ANT OCCUPANCY AND ANTI-HERBIVORE DEFENSE OF *Cordia alliodora*, A NEOTROPICAL MYRMECOPHYTE

By

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ANT OCCUPANCY AND ANTI-HERBIVORE DEFENSE OF *Cordia alliodora*, A NEOTROPICAL MYRMECOPHYTE

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Chair: Emilio M. Bruna
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I studied patterns of ant occupancy, herbivory and anti-herbivore defense in *Cordia alliodora* (Boraginaceae) (Ruiz and Pavon) Oken, a common neotropical myrmecophyte. Specifically, I investigated patterns in ant community composition in 1-, 2-, and 5-yr-old plants and tested whether changes in ant occupancy with plant age affect the amount of herbivory sustained by the host plant.

Although 11 ant species were present in the plants studied, four species—*Azteca pittieri* Forel, *Cephalotes setulifer* Emery, *Crematogaster carinata* Mayr and *Pseudomyrmex fortis* Forel—accounted for the vast majority of occupied domatia. The relative abundance of these species varied according to plot-level environmental variation, domatia position within plants and plant age. The two most abundant ant species, *A. pittieri* and *Cr. carinata*, were nearly mutually exclusive in the 5-yr-old plants and the interaction between these species affected the distribution and abundance of the third most abundant species, *Ce. setulifer*. Coexistence of multiple ant species on the

same host plant in this system appears to be promoted by heterogeneity in nest site availability, competitive interactions among species, different life history strategies and interactions with other organisms.

The amount of herbivore damage on leaves surrounding domatia was affected by random environmental variation, plant age, fertilization and the number of ants present. For the 5-yr-old plants, local herbivory was not affected by fertilization but was lower as a function of the number of workers in a domatium. However, fertilization reduced herbivory and ant abundance had no effect for the 1-yr-old plants. A palatability trial with a specialist herbivore, the beetle *Coptocycla leprosa* Boheman, showed that fertilized plants were less palatable regardless of plant age. This suggests that *C. alliodora* likely has a mixed defensive strategy in which young plants are chemically defended or tolerant to herbivory, with the effectiveness contingent upon resource availability, whereas older plants appear to be defended by their resident ant colonies.

CHAPTER 1
INTRODUCTION

Ant-plant protection mutualisms are common throughout the tropics and have fascinated naturalists and ecologists for over a century (reviewed in Beattie 1985 and Heil and McKey 2003). Amongst ant-plant protection mutualisms, the symbioses between myrmecophytes, plants that produce cavities in their stems or leaves in which ants nest, and their resident ants are considered to be the most specialized (Beattie 1985). Although there is substantial variation in myrmecophyte-ant relationships, these mutualisms are maintained by the reciprocal benefits afforded to both parties from participating in the symbiosis. The ants receive housing and often food from the plant, whereas the benefits to the plants include protection from herbivores, removal of encroaching vegetation and, in some cases, fertilization from ant waste. At least 400 plant species in over 100 genera produce structures for housing ants, and at least that many ant species are myrmecophyte-nesting specialists (Beattie 1985, Davidson and McKey 1993). Myrmecophytes have served as model systems for studying diverse topics in ecology and evolution, including plant defense strategies, conditional outcomes of interspecific interactions, the evolution and maintenance of mutualisms, trophic cascades, and mechanisms of species coexistence (reviewed in Bronstein 1998 and Heil and McKey 2003).

Most myrmecophyte species are occupied by more than one ant species (Fonseca and Ganade 1996). Ant communities of myrmecophytes often vary temporally according to plant age or random variation (e.g., Alonso 1998, Palmer et al. 2000) and spatially due

to the interactions between habitat heterogeneity, habitat preferences and interspecific competition (e.g., Vasconcelos and Davidson 2000, Yu et al. 2001, Palmer 2003). Nesting space may be a limiting resource in these relationships (Fonseca 1999), and therefore ants commonly compete to maintain occupancy of a plant, often to the point of mutual exclusion, as the host plant grows (Janzen 1966, Davidson et al. 1989, Stanton et al. 2002). Because ant species often differ dramatically in their ability to defend their host plants, variation in occupancy could significantly influence plant performance (Janzen 1975, McKey 1984, Itioka et al. 2000, Heil et al. 2001a, Bruna et al. 2004). Addressing the factors that affect ant community composition as myrmecophytes grow and the consequent effects on herbivore damage sustained by the host plant is a critical component of understanding the temporal dynamics of ant-plant protection relationships and is the focus of this study.

Cordia alliodora is a fast-growing myrmecophytic tree common in secondary forests and fields throughout much of Central America and northern South America. The ant associates of *C. alliodora* inhabit naturally hollow swellings, known as domatia, produced by the plants at most branch nodes (Figure 1-1). In Costa Rica, more than ten different ant species have been recorded occupying the domatia of *C. alliodora*, including both specialists to that myrmecophyte and stem-nesting generalists (Wheeler 1942, Longino 1996, Tillberg 2004). Unlike many other myrmecophytes, mature *C. alliodora* trees often host colonies of multiple ant species simultaneously, making this system particularly suitable to studies of species coexistence (Longino 1996; Tillberg 2003, 2004).

The leaves of *C. alliodora* are eaten by a number of insect herbivores including lepidopteran and dipteran larvae, plant hoppers, scales and mealybugs, beetles and leaf-cutter ants (Wheeler 1942, Flowers and Janzen 1997, Möser 2000, Tillberg 2004). The high levels of herbivory and large number of insect herbivores on *C. alliodora* led Wheeler (1942) to conclude that the ant occupants provided no substantive benefit for the plant and, perhaps, were even parasites. However, behavioral studies, analysis of herbivore damage and analyses using stable isotopes suggest that at least some of the ant species attack and eat herbivorous insects (Möser 2000, Tillberg 2004).

The objectives of this study are to describe patterns in ant occupation of *C. alliodora* and examine the importance of ants and other factors in the anti-herbivore defense of 1- and 5-yr-old plants. In the second chapter I present results from a survey of ant occupation of *C. alliodora* and attempt to identify potential mechanisms of coexistence. In the third chapter I describe the effects of plant age, fertilization and ant occupancy on the level of insect herbivory sustained by *C. alliodora* plants and interpret these results in light of plant defensive strategies. In the fourth chapter I integrate the results of these studies and interpret their results with respect to the evolution and maintenance of the *Cordia*-ant relationship.

Figure 1-1. The inside of a *Cordia alliodora* domatium containing part of a *Cephalotes setulifer* colony. Larvae and pupae are cylindrical and whitish. Cohabiting scale insects are smaller, pink and attached to the wall of the domatium.

CHAPTER 2
ANT SPECIES COEXISTENCE IN *CORDIA ALLIODORA*, A NEOTROPICAL MYRMECOPHYTE

Introduction

The co-occurrence of species with similar ecological requirements is ubiquitous in natural communities, and the mechanisms promoting coexistence are a major focus of ecological inquiry. Both the intrinsic properties of organisms (e.g., behaviors and life history traits) and characteristics of the environment (e.g., habitat structure, disturbance regimes, interactions with other organisms and stochastic events) allow the persistence of species with overlapping resource needs (Tokeshi 1999, Chesson 2000, Amaresekare 2003). Because patterns of species coexistence differ across space and through time, single factors rarely account for the observed variation in the presence and abundance of species (Amarasekare 2003). Consequently, field studies examining the composition of natural communities benefit greatly from the consideration of multiple, often complementary, mechanisms of coexistence.

Ant-plant symbioses are ideal model systems in which to study the patterns of coexistence of species with similar resource requirements (Davidson and McKey 1993, Bronstein 1998, Heil and McKey 2003, Palmer et al. 2003). In specialized ant-plant symbioses, myrmecophytes (i.e., ant-plants) provide nesting space and food resources for ant inhabitants. Ant inhabitants often provide some benefit for the plant in return, such as protection from herbivores, thereby maintaining the mutualism (Hölldobler and Wilson

1990, Bronstein 1998, Heil and McKey 2003). Whereas space is rarely a limiting factor for terrestrial ant communities (Albrecht and Gotelli 2001), ants inhabiting myrmecophytes may face strong inter- and intra-specific competition for limited nest sites (Janzen 1966, Davidson et al. 1989, Fonseca and Ganade 1996, Fonseca 1999, Stanton et al. 2002). Therefore, although young myrmecophytes may experience multiple colonization events by queens of different species or undergo a succession of ant species as they plant grow, mature plants are usually dominated by a single ant colony (Davidson et al. 1989, Longino 1991, Vasconcelos 1993, Young et al. 1997, Palmer et al. 2000, Feldhaar et al. 2003).

Recent studies on ant-plant relationships have provided empirical models for both spatial (e.g., Yu et al. 2001, Palmer 2003) and temporal (e.g., Young et al. 1997, Alonso 1998) habitat partitioning among ant species that inhabit the same plant species. In some systems, variation in host-plant characteristics, often related to underlying habitat heterogeneity, allows fine-scale partitioning of resources among ant species (Davidson et al. 1989, Longino 1989, Vasconcelos and Davidson 2000, Palmer 2003). In more homogeneous conditions, coexistence may result from interspecific differences in ant life histories and behaviors, including trade-offs between competitive dominance and dispersal capability (Stanton et al. 2002), fecundity and dispersal ability (Cole 1983, Vasconcelos 1993, Yu and Wilson 2001, Yu et al. 2004), or interference and exploitation competition (Fellers 1987, Davidson 1998, Holway 1999). Positive priority effects (the continued occupation of the species that colonizes first) may also allow ant species that are poor competitors or even poor colonizers to occupy sites following colony

establishment despite the presence of otherwise dominant species (Longino 1989, Palmer et al. 2002).

