with no ants observed over 1 year of censuses (year 1: n=167 plants, year 2: n=199), and the high overdispersion ($\theta=0.474$, $LR=1557$, $df=1$, $P<0.001$), a zero-inflated negative binomial model fit the data best (pscl package in R, Zeileis 2008), using stem diameter as the independent variable for the binomial zero-inflation, and stem diameter and year as independent variables for the negative binomial model. I adopted the model with a linear stem diameter term because it had a lower AIC value than a model without stem diameter (i.e., a size independent model) or a model with both linear and squared diameter terms (Appendix B, Table B-1). I then used this model to predict ant density for measured plants in all treatments (n=1032) and computed the residual (observed minus predicted ant density) to give the “residual ant density.” Residual ant density is therefore a size-independent measure of ant attendance on plants, with negative values for plants that have a lower density of ants than expected for their size, and positive values for those with higher values than expected. Residual ant density, rather than treatment, was used as the independent variable in all analyses of how ant density affected *C. spinosa* growth and survival.

To determine effects of residual ant density on growth, survival, and herbivory damage, I fit linear or general linear mixed models, with random intercepts of sites and plots nested within sites. Survival was fit as a logistic regression of fate (alive or dead)

against residual ant density, squared residual ant density ($[\text{residual density}+2.5]^2$, adding the constant 2.5 to remove negative residual values), plant size (stem diameter), year, and an interaction between plant size and year, with random effects of sites and plots (package lme4 in R, Bates and Maechler 2009). I measured growth as the proportional change in stem diameter over one year: $[\text{diameter in year } t+1 - \text{diameter in year } t] / \text{diameter in year } t$. Using change in height or in number of new leaves instead of change in diameter produced qualitatively similar growth results. I fit proportional growth as a linear mixed effects regression against residual ant density, squared residual ant density ($[\text{residual density}+2.5]^2$), plant size (stem diameter), and year, with random effects of sites and plots. Variance in proportional growth often decreases with plant size, so I fit a model in which log residual variance is a linear function of plant size (Zuur et al. 2009).

Very small *C. spinosa* individuals do not generally receive ant visitors (pers. obs.), and it is possible that plants receive no benefit from ants until they reach a certain size. In this case a model with no benefit below a size threshold might be more reasonable than a linear model with a size-by-benefit interaction term, which might force ant effects to be negative at small plant sizes. To consider both possibilities, I fit piecewise models in which ant effects were only estimated for plants above a diameter threshold. The minimum size was determined by varying the threshold from 1mm to 5mm, at 0.25mm intervals, and comparing model fits using AIC. It is appropriate to use AIC to compare

these models with different diameter thresholds, because they use the same data but vary in a discrete factor (0 or 1 for larger or smaller than the threshold).

To assess the effect of ant density on herbivore abundances, I used a zero-inflated negative binomial model as for ant effects above, due to the high number of observations of zero herbivores and overdispersion of the data (package `pscl` in R, Zeileis 2008). Herbivory damage, measured as the proportion of leaf damaged or missing, was arcsine square root transformed for statistical analyses to ensure a normal error distribution. I then fit transformed herbivory damage as a linear mixed effects regression against residual ant density, squared residual ant density, plant size (stem diameter), and year as fixed effects, and random effects of sites and plots blocked across sites (package `lme4`, Bates and Maechler 2009). As with growth and survival, the best fit was a piecewise model with no effects of ants on herbivory damage on very small plants.

Due to the large number of ant species observed, and low numbers of individuals observed for each species, it was not feasible to look at effects on plant performance of each ant species or even each genus separately. Instead, I used mixed effects linear regressions (growth) and general linear regressions (survival) to look at effects of the four ant taxa with the most total observations on all plants (treated and control): *Ectatomma ruidum*, *Pheidole* spp., *Brachymyrmex* spp. and *Paraponera clavata*. Models were fit with independent variables for the abundance of ants in each taxon and

the abundance of ants in all other taxa combined, and compared using AIC to a simpler model combining ants in all taxa. I also divided ant species into two groups by body size. Ant species were split into “large-bodied” (individual dry biomass $>1.74 \times 10^{-4}$ g) and “small-bodied” (individual dry biomass $\leq 1.74 \times 10^{-4}$ g) groups, so that there was an approximately equal number of observations of the large-bodied and small-bodied ants (n=546 observations with small ants, n=572 observations with large ants). One observation was an instance in which ants were observed on a plant, disregarding the number of ants present. Presence of large or small ants was used, rather than residual ant density, because residual ant density was calculated for all ant species combined.

To ask how the frequency of ant visits changed with plant size, I used a linear regression of the proportion of observations with ants present (arcsin square root transformed) on plant stem diameter, with random intercepts of sites and plots blocked across sites. I also considered variation in the size of ant visitors with plant size using the same model but with the proportion of observations with either large-bodied or small-bodied ant species as the response variable.

2.3 Results

2.3.1 Ant effects on plant growth

Plant growth was positively related to residual ant density (Figure 2-1). The effect of ants on plant growth appeared to be stronger in year two, but the difference between years was not significant (year by ant interaction: 2.66×10^{-4} , $LR=1.93$, $df=1$, 1396,

$P=0.16$; linear mixed effects regression).

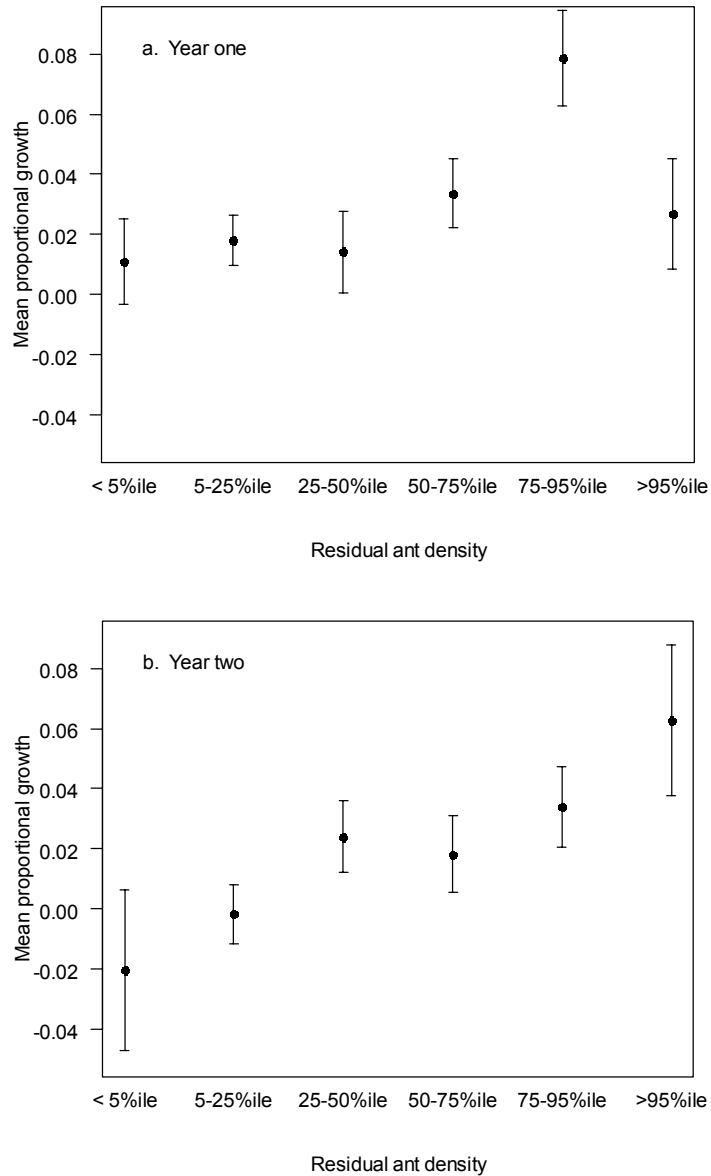


Figure 2-1: Effects of ants on proportional change in stem diameter over one year (means \pm SE) for *Colubrina spinosa* juveniles. a) Year one; b) Year two. The quantiles of residual ant densities were calculated for each year separately. Only plants >3.5 mm in diameter are shown here, as there was no effect of ants on growth of smaller plants.

Ant benefit is dependent on plant size, with no effect of ants on growth of very small plants; the best model was a piecewise linear model which set the ant effect to zero for plants smaller than 3.5mm in diameter (ant coefficient: 0.0523, $LR=10846$, $df=2$, 1397, $P<0.001$). For plants larger than 3.5mm, ant effects on growth increased linearly with plant size (ant by diameter interaction: 6.16×10^{-4} , $LR=247.1$, $df=1$, 1397, $P<0.001$). Adding a squared ant density term did not significantly improve model fit (squared ant coefficient: 8.61×10^{-5} , $LR=3.83$, $df=1$, 1396, $P=0.0505$).

2.3.2 Ant effects on plant survival

Increased ant densities also resulted in higher plant survival (Figure 2-2). As with growth measures, the best-fitting model was one in which ants affect survival only for plants above a size threshold, which was again 3.5mm in diameter (ant coefficient: 0.201, $LR=10.21$, $df=2$, 1523, $P=0.001$, logistic mixed effects regression). Adding a squared ant abundance term did not significantly improve model fit (squared ant coefficient: 0.011, $LR=0.824$, $df=1$, 1522, $P=0.36$). Survival increased with plant size, independent of ant effects (size coefficient: 0.116, $LR=83.76$, $df=3$, 1523, $P<0.001$). In addition, for plants above 3.5mm, ants had a greater effect on survival of larger plants (Appendix B, Figure B-2; ant by size interaction: 0.0752, $LR=7.31$, $df=1$, 1523, $P=0.007$).

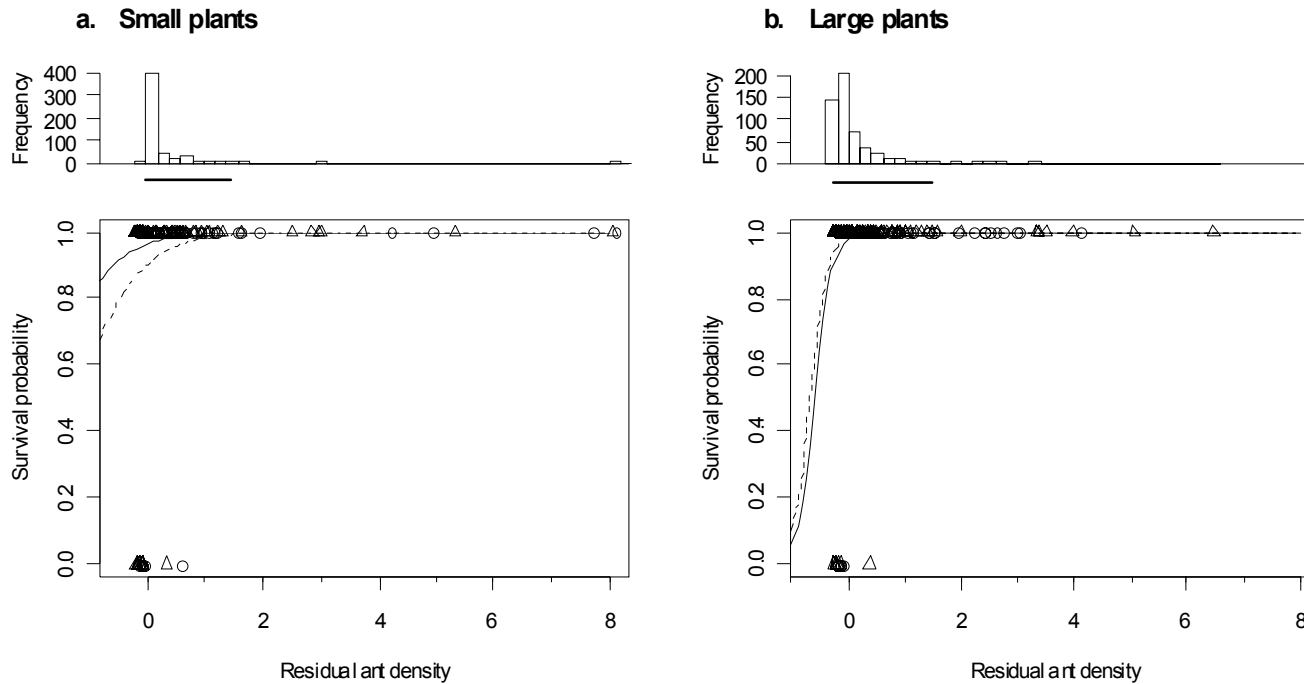


Figure 2-2: Survival increased with residual ant density for a) small plants (stem diameter < median, 8.9mm), and b) large plants (stem diameter > 8.9 mm). Very small plants (diameter < 3.5mm) are not shown because there was no ant effect on survival. Circles show data from year 1 (n=274 small plants, 261 large plants), triangles show year 2 data (n=267 small plants, 273 large plants). Lines show the logistic regression for year 1 (solid line) and year 2 (dotted line). Histograms show frequencies of all plants with different observed residual ant densities, and the horizontal bars show the natural range of ants for unmanipulated control plants (n= 223 small plants, 182 large plants).

2.3.3 Ant effects on herbivory and potential herbivores

Both growth and survival increased with increasing residual ant density on plants, thus presenting the question: by what mechanism do ants benefit *C. spinosa*? Specifically, I assessed whether differences in herbivory damage or herbivore abundances explain the observed effects on plant performance. Abundance of potential herbivores decreased with increased adjusted ant abundance, both for total herbivores (ant coefficient: -5.38×10^{-3} , $LR=6.37$, $df=2$, 1571, $P=0.04$, general linear regression with a zero-inflated negative binomial distribution) and for leaf-chewing herbivores (ant coefficient: -5.32×10^{-3} , $LR=6.69$, $df=2$, 1571, $P=0.035$). However, there was no significant relationship between residual ant density and decreased herbivory damage on new leaves ($LR=1.305$, $df=2$, 1306, $P=0.52$; linear mixed effects regression), or between residual ant density and mean herbivory damage after two years, as compared to pretreatment levels ($LR=0.1125$, $df=1$, 1307, $P=0.74$). Herbivory damage to new leaves increased with plant size, independent of ants (8.06×10^{-3} , $LR=237.1$, $df=2$, 1306, $P<0.001$).

2.3.4 Effects of ant species on plant growth and survival

Classifying plants by the presence of different ant taxa shows variation in growth and survival among different ant species (Figure 2- 3). In particular, growth benefit is lower for plants visited by ants of small body size. Densities of the four most commonly observed ant taxa (*E. ruidum*, *Brachymyrmex* spp., *Pheidole* spp., and *P. clavata*) did not explain plant growth better than using densities of all ant taxa combined. Four growth

models that included separate terms for one of these taxa and for all other ants combined showed no significant differences in growth effect sizes among taxa, and were not an improvement over considering all ants together (Appendix B, Table B-2). Survival results are similar: considering *E. ruidum*, *Brachymyrmex spp.*, or *Pheidole spp.* separately from all other ant taxa does not improve the survival model (Appendix B, Table B-2). The best survival model completely excluded *P. clavata*, and used only the density of ants from all other taxa (AIC=786.9 excluding *P. clavata*, AIC= 787.5 with all ant taxa combined).

Classifying ants by body size, benefits of large and small ants vary with plant size, for both growth and survival (Figure 2-4). Rather than adjusted ant abundance, presence or absence of small or large ants was used; larger plants were more likely to have ants present so small and large plants (smaller or larger than the median diameter) were analyzed separately. For small plants, only the presence of small ants was significantly associated with increased growth (small ant coefficient: 0.0429, $LR=5.41$, $df=1$, $P=0.02$; linear mixed effects regression) and increased survival (small ant coefficient: 16.62, $LR=13.12$, $df=1$, $P<0.001$; general linear mixed effects regression). For large plants, there was a positive effect of large ants on growth, but no significant effect of small ants (large ant coefficient: 0.0414, $LR=10.85$, $df=1$, $P= 0.001$). In addition, there was an interaction between the presence of large ants and small ants on large plants.

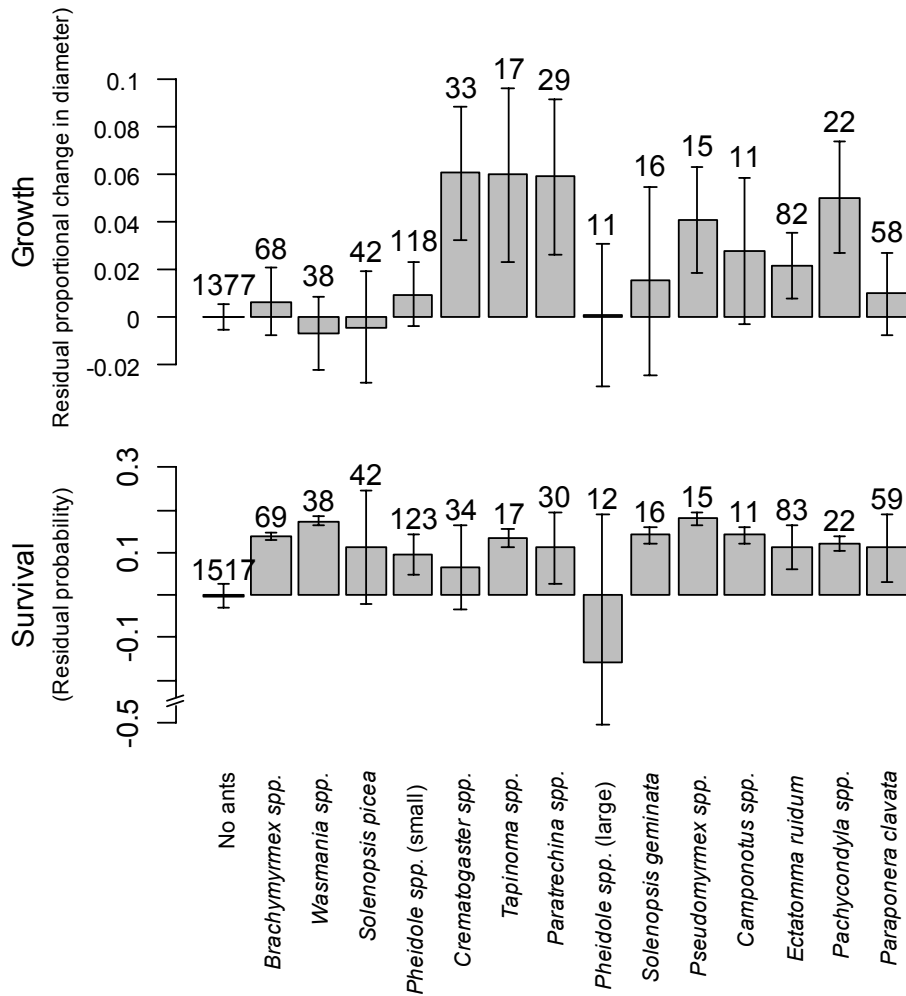


Figure 2-3: Growth and survival with presence of different ant taxa (mean \pm SE one-year proportional change in stem diameter and one-year survival probability, both corrected for plant size). Each bar shows plants in which the ant genus or species indicated was present for at least one observation in one year (years 1 and 2 pooled). Numbers above bars give the number of plant-observations; if a plant was visited by more than one taxon in a year, it appears in more than one bar (141 plant-obs. with >1 spp. in 1 year). Groups are therefore not independent and differences among means were not tested. Ant taxa are arranged in order of ascending individual body size, and only groups with > 5 observations are included. *Pheidole* (16 spp.) was split into large- and small-bodied *Pheidole* species, as species size varies substantially within the genus. Note the break in the y-axis of the survival plot to show error bars for large *Pheidole* spp.

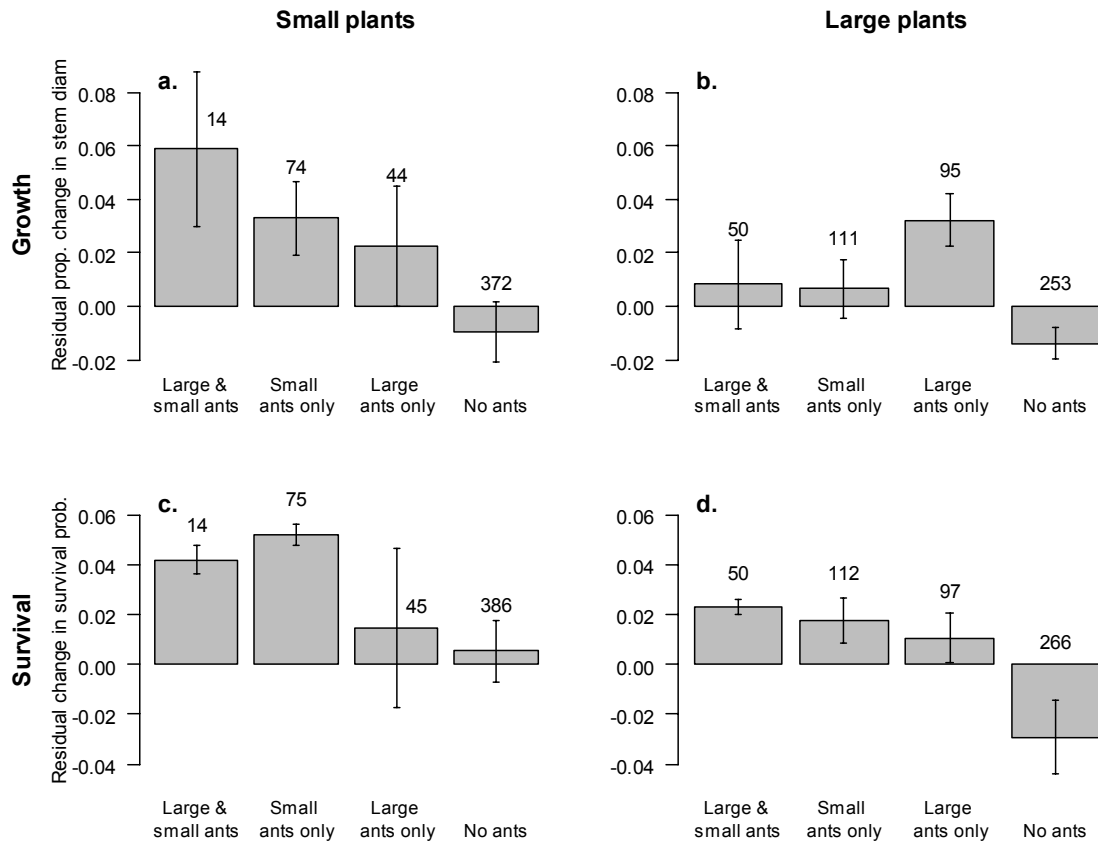


Figure 2-4: Effects of large and small-bodied ants on plant growth (a,b; mean \pm SE proportional change in diameter over one year) and survival (c,d; mean \pm SE one-year probability of survival). Plants are divided into small and large plants as in Figure 2-2. All bars (growth and survival) are adjusted for plant size by taking the residuals of a mixed effects linear regression of growth or survival (logistic regression) against plant stem diameter and year. Numbers above bars give the number of plant-observations in each group (both years pooled); only plants that survived to the end of the year were used in growth analyses.