Characteristics of the host plants and the resident ant colonies change over time as the plant develops and the ant colonies grow, senesce or are replaced by other species (Vasconcelos and Casimiro 1997, Young et al. 1997, Alonso 1998, Itino and Itioka 2001, Del Val and Dirzo 2003). The mechanisms listed above are rarely manifested at one point in time at a single locale, but rather promote species coexistence at larger spatial and temporal scales (Young et al. 1997; Alonso 1998; Yu et al. 2001, 2004). The early ontogeny of myrmecophytes may be particularly important for species-sorting of ant occupants (Davidson et al. 1989, Vasconcelos and Davidson 2000, Palmer et al. 2002). A comprehensive understanding of ant species coexistence in ant-plant symbioses therefore must encompass both differences in the life history strategies of the ant species and the temporal dynamics of the symbiosis.

Cordia alliodora is a neotropical myrmecophytic tree inhabited by several ant species, including both specialists and stem-nesting generalists. Although *C. alliodora* has an extensive geographic range and is often locally abundant, little community-level research has been conducted on the ants that inhabit this myrmecophyte (but see Longino 1996 and Tillberg 2004). Here I describe patterns of ant occupation in *C. alliodora* during the course of the host plant's ontogeny from sapling to young mature tree. Specifically, I investigated the following questions: 1) How does ant community composition change with plant age? 2) Is there spatial variation in ant species occupancy? 3) Are there interspecific differences among ant species in colony founding and

expansion? These data are then used to identify potential mechanisms that account for the maintenance of ant species diversity in the *C. alliodora* system.

Methods

Study System

Cordia alliodora (Boraginaceae) is widespread and abundant in the secondary forests, clearings and forest edges at La Selva Biological Station (Costa Rica, Heredia Province, 10° 26' N, 83° 59' W), where I conducted this study. I collected data from trees that were planted in three replicated, monospecific blocks as part of the Huertos Project, a long-term ecological study established at La Selva in 1991 in which *C. alliodora* was one of the focal tree species. In the Huertos Project plots, single-age stands were planted in rows at a very high initial density (2887 trees ha^{-1}), periodically thinned, and regularly weeded to prevent the establishment of other vegetation. Each block had three adjacent monoculture plots of C. alliodora in 1-, 4- and 16-yr planting cycles; the results presented here are from 1-, 2- and 5-yr-old trees sampled evenly from all three blocks. Within plots, rows were 1.73 m apart and plants were placed at 2 m intervals within rows, such that each individual was at the center of a hexagon 2 m from the six closest plants. Haggar and Ewel (1995) provide further details about the site, the planting techniques and the design of the Huertos Project. *Cordia alliodora* individuals were common in the secondary forest surrounding the Huertos Project plots.

The ant associates of *C. alliodora* inhabit naturally hollow cauline swellings (i.e., domatia) that the plants produce at most branch nodes. Wheeler (1942) reported 44 ant species from *C. alliodora* domatia in Panama, and more than ten ant species have been recorded as occupants of these swollen nodes in Costa Rica (Longino 1996; Tillberg 2003, 2004). Most of these ant species are stem-nesting generalists with no particular

affinity for *C. alliodora*, but there are also a number of specialist ant species that are only found in *C. alliodora* (Wheeler 1942, Longino 1996, Tillberg 2004). Unlike many other myrmecophytes, individual *C. alliodora* trees often host colonies of multiple ant species, making this system particularly amenable to studies on species coexistence (Longino 1996, Tillberg 2004).

Ant Community Composition

I inventoried all domatia from 18 one-year-old and 18 five-year-old *C. alliodora* trees in May and June 2004. The two ages were in adjacent plots in each of the three blocks. Additionally, Tillberg (2003) determined ant occupancy from all domatia of nine 2-yr-old trees following similar methodology in May-July 2001 and shared these data. The 2- and 5-yr-old-trees belonged to the same cohort but individual trees were not resampled because the domatia collection required felling the tree. Trees were sampled evenly from three replicate blocks in the Huertos Project. The occupation status of the small proportion of domatia encased in trunks or large branches was determined by identifying the ants passing through the entrance or by opening the domatium in the field. All other swollen nodes were excised from the trees and then frozen to kill ant occupants. I then dissected the domatia in the laboratory, recorded whether they were occupied and identified the ant inhabitants. Although dead queens were common in the domatia of 1-yr-old trees and some empty swollen nodes showed signs of past occupation, I considered only those domatia that contained live ants at the time of collection to be inhabited.

I tested for relationships among the number of domatia, the proportion of domatia occupied and ant species richness using regression analysis. I compared ant species richness among the three age classes using ANCOVA, with the number of domatia of each plant as the covariate.

Spatial Variation in Ant Species Occupancy

To assess within-tree habitat partitioning, Tillberg (2003) classified domatia according to their relative vertical position on the plant for 2-yr-old trees (i.e., low-, mid- and high-level branches) and I classified domatia according to their relative age for the 5-yr-old trees (i.e., younger domatia from terminal and subterminal branches vs. older domatia from large branches and the bole). The domatia from higher branches of 2-yr-old plants were similar in age and physical condition to the domatia from the terminal or subterminal branches of the 5-yr-old plants, making these categories somewhat comparable. Because the 1-yr-old plants did not have sufficient numbers of domatia or the branching structure required to make such classifications, they were excluded from the analysis.

I used chi-square tests to examine the frequency of species occurrence in domatia from different parts of the trees, with expected values for the test of within-tree habitat partitioning derived from the total proportion of domatia occupied by each species. To examine relationships among the occupation patterns of the three most abundant species I performed ANOVA on the proportion of domatia they occupied in 2- and 5-yr-old trees.

Colony Founding and Expansion

Although there were other mature *C. alliodora* individuals surrounding the Huertos Project, I assumed that the dozens of 5-yr-old plants in the immediately adjacent planting were the most likely source population from which foundress queens would colonize the 1-yr-old plants. Therefore, I used chi-square tests to analyze the ant occupation of 1-yr-old trees, with the expected values for frequency of ant occupation derived from the proportion of domatia occupied by each species on the 5-yr-old trees. These proportions were pooled across the three blocks.

In June 2004, I also identified the ant occupants and phase of colony development in the distal, most recently produced, two or three domatia from a single branch haphazardly selected from 5-yr-old trees (n = 60) that were not used in the whole-tree analysis. I tested for interspecific differences in colonization frequency of these domatia using chi-square tests, for which I assumed a null hypothesis that newly produced domatia would have an equal likelihood of being empty, being colonized by the same species that occupied the nearest node down the branch, or being colonized by a different species.

Although the Huertos Project replicated plant age treatments at the plot level, in this study I treated plants as independent replicates because ant species were patchily distributed among the plots and I was primarily concerned with variation in ant communities at the level of individual trees or domatia within trees. Data were transformed to satisfy the requirements of parametric tests when appropriate. Analyses were conducted with SPSS 13.0 and R 2.2.0.

Results

Ant Community Composition

The whole-tree inventory included 9051 domatia (n = 664 from 1-yr-old trees, n = 3430 from 2-yr-old trees and n = 4957 from 5-yr-old trees) in which we identified 11 ant species. The four most abundant species across all plant ages, together accounting for over 97% of occupied domatia, were *Azteca pittieri* (35 trees, 3113 domatia), *Crematogaster carinata* (19 trees, 1677 domatia), *Cephalotes setulifer* (23 trees, 714 domatia), and *Pseudomyrmex fortis* (7 trees, 235 domatia). Others species found were *Cephalotes multispinosus*, *Crematogaster curvispinosa*, *Wasmannia auropunctata*,

Pachychondyla crenata, *Pheidole caltrop* and unidentified species of the genera *Brachymyrmex*, *Pheidole* and *Pseudomyrmex* (Tillberg 2003, pers. obs.).

When data from plants of all three ages were pooled, ant species richness was positively related to the number of domatia present ($R^2 = 0.331$, n = 45, P = 0.012). The 2-yr-old plants had the most domatia (Fig. 1a), and also hosted the highest richness of ant species (Fig. 1b), even with the number of domatia included in the model as a covariate (Table 1). However, higher ant species richness in trees did not translate to higher proportions of occupied domatia ($R^2 = 0.065$, n = 45, P = 0.31). Indeed, approximately one-third of the domatia in the 1- and 2-yr-old trees were occupied (mean = 34.2%, std. dev. = 23.7% and mean = 31.1%, std. dev. = 20.2%, respectively), whereas nearly all of the domatia on 5-yr-old trees (mean = 94.7%, std. dev. = 5.6%) contained ants.

Spatial Habitat Partitioning

The presence and abundance of ant species varied among the three replicate Huertos Project blocks, among trees within blocks and among domatia within trees. Three species – *A. pittieri*, *Cr. carinata* and *Ce. setulifer* – were present in all three blocks. *Crematogaster carinata* showed a highly clumped distribution, occurring in 2-yr-old trees in all three blocks and dominating most 1- and 5-yr-old trees in the one block where it was most common. *Azteca pittieri* was present on at least some plants of all ages in all blocks. *Cephalotes setulifer* was present in 2- and 5-yr-old trees from all three plots, but was absent from 1-yr-old trees in two blocks. *Pseudomyrmex fortis* was present in 5-yr-old trees in two of the three blocks but was not found on any of the 1-yr-old plants and was very uncommon in the 2-yr-old plants included in the whole-tree occupancy survey. Although either *A. pittieri* or *Cr. carinata* occupied the majority of domatia on nearly all 5-yr-old trees, the smallest tree in this age class was completely inhabited by

Ce. setulifer which occupied 48 of the 49 domatia on the plant; one domatium on this tree was uninhabited.

Ant species displayed non-random within-plant microhabitat occupancy in both the 2- and 5-yr-old plants (χ^2 = 133.1, df = 4, P < 0.0001 and χ^2 = 76.81, df = 3, P < 0.0001, respectively). In the 2-yr-old plants, *A. pittieri* had a significantly higher habitation frequency in branches from the upper stratum of *C. alliodora* plants (χ^2 = 32.35, df = 2, P < 0.0001), whereas *Cr. carinata* had a higher habitation frequency for the lower, older branches (χ^2 = 90.26, df = 2, P < 0.0001). *Cephalotes setulifer* displayed a non-random pattern of occupancy in the three strata (χ^2 = 10.43, df = 2, P = 0.005), but no clear directional trend was evident (Fig. 2-2a). However, in the 5-yr-old plants, *Ce. setulifer* was significantly more common in the younger terminal or subterminal domatia than expected by chance (χ^2 = 56.47, df = 1, P < 0.0001). In these older plants, *Cr. carinata* exhibited no difference in occupation frequency in different parts of the tree (χ^2 = 0, df = 1, P = 1), and both *A. pittieri* and *P. fortis* were less common than expected in terminal or subterminal domatia (χ^2 = 4.64, df = 1, P = 0.033 and χ^2 = 15.69, df = 1, P < 0.0001, respectively; Fig. 2-2b).

The relationship among the occupation patterns of the two numerically dominant species, *A. pittieri* and *Cr. carinata*, and the third most abundant species, *Ce. setulifer*, changed substantially over the ontogeny of the symbiosis. *Azteca pittieri* and *Cr. carinata* coexisted in many 1-yr-old plants and on eight of the nine 2-yr-old plants. However, they were mutually exclusive, with the exception of a single foundress queen of *A. pittieri* on one tree dominated by *Cr. carinata*, by the time trees were 5 years old. These two dominant ant species showed markedly different occupation strategies (Fig. 2-

3a), with the proportion of occupied domatia differing according to tree age and species (Table 2-2). When it was the dominant species, *A. pittieri* occupied a significantly higher proportion of the host plants' domatia than when *Cr. carinata* was the dominant species. The proportion of domatia occupied by *Ce. setulifer* was dependent upon whether *A. pittieri* or *Cr. carinata* was the dominant ant species in the tree (Fig. 2-3b). Specifically, trees dominated by *Cr. carinata* had more domatia occupied by *Ce. setulifer* regardless of tree age, and the changes in proportional occupancy of *Ce. setulifer* differed among trees dominated by the two species as the plants aged. The significant "Plant age x Ant species" interaction term suggests that *A. pittieri* increasingly excluded *Ce. setulifer* as the plants aged, whereas *Ce. setulifer* occupied an increasing proportion of domatia on trees dominated by r*C. carinata* as the plants aged (Table 2-3).

Colony Founding and Expansion

Most ant species inhabiting *C. alliodora* were present on 1-yr-old trees only as founding queens or very small colonies. The 2-yr-old trees were always occupied by multiple con- and heterospecific colonies, most of them with small numbers of workers. In these trees, domatia were colonized both through expansion of growing colonies and by establishment of new colonies by foundress queens in unoccupied domatia (Tillberg 2003). When the plants were 5 years old and fewer unoccupied domatia were available, the occupation of newly produced domatia appeared to occur primarily by colony expansion. However, although almost all of the domatia of 5-yr-old trees contained ants from established colonies, I also found some evidence of continued attempts by mated queens to establish new colonies in most recently produced domatia of these older plants.

Chi-square analysis showed that ant occupation frequency of 1-yr-old trees was significantly different than would be expected based on the relative abundance of the four

most abundant species in 5-yr-old trees ($\chi^2 = 44.34$, df = 3, $P < 0.0001$). Chi-squared tests for each species showed that *A. pittieri* was represented proportionally in 1-yr-old plants, but *Ce. setulifer* and *P. fortis* were underrepresented and *Cr. carinata* was overrepresented (Fig. 2-4).

Colonization of apical domatia in 5-yr-old trees was not random ($\chi^2 = 78$, df = 2, $P < 0.0001$), and the frequency of different states of domatia habitation did not differ among the four most common ant species ($\chi^2_{heterogeneity} = 8.17$, df = 6, $P = 0.23$). Rather, new domatia produced by plant growth were most likely to be colonized by expansion of the ant colony in the adjacent, more basal, domatium regardless of the species present (71 of 93 cases). Of the remaining apical domatia not colonized by expansion of the neighboring ant colony, 14 (15.1%) were inhabited by workers of species nesting elsewhere in the same plant or had been colonized by heterospecific queens and only eight (8.6%) were not yet colonized at the time of sampling.

The parasitoid wasp *Conoaxima affinis* (Eurytomidae) was abundant in one of the three blocks, where I found 13 larvae or pupae in domatia from the 1-yr-old plants with paralyzed or dead *A. pittieri* queens. There was never more than one larva or pupa on a queen. In this plot there were only 56 domatia in which I found live *A. pittieri* queens, indicating an attack rate of 18.8% (13 of 69) on foundresses. This may be an underestimate, as I frequently found domatia on 1-yr-old plants that contained only the legs and head capsules of queens that were presumably consumed by the wasps.

Discussion

The patterns of ant occupation I observed suggest that spatial heterogeneity of nesting space among and within plants, temporal variation in habitat characteristics related to changes in plant growth form, and interspecific competition among ant

occupants for nest space promote ant species coexistence in *Cordia alliodora*. Competition, both through preemptive discovery of resources and through physical conflict, is considered to be important for structuring many ant communities (Davidson 1980, Hölldobler and Lumsden 1980, Hölldobler and Wilson 1990). I did not examine behavioral interactions among the ant species in this study, but the patterns of colony initiation, growth and tree habitation suggest that the ant species occupying *C. alliodora* engage in competitive interactions for nesting space. It appeared that the outcome of these interactions varied according to plant age, random environmental variation among the three blocks and differences in the life-history strategies of the most common ant species.

In contrast to the conclusions of Fonseca (1999), the results of the whole-tree occupation analysis suggest that in the *C. alliodora* system the availability of nesting space does not limit ant colony size when the plants are 1 or 2 years old. Indeed, more than 60% of the domatia were unoccupied in both 1- and 2-yr-old trees. However, this was likely the result of two very different processes. The low frequency of ant occupation in the 1-yr-old plants was probably due to the shorter period that the domatia had been available for colonization combined with the small number of domatia, which may make trees less attractive for colony-founding queens. Conversely, the low frequency of ant occupation in the 2-yr-old plants was likely due to the superabundance of domatia and inability of young ant colonies to expand into available habitat. In fact, the 2-yr-old plants had significantly more domatia than the 5-yr-old plants included in this study, despite the fact that crown volume increases as the plants age (Menalled et al. 1998). It is possible that younger *C. alliodora* plants have denser branching that

produces relatively more domatia compared to the tiered, open crowns typical of mature *C. alliodora* trees. This counterintuitive pattern of domatia production may be an artifact of the planting design in the Huertos Project: because there was little competition for light among young *C. alliodora* plants, they displayed thick, bushy growth prior to dramatic increases in height accompanied by shedding of the lower branches as competition for light increased (J.J. Ewel, pers. comm.).

Regardless of the generality of *C. alliodora* crown geometry as the plants age, in this study the abundance of available nesting space in 2-yr-old trees may largely explain why ant species richness was highest at this age. The changes in crown structure may also partially explain why many of the generalist ant species found in the 2-yr-old plants were not present in the 5-yr-old trees. If interspecific competition for nest sites limits species coexistence as the plants age, then the presence of more unoccupied domatia in the 2-yr-old plants may have promoted species diversity at this stage of plant development. This could be particularly true when the ant colonies were small and unable to wage territorial wars typical of arboreal ant communities (Hölldobler and Lumsden 1980). Tillberg (2003) showed that the lower branches of 2-yr-old plants were inhabited by a diversity of generalist ants, which presumably did not recolonize after these branches senesced as the plants developed the crown structure typical of mature trees. Additionally, since many of the species found on 2-yr-old trees were generalist live-stem nesters, they may have been only opportunistic inhabitants of the younger *C. alliodora* plants and either died or moved as the plants aged and competition for nest sites increased (Alonso 1998, Longino 1996, Palmer et al. 2000).

I found evidence of within-tree habitat partitioning among ant species in both the 2- and 5-yr-old trees. There is little basis for comparison with other ant-plant systems because *C. alliodora* is unusual in housing multiple ant species in mature plants (but see Young et al. 1997 and Palmer et al. 2003). However, many myrmecophytes can be occupied by multiple ant species at early stages of their development and arboreal ants in other systems have been shown to divide space based on the distribution of resources (Cole 1983, Bluthgen et al. 2004). In the *C. alliodora* system, honeydew-producing hemipteran symbionts (Pseudococcidae and Coccidae) appear to be an important food for some of the ant species (Tillberg 2004). Because these insects rely on access to plant vascular tissue to feed, they are likely not distributed evenly among domatia of different ages. Therefore, variation in the ant-coccoid relationship may account for within-tree habitat selection. *Azteca pittieri* was more abundant in the younger domatia in 2-yr-old plants but more abundant in older domatia in the 5-yr-old plants. Pseudococcids and coccids were common in *A. pittieri* domatia, but this species does not appear to rely solely on honeydew for nutrition (Tillberg 2004). *Crematogaster carinata* showed no preference for domatia microhabitat in the 5-yr-old trees, inhabited older branches more frequently in 2-yr-old trees, and also only rarely tended coccids and pseudococcids inside its domatia (Tillberg 2004). In contrast, *Ce. setulifer*, which disproportionately occupied younger domatia, commonly tended hemipterans and the apparently depends primarily on plant sources of nutrients such as the concentrated fluid excreted by the coccoids (Tillberg 2004). *Pseudomyrmex fortis* was more abundant than expected in the older domatia from large branches and trunks, which are not surrounded with the plant vascular tissue required for coccoids to feed. The relative importance of plant or animal food

sources for *P. fortis* is unknown, but its occupation pattern suggests that honeydew-producing coccoids may not be a primary source of nutrition. Work in other systems has shown complex relationships among ants, myrmecophytes and ant-tended Hemiptera (Gaume et al. 1998, Lapola et al. 2005), but the importance of these interactions in most systems is unknown.