The presence of large ants does not provide as much benefit to growth when small ants are also present (large by small ant interaction: -0.0205 , $LR= 5.03$, $df=1$, $P=0.03$). Both large and small ants increased survival of large plants, with no interaction (large ant

coefficient: 1.93, $LR=5.65$, $df=1$, $P=0.02$; small ant coefficient: 2.00, $LR=6.03$, $df=1$, $P=0.01$).

2.3.5 Variation in ant presence with plant size

As juvenile plants grow, they are more likely to have ants present at a given census (Figure 2-5a; size coefficient: 0.020, $LR=62.80$, $df=1$, 498, $P<0.001$). This difference is due primarily to an increase with plant size in the presence of large-bodied ant species (plant size coefficient: 0.0113, $LR=486.4$, $df=1$, 498, $P<0.001$), although the presence of small-bodied ant species also increased with plant size (size coefficient: 8.89×10^{-3} , $LR=214.5$, $df=1$, 498, $P<0.001$). Ant manipulations changed this progression; for ant exclusions, ants were present at very low levels for all sizes, with only a slight increase for the largest plants, due to an increased difficulty in maintaining exclusions at those sizes (Figure 2-5b). In contrast, even very small plants with supplemental nectar attracted similar levels of both large-bodied and small-bodied ants as large plants (Figure 2-5c).

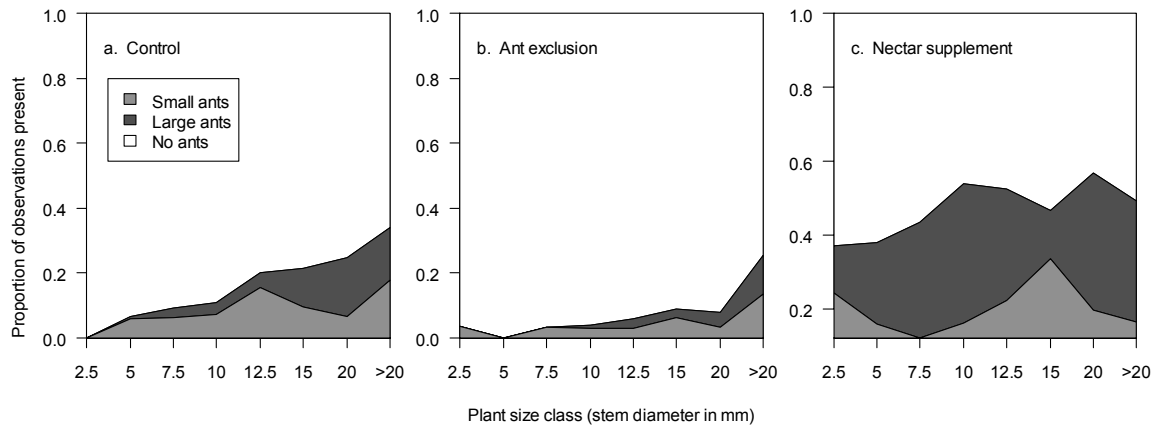


Figure 2-5: Change in visitation of different-sized ant species with plant size, for unmanipulated plants (a, year 1: n=255 plants, year 2: n=257), ant exclusion plants (b, year 1: n=96, year 2: n=87), and nectar supplement plants (c, year 1: n=90, year 2: n=83). The x axis gives the upper limit of the interval for each size class. All data combine both years of ant censuses (n=9 observations per plant).

2.4 Discussion

Benefit to *C. spinosa* plants was found to vary with mutualist ant densities, ant species identity, ant body size, and plant size. The experimental demonstration of these several different sources of variation supports the importance of considering variation in benefit to understand multispecies facultative mutualism and its effects on the population and community dynamics of the species involved. Higher levels of ant visitation were associated with increased plant performance in *C. spinosa* juveniles, when performance was measured by growth and survival. This study is unique in using experimental manipulations to induce a range of ant densities and thus determine the relationship between mutualist density and plant benefit in a diffuse, facultative mutualism. In addition, manipulations were maintained for a relatively long time period (2 years), to detect growth differences even in a slow-growing tropical rainforest understory species. In the following sections, I further explore the relationship between ant densities and plant benefit, including why benefit was not observed to saturate, possible mechanisms of benefit to plants, variation in benefit with ant species and plant ontogeny, and potential consequences of this variation for *C. spinosa* populations.

2.4.1 Variation in plant benefit as a function of mutualist densities

Higher mutualist densities were correlated with more benefit to plants as measured by growth and survival. Ant abundances on plants were determined by ant foraging choices rather than by experimental addition of different numbers of ants.

Therefore it is possible that as plant 'quality' improves due to underlying environmental factors, both plant performance measures and ant attendance increase, but ants do not benefit plants. However, observed ant abundance levels were induced by experimental manipulations, and treatment effects show the same patterns: lowest growth and survival in ant-excluded plants, higher in control plants, and highest on nectar-supplemented plants, although small plants had higher growth with ant exclusion, and only large supplement plants showed improved performance compared to control plants (Appendix B, Table B-3). This supports the interpretation that ant densities are influencing plant performance, and not that plant performance is driving ant abundance or that both are correlated with a third environmental factor.

Many previous studies of mutualism have measured benefit as the difference in plant performance with the presence and absence of mutualists; in other words, structuring the analysis as an ANOVA to compare two groups rather than using regression to determine the numerical relationship between mutualist density and plant benefit. Studies that look for density-dependent effects of mutualists on a partner are rare (reviewed in Holland et al. 2002, Ness et al. 2006), compared to those that simply compare performance in the presence and absence of a mutualist (reviewed in Morris et al. 2007).

Although it is possible to use these data to show differences in plant performance among treatments (see above), a goal of this study was to determine the relationship

between mutualist density and plant benefit. The difference in observed ant densities on control and ant-excluded plants was small (mean residual ant density \pm SE: -1.03 ± 0.158 for ant-excluded plants, -0.304 ± 0.185 for control plants, $n=100$ plants per treatment), and the nectar supplement treatment induced ant densities that were, in some cases, far beyond those observed in nature. However, the mean levels of ants observed on control plants were low due to the many small plants with no ants, together with a large range in ant densities. The distribution of residual ant densities on all plants is right-skewed, with most residual ant density values around zero and a long right tail (Figure 2-2 histograms). Simply comparing control plants to exclusion would include in the 'ants-present' group all those plants that, although ants are allowed, have no or very few ants.

There is no reason to expect plants that naturally have no ants would demonstrate increased performance compared to ant-excluded plants. A more relevant question is whether plants with higher densities of ants show increased growth and survival. The nectar supplement treatment allowed me to induce the presence of ants on plants where they might usually be absent. This is a less-used technique than removing ants from plants where they would usually be present, but is equally useful in decoupling plant quality from ant density. Although nectar supplement plots induced levels far beyond those observed on control plots, the positive relationship between ant density and plant benefit occurs at ant densities within the ranges observed on unmanipulated plants. For example, the transition from low to high survival with

increasing ants occurred at low residual ant densities, well within the range of residual ant densities observed in control plots (Figure 2-2, horizontal lines show control range).

I observed a linear relationship between residual ant density and benefit to plants for both growth and survival. Clearly, at some point net benefit has to saturate, or there would be limitless growth of mutualist populations (May 1982), and it is likely that benefit to plants eventually saturates at some level of ants. It is possible that nonlinearly increasing costs drive the saturation of net benefit. In this experiment, neither excluding ants nor adding artificial nectar is likely to have a large effect on nectar production costs to the plant, as ant-excluded *C. spinosa* individuals continue to produce at least some nectar (pers. obs.), and artificial nectar is 'free.' However, even without considering costs of nectar production, at some point adding individual ants to a plant must no longer provide additional benefit to that plant. Fitting a quadratic term of ant density did not improve either the growth or survival model; the quadratic term was nearly significant in the growth model ($P=0.0505$), but the coefficient for the quadratic was in fact positive, so this would cause benefit to increase more rapidly, not saturate. It is possible that a saturation of benefit to plants was not observed here because most data points were clustered at low levels of ant abundance, with few towards the upper end of mutualist densities.

2.4.2 Mechanism of benefit to plants

The reduction of herbivory damage, as measured in this study, could not

completely explain benefit to plants. Higher ant densities were correlated with lower presence of both leaf-chewing and sap-sucking potential herbivores, but this did not result in significantly lower herbivory damage to leaves. However, positive effects of ants on plant growth and survival were observed even without a significant effect on herbivory damage. There are several reasons that might explain why I did not observe a significant influence of ant density on damage. New leaves were not followed individually, so damage might have been underestimated for greatly-damaged leaves that could have dropped off or been lost when a branch tip was broken between measurements. Ants might also have the greatest effect on guilds of herbivores that do not damage leaves, such as sap-sucking insects. This benefit would not be detected by measures of leaf herbivory damage. Neither herbivore censuses nor measures of herbivory damage are a perfect measure. Herbivore abundances can vary greatly with time of day, and in this case only potential herbivores were identified, and I did not record herbivore body size. Ants may also benefit plants by reducing fungal pathogen attack (de la Fuente and Marquis 1999). I observed *C. spinosa* individuals with symptoms of apparent fungal pathogen infection, including some that later died (pers. obs.), but causes of mortality were not followed in this study. Much of previous work on the benefit of ants to EFN plants has measured herbivory damage but not more direct measures of fitness (but see Horvitz and Schemske 1984, Rudgers 2004, Rutter and Rausher 2004). However, the differences in growth and survival observed here without

a significant herbivory effect suggests that it is useful to measure plant performance directly.

Variation in herbivory damage with plant size, independent of ant effects, might explain why larger plants benefit more from the presence of ants. Smaller plants suffer less herbivory damage than large plants even when ants are absent. Therefore the presence of ants, even if it further reduces already-low levels of damage, might not provide any benefit. It is possible that smaller plants provide a smaller target to attract potential herbivores, or that leaves on small plants are more heavily defended with secondary compounds than larger plants (reviewed in Boege and Marquis 2005). In some myrmecophyte species, plants switch from chemical to ant-defense at different stages of growth (Coley 1987, but see Del Val and Dirzo 2003).

2.4.3 Variation in ants and benefit with plant ontogeny

Ant effects on *C. spinosa* growth and survival changed with plant size, with larger juveniles receiving more benefit. Ants had no effect on very small individuals (< 3.5mm in diameter). Ant visits are infrequently observed on very small unmanipulated *C. spinosa* plants in the field, and even when small plants were manipulated with artificial nectar to receive more ants, they did not benefit. If very small plants receive no benefit from ants, an optimal strategy might be not to produce nectar until they are large enough to benefit. Although very small juveniles do possess EFNs, they do not appear to actively produce nectar; in 1233 observations of very small plants (n= 282 plants < 3.5mm

diameter), I never observed a visible drop of nectar or an ant removing nectar from an EFN (pers. obs.). Slightly larger juveniles (>3.5 mm in diameter) did benefit from ant visits, but received a smaller amount of benefit than larger plants with a similar level of ant presence, as measured by both growth and survival.

Differences in which ant species are present on plants of different sizes might also contribute to size-based differences in benefit. As juvenile plants grow, they are more likely to be visited by larger-bodied ant species (Figure 2-5a). This is consistent with observations in several different myrmecophytes that the ant species occupying the plant changes with plant ontogeny (Davidson et al. 1989, Palmer et al. 2000, Vasconcelos and Davidson 2000, Fonseca and Benson 2003). In addition, different ant species assemblages forage at different levels of tropical rainforest canopy, understory, and leaf litter (Bruhl et al. 1998), so it is not surprising that juveniles of different heights would tend to be visited by different ant species. However, adding artificial nectar removes the correlation of ant species and plant size. Nectar supplement plants of all sizes resemble large control plants in that they receive many ant visitors, in particular large-bodied species (Figure 2-5c). This result suggests that differences in the amount of nectar resource produced by plants may drive changes in ant species with plant size, as larger-bodied ant species are attracted to the larger quantities of nectar produced by larger plants. This would require that the ants consider the whole plant as one resource when making foraging decisions, rather than seeing each individual nectary as a resource.

Alternatively, individual EFNs on larger plants may produce greater volumes of nectar and therefore attract larger ants, even if nectary size does not vary with plant size.

Nectar production was not measured in this study. It is quite likely that larger plants produce more nectar, even if they do not produce more nectar per EFN, as they have more leaves and therefore more potentially active EFNs.

If different ant species provide different levels of benefit to plants, changes in ant species with plant ontogeny could explain the interaction between plant size and benefit. Specifically, if larger-bodied ant species benefit plants more, and larger juveniles are more likely to attract large-bodied ants, this would explain higher levels of benefit to larger juveniles. However, breaking down benefit by ant species size did not consistently explain plant size differences. In large plants there was an interaction between the effects of large and small ants on growth, in which the presence of large ants did not provide as much benefit when small ants were also present. This negative interaction term between large and small ants is not due to competitive displacement: the number of observations with both large and small ants present is not lower than expected from the probabilities of having large or small ants alone (G-test: $G = 11.09$, $df = 9$, $P = 0.27$; R function `g.test`, Hurd 2001). It is also not due to fewer large ants when small ants are also present (with small ants: 0.648 ± 1.87 mean \pm SD large ants per plant-observation; without small ants: 0.417 ± 2.16). It is possible that behavioral changes make large ants less effective defenders of plants when in the presence of small ants.

Considering the wide range of ant species observed to visit *C. spinosa*, it is likely that individual ant species, even those of similar size, vary greatly in the benefit they provide to plants, ranging from true mutualists to parasites. For example, the largest ant species, *P. clavata*, had no observed benefit on *C. spinosa* juveniles. Ant body size, although related to plant benefit, is perhaps not the best measure of differences among species. Other characteristics of ant species might better explain changes in benefit with plant ontogeny.

2.4.4 Consequences of variable benefits in *C. spinosa*

Variation in ant species identity and density has important consequences for the benefit received by *C. spinosa*. The presence of parasitic ant species within the guild of potential partners might act to set an upper limit on benefit, if parasite visits increase faster than mutualist visits with greater nectar production. For example, *P. clavata*, the largest ant species observed, does not appear to benefit *C. spinosa*. It is interesting to note that *P. clavata* is rarely observed on unmanipulated *C. spinosa* juveniles (Appendix A), and only occasionally on very large juveniles or adults (pers. obs.). I observed *P. clavata* most frequently on supplemented-nectar plants, suggesting that *P. clavata* may be attracted to plants with nectar resources greater than some minimum level. If this species is effectively a parasite on *C. spinosa*, then this might act to create an optimal upper limit for EFN production in *C. spinosa*, as a strategy to limit visitation by *P. clavata*. More work is needed to further separate the effects of individual ant species in this

highly diverse, facultative mutualism.

The overall low levels of ant attendance on unmanipulated juveniles, together with the linear function of plant benefit with ant density, suggest that ants are a limiting resource for *C. spinosa*. If ants are limiting, *C. spinosa* individuals might compete for mutualists. This would occur if the presence of other EFN plants (of the same or different species) affects ant abundance on a given *C. spinosa* individual. Benefit of ants to *C. spinosa* depends on plant size. Size-dependent benefits of mutualists could have interesting population-level effects, if those size classes that benefit most (or least) from ants disproportionately contribute to population growth.

This study establishes a relationship between variation in ant density on *C. spinosa* and plant performance, as a first step in determining population-level effects of this mutualism. Further work is needed to address whether these effects of ants on plant vital rates lead to significant differences in population density or growth rates.

3. Intraspecific competition and facilitation for mutualist ants among extrafloral nectary bearing plants: contrasting effects at different spatial and temporal scales

3.1 Introduction

Although we often study mutualisms (interactions in which both species benefit) at the level of the individual partners, mutualistic interactions take place in the context of populations and communities (Bronstein 1994, Stanton 2003). For populations above some minimum density, most individuals will share mutualists with others in the population. The shared mutualists may be from either a single partner species or of a guild of potential partners. Sharing a common mutualist among individuals within a population, or even among different species, could cause indirect interactions between those individuals or species. For example, if the resources or services provided by the common mutualist are a limiting factor for the partner species, then those partners might compete for mutualists (Addicott 1985). Facilitation might also occur, in which the presence of several partners increases the number of mutualists visiting an area (short-term facilitation) or the population of the shared mutualists (long-term facilitation) (Waser and Real 1979, Rathcke 1983). Therefore being near other conspecific or heterospecific individuals may be advantageous, if their presence increases the numbers of the shared mutualist partner.

Both facilitation and competition could occur in the same system, at different spatial or temporal scales, or at different population densities (Addicott 1985). For example, in the mutualism between extrafloral nectary (EFN) plants and their ant defenders, over a short time scale there is a finite number of ants in a given area. These ants may be divided evenly or unevenly among the local plants, but if one plant receives more mutualists than another must necessarily receive fewer. Short-term effects must be focused over a relatively small spatial scale, as plants that share mutualists must be within ant colonies' foraging ranges. However, over longer time periods, facilitation effects could occur over larger spatial scales. If high densities of EFN plants lead to increased density of ant colonies, the underlying processes such as recruitment of new colonies or nest movement of older colonies are likely to occur at spatial scales larger than foraging ranges (Hölldobler and Wilson 1990). Thus neighboring EFN plant density at smaller and larger spatial scales could have contrasting effects on a focal individual, for example if close neighbors draw ants away, but high neighbor densities at slightly larger spatial scales contribute to high local ant densities. Either competition or facilitation effects might dominate over different temporal and spatial scales.

In this study I looked for mutualist-mediated competition and facilitation between plants that produce extrafloral nectaries (EFNs). Ants and parasitoid wasps are attracted to EFN plants by nectar resources, and in exchange often protect plants from

herbivory (Bentley 1977). Plants with EFNs are found in over 90 families (Koptur 1992) and contribute up to one third of plant species in tropical forests (reviewed in Blüthgen and Reifensrath 2003). Interactions among EFN plants may be especially important in disturbed high-light habitats such as forest edges, secondary forests, and treefall gaps where EFN species are particularly abundant (Bentley 1976, Schupp and Feener 1991, Fiala and Linsenmair 1995). Unlike myrmecophytes, most EFN plants do not produce specialized structures to house ant colonies, and most ants do not nest on the plant. This suggests that individual plants are likely to share the same mutualist ants, and that competition or facilitation effects could occur over relatively short time scales, since plant benefit is affected by daily foraging decisions by ant visitors. There is evidence that EFN plants compete with homopterans for ant attendance (Buckley 1983, Sakata and Hashimoto 2000), and that ants compete with other arthropods for EFNs (Heil et al. 2004). However, competition or facilitation for ants among EFN plants has not previously been studied.

Characteristics of the partners that share mutualists could influence whether facilitation or competition occurs. For example, larger individuals might draw mutualists away from smaller individuals, but several small individuals might collectively attract more mutualists. Many pollination and seed dispersal mutualisms show density-dependent effects of sharing mutualists, with facilitation of neighboring plants at low plant densities, but competition at high densities (Moore and Willson 1982,

Manasse and Howe 1983, Rathcke 1983, Feldman 2008).

In addition to changes in local ant populations, ant dietary changes could be another mechanism of facilitation for mutualists among EFN plants. Some ant species exhibit a stronger preference for protein over sugar resources when allowed access to EFNs (Ness et al. 2009). This could make ants better defenders, if a preference for protein increases their aggression towards herbivores (Ness et al. 2009). Therefore if high densities of EFN plants increase preference for protein in the local ants, this could also lead to an indirect, mutualist-mediated benefit to being near other EFN plants. EFN plant-ant mutualisms are generally multispecies facultative interactions, with each ant species visiting many different plants, and each plant species visited by multiple ant species. Different ant species may respond differently to changing nectar levels, both in their dietary preference response as well as changes in their abundance.

Demonstrating competition or facilitation for mutualists requires two pieces of experimental evidence: 1) that conspecific or heterospecific neighbor densities affect mutualist visits to an individual, and 2) that the change in mutualist visits results in reduced benefit to that individual (or in the case of facilitation, increased benefit). Many previous studies on competition for mutualists found neighbor effects on mutualist densities, but did not show whether this change in mutualist densities affected the benefit received from mutualists (but see Rathcke 1983, Cushman and Addicott 1989). In previous work, I found that plant growth and survival benefit

increased with increasing ant density for the EFN species *Colubrina spinosa* (see Chapter 2). In this study, I present evidence that conspecific EFN-bearing neighbors affect ant density on a focal individual. I manipulated nectar resources and conspecific neighbors in order to ask the following questions: 1. Does short-term intraspecific competition or facilitation for mutualist ants occur in *C. spinosa*? 2. How does neighbor influence on ant abundance change with size and distance to a focal plant? 3. Are long-term effects of neighbor plants consistent with short-term effects, or do changes in local nectar resource introduce facilitation as well? 4. Do increased nectar resource levels lead to strong preference for protein by ants?

3.2 Methods

3.2.1 Study system and site

I conducted all research presented here at La Selva Biological Reserve, Heredia Province, Costa Rica, in secondary or disturbed tropical rainforest areas with high densities of EFN plants. The focal species, *Colubrina spinosa* (Rhamnaceae), is an understory tree with extrafloral nectaries, and is visited by at least 69 ant species at La Selva (See Appendix A for complete ant species list). Work at La Selva suggests low overall levels of EFN plants, but higher local abundance in secondary forest (Marquis and Braker 1994). In surveys of *C. spinosa* juveniles along trails, 78 of 120 were within 2 m of another EFN plant species (pers. obs.). The most common neighboring EFN plant species included juveniles of the canopy trees *Pentaclethra maculosa* and *Inga* spp.

(Fabaceae), as well as understory herbs *Costus* spp. (for complete species list see Appendix B, Table B-1). *C. spinosa* fruits lack a fleshy mesocarp and are not known to be dispersed by animals (Johnston 1971); dry fruits dehisce explosively, leading to clumps of high juvenile density near parent trees (observed range: 1.00 to 5.36 plants/m², n=15 5x5m plots chosen to contain at least 20 individuals). High local conspecific and heterospecific EFN plant densities suggest that *C. spinosa* juveniles are likely to share mutualist ants with neighbors.