The presence of a large, polydomous colony of the habitat generalist *Cr. carinata* in one of the plots apparently reduced the colonization frequency of *Azteca pittieri* in 1-yr-old plants and completely prevented colonies of that otherwise dominant species from occurring in 5-yr-old trees. The dominance of *Cr. carinata* in this plot could be attributable to a number of factors, but certainly this species' mode of colonizing the 1-yr-old trees (colony expansion from the leaf litter) allowed it to exploit the empty domatia prior to discovery by foundress queens of *A. pittieri*. *Crematogaster carinata* displayed a markedly different occupation strategy than *A. pittieri* on older plants as well, in which the former species left significantly more domatia uninhabited on 5-yr-old trees where it was the numerically dominant species (Fig. 3a). It appeared that the competitive interactions between the two dominant ants resulted in available habitat (uncolonized domatia) that *Ce. setulifer* was able to occupy and then successfully defend against the numerically dominant colonies of *A. pittieri* and *Cr. carinata*.

Interaction with natural enemies can alter the outcome of competitive interactions, thereby promoting coexistence (Worthen 1989, Pacala and Crawley 1992, Tokeshi 1999, Chesson 2000). The high mortality of *A. pittieri* queens due to attacks by the parasitoid wasp, *C. affinis*, may have mediated the success of founding queens of other species. In his description of this genus, Brues (1922) stated that a congener wasp, *C. aztecicida*, was

responsible for the death of many *Azteca* queens colonizing *Cecropia* plants in Guyana. Yu and Davidson (1997) also found that *C. aztecicida* accounted for a high proportion of colony failures in two of the *Azteca* species in their study. Although the effects of parasitoids on myrmecophyte ant community composition have not been tested directly, there is evidence that wasp abundance varies across habitats and can facilitate colony success of non-host species (Yu and Davidson 1997). In my study, *A. pittieri* had the lowest proportional occupancy in the plot where *C. affinis* was most abundant. This was also the plot where *Cr. carinata* was abundant in the 1-yr-old trees and was the only plot where founding queens of *Ce. setulifer* were found in domatia of 1-yr-old plants. Longino (1996, pers. comm.) found similar wasps killing *Azteca* queens in both *C. alliodora* and *Cecropia* species. If attack by parasitoid wasps diminishes the survivorship of *A. pittieri* queens to the extent that it increases the availability of nesting habitat for other ant species, then these wasps may be an important equalizing factor in interspecific competition (Chesson 2000).

Although I did not conduct the experiments necessary to test for interspecific trade-offs in competition and colonization or fecundity and dispersal, the observed patterns of ant occupation suggest that the ant species did differ in their ability to colonize domatia and compete with other species for nest sites. The same two species, *A. pittieri* and *C. carinata* were numerically dominant at all three plant ages included in this study, but their proportional occupancy varied significantly between 2- and 5-yr-old trees. Both *P. fortis* and *C. setulifer* occupied fewer domatia in 1-yr-old trees than expected based on their relative abundance in 5-yr-old trees. This could indicate a low resource allocation to reproductive castes in these species paired with a higher success

rate of colony founding for the few queens produced, as Cole (1983) suggested for other species of *Pseudomyrmex* and *Cephalotes* (= *Zacryptocerus*) that inhabit mangrove islands. Positive priority effects for young colonies of these subdominant species, namely the ability to resist eviction by the larger colonies of *A. pittieri* or *Cr. carinata* following colony establishment, may explain this phenomenon. Workers of both *C. setulifer* and *P. fortis* are much larger than those of *A. pittieri* and *Cr. carinata*, which could result in favorable one-on-one success in conflict for the former two species, or at least for the aggressive *P. fortis* (McGlynn 2000). Both ant species also have formidable, though very different, defenses: queens and major workers of *Ce. setulifer* effectively block the entrances to their domatia with their phragmotic heads, and *P. fortis* workers possess a powerful sting. Colony size alone has proven to be a strong predictor of competitive outcome in other ant communities (Fellers 1987, Palmer 2004), but, as shown in this and other systems, morphological and behavioral adaptations of ant species are also important in determining the outcome of competitive interactions (Davidson 1998, Holway 1999).

In order to understand the effects of microhabitat occupation and ant competition in the context of the mutualism, it is useful to explicitly consider the life history of the plant that provides the resources for which the ant species presumably compete (Bronstein 1998, Heil and McKey 2003). If one or more of the ant species provide fitness benefits for *C. alliodora* individuals, then selection on plant life history traits would favor allocation for ant-related traits (i.e., domatia production and lack of chemical defenses against ant-tended coccoids) at the stage where acquiring a protective ant colony is most beneficial and least costly (Brouat and McKey 2000). Because the ant species

may affect the plant differentially, plant traits should evolve to maintain the relationship with the ant partners that provide the greatest net benefit (Stanton 2003). Although virtually all *C. alliodora* plants beyond the seedling stage produce domatia at branch junctures, most of the 1-yr-old plants in this study were still small and had only a few domatia. However, the 2-yr-old trees had a large number of domatia—significantly more than the 5-yr-old plants—that housed young colonies of many ant species. If rapid plant growth during the first 2 years of development results in the production of more domatia, then the probability of mutualistic ant species, such as *A. pittieri* or *Cr. carinata*, would increase (Tillberg 2004). The ant community composition data showed that as the plant cohorts aged, these two ant species were also competitively superior and dominated nearly all the trees within 5 years of establishment. If they are indeed mutualists (see Chapter 3 of this thesis, Tillberg 2004), then their numerical dominance over other ant species likely provides significant fitness benefits for *C. alliodora*. The benefits to the host plant afforded by mutualist ant species in turn could have affected the evolution of allocation for domatia production and perhaps other traits that benefit these ant species in particular (Brouat and McKey 2000).

In this study, I have described the patterns of ant occupancy in *C. alliodora* and attempted to explain them through invoking the life-history characteristics of the ant species, changes in branching structure related to the age of the host plants, the differential abundance of honeydew-producing coccoids in different parts of the tree, the effect of parasitoid wasps attacking one of the dominant ant species, and the interaction of these mechanisms across space and through time. Although coexistence and competition have been studied in ant-plant mutualisms, most research has focused on

single mechanisms that structure the relationship. However, mutualist systems often involved multiple guilds of interacting species that likely vary in their interactions and responses to environmental variation (Stanton 2003). As such, investigations of ant species coexistence in myrmecophytic hosts clearly benefit from the incorporation of multiple factors, and such approaches likely provide a better understanding of these apparently simple systems.

Table 2-1. Results of ANCOVA testing the effects of plant age on ant species richness, with the number of domatia on each plant (log_{10}-transformed) included as a covariate.

Source of variation	df	MS	F	P
Plant age	2	25.107	53.129	< 0.001
No. domatia	1	4.514	9.551	0.004
Error	41	19.375	0.473	

Table 2-2. Results of ANOVA examining the effects of plant age and the identity of the dominant ant species on the proportion of domatia occupied. Proportional occupancy was arcsine (square root) transformed to improve normality. Only 2- and 5-yr-old trees primarily occupied by either *Azteca pittieri* or *Crematogaster carinata* were included in this analysis (only one tree within these age groups, which was dominated by *Cephalotes setulifer*, was excluded).

Source of variation	df	MS	F	P
Plant age	1	3.24	51.92	< 0.001
Ant species	1	0.38	6.07	0.022
Plant age x Ant species	1	0.10	1.63	0.215
Error	22	0.062		

Table 2-3. Results of ANOVA testing the effects of plant age and the presence of the two numerically dominant ant species (*Azteca pittieri* and *Crematogaster carinata*) on the proportion of the plant occupied by the third most abundant species, *Cephalotes setulifer*. Proportional occupancy was arcsine (square root) transformed to improve normality. Only 2- and 5-yr-old trees primarily occupied by either *A. pittieri* or *Cr. carinata* were included in this analysis (only one tree within these age groups, which was dominated by *Ce. setulifer,* was excluded).

Source of variation	df	MS	F	P
Plant age	1	0.009	5.43	0.029
Ant species	1	0.080	50.53	< 0.001
Plant age x Ant species	1	0.022	14.03	0.001
Error	22	0.002		

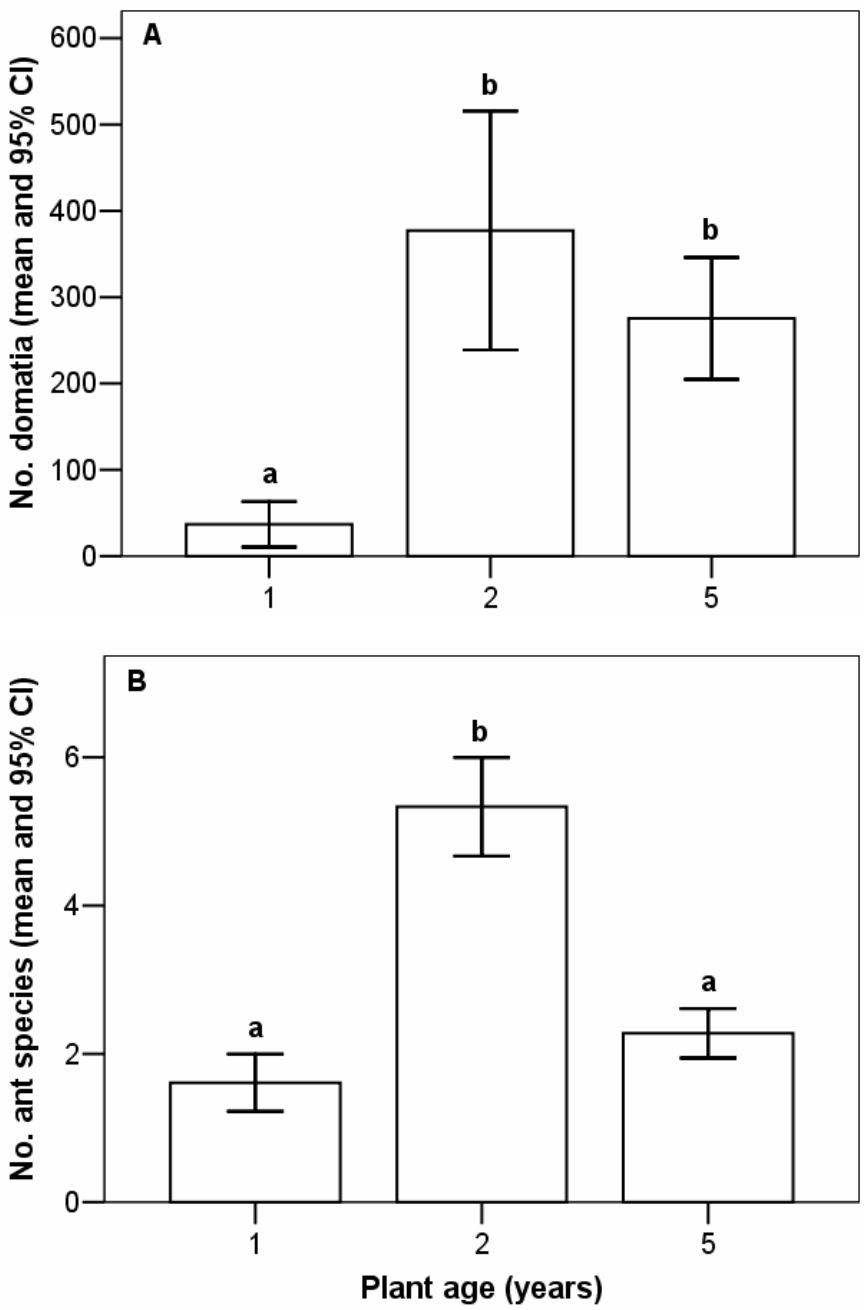

Figure 2-1. Plant age significantly affected both (A) the number of domatia on a plant and (B) the number of ant species present. Means and 95% confidence intervals are shown, with pairwise differences (calculated with Tukeys HSD) indicated with lowercase letters.

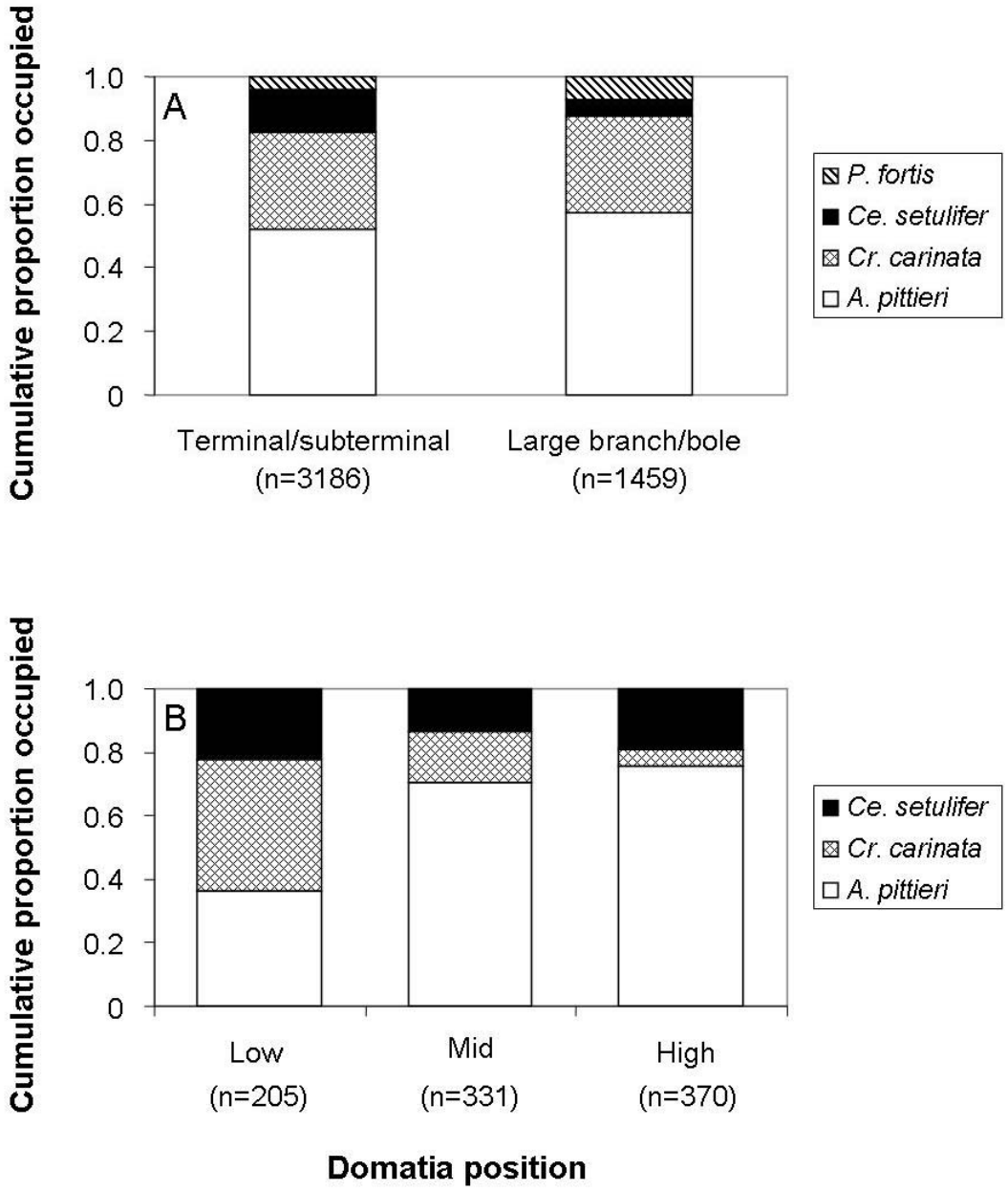

Figure 2-2. Proportional occupancy of domatia in different tree microhabitats varied among species in both (A) the 2-yr-old trees and (B) the 5-yr-old trees. Although a number of other species were present in the 2-yr-old trees, including *Pseudomyrmex fortis*, none were present in either a large number of trees or a large number of domatia and so were not included.

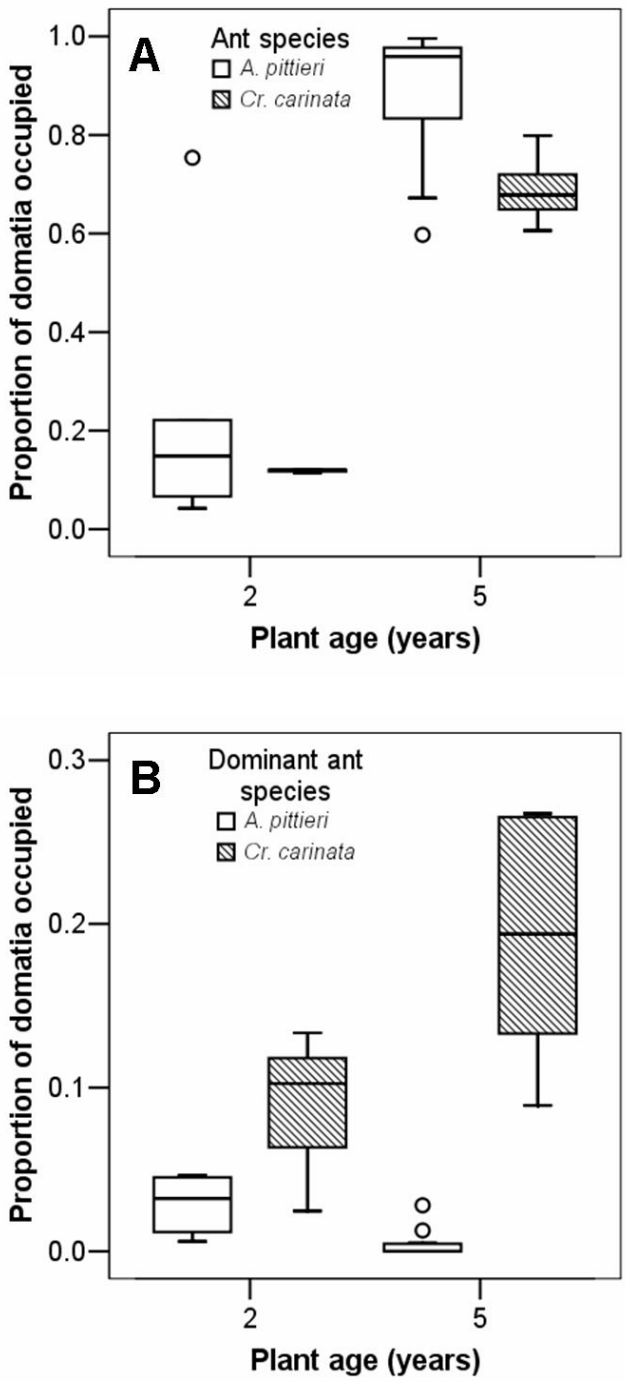

Figure 2-3. The two most abundant species, *Azteca pittieri* and *Crematogaster carinata*, differed in their occupation patterns and effects on other species. (A) *A. pittieri* occupied relatively more domatia in trees where it was the dominant species compared with *Cr. carinata.* This was true regardless of plant age. (B) The proportional occupancy of *Cephalotes setulifer* was affected by the species of dominant ant in the tree, the age of the plant and the interaction of these two factors (Table 2-3).