Ant activity has been found to depend on temperature and moisture in neotropical forests; ant foraging increases with increasing moisture (Kaspari and Weiser 2000). In order to consider these environmental effects as covariates in ant response to nectar manipulations, I used daily rainfall and temperature data collected at La Selva Biological Station's meteorological station (compiled by D.A. Clark). As a lowland rainforest site, La Selva receives 4m of rain each year, with lower rainfall typically from February to April and August to September, but no true dry season as in tropical dry forest (McDade 1994). Therefore mean monthly rainfall rather than calendar month was used to designate dry or wet observational periods.

3.2.2 Short-term neighbor manipulation experiment

To isolate the effects of competition for ants, I censused ants on focal plants with neighboring conspecifics that were either accessible or temporarily inaccessible to ants. The prediction tested by this experiment is that if neighboring plants compete for ants,

focal plants with neighbors that are inaccessible to ants should receive more ant visits. In order to ask whether neighbor size influences competition for ants, accessible neighbors were chosen to be either all larger or all smaller than the focal plant. If size-based competition occurs, focal plants with larger neighbors should receive fewer ant visits compared to focal plants with smaller neighbors.

I located 30 groups of *C. spinosa* juveniles in which a focal plant was surrounded by at least four conspecific neighbors (two larger and two smaller than the focal plant) growing within 1.25m of the focal plant. The focal plants ranged in size from 6.46 to 13.0 mm in stem diameter, the 0.55th to 73rd percentile of juvenile plant size (distribution based on n=791 plants). Small neighbors ranged from 4.06 to 8.54 mm diameter, and the large neighbors from 10.40 to 40.20 mm diameter. Although the size intervals overlap, within each group of plants the focal was of intermediate size with two larger and two smaller neighbors. For groups containing more than 4 neighbors (n=27), two large and two small neighbors were selected randomly, and all other *C. spinosa* plants were removed. I recorded distances from focal plants to neighbors.

Of the 30 focal plants, 10 were designated control plants, and ant abundance on neighbors was not manipulated. On the other 20 groups I applied four treatments (5 groups/treatment) in a factorial design: ants excluded with Tanglefoot (a sticky resin ants cannot cross) from all but 1 or 2 neighbors within 1.25 m, with all ant-accessible neighbors either larger or smaller than the focal. Tanglefoot was applied to tape placed

on the base of stems, and untreated plants had only tape applied. The resulting treatments were: 1. Control (4 neighbors accessible to ants) 2. One larger ant-accessible neighbor 3. Two larger accessible neighbors 4. One smaller accessible neighbor 5. Two smaller accessible neighbors. Leaving neighbors in place to compete for all other resources besides ants assures that any changes in ant abundance on the focal plant with manipulations of neighbors are due to competition for ants, and not due to changes in focal plant quality mediated by competition for other resources.

I censused ants and potential herbivores on all plants for one month following application of treatments (n=6-9 observations). I repeated this experiment four times: in the dry season (February to March) and wet season (May to June) in 2007 and 2008. Each time experimental treatments were applied randomly to groups without replacement (not allowing repetition of the same treatment on a group). At the start of the experiment, I censused ants on all plants for one month prior to the first application of treatments (March-April 2007, n=8 observations).

All statistical analyses were performed in R (version 2.9.2, R Development Core Team 2009). Ant responses on plants were computed as a plant-size-adjusted 'residual ant density,' which I found by taking the residual of a regression of ants per mm plant stem diameter by plant size, using only unmanipulated plants (see Chapter 2.2 Methods). Residual ant density is thus a size-adjusted measure, with a value of zero indicating that a plant has the expected ant density given its size. Using residual ant

density as the response variable allowed me to directly compare results of the short-term neighbor manipulations with the long-term nectar manipulations. To look for competition for ants in the short-term experiment, I used linear mixed effects models to regress residual ant density on total numbers of accessible large and small neighbors, with focal plant size (stem diameter) and experimental replicate as fixed effects and a random intercept for plant group (lmer, lme4 package, Bates and Maechler 2009). I also included a neighbor density by focal size interaction to look for size-based competition effects. In addition, to consider variation among plant groups in the sizes of neighbors and their distances to the focal plant, I used the same model but replaced number of neighbors with a weighted neighbor density term, calculated as the sum over all accessible neighbors of their stem diameter (mm) divided by their distance to the focal (cm).

If short-term competition occurs, then higher ant abundances should be observed on focal plants with more ant-excluded neighbors (and thus fewer neighbors potentially competing for ants). In contrast, if short-term facilitation occurs, then focal plants with fewer ant-excluded neighbors should have higher ant abundances. In addition, if large plants draw ants away from smaller ones, then focal plants with large ant-accessible neighbors should have fewer ants than focal plants with small accessible neighbors.

3.2.3 Long-term nectar manipulation experiment

To determine if long-term facilitation was occurring, it was necessary to observe ant abundances over larger time and spatial scales than those measured in the short-term small group experiment above. I manipulated nectar resources in 5x5m plots, chosen to contain at least 20 *C. spinosa* juveniles (observed range of densities: 1.00 to 5.36 plants/m², n=15 5x5m plots). These plant densities are typical for *C. spinosa* at La Selva Biological Station (pers. obs.), and thus any competition and facilitation effects observed in this experiment occurred over a biologically relevant range of conspecific densities. Within these plots, some plants contained no neighbors within a 1m radius, suggesting that they sample the lower range of potential *C. spinosa* densities and not only the upper range.

I randomly applied three treatments to individuals in these plots: ants excluded from 20 *C. spinosa* juveniles per plot (reduced nectar levels), artificial nectar (50 mL tubes of 15% sucrose solution) added to 20 *C. spinosa* individuals per plot (increased nectar levels), or control (unmanipulated nectar levels). Three plots, one of each treatment, were nested in each of five sites, for a total of 15 plots. See Chapter 2.2 Methods for more details on experimental design. Treatments were maintained for two years, from September 2007 to August 2009. I mapped all *C. spinosa* plants in each plot, as well as in a 2 m border around the edges of the plot. Local ant abundance was censused on all *C. spinosa* in the plots nine times over the two years of treatments, and by baiting once prior

to application of treatments and eight times over the two years of treatments. An increase in local ant abundance in plots with increased nectar resources and a corresponding decrease with decreased nectar resources would provide evidence of long-term facilitation.

To look for competition or facilitation effects over a longer time scale, I used data from the two-year nectar manipulation experiment, including both ant censuses on *C. spinosa* plants and ant baiting data. Ant responses on plants were computed as a plant-size-adjusted 'residual ant density,' as in the short-term competition experiment above (see Chapter 2.2 Methods). To consider small-scale effects using the nectar manipulation plot data, I computed a local weighted neighbor density by summing stem diameter divided by distance from the focal for all conspecifics within a one meter radius. Ant-excluded plants were not included in the summed 1m neighbor density. This allowed me to create a metric comparable to that used in the short-term neighbor group manipulations. I also summed the number of neighbors within a 1m radius.

I also computed an index of plant density on the plot scale, by summing stem diameters of all *C. spinosa*. To consider any potentially confounding effects of heterospecific EFN plant density, I included a separate term summing all heterospecific EFN plants in each plot (observed range: 0 to 1.48 plants/ m², n=15 5x5m plots, see Appendix C, Table C-1 for species list). Herbaceous EFN species (mainly *Costus* spp.) were not included in the summed heterospecific density, as their stem diameter

measures are not equivalent to those of the other woody EFN species. In addition, *Costus* spp. EFNs are located on inflorescences, so the observed *Costus* spp. neighbors would share ants with *C. spinosa* only during the approximately four months of the year when *Costus* spp. may flower (Kay and Schemske 2003), and then only with plants that actually flower.

I used a linear mixed effects model to regress residual ant density against plant treatment, plot-level nectar manipulation treatment, *C. spinosa* and heterospecific EFN plant density on the 5x5m plot scale, number of conspecific neighbors in a 1-m radius, and interactions between 1-m neighbors, plant size and plant treatment (function `lme` in package `nlme`, Pinheiro et al. 2007). Random intercepts were fit for sites, plots nested within sites, and years nested within plots. Variance in ant density decreased with plant size, so I used an exponential variance structure with $\log(\text{variance})$ as a function of plant size (Zuur et al. 2009). I included a Gaussian spatial autocorrelation term to account for non-independence of neighboring plants within plots (Legendre and Fortin 1989). Very small juveniles (<3.5mm stem diameter) do not receive ant visits or benefit from ants (see Chapter 2), so only plants greater than 3.5mm in diameter were included in the regression. I also fit this same model using size-weighted neighbor density instead of numbers of neighbors.

3.2.4 Ant baiting

To measure overall levels of ant abundances in plots, rather than abundance on EFN plants, I used protein (canned tuna) and sugar (honey) baits, a standard method of measuring ant abundance (Bestelmeyer 2000). Four bait stations were placed at the corners of a 2.5 by 2.5 m square in the center of each plot. Baits were placed in the center of plots to allow a buffer zone to control for edge effects. In order to survey a similar assemblage of ant species to those that visit EFN plants, baits were placed on plants rather than on the ground, at heights of 5 to 150 cm from the ground (mean \pm SD: 57.6 \pm 30.0 cm). Only plants without EFNs were used, and bait height and plant identity (to genus, or species where possible) were recorded as covariates. Each bait station comprised a protein (canned tuna, Atún Azul brand) and a sugar (honey, Del Panal brand) bait of approximately equal volumes, each on a 3x3 cm plastic square, and both placed on the same 100 mm plastic petri dish. All baiting was conducted from 9:00 to 11:45 am, on mornings with no rain. All three plots in a location were baited on the same morning, and all 5 locations were baited within one week for a given observation, as allowed by weather conditions. I checked each bait station once every 15 minutes for the 90 minutes following bait placement (typical time to discovery by dominant ant species; Bestelmeyer 2000), and recorded the numbers of individual ants of different species on tuna and honey baits. Ants were collected in 95% ethanol and identified to

species (using keys in Longino 2009). When possible, I collected ants at the end of the baiting period, to avoid disturbing foraging behavior on baits.

Baiting data were analyzed using a linear mixed effects model with mean total ant biomass (all species pooled) as the response variable, fixed effects of nectar manipulation treatment, rainfall, and the interaction between the two, and random effects of site, plots nested in sites, and bait stations nested in plots. To summarize changes in ant species abundance across nectar treatments, for each ant species I computed the rank correlation coefficient (Spearman's rho) between abundance and plot-level nectar treatment as an ordinal variable (1=reduced-EFN, 2=control, 3=added-nectar). A rank correlation coefficient close to one indicates an increasing abundance response to nectar levels (i.e. evidence of facilitation) while a value of negative one would indicate the opposite pattern, with highest abundance in reduced-EFN plots.

3.3 Results

3.3.1 Short-term competition for ants

The best model to explain ant density on a focal plant used the sum of distance-weighted sizes of ant-accessible neighbors. Observed ant density on focal plants decreased with increasing weighted neighbor density (Table 3-1, general linear mixed effects model). In addition, there was a positive interaction between focal plant size and neighbor density effect. If the focal is large enough (>9.86 mm in diameter), it may gain ants from the presence of neighbors (i.e. it has a net positive neighbor effect, summing

the neighbor and the size by neighbor interaction terms; Table 3-1). This suggests size-based competition for ants in which smaller plants donate ants to larger plants. Using the number of large and small neighbors instead of weighted neighbor density produced similar results, with negative effects of number of neighbors and positive interactions of neighbors by focal size, but these effects were not statistically significant (Appendix C, Table C-2). There was no correlation between focal plant size and mean neighbor plant size (Pearson's correlation: 0.0200, nonparametric bootstrapped 95% CI: [-0.0955, 0.133]).

Table 3-1: Effects of manipulated neighbor plants on focal plant ant density in the short-term experiment: fixed effects coefficients and likelihood ratio tests for the best-fitting linear mixed effects model. Weighted neighbor density was computed as the sum over all neighbors of the neighbor's diameter divided by its distance from the focal plant. Ant-excluded neighbors were not included in neighbor density since they did not share ants with the focal plant. Experimental replicate (Exp.) was included as a fixed effect, and focal plant identity was included as a random intercept (one control and 4 treatment replicates per focal plant group, n=30 groups)

	Estimate	LR	df	P
(Intercept)	9.9093		1	
Neighbor density	-8.5001	20.24	1	<0.001
Focal size (mm diameter)	-0.7228	14.64	1	<0.001
Exp. 1	1.0922	13.40	4	<0.01
Exp. 2	-0.459			
Exp. 3	-1.6764			
Exp. 4	-0.8725			
Neighbor density x focal size	0.8618	18.77	1	<0.001
Residual df			107	

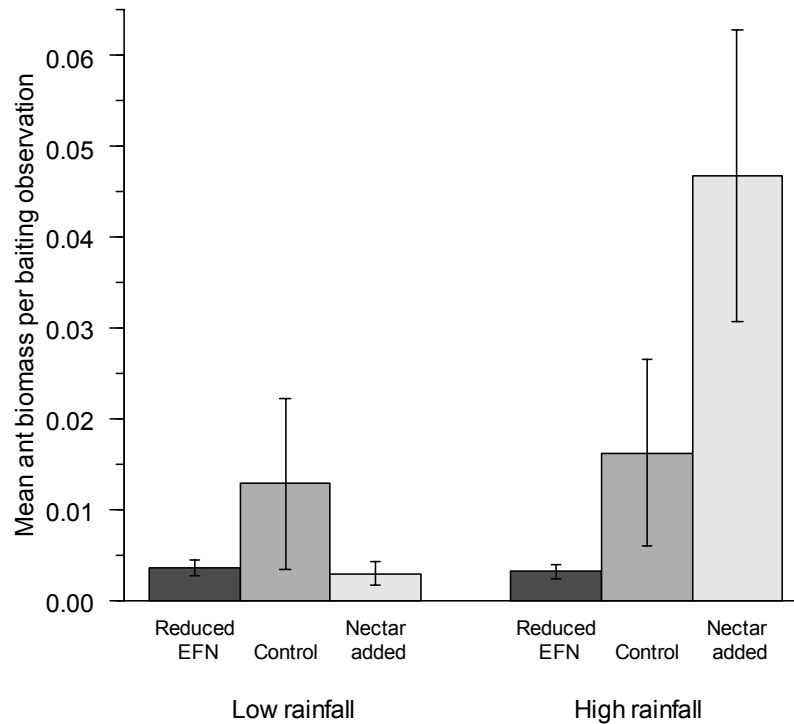


Figure 3-1: Rainfall affects response of local ant abundance to changes in nectar resources. Bars give mean ant biomass \pm SE for all observations combined (n=5 sites, 4 baits per treatment plot, plots blocked across sites, 8 observations over 2 years), in different nectar resource treatments: ants excluded from 20 EFN plants per plot, control, or artificial nectar supplemented on 20 EFN plants per plot. The left set of bars uses observations in which the mean daily rainfall for 4 weeks prior to baiting was less than the median of 4-week mean daily rainfall for all observations combined (median= 12.17 mm, n=4 obs. for each group); the right set of bars uses those observations with 4-week mean daily rainfall higher than the median.

3.3.2 Long-term facilitation: changes in ant abundance with nectar manipulation

Local ant abundance in nectar manipulation plots, as measured by mean total ant biomass per baiting observation, increased with increasing nectar resources as expected with facilitation for ants, but only in observations during periods of high rainfall (Figure 3-1; linear mixed effects model, reduced EFN coefficient: -0.0147, interaction with

rainfall: 3.16×10^{-3} , added nectar coefficient: -0.0289 , interaction with rainfall: 3.39×10^{-2} ; nectar treatment $LR=6.08, df=2, 391, P=0.048$; rainfall by treatment interaction $LR=13.83, df=2, 389, P<0.001$).

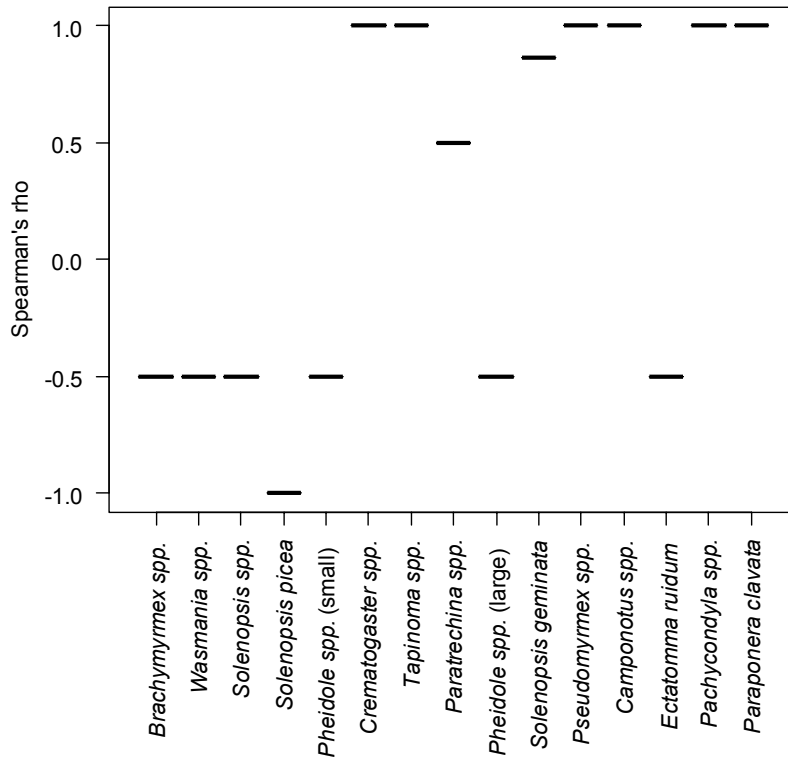


Figure 3-2: Rank correlations of ant abundances with plot nectar treatment for different ant taxa. Ant taxa are listed in order of ascending body size. The y axis gives the ranked correlation coefficient (Spearman's rho) for ant abundance on plants with plot treatment; a value of 1 would indicate highest ant abundances in added nectar plots, intermediate abundances in control plots, and lowest in plots with reduced ant access to EFN plants, as predicted with facilitation for ants among EFN plants. A value of -1 would indicate the opposite pattern. With two exceptions (*E. ruidum* and large *Pheidole* spp.), those taxa with larger body size appear to show increased abundance with increased nectar resources, while the smaller taxa do not demonstrate this pattern.

When ants are grouped by taxa, all possible outcomes of ant abundance were observed in at least one ant taxon (highest in added nectar, control, or plots with reduced EFN plant access; Appendix C, Table C-3). This appears to be related to ant body size; larger ants were more likely to have highest abundances in nectar added plots (Figure 3-2). Using ranked correlation coefficients to describe ant species' baiting response and ranking ant taxa by individual body mass, I found that ant taxa with larger body size were more likely to exhibit a facilitation response to nectar manipulations (Spearman's ranked correlation of body size with facilitation response: 0.607; non-parametric bootstrapped 95% CI: [0.157, 0.862]).

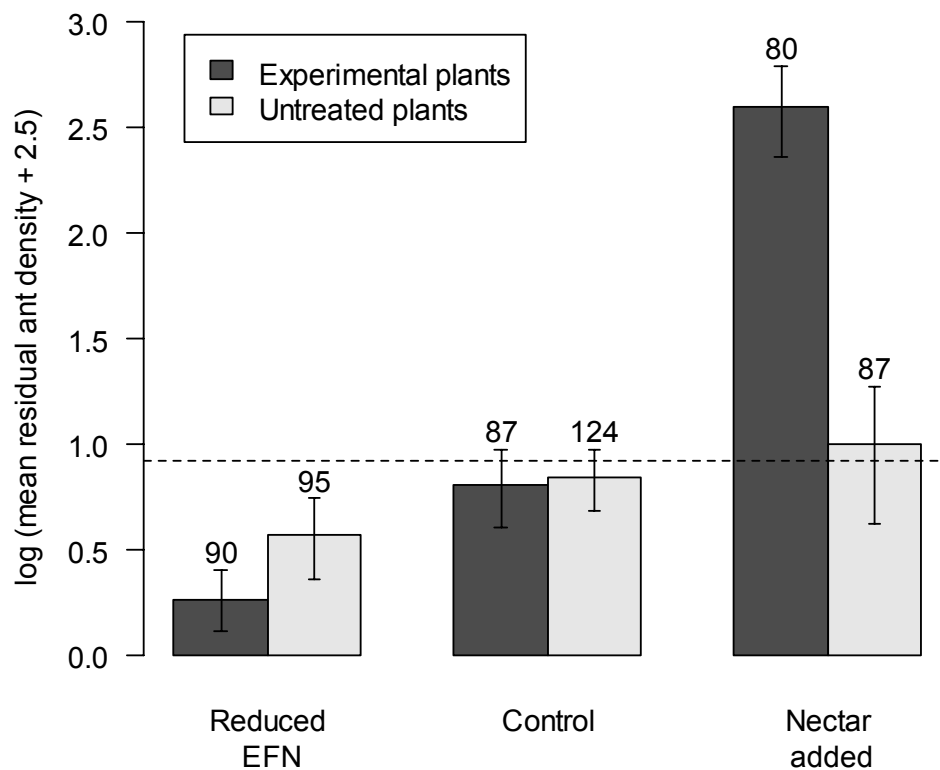


Figure 3-3: Changes in ant abundances on *C. spinosa* juveniles in response to nectar resource manipulations Bars are logs of the residual ant density (means ± 2 SE), a size-adjusted measure of ant density to control for differences in mean plant size across plots. The dotted line shows the expected value if every plant has the ant density expected for its size ($\log(2.5)$). Bars in which the confidence intervals do not overlap the dotted line differ significantly from the expected ant density at the $P=0.05$ level. Experimental plants (shaded bars) had treatments applied: ants excluded from the plant (reduced EFN resource), control, or an artificial nectar source added to the plant (added nectar). Unmanipulated plants (unshaded bars) are located in the different nectar manipulation plots, but with no treatments applied to those plants. Numbers above each bar give the number of plants (years one and two pooled, plants larger than 3.5mm diameter).