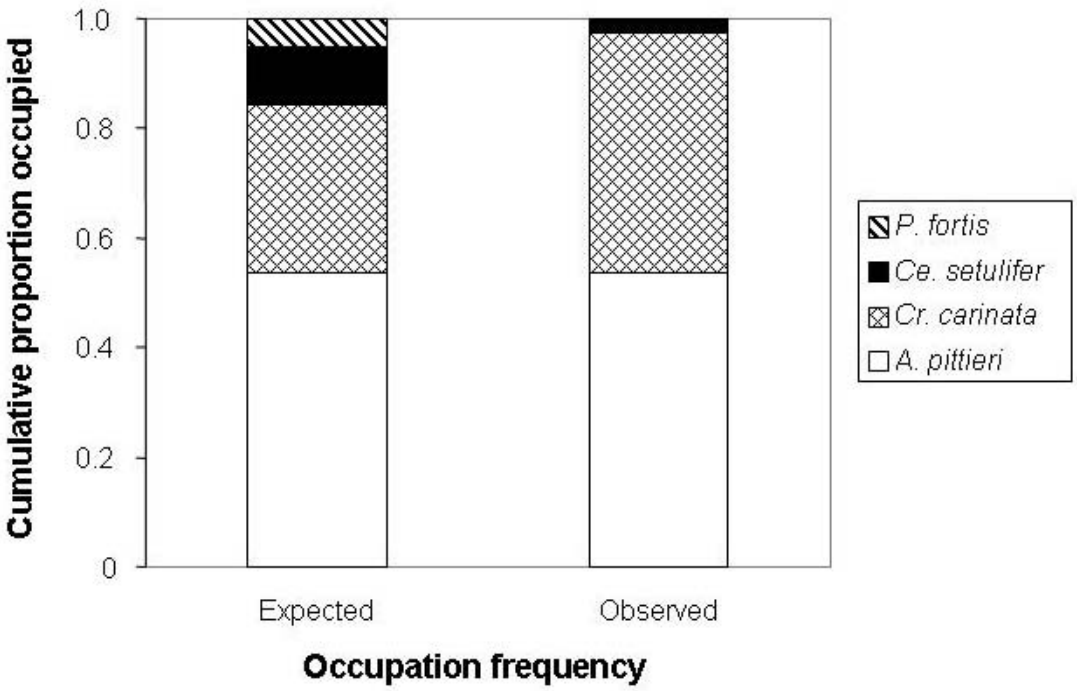

Figure 2-4. Stacked bar graph showing the expected occupation of the four most abundant ant species in the 1-yr-old trees—based upon their occurrence in the 5-yr-old trees—and the observed occupation frequency. All species except *Azteca pittieri* had significantly different occupation patterns than expected.

CHAPTER 3
HERBIVORY AND ANTI-HERBIVORE DEFENSE OF *CORDIA ALLIODORA*: HOW IMPORTANT IS ANT DEFENSE?

Introduction

The diverse chemical and physical defenses that plants have evolved in response to herbivory can be physiologically costly and often are produced at the expense of growth and reproduction (Herms and Mattson 1992, Sagers and Coley 1995, Coley et al. 2005). Although there are many theories explaining plant defensive strategies (reviewed in Coley and Barone 1996 and Stamp 2003), in general plants should produce the type and amount of defenses that optimize their fitness given limited resources and trade-offs in their allocation (Coley et al. 1985, Zangerl and Rutledge 1996). Tropical forests often have exceptionally high rates of herbivory and, therefore, selection for effective anti-herbivore defense strategies may be particularly strong for tropical plants (Coley and Aide 1991, Coley and Barone 1996).

Variation in anti-herbivore defenses among individuals within a plant species may be influenced such as age, size, genotype, season, habitat, previous herbivory and plant condition (Feeny 1970, McKey 1974, Ernest 1989, Mihaliak and Lincoln 1989, Bowers and Stamp 1993). Additionally, nutrient availability can affect both the evolution of defensive strategies of species and the ability of individual plants to synthesize deterrent chemicals or continue growth despite herbivore damage (Coley et al. 1985, Nichols-Orians 1991, Bryant et al. 1992, Folgarait and Davidson 1995, Wilkens et al. 1996, Strauss and Agrawal 1999). Determining the net costs or benefits of chemical anti-herbivore defense can allow the formulation of testable predictions regarding the

distribution of herbivore damage among and within plants (McKey 1974, Zangerl and Rutledge 1996). Conversely, studying patterns of herbivory in experimental or natural systems can elucidate the effects of plant characteristics and environmental variation on the production and relative importance of different types of anti-herbivore defense (Coley et al. 1985, Ernest 1989, Coley et al. 2005).

Myrmecophytes (ant-plants) are particularly interesting subjects with which to examine plant defense strategies (Folgarait and Davidson 1994, 1995; Dyer et al. 2001, Heil and McKey 2003). In these systems, plants provide nesting space and often food for ant occupants, which in turn usually protect the plants from herbivory and encroaching vegetation (reviewed in Davidson and McKey 1993, Bronstein 1998 and Heil and McKey 2003). The degree of protection afforded by the ants varies among ant-plant partnerships (Vasconcelos and Casimiro 1997, Itioka et al. 2000, Nomura et al. 2000, Heil et al. 2001a), and within systems according to environmental factors and individual plant and ant characteristics (Koptur 1985, Michelangeli 2003, Fáveri and Vasconcelos 2004). Most myrmecophyte species can be occupied by multiple ant species (Fonseca and Ganade 1996, Alonso 1998, Bruna et al. 2005), which range in their ability to defend the plant from herbivorous insects from very efficient to completely ineffective (Janzen 1966, McKey 1984, Heil et al. 2001a, Bruna et al. 2004). Identifying this variation in partner quality and determining the effects on the host plant are important aspects of understanding the evolution and maintenance of ant-plant mutualisms involving guilds of ant occupants (Stanton 2003).

Because the defensive ability of ant symbionts varies, it could benefit plant performance to increase production of chemical or other defenses when the ants do not

prevent herbivory. Conversely, in circumstances where ants do provide effective anti-herbivore defense, allocation for ant-related traits should increase and allocation for other defenses should decrease. Housing and provisioning ants may bear substantial costs for the plant, and in some ant-plant systems the plants only make these investments when the benefits of defensive ants are realized (Heil et al. 1997, Heil et al. 2001b, Dyer et al. 2001). For example, work on *Piper cenocladum*, has shown that experimental removal of defensive ants resulted in reduced investment by the host plant in food bodies for ants and increased production of defensive chemicals. This finding supports the idea of a trade-off between alternative defensive strategies at the level of individual plants of myrmecophytic species, with the allocation to different defenses contingent upon both resource availability and presence of ant defenders (Dyer et al. 2001, 2004).

In addition to ant presence, nutrient availability also affects the defensive mechanisms of at least some ant-plants. Myrmecophytes that feed resident ants depend on light and soil resources for both the production of food bodies and the synthesis of defensive chemicals. Some work has shown that abundant resources may break the apparent trade-off between these two investments in anti-herbivore defense (Dyer et al. 2004). Indeed, Folgarait and Davidson (1994) found that in *Cecropia*, higher light levels resulted in increased production of both food bodies for ants and carbon-based defensive chemicals, suggesting that defense-related traits may co-vary and correlate with plant growth when resources are abundant. However, work on the same species of *Cecropia* found that nutrient augmentation increased the production of food bodies, but concentrations of carbon-based chemical defenses were significantly higher under low nutrient treatments (Folgarait and Davidson 1995). For myrmecophyte species that do

not produce food bodies, nutrient availability could directly affect the rate of leaf damage and total losses to herbivory through either the production of defensive chemicals or variation in tolerance and resilience to leaf damage. The production of domatia, usually associated with plant growth in general, is a key component of myrmecophyte growth (Brouat and McKey 2000) and may be an important indirect effect of nutrient availability in species that do not provision ant occupants.

Seedlings and very young individuals of myrmecophytic species usually are not colonized by ants or house only very small colonies (Feldhaar et al. 2003, Dejean 2004). This has led to the suggestion that young myrmecophytes may be more reliant upon chemical and physical defenses than older individuals that are protected by mutualist ant colonies. Nomura et al. (2001) found that myrmecophytic species of *Macaranga* produced more chemical defenses as saplings prior to ant occupation than as ant-occupied adults. However, even small individuals of some myrmecophyte species may be protected by mutualistic ants (Schupp 1986, Itino and Itioka 2001). Furthermore, young individuals of fast-growing myrmecophytes may have high tolerance to herbivory, allocating more resources to growth than to defense (Del Val and Dirzo 2003).

Determining the relative importance of ant defenders for preventing herbivory as myrmecophytes grow from seedlings to mature plants is critical to understanding the evolution and ecology of ant-plant mutualisms (Bronstein 1998, Heil and McKey 2003). Additionally, nutrient availability is known to be important for the anti-herbivore defense of several myrmecophytic species and should be considered in any examination of potential trade-offs or ontogenetic shifts in plant defensive strategies. The primary objective of this study was to investigate how the presence and identity of ant occupants,

plant age, and nutrient availability affect herbivory of *Cordia alliodora*, a common neotropical myrmecophyte. *Cordia alliodora* is occupied by a diversity of ant species, which are non-randomly distributed among plant ages (Chapter 2, this thesis) and likely vary in their ability to deter herbivores, making this an ideal system to investigate the sources of inter-plant variation in herbivory and anti-herbivore defense by ants (Stanton 2003).

Methods

Study System

Cordia alliodora (Boraginaceae) is widespread and abundant in the secondary forests, clearings and forest edges at La Selva Biological Station (Costa Rica, Heredia Province, 10° 26' N, 83° 59' W), where I conducted this research. I studied trees planted in monospecific plots as part of the Huertos Project, a long-term ecological study established at La Selva in 1991 in which *C. alliodora* was one of the focal tree species. In each of the three replicated blocks of the Huertos Project, single-age stands of *C. alliodora* were planted in rows at a very high initial density (2887 trees ha^{-1}), periodically thinned, and regularly weeded to prevent the establishment of other vegetation. Each block had three adjacent plots of trees in 1-, 4- and 16-yr planting cycle; the results presented here are from 1- and 5-yr-old trees sampled evenly from all three blocks. In each plot, parallel rows were offset and placed 1.73 m apart, with plants placed at 2 m intervals within rows, such that each individual was at the center of a hexagon 2 m from the six closest plants. Haggar and Ewel (1995) provide further details about the site, the planting techniques and the design of the Huertos Project. The plots included in this study were divided into fertilized and unfertilized treatments. The nutrient-supplemented

plants received a liquid fertilizer of urea and NO_3- (31% N volume:volume) every two weeks, totaling 320 $kg*ha^{-1}*y^{-1}$ (Silver et al. 2005).