Plot-level mean densities of ants on *C. spinosa* juveniles, adjusted for plant size, were highest in added nectar plots, intermediate in control plots, and lowest in reduced EFN (Figure 3-3). These differences were driven by changes in ant densities on

manipulated plants, which is somewhat trivial; in the reduced EFN treatment ants were excluded from manipulated plants, so a lower ant density on those plants demonstrates that the treatment was successful but does not provide evidence for or against facilitation effects. However, different plot-level mean ant densities are not predicted in the absence of facilitation for ants. If only competition had occurred, then ants would merely be redistributed among plants within the reduced-EFN and added nectar plots, and the mean ant densities in each plot (averaged over treated and untreated plants) should be the same across treatments. This is not the case; ant-excluded plants had fewer ants than unmanipulated individuals in the reduced EFN treatment plots, but the overall mean ant abundance in reduced-EFN plots was lower than in control plots. Likewise, although manipulated individuals in the added-nectar plots were visited by more ants than unmanipulated plants in those plots (Figure 3-3), the mean number of ants on all individuals in those plots was significantly higher than in control plots, so the result of treatments was not simply a redistribution of ants (treatment coefficients: reduced EFN=-0.894, added nectar= 4.60, $LR=39.84$, $df=2,1029$, $P < .0001$, linear mixed effects model with spatial autocorrelation, plants larger than 3.5mm in diameter).

Ant densities on unmanipulated plants were highest in added nectar plots, lower in control plots, and lowest in reduced EFN plots, as expected with facilitation (Figure 3-3; treatment coefficients: reduced EFN= -0.651, added nectar= 0.284, $LR= 5.96$, $df=2, 537$,

$P = 0.05$, linear mixed effects model with spatial autocorrelation and variance by year; only unmanipulated plants larger than 3.5mm diameter).

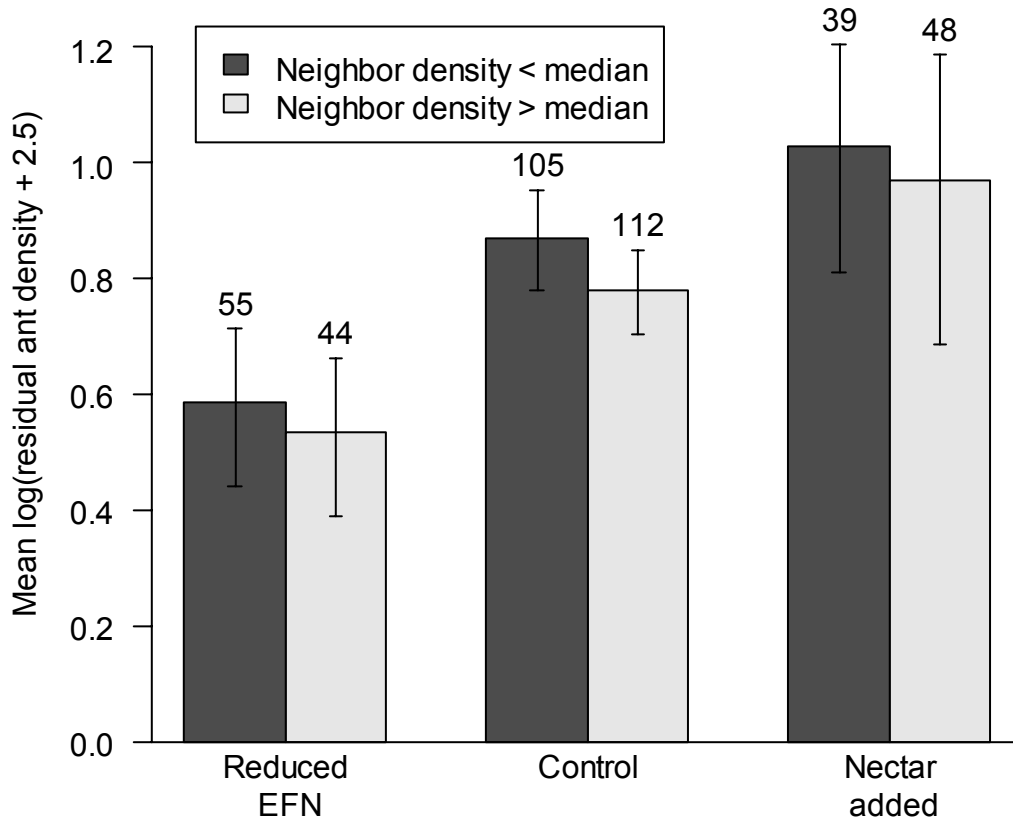


Figure 3-4: Nectar resource and local neighbor density effects on ant density on plants. Bars are log (means \pm SE) of the residual ant density (adjusted for plant size, see methods), for unmanipulated plants in each of the three nectar manipulation plots. Dark bars show plants with lower than the median conspecific neighbor density (summed neighbor sizes/distance within 1m), light bars, higher than the median neighbor density. Numbers above the bars give the number of plants (years one and two pooled, plants larger than 3.5mm diameter).

3.3.3 Contrasting competition and facilitation effects at different spatial scales:

Considering local neighbor density at both the 1m radius and 5x5 m plot level, ant densities on *C. spinosa* plants showed evidence for a small-scale competition effect, as well as a trend towards a contrasting larger scale facilitation effect at the plot level (Figure 3-4). The best-fitting mixed linear effects model included both a negative neighbor density term, summarizing neighbors in a 1m area surrounding an individual, and a positive neighbor density by focal size interaction (Table 3-2), similar to results of the short-term neighbor manipulation experiment. The coefficient sizes for the neighbor density term and its interaction with plant size were smaller than those found for the short-term neighbor manipulation. The neighbor plant effects also depended on the treatment applied to the focal individual; in contrast to unmanipulated individuals, plants with added artificial nectar experienced increased ant density with increasing neighbor density.

In addition to local neighbor effects at the 1m scale, there was a nonsignificant trend towards plot-level facilitation, with the lowest ant density in reduced-EFN plots, intermediate ant density in control plots, and highest in added-nectar plots, even for unmanipulated individuals (Table 3-2; coefficients for reduced EFN plot and nectar supplement plot effects). Adding a plot-level estimate of conspecific plant density (summed stem diameters of all conspecific individuals in the plot) did not improve the fit of the model ($LR=-14.2$, $df=1,1020$, $P=1$), nor did a plot-level estimate of heterospecific

density ($LR=-9.69$, $df=1,1020$, $P=1$). Results using number of neighbors within 1m instead of size-weighted neighbor density were qualitatively similar (Appendix C, Table C-4).

Table 3-2: Contrasting competition and facilitation effects in 2-year plots: table of coefficients from linear mixed effects model. Fixed effects coefficients and likelihood ratio tests for the best-fitting linear mixed effects model. The model included random intercepts of sites, plots nested in sites and years, variance as an exponential function of plant size, and Gaussian spatial autocorrelation within plots. Neighbor density was computed as for the small-group neighbor manipulation (see Table 3-1). Both manipulated and unmanipulated plants were used, and plant treatment was included as a fixed effect.

	Coefficient	<i>LR</i>	<i>df</i>	<i>P</i>
(Intercept)	0.047156		1	
Ants excluded from plant	-0.623215	184.6	6*	<0.0001
Nectar added to plant	6.024581			
Reduced EFN plot	-0.579239	3.53	2	0.17
Nectar added plot	0.358365			
1m weighted neighbor density	-0.161575	71.09	6*	<0.0001
Neighbor density x plant diameter	0.002102	7.8	1	0.0052
Neighbor density x ants excluded	-0.289074	11.05	2	0.004
Neighbor density x nectar added	7.40075			
Neigh. density x ants exc. X diam.	0.047613	64.89	2	<0.0001
Neigh. density x add. nectar X diam.	-0.327701			
Residual df			1021	

* *df* used to compare models, includes factor and all interactions of that factor

3.3.4 Changes in ant dietary preferences with nectar manipulation

Considering baiting data for all ant species together, ants generally preferred sugar baits, but the strongest preference for protein was observed in added-nectar plots (lowest log ratio of numbers of ants on sugar to numbers of ants on protein), compared to a weaker protein preference (larger log ratios) in control plots and in plots with

reduced access to EFNs, which did not differ from each other (Figure 3-5; comparing across all three treatments: reduced EFN effect 0.197, added nectar effect -0.612, $LR=4.233$, $df=2, 7$, $P=0.12$; comparing added nectar to others: added nectar effect -0.513, $LR=3.753$, $df=1,8$, $P=0.05$). Protein preference also varied with rainfall, with the strongest protein preference observed in added-nectar plots in times of low rainfall (Figure 3-6; low rainfall effect -0.826, $P=9.66$, $df=1, 23$, $P<0.01$; reduced EFN effect 0.149, added nectar effect -0.814, $P=10.05$, $df=2, 23$, $P<0.01$). There was no evidence for an interaction between nectar plot treatment and rainfall (interaction coefficient=2.20, $df=2, 22$, $P=0.33$).

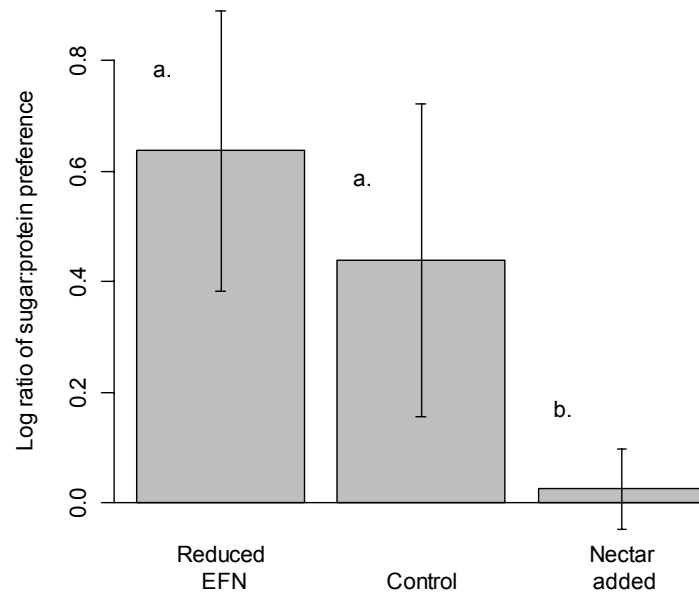


Figure 3-5: Ant preferences for protein or sugar baits across nectar manipulation treatments. The bars give the mean log ratio of total numbers of ants on sugar baits to ants on protein baits: $\log(\text{number of ants on sugar}) - \log(\text{number of ants on protein})$. Each bar is the mean \pm SE log ratio across 5 sites in each treatment, calculated from numbers of ants totaled across 8 observations (over 2 years) of 4 baiting stations per site. A positive value indicates a preference for sugar; a negative value, preference for protein. Letters show significantly different ratios at the $P=0.01$ level.

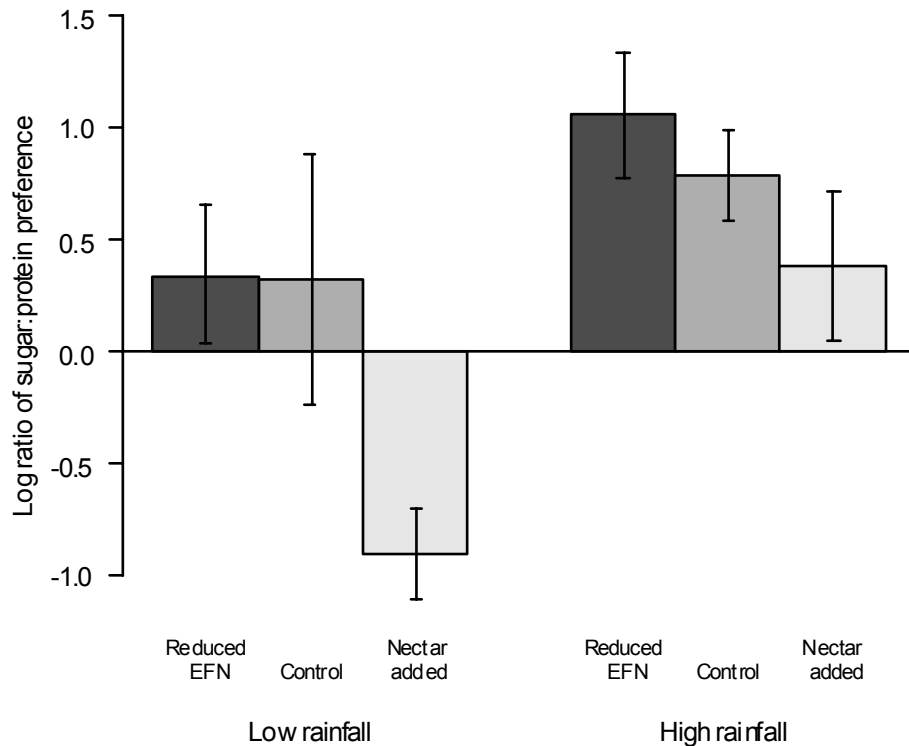


Figure 3-6: Ant preferences for protein or sugar baits by rainfall. The bars give the mean \pm SE log ratio of total numbers of ants on sugar baits to ants on protein baits, as in Figure 3-5 (n=5 sites/treatment). The left set of bars uses observations with 4-week mean daily rainfall less than the median, the right set those with 4-week mean daily rainfall greater than the median, as in Figure 3-3.

The pattern in dietary preference across treatments when considering all ant species together could result from changes across treatments in ant species composition, or from changes in within-species protein:sugar preferences. When ants are grouped by taxon, some taxa show an increased relative preference for protein in added nectar plots, while others show the opposite pattern (Appendix C, Figure C-1). Unlike ant species patterns of abundance with nectar manipulation, differences in dietary preference (sugar:protein log ratio) across nectar treatments do not appear to correlate with ant

body size (Spearman's ranked correlation coefficient: -0.175, nonparametric bootstrapped 95% CI: [-0.745, 0.486]). Ant species abundance responses to nectar manipulations also do not explain the different dietary preference patterns among species: there is no correlation among species between facilitation response and changes in sugar:protein preference across treatment (correlation coefficient: 0.0859, nonparametric bootstrapped 95%CI: [-0.708, 0.605]).

3.4 Discussion

This study has shown that ant density on *C. spinosa* juveniles depended on the local conspecific plant density, with evidence for both competitive and facilitative effects at different spatial scales. Previous work provided evidence that ant density affects growth and survival of *C. spinosa* juveniles (see Chapter 2). Therefore effects of neighbors on ant density can be expected to lead to changes in plant growth and survival, as a mutualist-mediated competition or facilitation effect.

To my knowledge, this is the first study to show evidence of mutualist-mediated competition or facilitation in an EFN plant. Competition for mutualists has been found in ant-aphid interactions (Cushman and Addicott 1989, Fischer et al. 2001), pollination systems (Free 1968, Pleasants 1980, Kephart 1983), seed dispersal mutualisms (Davidson and Morton 1981), and obligate ant-plant interactions (Davidson et al. 1989, Young et al. 1997, Palmer et al. 2000). Most work has focused on interspecific competition (but see Moore and Willson 1982, Manasse and Howe 1983, Moeller 2004).

In this study I found no significant effect on *C. spinosa* of heterospecific EFN plant density at the level of 5x5m plots. However, heterospecific neighbors were not manipulated, and it is possible that *C. spinosa* only shares ants with certain other EFN species, or that interspecific competition or facilitation occurs at a different spatial scale.

Previous evidence of facilitation for mutualists comes mainly from pollination biology (but see Holbrook and Schmitt 2004). Co-flowering plants may increase total pollinator visits to an area, thus creating a benefit to being near other flowering individuals (Lavery 1992, Moeller 2004). Long-term facilitation may occur when sequentially flowering plant species benefit one another by maintaining pollinator populations (Waser and Real 1979, Rathcke 1983). Pollination systems are not representative of most other mutualisms, in that sharing mutualists with conspecifics is frequently necessary in order for plants to benefit from the interaction. In EFN plant-ant protection mutualisms, however, there exists a wider range of possible outcomes, including the possibility that mutualist visits are highest when no conspecific neighbors are present. Therefore findings from EFN plant-ant interactions might be more readily generalized to intraspecific facilitation in other non-pollination mutualisms.

The result that EFN plants compete for ants does not support the theoretical prediction that the shorter-lived partner will tend to compete for the service of the other species in the mutualism (Stanton 2003). This hypothesis is based on the idea that populations of the partner with the longer generation time are not able to respond

quickly to changes in density of the other partner, and would predict competition between ants rather than between plants, as has been observed in several myrmecophytic systems (Davidson et al. 1989, Young et al. 1997, Palmer et al. 2000). Unlike those myrmecophytes, many *C. spinosa* plants experience very low levels of ant visitors, suggesting that ants are a limiting resource (pers. obs.; proportion of plants > 3.5 mm diameter with no ants observed in one year of censuses: 0.56; n=198 in year 1, 202 in year 2). Due to the generalized and facultative nature of EFN plant-ant interactions, ants that visit EFNs might not be limited by any one EFN source to the same degree that obligate myrmecophytic ants are limited by their host plants.

In the following sections I will discuss plant size effects on competition for ants, how ant dietary requirements might explain seasonal facilitation effects, contrasting competition and facilitation responses at different spatial scales, variation among ant taxa, and potential consequences of competition for and facilitation via mutualists.

3.4.1 Size-dependence of competition effects

Both short-term neighbor manipulations and 2-year observational data from mapped nectar manipulation plots showed evidence of size-based competition for mutualist ants at small spatial scales (1-1.25 m radius around focal plant). Small juveniles received fewer ant visits in the presence of more neighbors, whereas larger juveniles ($\sim \geq 1$ cm in diameter) experienced no effect of neighbors or even received more ants (i.e. a net zero or positive coefficient for total neighbor effect). In addition, the effect

on the focal plant also depended on neighbor plant size, with larger neighbors having a larger magnitude impact; size-weighted neighbor density predicted ant density on the focal better than number of neighbors. In the data from the nectar manipulation plots, plants with added artificial nectar experienced a positive effect of neighbor density, even for small nectar-added plants. This suggests that ant preference for larger nectar resources might be the mechanism behind size-based competition for mutualists in *C. spinosa*. This is one of only a handful of studies (cf. Manasse and Howe 1983, Davidson et al. 1989) to find that individual characteristics such as size affect the outcome of competition for mutualists.

3.4.2 Ant dietary requirements and response to nectar resources

Nectar manipulation plots showed evidence of long-term facilitation for ants, both in censuses of ants on *C. spinosa* plants and in baiting data. The increase in local ant abundance with increasing nectar treatments was only seen on baits during times of high rainfall throughout the 2-year period. Therefore plot-level changes are less likely the result of increased colony growth or recruitment of new ant colonies, as those processes would cause a continual increase in ant abundance over time, and more likely due to changes in foraging behavior or nest relocation of existing colonies over shorter time scales. The most commonly observed ant species, *E. ruidum*, moves nests over time periods much shorter than the two years of the experiment (McGlynn et al. 2010). Although *E. ruidum* did not exhibit a facilitative response to nectar manipulations, it is

possible that other common ant species might also have moved nests in and out of plots in response to nectar manipulations (McGlynn 2006). Baiting data are not a completely neutral measure of ant abundance, as they result from a combination of ant foraging choices and local abundance. However, the baiting results agreed with censuses on *C. spinosa* plants, and baiting on plants rather than using ground pitfalls sampled an ant community very similar to that found foraging on *C. spinosa*, and also provided data on protein:sugar preference.

Baiting data suggest that facilitation of ants by high densities of EFN plants is more likely in times of high rainfall. This is also when *C. spinosa* plants are most likely to be producing new leaves (pers. obs., data not shown) and might most need defense from herbivores. Different dietary requirements at different stages of ant colony growth could explain seasonal changes in ant responses to nectar manipulations. Ant colony ontogeny is seasonal in most species, with reproductives produced at one time of year and workers at another (Hölldobler and Wilson 1990). The strongest shift towards a preference for protein was observed in added nectar plot at times of low rainfall (Figure 3-6), suggesting that higher dietary requirements for sugars during the high-rainfall season could explain the greater increase in ant abundance with nectar addition at that time of year.

The artificial nectar used in this study was pure sucrose; true extrafloral nectar contains many different sugars such as glucose and fructose, and often an array of

amino acids as well (Bentley 1977). Ants may respond differently to glucose or fructose than to sucrose. However, manipulating only sugars makes this study a conservative test of the importance of sugar resources like EFN for ants (and thus potential facilitation of ants); if local ant abundance increases in response to pure sugar resources, then it is likely that they would respond even more strongly to the amino-acid-rich EFN.

3.4.3 Contrasting competition and facilitation effects at different spatial scales

Over two years of observations of nectar manipulation plots, there was evidence for both a small-scale competition effect as well as a larger facilitation effect. The plot-level nectar treatment effect was statistically significant in an analysis using only unmanipulated plants, but not in the analysis that used all plants and incorporated a small-scale neighbor term ($P=0.16$, Table 2). This difference might result from including treated plants in the second model; a plot-level nectar treatment effect separate from individual treatment effect is not meaningful for treated individuals. In addition, results from ant baiting are consistent with increasing ant densities with ascending nectar treatments in plots, further supporting a plot-level facilitation effect.

Although the small scale (1m radius) neighbor plant effect in the long-term experiment supported size-based competition for mutualists as observed in the small-scale neighbor group manipulations, the magnitude of the observed effects was much smaller in the long-term nectar manipulation plots. This difference might be due to the different time scales of the two experiments; over longer time periods facilitation effects

at a larger spatial scale might dominate and obscure smaller scale competitive effects. In addition, the individuals in the nectar manipulation plots experienced a much larger range of neighbor plant densities than the neighbor group experiment. If neighbor effects were nonlinear, with the strongest competition effects occurring at low neighbor densities, this would result in a lower linear neighbor effect in data over a larger range of neighbor densities. However, there was no evidence of a nonlinear effect of neighbors in the nectar manipulation plot data (adding a squared neighbor density term does not improve the linear model; $LR=-3.63$, $df=1$, 1021 , $P=1$).

3.4.4 Variation in facilitation response among ant taxa

Most results in this study pooled all ant species together, despite the fact that the 69 ant species observed almost certainly differ in both their responses to plant-density manipulations and in the degree of benefit they provide to the plants. Previous work has shown that total ant density of all species together predicts benefit to *C. spinosa* juveniles (see Chapter 2). Splitting results from the most common ant taxa, not all showed evidence of facilitation. Ant species differ in their preferences for different types of sugars in EFN (Blüthgen and Fiedler 2004), which could contribute to the observed variation in facilitation response among ant taxa. Differences in response to nectar manipulations among ant taxa, as well as a tendency for larger-bodied ants to exhibit a stronger facilitation response, could be due in part to interactions among ant species. Interspecific competition is a key factor in structuring ant communities

(Hölldobler and Wilson 1990). Some ant species might have decreased in abundance with increasing nectar because they were excluded by other more competitive ant species that were present in high numbers in the nectar addition plots. For example, *Solenopsis geminata*, an aggressive ‘fire ant’ species, showed large increases in abundance in added nectar plots, and built external nest tunnels up to the artificial nectar tubes, preventing access by other ants. These nest tunnels were not observed on any unmanipulated juveniles in this study, but are found on mature *C. spinosa* trees (pers. obs.), suggesting that *S. geminata* colonies might defend nectar sources above a certain minimum resource size. The relationship between body size and a facilitation response may also partly be an artifact of the fact that smaller-bodied ants are more able to avoid exclusion treatments, and are therefore not as affected by the ‘reduced-EFN-access’ treatment.