The domatia inhabited by the ant associates of *C. alliodora* are naturally hollow cauline swellings that the plants produce at most branch nodes. In Costa Rica, *C. alliodora* is most commonly occupied by the specialist ants *Azteca pittieri* and *Cephalotes setulifer*, as well as several generalist live stem inhabiting ants including species of *Crematogaster*, *Pseudomyrmex*, *Cephalotes* and *Pheidole* (Wheeler 1942, Longino 1996, Tillberg 2004). Stable isotope and behavioral studies suggest that *A. pittieri* and *C. carinata*, the two most abundant ant species occupying *C. alliodora* in the Huertos Project plots at La Selva, patrol the plant regularly and consume insect prey (Möser 2000, Tillberg 2004). In contrast, the isotopic profile of *Cephalotes setulifer* indicates that this species subsists primarily on honeydew secreted from coccoid hemipterans (Coccidae and Pseudococcidae) that cohabit the domatia they occupy (Tillberg 2004). However, the relative effects of occupation by different ant species on the amount of leaf damage sustained by their host plants are unknown.

The leaves of *C. alliodora* are eaten by a number of generalist and specialist insect herbivores (Wheeler 1942, Flowers and Janzen 1997, Möser 2000, Rojas et al. 2001, Tillberg 2004). Many of the resident ant species also tend honeydew-producing Hemiptera inside the domatia, which could negatively affect plant performance (Möser 2000, Tillberg 2004). The most abundant and most damaging insect herbivore present on the trees at the Huertos Project plantings of *C. alliodora* was the tortoise beetle *Coptocycla leprosa* (Chrysomelidae: Cassidini; Fig. 3-1). This beetle spends its entire life cycle on *C. alliodora*, appears to be a specialist on the genus *Cordia*, and can

extensively defoliate the plant when population densities are high (Wheeler 1942, Flowers and Janzen 1997). The chronically high levels of leaf damage and the large number of insect herbivores on *C. alliodora* led Wheeler (1942) to conclude that the ant occupants provided no substantive benefit to the plant.

Field Survey of Herbivory

To determine the effects of plant and ant factors on herbivory, I collected one subterminal domatium from a single haphazardly selected branch from 115 *Cordia alliodora* plants (N = 8-10 individuals from each plant age x fertilization x block combination). I identified the ant species present in each domatium, counted the number of workers present, and collected all fully expanded leaves within 10 cm of the focal domatium. I then produced digital images of these leaves with a flatbed scanner and measured the leaf area missing with Scion Image (v. 4.02, Scion Corporation) following the protocol described by O'Neal et al. (2002). The total area of collected leaves varied considerably among plants, so for the analysis of herbivory I used the proportion of leaf area missing, logit transformed to improve the distribution for parametric tests. Although measuring leaf area missing only once provides a static view of herbivore damage and may underestimate the impact of herbivores on longer time scales, it is nevertheless useful for comparisons of relative herbivore damage within species (Lowman 1984, Brown and Allen 1989, Coley and Barone 1996).

To test for the effects of plant age and fertilization on the number of leaves, total leaf area, and the number and identity of ants in the focal domatium, I used a linear mixed model analysis. Because plant age and fertilization were replicated at the level of the three blocks, individual plants (treated as subplots) were nested within block x plant age x fertilization combinations. There were no subplot-level factors or covariates in

these analyses. I also used a more complex linear mixed model procedure to test for the effects of plot-level variation (the block x plant age x fertilization combinations) and subplot-level variation (the number of worker ants on each plant) on the amount of leaf herbivory:

$$y_{ijkl} = \mu + b_i + \alpha_j + \beta_k + \varepsilon_{ijk} + \gamma x_{ijkl} + \varepsilon_{ijkl}$$

In this model, y_{ijkl} indicates the measure of herbivory, b_i is the random block effect, α_j is the fertilization effect, β_k is the plant age effect, ε_{ijk} is the whole-plot error, γx_{ijkl} is the effect of the covariate ant number within individual plants and ε_{ijkl} is the subplot (plant-level) error. In this analysis, individual plants were nested within the plots, which were defined as plant age x fertilization x block combinations.

Because the data were not balanced, I used maximum likelihood estimation methods to fit the models. The significance of the block effect, b_i, was determined using a likelihood ratio test and was treated as a random effect contributing to total variance in the final model rather than a fixed effect (Pinhiero and Bates 2000). Only the main effects of plant age and fertilization treatment were included in the final model following a likelihood ratio tests showing that interactive effects did not improve the model fit (Pinhiero and Bates 2000). Due to significant differences in ant occupancy and herbivory between 1- and 5-yr-old plants, I conducted analyses similar to the model above separately for the two ages. All mixed model analyses were conducted with the lme program in R 2.2.0 following the protocol of Pinhiero and Bates (2000). All P-values were calculated with marginal (Type III) tests for significance.

Leaf Palatability Bioassay

In July 2004, I tested the palatability of *Cordia alliodora* leaves for adult *Coptocycla leprosa* beetles with a three-factor, fully crossed laboratory trial. The factors

tested in this study were plant age (1- or 5-yr-old), leaf age (young or mature, which were easily distinguished based on leaf color and texture) and fertilization treatment (unfertilized or fertilized). Beetles were collected from 1-yr-old plants in the field and then starved for approximately 24 hr in the laboratory. I then placed each of 48 randomly selected individuals in a breathable plastic bag for 24 hr with one freshly collected *C. alliodora* leaf. All leaves were collected from the same plot and all had relatively little insect damage to control for herbivory-induced changes in palatability. I conducted three trials with identical treatments and experimental procedures, with each trial containing six replicates of the eight treatment combinations (N=144 beetles tested). To quantify herbivory I measured the initial and final leaf area with a Licor 3100 area meter. Because the young leaves lost area due to reduced turgor pressure over the course of the experiment, I used a correction factor derived from a linear regression equation based on the initial area to calculate their change in area due to shrinkage ($y = 0.9934x - 0.7378$, $R^2 = 0.9988$, $P < 0.001$). I used a block design ANOVA to test for main and interaction effects of the treatments on the amount of leaf material consumed, with the trial as the block factor to account for random temporal effects.

Results

Field Survey of Herbivory

One to 16 leaves were present within 10 cm of focal domatia (mean ± SD = 6.2 ± 3.1), and the total leaf area before herbivory ranged from 4.6 to 310.8 cm^2 (mean ± SD = 96.9 ± 60.9 cm^2). There were no significant effects of plant age or fertilization on the number of leaves (Table 3-1), but the total leaf area was marginally significantly larger for the 5-yr-old plants (Table 3-2). Although the leaf area varied within both ages, the 5-

yr-old plants tended to have greater leaf area than the 1-yr-old plants (mean ± SD = 111.7 ± 65.2 cm^2 and 82.8 ± 53.2 cm^2, respectively).

Most domatia (78.3%) contained ants. The frequency of unoccupied domatia was approximately twice as high for the 1-yr-old plants as for the 5-yr-old plants (28.8%, and 14.3%, respectively). *Azteca pittieri* was the most abundant species (43.3%), followed by *Crematogaster carinata* (33.3%), *Cephalotes setulifer* (14.4%) and *Pseudomyrmex fortis* (8.9%). Significantly more worker ants were present in domatia from 5-yr-old plants than in domatia from 1-yr-old plants, but there was no effect of fertilization on ant number (Table 3-3, Fig. 3-2). The number of worker ants present also varied substantially according to which species occupied the plant (Fig. 3-3). Overall, *Cr. carinata* had the most workers per domatium overall and was the only species to have more than founding queens and a few workers present in domatia from the 1-yr-old plants.

The proportion of leaf area missing from the leaves surrounding each domatium ranged from 0.02 to 0.45 (mean ± SD = 0.12 ± 0.09). The 1-yr-old plants tended to have higher proportion of leaf area missing, as did the plants that were not fertilized—both of these effects were marginally significant ($P < 0.10$). The number of ants, included as a plant-level covariate in the model, significantly affected herbivory (Table 3-4). Linear regression showed only a weak but significant negative relationship between ant number and herbivory ($R^2 = 0.24$, $P < 0.0001$), but the mixed model analysis showed that ant presence and abundance were clearly important when plant age and fertilization were included in the model. Although the effects of age and fertilization were not statistically significant at the $\alpha = 0.05$ level, examination of the data suggests that the 1-yr-old plants

experienced more proportional leaf damage than the 5-yr-old plants and that fertilization reduced herbivory for the younger plants (Fig. 3-4). When the two ages were analyzed separately, fertilization did not significantly affect the proportion of leaf area missing for either, but the number of ants had a significant negative effect on herbivory for the 5-yr-old plants (Table 3-5).

Leaf Palatability Bioassay

The results of the ANOVA for the leaf palatability trial showed that fertilization significantly affected the amount of leaf area consumed by *C. leprosa*, whereas leaf age had only a marginally significant effect and there was no effect of plant age (Table 3-6). Overall, the beetles consumed more material from young leaves than from mature leaves, and more from the leaves of unfertilized plants than from fertilized plants. However, these main effects were mainly due to the high leaf area consumed from young, unfertilized leaves, as indicated by the marginally significant interaction effect between fertilization treatment and leaf age (Fig. 3-6). None of the other interactions among factors were statistically significant.