3.4.5 Consequences of competition for mutualists

Competition for mutualist ants could have important consequences for plant populations and communities. It might affect population sizes and dynamics by introducing a new source of density dependence (Breton and Addicott 1992, Morales 2000). The size-based competition observed in this study, in which smaller plants donate ants to larger plants, could either minimize or accentuate the effects of competition for ants on *C. spinosa* populations, if larger plants contribute disproportionately to population growth. Facilitation for mutualist ants might result in

Allee effects such as those observed in pollination mutualisms, in which population growth rate increases with population density at the low end of the density range (e.g. Groom 1998, Hackney and McGraw 2001). Facilitation effects must logically saturate at some plant density, or else be overwhelmed by negative density-dependence, since population size cannot increase indefinitely. Further work is needed to model the effects of competition for and facilitation via ants on population dynamics of *C. spinosa*.

Competition for mutualist ants could also contribute to plant community composition, particularly in areas with high densities of EFN plants. If intraspecific competition for mutualists is stronger than interspecific competition, this could help maintain high plant diversity in tropical forests, where EFN plants are often abundant (Schupp and Feener 1991, Fiala and Linsenmair 1995). Intraspecific competition for ants is likely to be stronger than interspecific competition, because different EFN species vary in their nectar composition (Bentley 1977), and different ant species vary in their preferences for different nectars (Blüthgen and Fiedler 2004). Therefore two plants of the same species are more likely to share and potentially compete for the same ant visitors. Further work is needed to determine the relative strengths of intraspecific and interspecific competition for mutualists among EFN plants.

4. Facilitation but not competition for mutualists affects long-term population growth of an extrafloral nectary bearing plant

4.1 Introduction

Resource-based competition has long been viewed as an important factor affecting populations and communities (e.g. Lotka 1932, Hutchinson 1957). Competition for the resources or services provided by a mutualist partner might also affect population dynamics of those species engaged in mutualistic interactions (Addicott 1985, Cushman and Addicott 1989). Alternatively, population-level effects may also result from facilitation, in which the presence of several partners increases local mutualist density and thus creates a benefit to being near other individuals that share the same mutualists (Waser and Real 1979, Rathcke 1983).

In the case of interspecific competition for mutualists, it is relatively straightforward to predict population-level effects. As with competition for any other resource, population density in the presence of a competitor for mutualists will tend to be lower than in its absence, although if simultaneous facilitation effects are also allowed then population dynamics become more complex and context-dependent (Stanton 2003). It is more difficult to predict the effects of intraspecific competition for mutualists on population dynamics, because the total number of mutualists interacting with the population does not necessarily change as in interspecific competition. Decreasing mutualist density per individual may reduce individual benefit received from

mutualists as population density increases and thus introduce a new source of negative density dependence (Cushman and Addicott 1989, Breton and Addicott 1992). If the outcome of competition depends on differences in size or other characteristics of competing mutualists, competition may result in an uneven distribution of mutualists within the population. This could have effects on long-term population dynamics if individuals of different sizes (or different qualities or other traits) contribute disproportionately to population growth. For example, if larger individuals draw mutualists away from smaller individuals, and larger individuals contribute relatively more to population growth, then intraspecific competition for mutualists could result somewhat paradoxically in a higher population growth rate.

Intraspecific facilitation via mutualists may introduce a source of positive density dependence, in which mutualist visits increase with population density, thus causing population growth rate to increase with increasing population densities (Groom 1998, Hackney and McGraw 2001). Positive density dependence from facilitation must saturate at some point or it would cause populations to increase indefinitely, and most intraspecific facilitation effects on population growth have been observed as Allee effects occurring at low density ranges (Groom 1998, Hackney and McGraw 2001). If both competition for mutualists and facilitation occur in the same system, it is possible that one or the other dominates at different temporal or spatial scales. For example, if

facilitation effects occur over longer time periods than competition effects, facilitation may be more important for long-term population growth.

Population projection models are useful in predicting long-term effects of ecological processes such as interspecific interactions. While it would be ideal to observe population-level effects over a long time period, this is not always feasible, particularly for slow-growing species such as trees. Manipulating mutualist or antagonist partners on the scale of populations can also be logistically difficult. Projection matrix models are one of the most commonly used tools for predicting long-term population dynamics using experimentally measured vital rates (Caswell 2001, Morris and Doak 2002). Using a projection matrix model, it is possible to manipulate individuals in a population, measure their vital rates, and then extrapolate those individual-level effects to determine the effects of interspecific interactions on populations. Integral projection models (IPMs) are similar to projection matrix models but instead of using discrete stages or size classes, they model populations as a continuous distribution along a variable such as size (Easterling et al. 2000, Ellner and Rees 2006). The advantage of IPMs is that they use fewer parameters, which is useful with limited data. Projection matrix models have been used previously to investigate population effects of species interactions (e.g. Ehrlén 1995, Maron et al. 2010). IPMs have recently been used to model effects of herbivory treatments (Williams et al. 2010) and naturally occurring herbivory gradients (Hegland et al. 2010), but no IPM has yet

directly incorporated densities of another species in order to model an interspecific interaction. Here we modify the IPM approach to include mutualists and neighbor plant densities.

The mutualism between plants that produce extrafloral nectar (EFN plants) and the ants that visit them is a good study system to assess the effects of competition and facilitation for mutualists on population dynamics. EFN plants are often present at high local densities in tropical forests (Schupp and Feener 1991), making interactions among individuals likely. Previous work has provided evidence of both small-scale competition and larger-scale facilitation for mutualists in the EFN plant *Colubrina spinosa* (see Chapter 3). Small-scale competition (at a 1m radius around a focal plant) was observed as both a negative effect of neighboring conspecifics on the density of ants visiting a focal plant and a positive interaction of focal size with neighbor density. This suggests size-based competition, in which larger plants draw ants away from smaller plants. Competition for ants could thus lead to the redistribution of ants within a plant population, with larger individuals receiving more ants. Larger juveniles also receive more growth and survival benefit from ants than smaller juveniles do (see Chapter 2), so redistribution of ants from smaller to larger juveniles might cause an increase in the rate of population growth. Facilitation of EFN plants was also observed at the larger scale of 5x5m plots; increased conspecific density at the plot-scale was associated with increased mean ant density on plants within the plot. Increased mean ant density could contribute

to increased growth and survival, which might lead to an increased population growth rate as well.

Using these data on competitive and facilitative neighbor density effects (Chapter 3), together with data on the effects of ants on growth and survival (Chapter 2), we assessed the effects of intraspecific competition and facilitation for mutualists on long-term population dynamics in an EFN plant. We used an integral projection matrix model (Ellner and Rees 2006) to ask the following questions about intraspecific competition and facilitation for mutualist ants: 1. What are the effects of competition and facilitation for mutualists on long-term population growth rate and the population's asymptotic size distribution? 2. How does the mean ant density affect long-term population growth?

4.2 Methods

4.2.1 Field experiment

We conducted all research presented here at La Selva Biological Reserve, Heredia Province, Costa Rica, in secondary or disturbed tropical rainforest areas containing high densities of EFN plants. The focal species, *Colubrina spinosa* (Rhamnaceae), is a tropical treelet that has extrafloral nectaries located in pairs at the base of the leaf blade. The largest adult tree observed in this study was 31.4 cm in diameter at the base. Juveniles in the forest understory grow slowly (yearly change in stem diameter: median 0.022, range -0.74 to 1.41 mm), so reproductive adults are likely to be decades old. We have

observed 69 species of ants from 22 genera visiting the nectaries of *C. spinosa* at La Selva (see Appendix A for complete species list). The total ant density of all species pooled was a good predictor of plant performance (see Chapter 2) so all demographic rates as a function of ants used the pooled total density and did not distinguish among different ant species.

In order to increase the range of variation in ant density, independent of local environment and among-plant differences in nectar production, we manipulated ant abundance on *C. spinosa* juveniles with three treatments: ant exclusion, artificial nectar supplementation, and control. We applied treatments to 20 plants in each of five 5x5m plots per treatment. Plots of each treatment were nested in five similar sites within La Selva. We marked and followed all other unmanipulated *C. spinosa* juveniles in each plot in the same way as manipulated individuals (n=1032 plants total across all plots, treated and untreated pooled). Treatments were maintained for two years, from September 2007 to August 2009. For more details on application of treatments, see Chapter 2.2 Methods. All *C. spinosa* plants were mapped, and we computed weighted local neighbor density by summing neighbor stem diameter divided by distance from the focal for all conspecifics within a one meter radius of the focal plant (see Chapter 3.2 Methods).

Adult trees were not manipulated due to the extreme difficulty of excluding ants from very large individuals, whose canopies contact those of many other plants, which would form bridges for ants even if the trunk were to be blocked with Tanglefoot.

Isolated *C. spinosa* trees are rarely observed at La Selva (pers. obs.), so we estimated ant-independent fecundity by summing all seedlings around groups of adults. In May 2008 we mapped all *Colubrina spinosa* trees larger than 44 mm in basal diameter (minimum size observed fruiting in 2006-2008, pers. obs.) in 8 groups of parent trees, with a total of 83 reproductive adults (size range: 44mm to 314mm stem diameter at base). *C. spinosa* is hermaphroditic (Bawa et al. 1985), so all individuals may produce fruits. The area of a group was defined as a circle extending to 1m beyond the farthest observed *C. spinosa* seedlings. Three 1m wide transects were drawn from the center of the circle, spaced evenly (60 degrees apart) starting from a random compass direction. All *C. spinosa* seedlings within a 1m² area were counted every 2 meters along the transects. The total number of seedlings in an area was then estimated by extrapolating seedlings for concentric rings 1 m wide, and summing over all rings. Mean seedlings per tree was calculated using all trees in a group, including those that did not produce fruits that year. The fecundity value thus incorporates both probability of producing fruits in a year and the expected number of seedlings given fruit production.

Ants were not manipulated on seedlings (which we define as plants less than 1.5 years old). Although even the first true leaves of *C. spinosa* have EFNs, they do not appear to produce nectar at small sizes (pers. obs.). We followed seedling survival in the 5x5m plots, marking new seedlings with plastic toothpicks in March 2008 and March

2009, and checking for surviving seedlings every six months (year 1: n=2243, year 2: n=1170).

4.2.2 Functions for vital rates and competition effects

The integral projection model we used integrates vital rates over two continuous variables, size and local neighbor density (cf. Ellner and Rees 2006). We used linear and general linear mixed models to determine vital rates as functions of size and neighbors, using data from the field experiment. To determine effects of neighbor plants on vital rates, we fit models of neighbor effects on ant density, and of ant density effects on vital rates. In addition, not all neighbor densities are equally common in the field, so we weighted the integral over neighbor densities by the probability distribution of neighbors, which was also fit using observational data.

To determine how vital rates varied with abundance of attending ants and plant size, we fit linear or generalized linear mixed effect models with random intercepts of sites and plots nested in sites. Survival probability was fit as a logistic regression of plant fate after one year (alive or dead), with fixed effects of year, plant size, ant density, and the interaction between size and ant density (package lme4 in R, Bates and Maechler 2009). Growth was fit as a linear mixed effects model of size (stem diameter) next year as a function of size this year, ant density, the interaction between size and ant density, and year. The growth model also incorporated an exponential variance term, with log variance as a linear function of plant size, ant density and a plant size by ant density

interaction (after Ellner and Rees 2006). For both growth and survival, ants had no effect on plants smaller than 3.5 mm in diameter, so growth and survival functions are piecewise functions with no ant effect for plants smaller than 3.5mm (see Chapter 2). For all vital rate and competition functions, the measure of ants used was a plant-size-adjusted residual ant density, found by taking the residual of a regression of ants/stem diameter on plant size (see Chapter 2.2 Methods for more details). A residual ant density of zero indicates that a plant has the expected ant density given its size; negative values indicate a lower ant density than expected, positive values a higher than expected ant density.

Because we could not exclude ants from large reproductive trees, we could not quantify ant effects on fecundity. To estimate fecundity as a function of adult size, we used data from groups of adults to perform a linear regression of total seedlings per group against summed basal diameter and squared basal diameter for each group, and compared the fits of models with linear or squared basal diameter terms. We then used this regression to assign estimated seedlings to each parent tree in the areas, by dividing total seedlings among adults in the area proportional to their squared basal diameter (the better-fitting model). We then regressed those estimated seedling values on individual tree sizes to give a fecundity function of seedlings produced by trees of different sizes for the population model. We used a non-parametric bootstrap to find 95% confidence intervals around the fecundity parameter. Survival of seedlings (plants

<1.5 years old) was independent of ants, and computed as the total number of seedlings alive after six months divided by the initial count of seedlings. Different seedling survival values were found for year one and year two, and for the first and second six-month intervals in the first year of life.

Growth and survival are functions of ant density on an individual plant, and thus indirectly functions of conspecific neighbor density, as ant density depends on neighbor density (see Chapter 3.2). To determine effects of small-scale (1 m radius) and larger-scale (5x5 m plot) neighbor density, we fit a linear mixed effects model of residual ant density, with fixed effects of small-scale and plot-level neighbor density and the interaction of small-scale neighbor density with plant size, and random intercepts of sites and plots nested in site, and a spatial autocorrelation term (package nlme in R, Pinheiro et al 2007). Small-scale neighbor density was computed for each individual in the plot by summing stem diameters (mm) divided by distance to the focal (cm) for all conspecifics within a 1m radius. The variable 'neighbor density' in the population model refers to this small-scale distance- and size-weighted neighbor density. See Chapter 3.2 Methods for more details.

Plot-level neighbor density was computed as the weighted sum of stem diameters (mm) of all conspecifics in a plot. In the work presented in Chapter 3, we used plot-level nectar treatment as the independent variable to look for facilitation effects; higher densities of EFN plants should increase local nectar levels, so if facilitation is

occurring then artificially manipulating nectar levels should induce changes in local ant abundance. For the population model it is convenient to use a measure of facilitation that directly incorporates local plant density, while taking into account the nectar manipulations. We thus weighted the plot-level neighbor density similarly to the small-scale density; ant-excluded plants were not included in the sum of stem diameters, and nectar-supplemented plants were weighted more heavily, by assigning them the stem diameter of an equivalent larger unmanipulated plant that would attract the same number of ants as the nectar-supplemented plant (see Chapter 2, Figure 2-5). This weighted plot-level plant density was used to determine facilitation effects in the linear mixed effects model described above. Thus the facilitation coefficient for the model was fit using a larger range of plant densities than those observed in the field, similar to the method we used to measure growth and survival effects of ants by inducing a larger range of ant densities with experimental manipulations. In contrast, we used unweighted summed stem diameter per plot to calculate the mean plot-level plant density, which was used in the population model to determine the expected level of facilitation based on current field conditions. Therefore potential effects of facilitation were considered over the observed range of plant densities in 5x5 m plots, and not over the larger range induced by nectar manipulations.

The population model integrates small-scale neighbor effects over the observed range of neighbor densities. In the field experiment plants with low neighbor densities

were more frequently observed than those with high neighbor densities, suggesting that the size-weighted neighbor density follows a gamma or exponential distribution. To determine if the distribution of neighbor densities varied with focal size, we fit exponential and gamma functions to the distribution of neighbor densities for plants of different sizes, by dividing plants into size classes (n=35 plants per class, 20 classes) and fitting parameters for each size class. We then regressed the exponential parameter or gamma parameters against median plant size per class. This gave us a probability density function for neighbor densities as a function of plant size, which we included in the population model in the integral over all neighbor densities (see below). This allowed us to weight the contributions of neighbor densities to demographic rates based on how commonly those neighbor densities are experienced by plants of different sizes. For example, high neighbor densities might have large effects on plant growth and survival, but if those densities are rarely observed in the field, then they will have a smaller effect on population dynamics than if they were commonly observed.

The plants used to estimate growth, survival, and neighbor effects and distributions did not represent the full range of sizes of *C. spinosa* (stem diameter of experimental plants: median= 5.89 mm stem diam., range [0.32, 71.28] ; range of all individuals recorded [0.32, 313.54]). This is due to the fact that smaller plants are abundant and very large individuals are rare. Consequently, functions that estimate growth or neighbor effects well at small plant sizes are not necessarily biologically

realistic when extrapolated to very large individuals. In order to avoid biologically unrealistic extrapolations of ant and neighbor effects, we used a piecewise function for ant density and neighbor distribution, in which plants greater than 44mm in diameter (the minimum reproductive size) were assigned the ant density or neighbor density distribution of a 44mm individual. In addition, we modified the growth function to add a negative diameter² term for plants > 44mm so that plants at the maximum size have zero probability of growing larger. We also used a piecewise function for the exponential function describing variance in growth, assigning a constant variance for plants > 44mm. No changes were needed to the survival function because as a logistic function it saturates at a survival probability very close to one for large plants within the experimental range; thus extending the upper range does not change survival values.

4.2.3 Population model

We used an integral projection model (IPM) to model *C. spinosa* population dynamics (Easterling et al. 2000, Ellner and Rees 2006). Using an IPM rather than a projection matrix model allowed us to model plant sizes and neighbor effects as continuous distributions instead of assigning arbitrary size classes and levels of neighbor density. This is particularly useful here because *C. spinosa* grows slowly and reaches a large maximum size, and thus might be poorly described by a matrix model unless the model included a very large number of size classes (and thus a large number of parameters), which would have required using a much large number of plants than

were included in the field study (Zuidema et al. 2010). Because an IPM includes fewer parameters than a matrix model, its parameters are estimated with greater precision using a limited set of data (Ellner and Rees 2006).

In the model we constructed, growth and survival are functions of ant density $a(x)$, which in turn is determined by local conspecific neighbor density (h), plant size (x), overall mean ant density (a_0), and intraspecific competition strength (c) (Table 4-1). To look at the effects of mean ant levels on population dynamics, we varied mean ant density (a_0) in different runs of the model from -2.5 (minimum observed residual ant density, including treated and untreated plants) to 10 (95th percentile of observed residual ant density, all treated and untreated plants), in increments of 0.5. In different runs we also varied intraspecific competition strength (c), which multiplies the coefficients for neighbor density and the plant size by neighbor density interaction (fit from observed data). A competition value (c) of 1 sets competition to the levels observed in the field, while $c=0$ would be no competition effect and $c=2$ would be twice the observed competition levels. Rather than vary neighbor density, we used the values of conspecific neighbor density (h) observed in the field, and varied the strength of competition (c) to look at the effects of intraspecific competition for ants on *C. spinosa* populations.

Table 4-1 Statistical models and parameter estimates for demographic and neighbor functions. Variable x is plant size (mm stem diameter), yr is a discrete factor for experiment year ($yr=0$ for year 1, $yr=1$ for year 2), a_0 is mean background ant density, c is competition strength, h is small-scale neighbor density, d is plot-level neighbor density, and a is ant density, which is a function of a_0 , x , h , c , and d . Mean growth (μ_g) is the predicted size next year (stem diam. in mm), fecundity is the number of seedlings produced per individual, mean size of 1.5 year olds (μ_y) is the mean stem diameter (mm) of a plant aging out of the seedling class, and ant density is size-adjusted ant density, which takes the value of 0 for a plant with the ant density expected for its size.

Demographic process	Model
Distribution of neighbor density	Exponential with $\lambda_h = \begin{cases} 0.5295 + 0.03329 * yr + (0.01556 - 0.00230 * yr) * x, & x < 44 \\ 0.5295 + 0.03329 * yr + (0.01556 - 0.00230 * yr) * 44, & x \geq 44 \end{cases}$
Ant density	$a = \begin{cases} a_0 + 0.0002871d - 0.05489c * h + 0.004832c * h * x, & x < 44 \\ a_0 + 0.0002871d - 0.05489c * h + 0.004832c * h * 44, & x \geq 44 \end{cases}$
Growth	$\mu_g = \begin{cases} 0.10918 + 0.03745 * yr + 1.008 * x - 0.0002402 * x^2, & x < 3.5 \\ 0.10918 + 0.03745 * yr + 1.008 * x - 0.0002402 * x^2 + (-0.005963 + 0.002687 * x) * a, & x \geq 3.5 < 44 \\ 0.10918 + 0.03745 * yr + 1.008 * x - (2.502 * 10^{-5} + 3.809 * 10^{-7} * yr) * x^2 + (-0.005963 + 0.002687 * x) * a, & x \geq 44 \end{cases}$
	$\log \sigma_g^2 = \begin{cases} 0.05461 + 0.1390 * x + (-0.01246 + 0.003264 * x) * a, & x < 44 \\ 0.05461 + 0.1390 * 44 + (-0.01246 + 0.003264 * 44) * a, & x \geq 44 \end{cases}$
Probability of survival	$Logit(p_s) = \begin{cases} 2.5918 - 2.5840 * yr + (0.1252 + 0.2604 * yr) * x, & x < 3.5 \\ 2.5918 - 2.5840 * yr + (0.1252 + 0.2604 * yr) * x + (-0.2805 + 0.08188 * x) * a, & x \geq 3.5 \end{cases}$
Fecundity	$f = \begin{cases} 0, & x < 44 \\ 0.03753 * x^2, & x \geq 44 \end{cases}$
Distribution of sizes for 1.5 year olds	Zero-truncated Normal with $\mu_y = 1.59 - 0.600 * yr$, $\sigma_y = 0.641 - 0.350 * yr$

All plants greater than 1.5 years old are described by a continuous size distribution, $n(x,t)$. The distribution of plants in year $t+1$ is predicted with:

$$n(y, t + 1) = \int_L^U \int_0^{U_N} g(y, x, h) \sigma(x, h) p(x, h) n(x, t) dh dx + \psi d(y) S_t$$

where growth $g(y,x,h)$ and survival $\sigma(x,h)$ are both functions of neighbor density h , because ants are a function of neighbor density h , and growth and survival are functions of ants. Integrating over all neighbor densities h (from zero to $U_N=1.1$ * maximum observed neighbor density) and all sizes x (from $L=0.9$ * smallest observed size to $U=1.1$ * largest observed size) at time t gives the contribution to sizes y at time $t+1$. The distribution function of neighbor densities $p(x,h)$ is an exponential distribution $p(x,h) \sim \text{Exp}(\lambda_h(x))$ in which λ_h is a function of plant size x . The second part of the equation, $\psi d(y) S_t$, is the contribution from the discrete seedling class, S_t . Seedlings in year t contribute to sizes y in year $t+1$ according to the distribution $d(y)$, where $d(y) \sim$ zero-truncated Normal(μ_y, σ_y) with mean μ_y and variance σ_y of the stem diameter of 1.5 year old seedlings. Seedlings are produced 6 months after the census date, so individuals in the seedling class are 6 months old at time t . To contribute to $n(y,t+1)$ they must survive from age 6 months to age 1.5 years with probability ψ . The new seedling class is:

$$S_{t+1} = \phi \int_L^U \int_0^{U_N} f(x^2) (\sigma(x, h))^{\frac{1}{2}} p(x, h) n(x, t) dh dx$$

Individuals larger than a minimum size produce seedlings as a function of their size $f(x^2)$. Fecundity $f(x^2)$ is a piecewise function that takes the value 0 for plants less than 44mm in diameter (Table 4-1), allowing integration over all sizes $[U, L]$ even though small plants produce no seedlings. The function $f(x^2)$ combines the probability of producing fruits in a year and the expected number of seedlings given that fruits are produced that year. Again, seedlings are produced 6 months after the census date, so for adults to contribute to the seedling class in the next census, they must first survive six months to the fruiting date with probability $(\sigma(x, h))^{(1/2)}$ (square root of one year survival), then produce seedlings, then the seedlings must survive the first 6 months with probability ϕ .

To find the stable population growth rate (λ) and the stable size distribution, we used a numerical approximation to iterate the population vector n_t over time (Ellner and Rees 2006). For plant size meshpoints $x_1=L+(i+0.5)w_1$, where $w_1=(U_2-L)*m_1$, and neighbor meshpoints $x_2=0+(i+0.5)w_2$, where $w_2=(U_N-0)*m_2$, the above projection equations are thus estimated as:

$$n(x_k, t + 1) = w_1 w_2 \sum_{i=1}^{m_1} \sum_{j=1}^{m_2} g(x_k | x_i, h_j) \sigma(x_i, h_j) p(x_i, h_j) n(x_i, t) + \psi \sum_{i=1}^{m_2} d(x_i) S_t$$

$$S_{t+1} = \phi \sum_{i=1}^{m_1} \sum_{j=1}^{m_2} f(x_i^2) (\sigma(x_i, h_j))^{\frac{1}{2}} p(x_i, h_j) n(x_i, t)$$

We used 150 meshpoints (m_2) for neighbor density, and 800 meshpoints (m_1) for plant size, which we chose as the minimum number at which adding more meshpoints did not change λ by more than 10^{-4} . The mesh size for plant size was 0.43 mm; for slow growing-tree species a mesh size of less than 1cm is recommended (Zuidema et al. 2010). All vital rates and neighbor functions were fit for years one and two separately, and we used either all year one or all year two values in each model run. We thus predicted long-term dynamics for the scenarios in which every year resembled year one, or every year resembled year two. Although not a realistic assumption, this allowed us to compare population dynamics under two different sets of observed conditions.

We used this numerical approximation to iterate the population vector $n(y,t)$ over time t until it reached the asymptotic population distribution, which we defined as the point at which change in total population size from t to $t+1$ was less than 10^{-9} (Ellner and Rees 2006). The population growth rate λ at that point was considered the asymptotic population growth rate. The IPM and all statistical models for vital rates and neighbor effects were run in R (version 2.9.2, R Development Core Team 2009; R code for the IPM was based on code provided by Ellner and Rees 2006).

4.3 Results

4.3.1 Functions for vital rates and neighbor density effects

Results for growth and survival probability as a function of plant size and ant density were previously reported (see Chapter 2) and will not be repeated here. Results

from the statistical models fit for all vital rates and neighbor density functions are summarized in Table 4-1. The best fitting model for fecundity (total seedlings produced per adult tree) used squared stem diameter (Figure 4-1; $LR= 126, df=1,74, P<0.001$). We used a zero-truncated normal distribution to approximate the size distribution of plants aging from the discrete seedling class into the continuous size distribution (Figure 4-2; year one mean \pm SD: 1.59 ± 0.641 , year two: 0.99 ± 0.291).

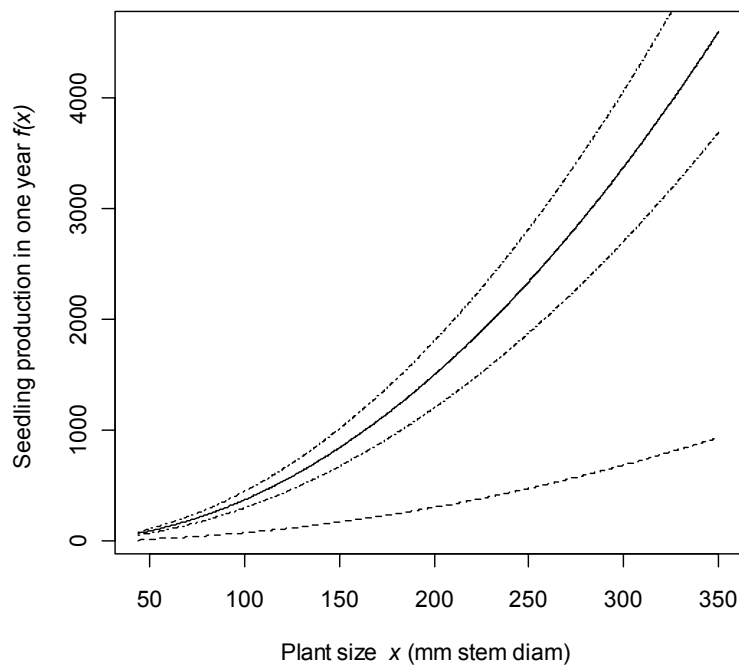


Figure 4-1: Fecundity values as a function of plant size The solid line gives the total number of seedlings produced by adults of different sizes in a given year, with 95% confidence intervals (dotted lines). The lower dashed line shows the contribution of plants of size x to the seedling class in the next year, incorporating 6-month adult survival (a function of size and ants) from the census date to the fruiting period and 6-month seedling survival from germination to the next census.

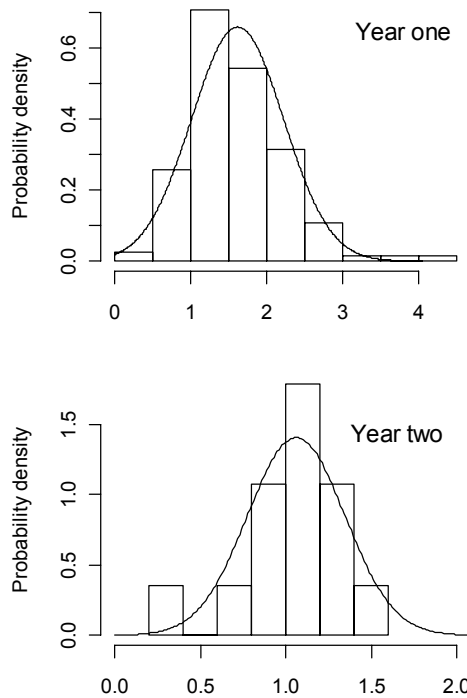


Figure 4-2: Size distribution of 1.5 year old juveniles, the point at which seedlings age into the continuous size distribution. Lines show the zero-truncated normal distribution (function $d(y)$ in the model); histograms give the observed distribution of sizes in year 1 ($n=147$) and year 2 ($n=14$). Both lines and histograms are normalized so that the area under the curve equals one.

The effects of local neighbor density on ant attendance were also previously reported (see Chapter 3), and include both a small-scale competition effect (within a 1m radius) and a larger scale facilitation effect at the 5x5 m plot scale (Table 4-1). The small-scale competition is size-based, with a positive interaction between focal plant size and neighbor density. Individuals greater than 11.36 mm in diameter actually gain ants from neighbors (i.e. experience a net positive coefficient for local neighbor density). The distribution of neighbor densities was found to vary with plant size, with larger individuals experiencing lower weighted local neighbor density (Figure 4-3, linear

model fitting exponential parameter λ by plant size, year and the interaction between the two, $F=9.118$, $df=3,36$, $P < 0.001$). We chose an exponential distribution over the gamma distribution on the basis of likelihood ratio tests comparing the two nested models (adding the extra parameter for the gamma does not improve the model; year 1: $LR = -34.07$, $df=1$, $P=1$, year 2: $LR = -29.72$, $df=1$, $P=1$).

Neighbor density incorporated both neighbor size and number of neighbors, but the decrease in neighbor density seems to be due to the presence of fewer neighbors for larger focal plants (correlation of number of neighbors and focal plant size: -0.261 in year 1, -0.271 in year 2).

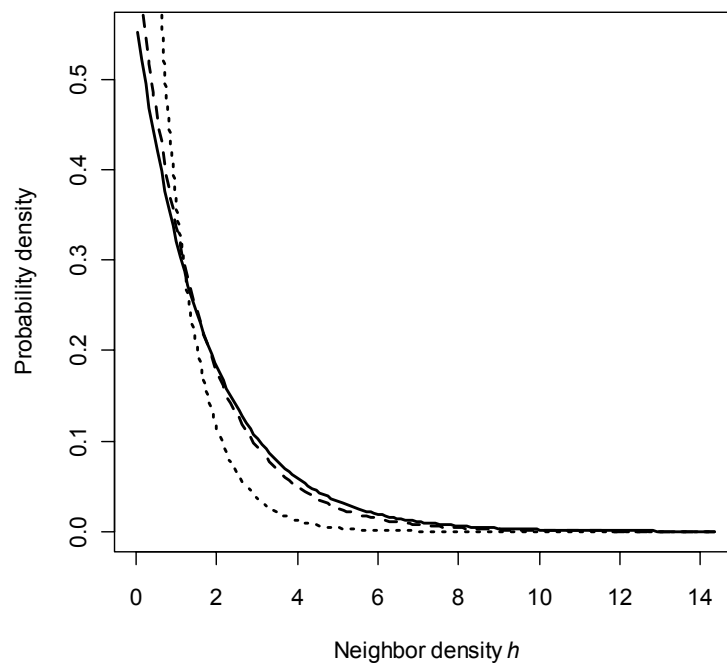


Figure 4-3: Exponential distributions of neighbor density. Lines show the fitted exponential distributions $p(x,h)$ for the minimum plant size ($x=0.32$ mm, solid line), the median plant size ($x=5.86$ mm, dashed line) and the maximum plant size ($x=313.5$ mm, dotted line).

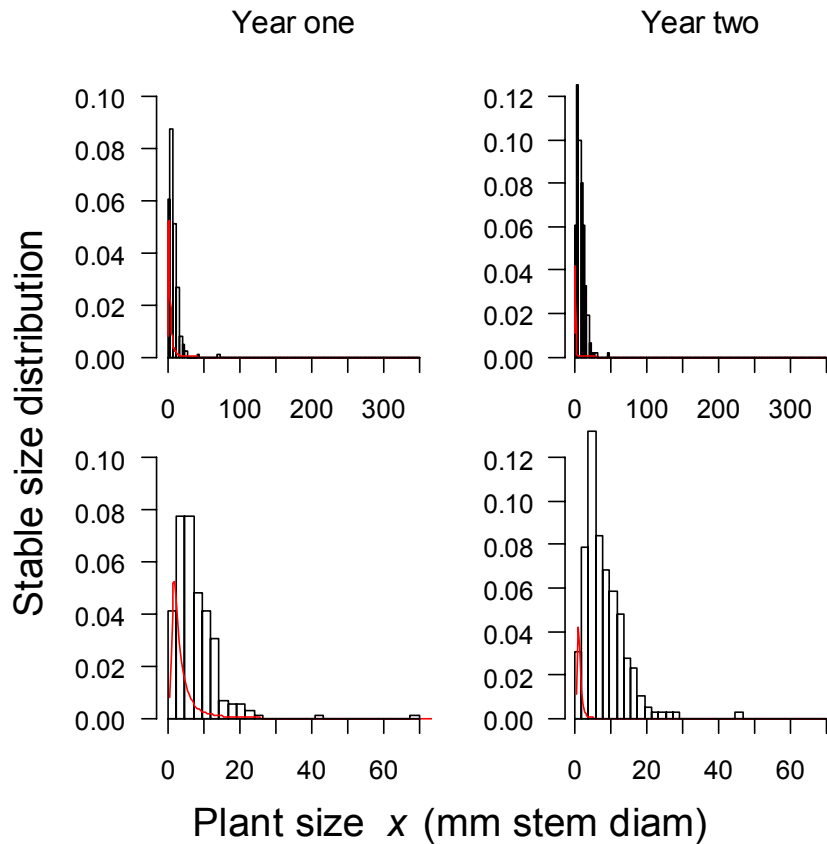


Figure 4-4: Stable size distribution, excluding seedling class. Lines show the stable size distribution predicted using vital rates from year one (left plots) and year two (right plot), excluding seedlings. Competition and facilitation for ants and mean ant densities were set at observed experimental levels. Histograms give the observed distribution of plants in control plots at the end of year one ($n=247$ plants) and year two ($n=228$ plants). The top set of plots uses the full range of plant sizes from the model; the bottom set shows the same data but focuses on the range of sizes observed in the experimental plots (max: 71.28 mm stem diameter). Both the predicted distributions and the histograms are normalized so that the area under the curve plus the seedling class sums to 1. Total area of the predicted distributions appears smaller than that of the histograms because the predicted seedling class was much larger than the observed seedling class, thus leaving a smaller proportion of the population to be distributed among plants in the continuous size class.

4.3.2 Population growth and stable size distribution

The model incorporating competition and facilitation for ants at experimentally observed levels predicted a similar distribution of sizes to that observed in the field, although values matched more closely for year one estimates than for year two (Figure 4-4). The predicted proportion of individuals in the seedling class differed from observed values, however. Using year one values, the seedling class was predicted to be 0.545 of the population, and the observed proportion was 0.782. With year two values the predicted stable proportion of seedlings was 0.781, compared to an observed value of 0.366. Predicted stable population growth (λ) varied slightly between models using vital rates from years one and two, with a slightly higher value of λ in year one. Both were close to $\lambda = 1$, which would indicate a population that is neither growing nor declining (Table 4-2).

Table 4-2: Effects of intraspecific competition for mutualists on stable population growth rate Population growth rates are the stable growth rates predicted using vital rates for year one and two. Strength of competition was varied (rows of table) by changing both the negative neighbor density coefficient and the positive size by neighbor interaction coefficient by the same factor c .

Strength of competition for mutualists	Population growth rate	
	Year 1	Year 2
No competition ($c = 0$)	1.02295	1.01019
Observed levels of competition ($c = 1$)	1.02334	1.01034
Increased competition ($c = 10$)	1.02621	1.01112

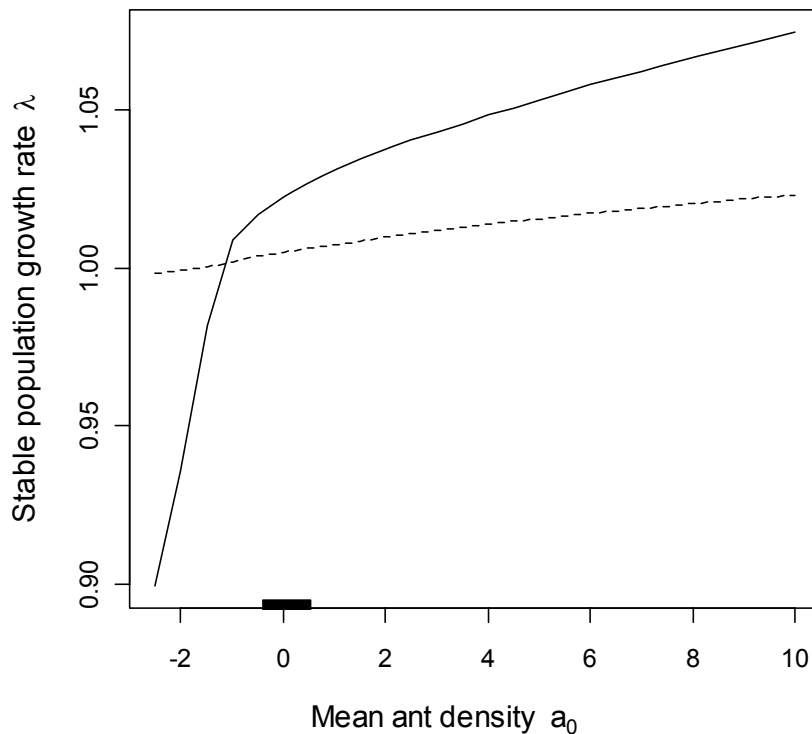


Figure 4-5: Effects of ants on stable population growth rate. Competition and facilitation effects were set to zero for the models shown here. Ant density is thus equal to the mean ant density (a_0) for plants of all sizes. Lines give the asymptotic population growth rate (λ) in year one (solid line) and year two (dashed line). The horizontal line on the x axis gives the range of mean ant densities in control plot (plot-level means; ant densities on individual plants in control plots were observed to take all values shown).

4.3.3 Effects of ants on long-term population growth

The stable population growth rate (λ) increased with increasing ant density (a_0) in the absence of competition or facilitation for mutualists, (Figure 4-5). This relationship differed between experimental years, with a much stronger response of population growth rate to increasing ant densities in year one than in year two. The relationship of population growth rate to ant density did not change when competition for mutualists

was added; competition had very little effect on population growth rate at all ant densities (Appendix D, Table D-1) and did not affect the nonlinearity of the relationship between ant density and population growth rate in year one (results not shown). For both year one and year two, population growth rates cross $\lambda=1$ (indicating a population that is neither growing nor declining) at intermediate ant densities (Figure 4-5).

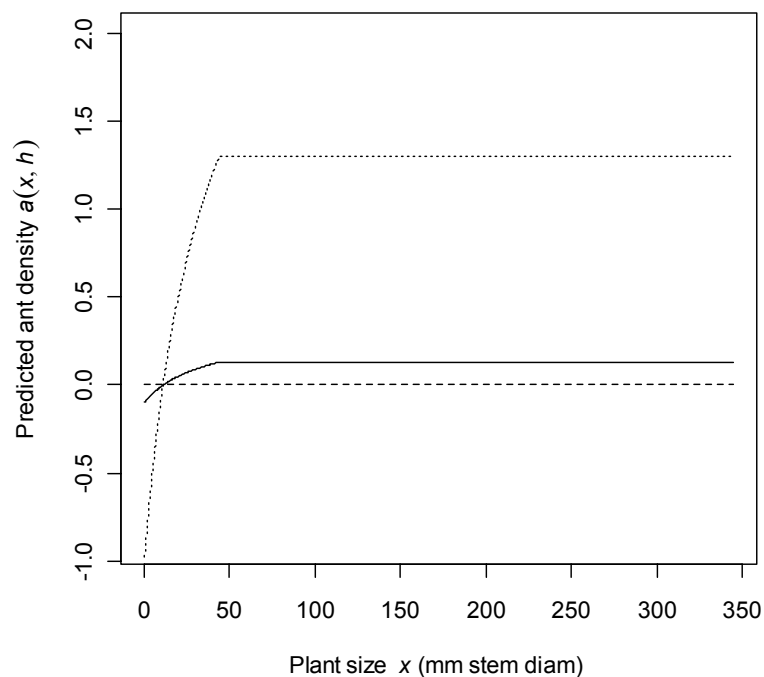


Figure 4-6: Effects of competition on ant density. Ant density is a function of plant size and conspecific neighbor density. An adjusted ant density of 0 indicates that an individual has the expected ant density given its size. Plot-level facilitation (at experimentally observed levels) is not included here, giving a constant adjusted mean ant density of 0 in the absence of competition (dashed line). Adding size-based competition at the magnitude observed in field experiments (solid line), or at 10 times the observed magnitude (dotted line) leads to a redistribution of ants, with larger individuals receiving more ants and smaller individuals receiving fewer. For this figure neighbor density h was set at the mean value of h for each plant size x ; in the model it was integrated over the probability distribution of all values of h

The range of ant densities used (-2.5 to 10) represented the range observed over all plants, manipulated and untreated, from the minimum value to the 99th percentile. The strongest effects of ant densities on population growth rates occurred over low values of ants (Figure 4-5). Control plots had a mean \pm SE plot-level ant density of -0.0417 ± 0.153 and a range of $[-0.401, 0.520]$ ($n=5$ plots; Figure 4-5, horizontal line on x-axis), while ant-exclusion plots had a mean \pm SE ant density of -0.866 ± 0.246 and a range of $[-1.65, -0.389]$ (plot-level means include both ant-excluded and untreated individuals in those plots).

4.3.4 Effects of competition and facilitation for ants on long-term population growth

Intraspecific competition affected the ant densities predicted for plants of different sizes, resulting in lower ant densities for smaller plants and higher ant densities for larger plants (Figure 4-6). The negative neighbor effect and the positive neighbor by size interaction summed to zero for a plant of size 11.36 mm (Figure 4-6, intersection point of three lines). Therefore plants greater than 11.36 mm in diameter benefit from competition for mutualists, whereas those smaller than 11.36mm lose ants when competition occurs. However, intraspecific competition for mutualists had little effect on long-term population growth (Table 4-2). There was little difference in population growth rate among models that set competition to either zero, experimental levels, or 10 times experimental levels. Stable population distributions were also very similar (Appendix D, Figure D-1).

Facilitation at the plot level raised the mean level of ants equally for plants of all sizes, so facilitation effects can be inferred from the results of changing mean background ant levels (Figure 4-5). Comparing asymptotic population growth rates λ among different plot-level plant densities, population growth rate increased with increasing plot-level plant densities, using year one parameters (Table 4-3). The magnitude of the facilitation effect depended on the background ant densities (a_0), with a stronger facilitation effect present at low background ant densities, as a result of the nonlinear relationship between ant density and stable population growth rate in year one (Figure 4-5).

Table 4-3: Facilitation effects on population growth rate (λ) at different background ant densities. Rows show a facilitation effect of increasing population growth as plot-level plant densities increase. The mean \pm SD observed plant density (summed mm stem diameter per 25 m²) was 978.71 \pm 382.5 (n=5 plots); the maximum observed density was 1729.42. Note that plant densities are observed densities unweighted by nectar manipulation treatments. Facilitation response varies with background ant density (columns), with a stronger facilitation effect at low ant densities. The columns give three levels of background ant density, from the minimum (equivalent to ant-excluded plots) to the ant density observed in unmanipulated plots to the 95th percentile of ant densities on all plants. All data are based on estimates from year one.

Plant density in 5x5 m plots	Mean ant levels (a_0)		
	Minimum observed ($a_0=-2.5$)	Control ($a_0=0$)	95%ile of all plants ($a_0=9.5$)
0 (No facilitation)	0.89969	1.02236	1.07239
Mean observed density	0.91993	1.02489	1.07349
Maximum observed density	0.93545	1.02684	1.07434

4.4 Discussion

This study is one of few to look at population-level effects of intraspecific competition or facilitation by mutualists (Breton and Addicott 1992, Morales 2000, Holbrook and Schmitt 2004). Intraspecific density effects on pollinator visits (e.g. Sih and Baltus 1987, Feldman 2008) and pollen limitation (reviewed in Knight et al. 2005) have been extensively studied in animal-pollinated plants, although fewer studies have looked at whether this in fact leads to demographic consequences for the plants (Groom 1998, Hackney and McGraw 2001, Horvitz et al. 2010). We found that although individuals in the population experienced both competition and facilitation via mutualists, facilitation had much stronger long-term demographic effects for *C. spinosa*. If facilitation effects tend to occur over longer temporal scales than competition, as might be expected if they are due to population responses of the partner species (Waser and Real 1979, Rathcke 1983), then facilitation effects might dominate demographic effects in other mutualisms as well.

This work is also unique in using an IPM rather than a projection matrix to model demography as a function of mutualists. IPMs have been used to model population growth as a function of environmental gradients and herbivory treatments and gradients (Hegland et al. 2010, Williams et al. 2010), but to our knowledge this is the first example of incorporating effects of mutualists into an IPM. Population-level studies of mutualisms in general are still relatively rare (Bronstein 1994, Stanton 2003), and even

fewer use the observed effects to then model long-term consequences for populations (Frederickson and Gordon 2009, Horvitz et al. 2010) .

In the following paragraphs we discuss differences between experimental years in population growth rate and the effects of ants on population growth, how density dependence was treated in our model, whether the relative strength of competition and facilitation effects might be due to differences in temporal scale, and the consequences of our results in terms of the importance of this mutualism for *C. spinosa*.

4.4.1 Interannual variation in population dynamics

Models using vital rates estimated from years one and two predicted different stable size distributions as well as different relationships between mean ant density and asymptotic population growth. In both years one and two, the predicted stable size distribution was skewed towards many small individuals and few large individuals, as observed in the field. However, the predicted densities were lower than the observed densities for individuals smaller than approximately 20mm in stem diameter. It is possible that the field population is not currently at its stable size distribution. In addition, very few large adults were sampled due to the small sample area and the rarity of the largest trees, so the field data might overestimate the frequency of small juveniles relative to their true frequency.

The year two models in particular predicted lower densities for all plants in the continuous size distribution (Figure 4-4), due to the fact that it predicted a much larger

seedling class than observed. The mean size of 1.5 year old individuals was lower in year two than year one (Figure 4-2) and seedling mortality was higher. Thus when the seedling class aged into the continuous size distribution it contributed less and to a smaller range of sizes compared to year one. We were unable to include different fecundity values by year, and it is possible that production of new seedlings is correlated with seedling survival. This model assumed constant vital rates (at year one or year two values) and constant mean local ant density over time. Adding stochasticity so that vital rates and ant densities are allowed to vary from year to year as the model is iterated over time would be more biologically realistic.

The response of population growth rate λ to background ant levels was much stronger in year one; in year two λ took a smaller range of values over the same range of ant densities. This may be due in part to the fact that ant-independent growth and survival of large plants are much higher in year two (intercept, size by year and year two coefficients, Table 4-1), and thus increasing ant densities might have little effect. For example, if ant-independent survival probability is already very close to one then increasing ant densities cannot further increase survival.

4.4.2 Density dependence in the IPM

Facilitation effects depended on plot-level plant density, but there were no other effects of plant density on vital rates in the model we used. The mean plot-level plant density was used to find the strength of facilitation in the model, rather than using

current population size at time t to set plant density at that time. When ant effects are not considered, population growth rate does not depend on the current population size. This is a reasonable assumption at low population sizes, but at some population size, population growth rate must begin to decrease with further increase in population size, independent of mutualist effects. A density-dependent model was not used here because sample sizes were too small to fit ant-independent density effects. Considering only ant-excluded plants, there was no evidence of non-mutualist-mediated effects of neighbor density on growth (local neighbor density coefficient: -0.0308 , $LR = -14.44$, $df = 1$, $P = 1$, linear mixed effects model). Seedling recruitment is one likely source of negative density dependence. Seedlings experienced extremely high mortality in the first year, and 6-month seedling survival decreased with increasing initial seedling density at time of germination, although there was no effect of juvenile density on seedling mortality (log initial seedling density coefficient: -0.120 , $LR = 8.24$, $df = 1, 4$, $P = 0.004$; linear mixed effects model, arcsine square root transformed 6-month survival probability regressed on log initial seedling density per plot and year). Adding density dependence of seedling survival might improve the population model.

The competition model also assumed that conspecific neighbor densities do not change with changing population size. The neighbor distribution is a function of plant size, so the size distribution may impact neighbor densities (populations with relatively more large individuals experience lower mean neighbor density). Over a short time

scale it is reasonable to assume that neighbor densities will remain constant. We use the model to quantify the current rate of population growth, and how it is affected by competition and facilitation for mutualists given the current distribution of neighbors.

4.4.3 Comparison of competition and facilitation effects: differences in temporal scale

Population growth rate increased with increasing mean background ant levels, which depended on facilitation strength and local conspecific densities at the 25m² scale. Therefore facilitation for ants, in which higher local plant densities leads to higher mutualist ant densities in an area, led to an increased population growth rate.

Facilitation is assumed to be intraspecific in this model, but the results could apply to conspecific or heterospecific facilitation for ants. Previous work on *C. spinosa* did not find a statistically significant effect of heterospecific EFN plant density (in 5x5m plots) on the ant density experienced by individuals in those plots (Chapter 3). However, heterospecific neighbors were not manipulated, and it is possible that some but not all neighboring EFN species impact ant density on *C. spinosa*. The strength of conspecific facilitation depended on background ant density, and if background ant density were affected by heterospecific neighbors, this might lead to interesting interactions between conspecific and heterospecific facilitation effects. As with conspecific facilitation, heterospecific facilitation effects must saturate at some heterospecific density, to avoid limitless population growth resulting from the positive density dependence.

In contrast to facilitation, size-based competition for mutualists had very little effect on long-term population growth, even when increased to levels 10 times those observed in the field. Although competition effects did cause a redistribution of mutualist ants among individuals of different sizes (Figure 4-6), this did not lead to changes in the stable size distribution (Appendix D, Figure D-1) or stable population growth rate (Table 4-2). The competition coefficients were found using observational data over the two years of plant censuses. Short-term (2 month) manipulations of small groups of *C. spinosa* juveniles found much stronger competition effects, approximately 150 times the long-term observational values (see Chapter 3). The short-term manipulations were for lower neighbor densities and smaller focal plants. If neighbor effects were nonlinear, with the strongest competition effects occurring at low neighbor densities, fitting a linear effect over a larger range would artificially depress the effects of competition. However, there was no evidence of a nonlinear effect of neighbors in the two-year manipulation experiment manipulation plot data (see Chapter 3).

It is perhaps not surprising that there was a stronger facilitation than competition effect on population dynamics of *C. spinosa*. The difference in strength of competitive effects between the two-month and two-year experiments (see Chapter 3) might be due to the different time scales; over longer time periods facilitation effects at a larger spatial scale might dominate and obscure smaller-scale competitive effects. We determined population effects using asymptotic population growth rates, so over those long time

scales it might be reasonable to expect facilitation effects to dominate. Transient population growth rates might be a more informative measure (Maron et al. 2010) of the effects of competition on the current field populations of *C. spinosa*, in particular because the population does not appear to be at its stable size distribution.

4.4.4 Consequences of the mutualism for *C. spinosa*

Another interpretation of the result that population growth rate increases with increasing mean ant density is that the mutualism with ants is important for *C. spinosa* populations. In the complete absence of ants, *C. spinosa* populations are expected to eventually decline to zero under this model ($\lambda < 1$ for $a_0 < -1$, equivalent to ant densities on ant-excluded plants). Although the mutualism between *C. spinosa* and its ant visitors is an extremely diffuse, generalized interaction, it is ecologically important for the persistence of the species. In fact, by the classic definition that obligate mutualism an interaction in which population size crosses zero at positive densities of the mutualist partner (i.e. stable population size is negative in the absence of mutualists; Vandermeer and Boucher 1978), this could be considered an obligate mutualism. Effect of ants varied strongly by year, and averaging over many years and different populations would be necessary to evaluate whether this EFN plant-ant mutualism might be obligate for the plant. However, this result does suggest how critical it is to consider population dynamics and not merely individual-level effects in the study of mutualism and other interspecific interactions.

Appendix A: Ant visitors to *Colubrina spinosa*

Table A-1: Ant visitors to *C. spinosa* by species. Ant species are listed from most abundant to least abundant on plants in control plots only (n=251 observations with ants present in control plots, out of 1834 total observations on 264 plants, observations pooled over 2 years). Ants that were not identified to species (usually because collection was not possible) are listed by genus at the bottom of the table; these ants are most likely from the observed species, not additional unknown species. Species listed as Longino ms. and Ward ms. have not yet been described (J.T. Longino pers. comm., P. S. Ward pers. comm.).

Ant species	Mass of 1 minor worker (major worker) in mg	Proportion of observations	Proportion of total number of ants
<i>Ectatomma ruidum</i>	10.0	0.187	0.208
<i>Solenopsis picea</i>	0.0941	0.104	0.146
<i>Wasmannia scrobifera</i>	0.08 ¹	0.072	0.087
<i>Brachymyrmex JTL-007</i> Longino ms.	0.02	0.068	0.084
<i>Pheidole specularis</i>	0.11 (0.633)	0.040	0.112
<i>Crematogaster carinata</i>	0.167	0.036	0.062
<i>Tapinoma ramulorum</i>	0.233	0.036	0.040
<i>Apterostigma collare</i> *	1.0	0.024	0.019
<i>Camponotus fastigatus</i>	6.15	0.024	0.019
<i>Pheidole olsoni</i>	0.11 (0.633) ³	0.020	0.025
<i>Brachymyrmex longicornis</i>	0.02 ²	0.016	0.019
<i>Pheidole flavens</i>	0.11 (0.633) ³	0.016	0.016
<i>Pseudomyrmex tenuis</i>	2.133	0.016	0.012
<i>Wasmannia iheringi</i>	0.08 ¹	0.016	0.012
<i>Pachycondyla lineaticeps</i>	12.7	0.012	0.012
<i>Pseudomyrmex oculatus</i>	0.45 ⁵	0.012	0.009
<i>Azteca</i> sp.	0.25	0.008	0.006

^a Observed on unmanipulated plants, but not in control plots

^b Observed only on treated plants (ant exclusion or nectar supplement); previously observed on *C. spinosa* at La Selva

^c Not observed in this study; previously observed on *C. spinosa* at La Selva

¹ estimated from *Wasmannia auropunctata*

² estimated from *Brachymyrmex JTL-007* Longino ms.

³ estimated from *Pheidole specularis*

⁴ estimated from *Crematogaster carinata*

⁵ estimated from *Pseudomyrmex simplex*

⁶ estimated from *Paratrechina steinhelli*

⁷ estimated from *Pheidole hirsuta*

⁸ estimated from *Pheidole subarmata*

⁹ estimated from *Solenopsis picea*

¹⁰ estimated from *Camponotus fastigatus*

¹¹ estimated from *Pachycondyla unidentata*

¹² estimated from *Pachycondyla lineaticeps*

* fungus-growing ant species; likely to be parasites

Ant species	Mass of 1 minor worker (major worker) in mg	Proportion of observations	Proportion of total number of ants
<i>Dolichoderus validus</i>	5.3	0.008	0.022
<i>Pachycondyla unidentata</i>	5.0	0.008	0.006
<i>Paratrechina JTL-006</i> Longino ms.	0.2375 ⁶	0.008	0.006
<i>Pheidole scaralis</i>	0.11 (0.633) ³	0.008	0.009
<i>Pseudomyrmex simplex</i>	0.45	0.008	0.006
<i>Wasmannia auropunctata</i>	0.08	0.008	0.009
<i>Aphaenogaster aranoides</i>	1.0	0.004	0.003
<i>Brachymyrmex JTL-010</i> Longino ms.	0.02 ²	0.004	0.006
<i>Cephalotes curvistriatus</i>	0.7	0.004	0.003
<i>Cephalotes multispinosus</i>	2.4	0.004	0.003
<i>Crematogaster linata</i>	0.167 ⁴	0.004	0.012
<i>Dolichoderus lutosos</i>	1.4	0.004	0.003
<i>Nesomyrmex pittieri</i>	0.3	0.004	0.003
<i>Pachycondyla carinulata</i>	5.5	0.004	0.003
<i>Pachycondyla striatinodis</i>	5.0 ¹¹	0.004	0.003
<i>Paraponera clavata</i>	140.6	0.004	0.003
<i>Pheidole cephalica</i>	1.3	0.004	0.003
<i>Pheidole simonsi</i>	0.35 (0.37) ⁷	0.004	0.003
<i>Tapinoma melanocephalum</i>	0.1	0.004	0.003
<i>Paratrechina steinheili</i>	0.2375	0 ^a	0 ^a
<i>Pheidole prostrata</i>	0.35 (0.37) ⁷	0 ^a	0 ^a
<i>Acanthoponera minor</i>	2.7	0 ^b	0 ^b
<i>Atta cephalotes</i> *	0.3	0 ^b	0 ^b
<i>Brachymyrmex pictus balboae</i>	0.02 ¹	0 ^b	0 ^b
<i>Camponotus atriceps</i>	6.2	0 ^b	0 ^b
<i>Camponotus pittieri</i>	NA (0.68)	0 ^b	0 ^b
<i>Crematogaster brasiliensis</i>	0.167 ⁴	0 ^b	0 ^b
<i>Crematogaster sotobosque</i>	0.25	0 ^b	0 ^b
<i>Pachycondyla crenata</i>	5.0	0 ^b	0 ^b
<i>Pachycondyla stigma</i>	0.7	0 ^b	0 ^b

^a Observed on unmanipulated plants, but not in control plots

^b Observed only on treated plants (ant exclusion or nectar supplement); previously observed on *C. spinosa* at La Selva

^c Not observed in this study; previously observed on *C. spinosa* at La Selva

¹ estimated from *Wasmannia auropunctata*

² estimated from *Brachymyrmex JTL-007* Longino ms.

³ estimated from *Pheidole specularis*

⁴ estimated from *Crematogaster carinata*

⁵ estimated from *Pseudomyrmex simplex*

⁶ estimated from *Paratrechina steinheili*

⁷ estimated from *Pheidole hirsuta*

⁸ estimated from *Pheidole subarmata*

⁹ estimated from *Solenopsis picea*

¹⁰ estimated from *Camponotus fastigatus*

¹¹ estimated from *Pachycondyla unidentata*

¹² estimated from *Pachycondyla lineaticeps*

* fungus-growing ant species; likely to be parasites

Ant species	Mass of 1 minor worker (major worker) in mg	Proportion of observations	Proportion of total number of ants
<i>Pachycondyla villosa</i>	12.7 ¹²	0 ^b	0 ^b
<i>Paratrechina longicornis</i>	0.32	0 ^b	0 ^b
<i>Pheidole hirsuta</i>	0.35 (0.37)	0 ^b	0 ^b
<i>Pheidole nitidicollis</i>	0.11 (0.633) ³	0 ^b	0 ^b
<i>Pheidole prattorum</i>	0.15 (0.90) ⁸	0 ^b	0 ^b
<i>Pheidole protensa</i>	0.11 (0.633) ³	0 ^b	0 ^b
<i>Pheidole subarmata</i>	0.15 (0.90)	0 ^b	0 ^b
<i>Pheidole synarmata</i>	0.15 (0.90) ⁸	0 ^b	0 ^b
<i>Solenopsis geminata</i>	0.594	0 ^b	0 ^b
<i>Solenopsis sp. 1</i>	0.0941 ⁹	0 ^b	0 ^b
<i>Brachymyrmex heeri</i>	0.02 ²	0 ^c	0 ^c
<i>Camponotus brettlesi</i>	3.5	0 ^c	0 ^c
<i>Camponotus novogranadensis</i>	6.15 ¹⁰	0 ^c	0 ^c
<i>Camponotus sp. 1</i>	2.2	0 ^c	0 ^c
<i>Ectatomma tuberculatum</i>	16.7	0 ^c	0 ^c
<i>Odontomachus erythrocephalus</i>	10.2	0 ^c	0 ^c
<i>Pachycondyla apicalis</i>	12.7 ¹²	0 ^c	0 ^c
<i>Pheidole boruca</i>	0.15 (0.90) ⁸	0 ^c	0 ^c
<i>Pheidole nebulosa</i>	0.11 (0.633) ³	0 ^c	0 ^c
<i>Pheidole nigricula</i>	0.11 (0.633) ³	0 ^c	0 ^c
<i>Pseudomyrmex PSW-36</i> Ward ms.	5.1	0 ^c	0 ^c
<i>Rogeria tonduzi</i>	0.45	0 ^c	0 ^c
<i>Pheidole spp.</i>	NA	0.135	0.177
<i>Brachymyrmex spp.</i>	NA	0.048	0.056
<i>Paratrechina spp.</i>	NA	0.008	0.006
<i>Crematogaster spp.</i>	NA	0.004	0.003

^a Observed on unmanipulated plants, but not in control plots

^b Observed only on treated plants (ant exclusion or nectar supplement); previously observed on *C. spinosa* at La Selva

^c Not observed in this study; previously observed on *C. spinosa* at La Selva

¹ estimated from *Wasmannia auropunctata*

² estimated from *Brachymyrmex JTL-007* Longino ms.

³ estimated from *Pheidole specularis*

⁴ estimated from *Crematogaster carinata*

⁵ estimated from *Pseudomyrmex simplex*

⁶ estimated from *Paratrechina steinheili*

⁷ estimated from *Pheidole hirsuta*

⁸ estimated from *Pheidole subarmata*

⁹ estimated from *Solenopsis picea*

¹⁰ estimated from *Camponotus fastigatus*

¹¹ estimated from *Pachycondyla unidentata*

¹² estimated from *Pachycondyla lineaticeps*

* fungus-growing ant species; likely to be parasites

Table A-2: Ant visitors to *C. spinosa* by genus. Ant genera are listed from most abundant to least abundant on plants in control plots only (n=251 observations with ants present, out of 1834 total observations on 264 plants, observations pooled over 2 years).

Ant genus	Number of species	Proportion of observations	Proportion of total number of ants
<i>Pheidole</i>	16	0.227	0.278
<i>Ectatomma</i>	2	0.187	0.168
<i>Brachymyrmex</i>	5	0.135	0.133
<i>Solenopsis</i>	3	0.104	0.118
<i>Wasmannia</i>	3	0.096	0.088
<i>Crematogaster</i>	4	0.044	0.063
<i>Tapinoma</i>	2	0.040	0.035
<i>Pseudomyrmex</i>	4	0.036	0.023
<i>Pachycondyla</i>	7	0.028	0.020
<i>Apterostigma</i>	1	0.024	0.015
<i>Camponotus</i>	6	0.024	0.015
<i>Paratrechina</i>	3	0.016	0.010
<i>Dolichoderus</i>	2	0.012	0.020
<i>Azteca</i>	1	0.008	0.005
<i>Cephalotes</i>	2	0.008	0.005
<i>Aphaenogaster</i>	1	0.004	0.003
<i>Nesomyrmex</i>	1	0.004	0.003
<i>Paraponera</i>	1	0.004	0.003
<i>Acanthoponera</i>	1	0 ^b	0 ^b
<i>Atta</i>	1	0 ^b	0 ^b
<i>Odontomachus</i>	1	0 ^a	0 ^a
<i>Rogeria</i>	1	0 ^b	0 ^b

^a Observed on unmanipulated plants, but not in control plots

^b Observed only on treated plants (ant exclusion or nectar supplement); previously observed on *C. spinosa* at La Selva

Appendix B: Supplemental Materials for Chapter 2

Table B-1: Model selection for computing residual ant density. Ant density (ants/mm stem diameter) was regressed against experimental year and plant stem assuming a zero-inflated negative binomial distribution. The models shown here are for the negative binomial, and all use the same zero-inflation model (binomial, with probability of a zero observation as a function of stem diameter). Models were compared using Akaike Information Criteria (AIC); the model with the lowest AIC is selected (bold text).

Model (fixed effects only)	AIC
Ant density ~ Year	6445
Ant density ~ Year + Diam	4534
Ant density ~ Year + Diam+ Diam ²	5953

Table B-2: Growth and survival by ant taxa. Models shown are linear (growth) or general linear (survival) mixed effects models. Different models separate each of the three of the most common ant taxa from all other ants, and are compared to a model using total ant density (null model, with asterisk). E.ruid is *Ectatomma ruidum*, Brachy is *Brachymyrmex spp.* and Pheid is *Pheidole spp.* Models were compared using Akaike Information Criteria (AIC); the model with the lowest AIC is selected (bold text). For both growth and survival, the best model considers all ant taxa together.

Model (fixed effects only)	AIC
Growth~Total Ants+Diam+Year *	-996.4
Growth~E.ruid + (Total Ants-Brachy) +Diam+Year	-992.9
Growth~Brachy + (Total Ants-Brachy) +Diam+Year	-993.2
Growth~Pheid + (Total Ants-Brachy) +Diam+Year	-991.1
Logit (Survival)~Total Ants+Diam+Year *	787.5
Logit (Survival)~E.ruid + (Total Ants-Brachy) +Diam+	788.6
Logit (Survival)~Brachy + (Total Ants-Brachy) +Diam+	787.9
Logit (Survival)~Pheid + (Total Ants-Brachy) +Diam+	789.2

*null model

Table B-3: Growth and survival by experimental treatment. The growth model (a) is a linear mixed effects model, with random effects of site and plot nested in site. The survival model (b) is a logistic regression of plant fate (alive or dead) after one year. As with the ant density model, ant effects were only fit for plants greater than 3.5 mm in diameter (see text). Ant-excluded plants which did not have significantly fewer ants than control plants (n=8 plants) were not used in the analysis. The growth model used fewer data points than the survival model because growth was only measured for plants that survived to the end of the year.

a) Growth model

	<i>Estimate</i>	<i>LR</i>	<i>df</i>	<i>P</i>
(Intercept)	0.02786		1	
Year	0.01195	1.33	1	0.25
Size	-0.0013	3.66	1	0.055
Ants excluded	0.03015	7.26	2	0.027
Added nectar	0.00642			
Size x ants excluded	-0.0029	64.51	2	<0.0001
Size x added nectar	0.00062			
Residual df			1325	

b) Survival model

	<i>Estimate</i>	<i>LR</i>	<i>df</i>	<i>P</i>
(Intercept)	3.1833		1	
Year	-1.6573	65.96	1	<0.0001
Size	0.1338	32.75	1	<0.0001
Ants excluded	-0.0487	8.92	2	0.011
Added nectar	-0.7518			
Size x ants excluded	-0.1037	19.345	2	<0.0001
Size x added nectar	0.1600			
Residual df			1445	

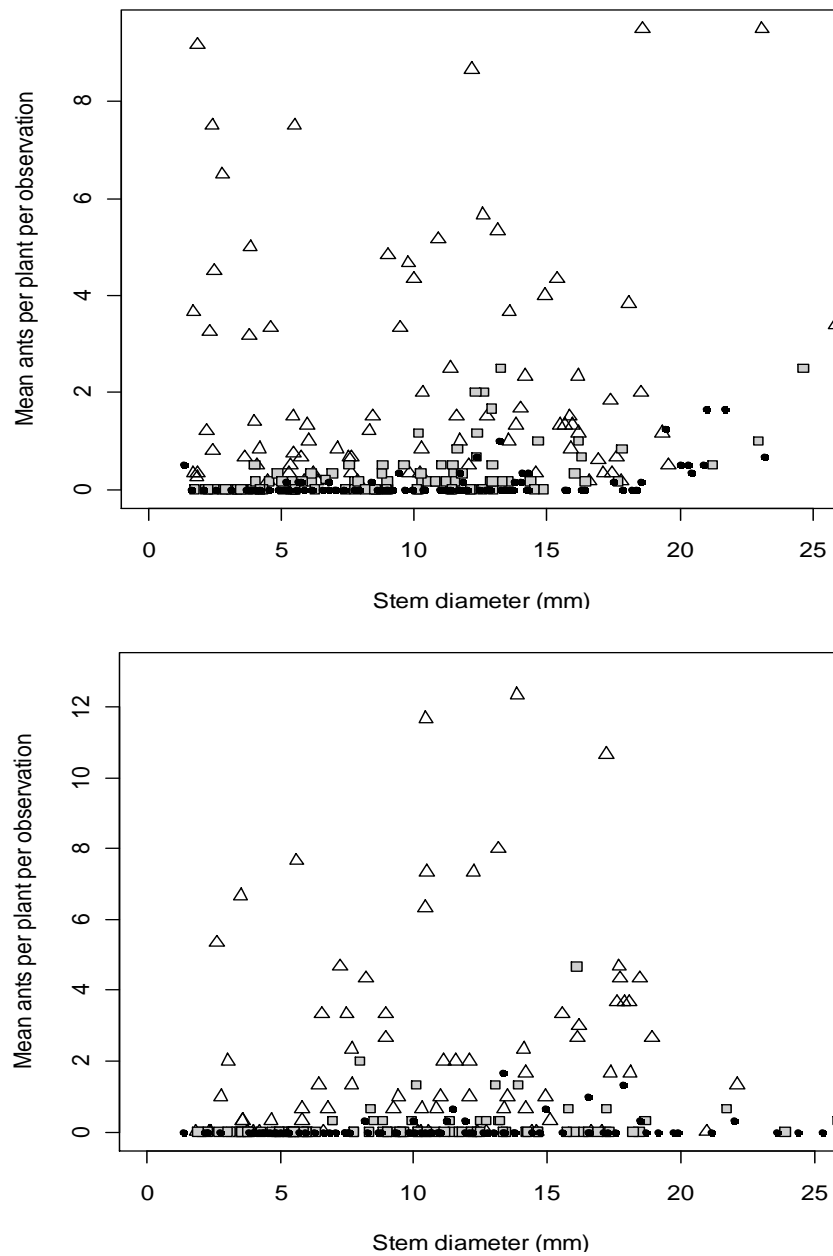


Figure B-1: Experimental treatments induced a continuous range of ant presence. For a given stem diameter, the natural range of ant visits to plants was observed on unmanipulated plants (grey squares), with more ants present on larger plants. Ant visits are near zero for all ant-excluded plants (black dots). Nectar supplementation (white triangles) increased ant visits to plants, independent of plant size. Each point is the mean number of ants observed per treated plant per observation over six observations in year 1 (top panel) or over three observations in year two (bottom panel, n=100 plants per treatment in both years).

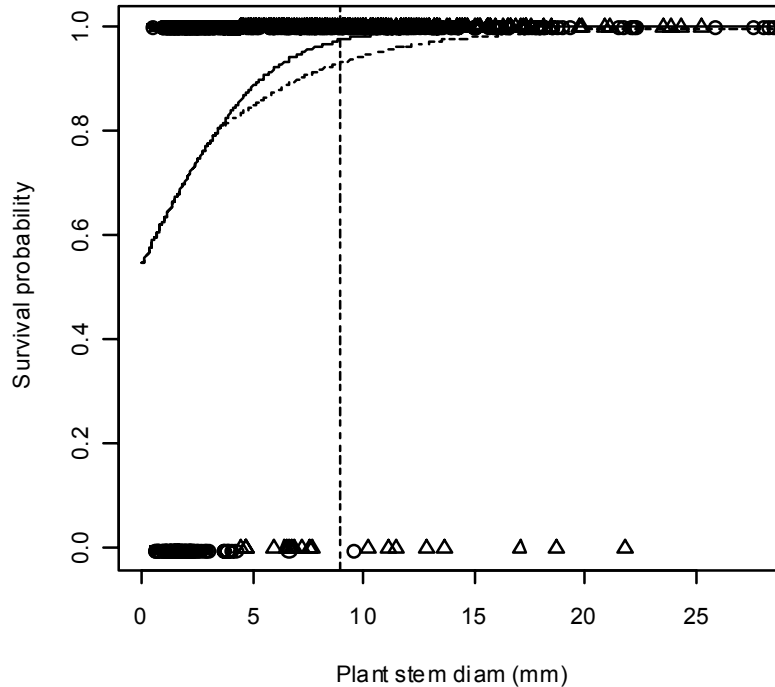


Figure B-2: Ant benefits to plant survival vary with plant size. Survival probability increases with plant size and residual ant density. Triangles show survival of plants with low residual ant density (less than the median, -0.103 , $n=328$ in year 2). Open circles show survival of plants with high residual ant density (greater than the median, $n=210$ in year 2). Filled circles are very small plants (stem diameter < 3.5 mm), and are not divided by ant abundance since there was no ant effect on survival for these plants ($n=258$ plants in year 2). Only year 2 data are shown, for clarity. Lines show results from the logistic regression at the 25% quantile of residual ant density (-0.178 , dotted line), and at the 75% quantile of ant abundance (0.0743 , solid line). The lines converge for plants smaller than stem diameter = 3.5 mm, as no ant effect was fit for those plants. The vertical dotted line shows the median diameter of plants > 3.5 mm (the division between the 'large' and 'small' plants in Figure 3).

Appendix C: Supplemental Materials for Chapter 3

Table C-1: Heterospecific EFN plants observed to co-occur with *C. spinosa* at La Selva Biological Reserve. Species listed were found growing within 2m of a *C. spinosa* individual in surveys of 120 juveniles, or observed in the 5x5m nectar manipulation plots. Note that this is not a complete list of the EFN species at La Selva, and many others could potentially co-occur with *C. spinosa*.

Family	Species
Costaceae	<i>Costus malortieanus</i> <i>Costus scaber</i>
Fabaceae	<i>Erythrina poeppigiana</i> ¹ <i>Inga jinicuil</i> <i>Inga leiocalycina</i> <i>Inga marginata</i> <i>Inga oerstediana</i> <i>Inga pezizifera</i> <i>Inga ruiziana</i> <i>Inga sapindoides</i> <i>Inga thibaudiana</i> <i>Inga tonduzii</i> <i>Inga umbellifera</i> <i>Leucaena multicapitula</i> ¹ <i>Pentaclethra macroloba</i> <i>Senegalia hayesii</i> <i>Stryphnodendron microstachyum</i> <i>Zygia gigantifoliola</i>
Passifloraceae	<i>Passiflora vitifolia</i>

¹ Introduced species at La Selva

Table C-2: Effects of large and small neighbor plants on focal plant ant density: fixed effects coefficients and likelihood ratio tests for the linear mixed effects model. This is equivalent to Table 3-1 but using number of neighbors larger and smaller than the focal plant instead of weighted neighbor density. Ant-excluded neighbors were not included in neighbor density since they did not share ants with the focal plant. Experimental replicate (Exp.) was included as a fixed effect, and focal plant identity was included as a random intercept (one control and 4 treatment replicates per focal plant group, n=30 groups).

	Estimate	<i>LR</i>	<i>df</i>	<i>P</i>
(Intercept)	14.40534		1	
N small neighbors	-3.27694	0.425	1	0.52
N large neighbors	-3.26047	1.24	1	0.27
Focal size (mm diameter)	-0.99324	0.295	1	0.59
Exp. 1	0.06672	5.90	4	0.22
Exp. 2	-1.32107			
Exp. 3	-2.63931			
Exp. 4	-1.75085			
Small neigh. x focal size	0.29759	1.16	1	0.28
Large neigh. x focal size	0.26721	0.916	1	0.34
Residual df			105	

Table C-3: Baiting abundance and dietary preference with nectar manipulation for different ant taxa Ant taxa are listed by increasing individual body size. The darkest shade highlights ant taxa which are most abundant on baits in the ant exclusion plots; the intermediate shade, taxa most abundant on control plot baits; and the lightest shade, taxa most abundant on baits in added nectar plots. Mean \pm SD ants per baiting observation were means of the 5 plots in each treatment, after summing all baiting sessions and baits in plots (15 plots, 4 baits/plot, 8 obs.) Dietary preference was computed as the $\log(\text{mean ants on sugar}) - \log(\text{mean ants on protein})$, using mean ant abundances for each treatment rather than for each plot, due to large numbers of zeros. A strong preference for protein gives large negative values; a strong preference for sugar, large positive values.

Taxon	Individual biomass (mg)	Mean ants \pm SE per baiting observation			Number of baiting observations with that taxon present			Dietary preference: $\log(\text{mean ants on sugar}) - \log(\text{mean ants on protein})$			
		Reduced EFNs	Control	Nectar added	Reduced EFNs	Control	Nectar added	Species mean	Reduced EFNs	Control	Nectar added
<i>Brachymyrmex spp.</i>	0.002	0.280 \pm 0.114	0.105 \pm 0.049	0.125 \pm 0.078	9	6	4	2.110	1.912	2.153	2.197
<i>Wasmannia spp.</i>	0.080	0.017 \pm 0.010	0.027 \pm 0.019	0.008 \pm 0.006	4	3	2	1.046	1.204	1.466	0.000
<i>S. picea</i>	9x10 ⁻³	1.583 \pm 0.701	0.603 \pm 0.323	0.210 \pm 0.114	17	13	8	-0.212	-0.170	-0.273	0.119
<i>Solenopsis spp.</i>	9x10 ⁻³	0.129 \pm 0.093	0.194 \pm 0.126	0.055 \pm 0.055	2	3	1	1.296	2.009	0.494	1.587
<i>Pheidole spp. (small)</i>	0.011	7.970 \pm 1.733	9.163 \pm 1.894	6.235 \pm 1.339	39	50	42	0.279	0.265	0.534	-0.016
<i>Crematogaster spp.</i>	0.017	0.064 \pm 0.043	0.088 \pm 0.088	0.331 \pm 0.301	3	1	4	-1.062	0.802	-1.514	-1.679
<i>Tapinoma spp.</i>	0.023	0.073 \pm 0.043	0.084 \pm 0.061	0.116 \pm 0.074	3	3	4	1.171	0.749	1.299	1.455
<i>Paratrechina spp.</i>	0.024	0.106 \pm 0.056	0.263 \pm 0.149	0.130 \pm 0.077	6	5	3	-0.146	-0.353	1.125	-0.717
<i>Pheidole spp. (large)</i>	0.035	0.172 \pm 0.137	0.215 \pm 0.185	0.025 \pm 0.018	3	2	2	0.491	1.164	-0.134	-0.167
<i>S. geminata</i>	0.059	0 ¹	0 ¹	0.403 \pm 0.283	0	0	3	-0.088	NA ¹	NA ¹	-0.088
<i>Pseudomyrmex spp.</i>	0.213	0.00208 \pm 0.00147	0.010 \pm 0.0044	0.017 \pm 0.00558	2	7	10	1.099	1.386	0.000	1.099
<i>Camponotus spp.</i>	0.615	0.030 \pm 0.022	0.032 \pm 0.019	0.056 \pm 0.034	3	3	5	1.322	0.460	2.603	1.482
<i>E. ruidum</i>	1.00	0.081 \pm 0.024	0.142 \pm 0.046	0.055 \pm 0.033	17	17	6	1.766	1.946	2.317	0.930
<i>Pachycondyla spp.</i>	1.27	0.00625 \pm 0.0036	0.016 \pm 0.0067	0.019 \pm 0.0066	4	6	10	0.580	1.386	0.693	0.000
<i>P. clavata</i>	14.06	0.00521 \pm 0.0031	0.078 \pm 0.048	0.152 \pm 0.056	3	7	13	2.227	1.012	Inf.	4.277

¹ no observations of that taxon

Table C-4: Contrasting competition and facilitation effects in 2-year plots: fixed effects coefficients and likelihood ratio tests using data from long-term manipulation plots. This is equivalent to Table 3-2 but using number of neighbors instead of size-weighted neighbor density as the measure of small-scale neighbor effects. The linear mixed effects model included random intercepts of sites, plots nested in sites and years, variance as an exponential function of plant size, and Gaussian spatial autocorrelation within plots. Both manipulated and unmanipulated plants were included, and plant treatment was included as a fixed effect.

	Coefficient	<i>LR</i>	<i>df</i>	<i>P</i>
(Intercept)	-0.018216		1	
Ants excluded from plant	-0.580266	169.2	6*	<.0001
Nectar added to plant	6.522073			
Reduced EFN plot	-0.582328	3.33	1	0.19
Nectar added plot	0.151791			
1m number of neighbors	-0.014724	49.0	6*	<.0001
Num. neighbors x focal size (diameter)	0.000127	-5.24	1	1
Num. neighbors x ants excluded	-0.060309	12.3	2	0.002
Num. neighbors x nectar added	1.022495			
Num. neigh. x ants exc. x size	0.007623	44.7	2	<0.0001
Num. neigh. x add. nectar x size	-0.050999			
Residual df			1021	

* *df* used to compare models, includes factor and all interactions of that factor

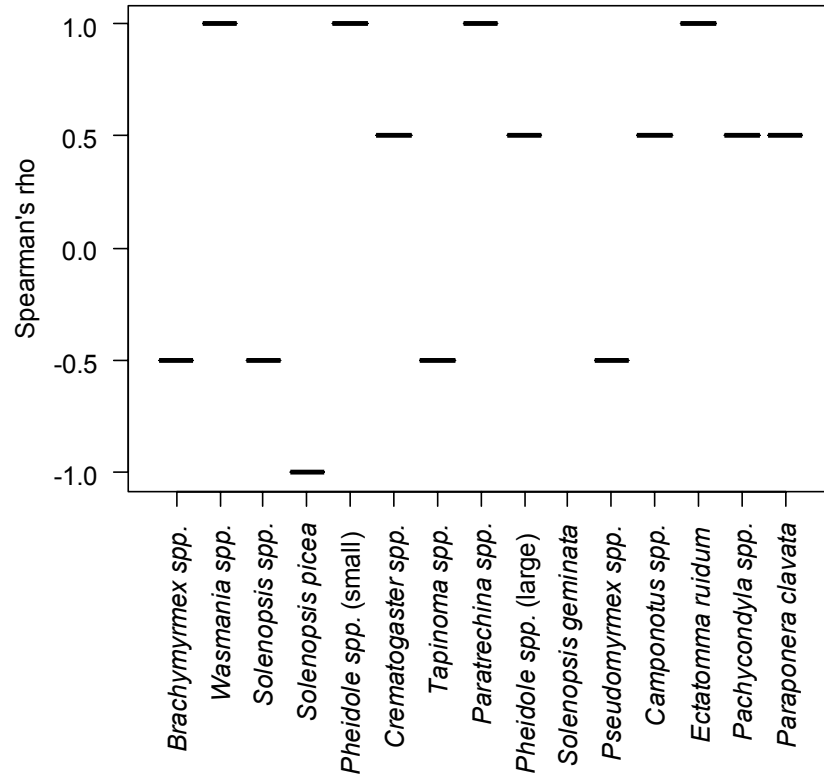


Figure C-1: Rank correlations of ant sugar:protein preference with plot nectar treatment for different ant taxa. Ant taxa are listed in order of ascending body size. The y axis gives the ranked correlation coefficient (Spearman's rho) for the change in sugar:protein preference with plot treatment. A value of 1 indicates the strongest preference for protein in the added nectar plots, intermediate preference in control plots, and weakest preference for protein in reduced EFN plots, as predicted by the hypothesis that ants will prefer protein when given increased access to sugar resources. A value of -1 indicates the opposite pattern, with strongest protein preference in reduced-EFN plots. Changes in protein preference with nectar resources varied greatly across ant species, and do not appear to correlate with ant body size or with changes in abundance across treatments.

Appendix D: Supplemental Materials for Chapter 4

Table D-1: Effects of competition on population growth rate at different background ant densities. Stable population growth rate (λ) is given for models with no competition ($c=0$) or experimentally observed competition ($c=1$) at different background ant densities (a_0 , in rows). Competition effects are minimal and do not appear to vary with background ant density.

Mean ant density (a_0)	Year 1		Year 2	
	No competition ($c=0$)	Competition ($c=1$)	No competition ($c=0$)	Competition ($c=1$)
-2.5	0.8997	0.9011	0.9980	0.9983
-1.5	0.9816	0.9919	1.0004	1.0006
0 (control)	1.0230	1.0233	1.0102	1.0103
5	1.0530	1.0533	1.0155	1.0155
10	1.0744	1.0745	1.0230	1.0230

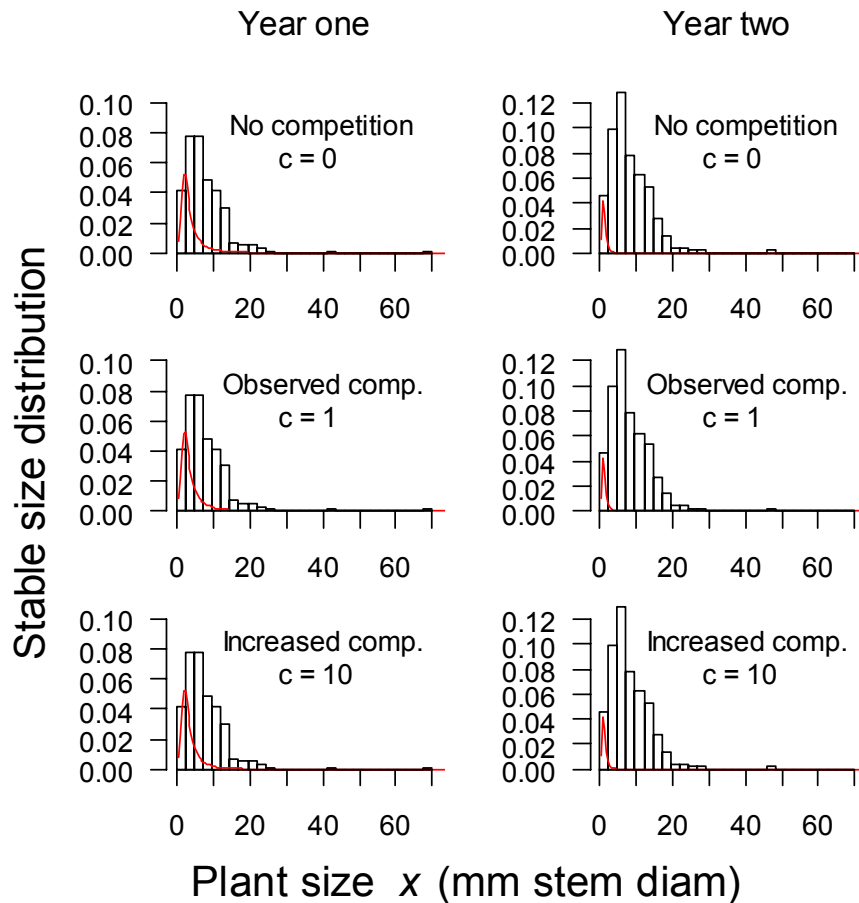


Figure D-1: Stable size distributions with increasing levels of intraspecific competition for mutualists. Histograms give the observed distribution of plants in control plots at the end of year one ($n=247$ plants) and year two ($n= 228$ plants); these data are repeated in all three graphs in a column to allow comparison with the stable size distribution (red lines) predicted by the population model at different competition strengths: 1. no competition for mutualists (top panels) 2. competition set to the levels observed in the field experiment (middle panels, repeated from Figure 4-4) 3. competition set to ten times experimental levels (bottom panels). Stable size distributions were very similar across all levels of competition strength. The y-axis gives plant sizes over the range observed in the experimental plots, for clarity, but stable size distributions were predicted over the full range of plant sizes in the model (max: 314 mm stem diameter).

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Biography

Arietta Elise Fleming-Davies

Born September 22, 1982 in San Diego, CA.

Education:

Duke University, Durham, NC. Ph.D in Biology, candidate defending Nov. 2010

Stanford University, Stanford, CA. B.S. with Distinction in Biological Sciences with Departmental Honors, June 2004

Publications:

Fleming-Davies, Arietta E. Sources of variation in a multispecies facultative plant-protection mutualism: ant density, ant species, and plant size influence plant benefit. In prep.

Scott R. Loarie , Suhkendu Chakraborty , Kyle Dexter , Arietta Fleming-Davies, Andrew D. Gronewold, Junhua Liu, Allen McBride , Emily Pollina. 2007. Density dependence and population growth: evaluating classical and Bayesian approaches to parameter estimation and model selection. In: J.S. Clark. Models for Ecological Data: An Introduction. Princeton University Press.

Honors and Awards:

National Science Foundation Doctoral Dissertation Improvement grant, 2007

Duke Graduate School International Travel grant 2007

Sigma Xi Grant-in-Aid, 2007

Duke Center for Latin American Studies Tinker grant, 2006

Organization for Tropical Studies pilot grant 2006

National Science Foundation Graduate Research fellowship, 2005

Duke University Scholars Fellow, 2004

James B. Duke Fellowship, 2004