Discussion

The relationships between different types of plant defenses are often complex and may vary over the ontogeny of the plant and according to resource availability and the efficacy of ant defense (Folgarait and Davidson 1994, 1995, Nomura et al. 2001, Del Val and Dirzo 2003, Dyer et al. 2004). In this study, I investigated the patterns of herbivory on *Cordia alliodora*, a common myrmecophytic tree, in relation to plant age, fertilization and ant abundance. Identifying variation in the benefits and costs associated with different partners in mutualist guilds and determining the consequences of such variation are major objectives of holistic examination of mutualism (Stanton 2003). The presence

of multiple ant species in *C. alliodora* plants, which appear to vary in their defensive behavior (Tillberg 2004), provided an ideal system in which to examine the effects of variation in partner quality in this system. Although I was unable to test the effects of different ant species on herbivory directly due to nonrandom distribution of the ants in the plants I sampled, I was able to infer that at least the two most abundant species, *Azteca pittieri* and *Crematogaster carinata*, appeared to defend the plant from insect herbivores. Future work on this system could include explicit tests of interspecific differences in the amount of leaf damage allowed by the different ant occupants.

The number of worker ants had no effect on the proportional leaf damage of the 1-yr-old plants, as might be expected given the very small number of individuals present in domatia collected from these young plants (Table 3-5). Young individuals of many tropical plants display a strategy of tolerance to herbivory rather than investment in anti-herbivore defenses (Strauss and Agrawal 1999, Coley et al. 2005), and this may be the case for *C. alliodora*. Del Val and Dirzo (2003) showed that leaves from young *Cecropia peltata*, another fast-growing myrmecophyte, were also more palatable to herbivores than leaves from older plants due to reduced investment in anti-herbivore defenses. However, despite high levels of leaf damage in the field, in controlled environments the beetle *C. leprosa* consumed marginally significantly less area from leaves taken from fertilized plants, particularly in the 1-yr-old plants (Table 3-6). This result suggests that even the 1-yr-old *C. alliodora* plants also produced chemical defenses that effectively deter at least one species of specialist herbivore. Whether these results extend to other herbivores is unknown.

By contrast, 5-yr-old trees appeared to rely on ants for anti-herbivore defense, with ant presence and the number of workers present in focal domatia significantly reducing the proportion of leaf damage in the immediate vicinity (Table 3-5). Due to the non-random distribution of the four ant species in this study, it was impossible to analyze the effects of each on leaf damage. However, since the number of workers was significantly negatively related to the proportional leaf damage, the variation among the ant species in the number of workers present is suggestive of interspecific differences in plant defense (Fig. 3-3). *Azteca pittieri* and *Crematogaster carinata* were the most abundant ant species in this study (together accounting for 69 of the 90 occupied domatia) and had the most workers per domatium (Figure 3-3), suggesting that they effectively reduced herbivore damage. This result is in accordance with behavioral and stable isotopic studies demonstrating that *A. pittieri* and *C. carinata* attack and consume insect herbivores on *C. alliodora* plants (Möser 2000, Tillberg 2004). *Azteca pittieri* and related *Azteca* species are the most abundant ants in *C. alliodora* throughout the range of the plant (Wheeler 1942, Longino 1996), and the protective effect of these specialist species may be a common result of occupation by these ants when they inhabit *C. alliodora*. Conversely, *C. setulifer*, which is another specialist inhabitant of *Cordia*, was only present in small numbers and appears to be at best a passive defender of *C. alliodora* (Tillberg 2004). Because it is rarely the dominant ant species on older trees and appears to be out-competed by *A. pittieri* on plants where the two species co-occur (Chapter 2, this thesis), whether or not *C. setulifer* effectively defends against insect herbivores may not affect plant performance at the individual or population level. Interestingly, *C. carinata*, which is only an opportunistic occupant of *C. alliodora* domatia, appears to

confer benefits to plants through reduction of herbivore damage. This species is not commonly found in *C. alliodora*, however, and therefore this ant species probably has little impact on the *C. alliodora*-ant relationship at the population level or across the range of the plant.

The results of the experimental leaf palatability trial with the specialist beetle herbivore, *Coptocycla leprosa*, corroborated those from the survey of natural herbivory in some ways but also differed on several important points. In the field, leaves from 1-yr-old plants had higher proportional leaf damage than those from 5-yr-old plants, but in the laboratory there was no difference between the two plant ages in leaf area consumed. Although the insects responsible for the leaf damage in the field are unknown, this difference could support the finding that ants limit *C. leprosa* damage in 5-yr-old trees because it was so abundant at the site. Whereas in the laboratory trial there was less herbivory on leaves from fertilized plants regardless of plant age, in the field the fertilization effect was only marginally significant and appeared to have no effect at all for the 5-yr-old plants. The contrast between the results of the leaf palatability trial and the observed patterns of leaf damage in the field suggest that the beetles do not base their foraging choices in nature solely on preference for the most palatable leaves. Rather, they are most effective at attacking young plants that do not house large colonies of defending ants.

Fertilization significantly reduced the leaf area consumed in the palatability trial and reduced herbivory of 1-yr-old, but not 5-yr-old, plants in the field survey of herbivory. Together, these findings suggest that nutrient augmentation increases the production of defensive chemicals in *C. alliodora*, and that the relative importance of

these chemicals for anti-herbivore defense varies according to plant age. Even if the chemical defenses of *C. alliodora* do not contain nitrogen, other work has shown that N fertilization can increase the production of carbon-based defenses (Mihaliak and Lincoln 1985, Wilkens et al. 1996). Relatively little is known of the secondary metabolites and anti-herbivore chemical defenses produced by *C. alliodora*. Chen et al. (1983) described several triterpenoid compounds isolated from the leaves of *C. alliodora* that repelled leafcutter ants in experimental trials. Gomez et al. (1999) found lower terpenoids in the leaves of a congener, *Cordia curassavica*, but did not find these compounds in *C. alliodora*. Additionally, a number of secondary metabolic compounds have been isolated from the bark and wood of *C. alliodora*, including some with fungicidal or insecticidal properties (Moir and Thomson 1973, Stevens et al. 1973, Manners and Jurd 1977, Ioset et al. 2000, Vanisree et al. 2002). However, it is unclear what role these chemicals or related compounds may have in plant defense against leaf herbivores. Identifying defensive chemicals from *C. alliodora* leaves, assessing their effects on herbivory, and determining how their production varies with plant age and environmental factors are critical areas of study for fully understanding the defensive strategy of this species.

The evolution and maintenance of ant-plant mutualisms is dependent upon net fitness benefits at the population level for both the ant and plant partners (Bronstein 1998, Heil and McKey 2003). The primary benefit for the plants is usually protection from herbivory, which often has negative short- and long-term fitness consequences (Marquis 1984, Ernest 1989, Doak 1992, Coley and Barone 1996). In this study, I found that the ants reduced herbivore damage on the 5-yr-old plants but not on the 1-yr-old plants. The increased nutrient availability marginally reduced herbivore damage overall but, at least

in the field, had no effect on herbivory of older plants (Table 3-4). However, there was no evidence of distinct trade-offs in defensive mechanisms over plant ontogeny in this system. Instead, it appeared that the reduction of herbivory resulting from the ant occupants was additive, and the palatability trial suggested that whatever chemical defenses *C. alliodora* produces were present in plants of both ages studied. Therefore, in the *C. alliodora*-ant system, and probably other ant-plant relationships, plant growth and the production of domatia to house mutualist ants likely represent important investments in anti-herbivore defense for the plant over both evolutionary and ecological time scales (Brouat and McKey 2000). This investment may not produce immediate benefits because the ants require time to colonize the plant and produce workers. Therefore, young plants may produce defensive chemicals if adequate resources are available or may simply invest in rapid growth that minimizes the effects of herbivory and promotes future ant protection.

Table 3-1. Results of mixed model analysis testing the effects of plant age and fertilization on the number of leaves surrounding focal domatia.

Source of variation	num. df	den. df	F	P
Plant age	1	6	3.46	0.11
Fertilization	1	6	1.31	0.30

Table 3-2. Results of mixed model analysis testing the effects of plant age and fertilization on the total area of leaves surrounding focal domatia.

Source of variation	num. df	den. df	F	P
Plant age	1	6	5.72	0.054
Fertilization	1	6	1.88	0.22

Table 3-3. Results of mixed model analysis testing the effects of plant age and fertilization on the number of worker ants within the focal domatia.

Source of variation	num. df	den. df	F	P
Plant age	1	6	24.35	0.0026
Fertilization	1	6	0.17	0.70

Table 3-4. Results of mixed model analysis testing the effects of plant age, fertilization, and the number of worker ants on the proportion of leaf area missing. The number of ants was $\log_{10}$-transformed and the proportion of leaf area missing was logit-transformed to improve the distribution for this analysis.

Source of variation	num. df	den. df	F	P
Plant age	1	6	3.90	0.096
Fertilization	1	6	4.98	0.067
No. ants	1	103	7.54	0.0071

Table 3-5. Results of mixed model analysis testing the effects of fertilization and the number of worker ants present on herbivory for 1-yr-old plants and 5-yr-old plants. The number of ants was log10-transformed and the proportion of leaf area missing was logit-transformed to improve the distribution for this analysis. Although the results are presented together, the analyses were conducted separately for the two ages.

Plant age	Source of variation	num. df	den. df	F	P
1	Fertilization	1	2	4.78	0.16
	No. ants	1	52	0.026	0.87
5	Fertilization	1	1	0.34	0.66
	No. ants	1	50	6.53	0.014

Table 3-6. Results of ANOVA testing the effects of plant age, leaf age and fertilization treatment on the leaf area consumed by one Coptocycla leprosa beetle in 24 hr, with trial as a random block effect.

Source of variation	df	MS	F	P
Plant age	1	6.25	2.03	0.16
Leaf age	1	10.99	3.58	0.061
Fertilization	1	18.41	5.99	0.016
Plant age x Leaf age	1	0.35	0.12	0.74
Plant age x Fertilization	1	5.40	1.76	0.19
Leaf age x Fertilization	1	11.38	3.70	0.056
Plant age x Leaf age x Fertilization	1	3.38	1.10	0.30
Trial	2	28.02	9.11	< 0.001
Error	134	3.08		

A.

B.

C.

Figure 3-1. The beetle *Coptocycla leprosa* spends its entire life cycle on *Cordia alliodora*. (A) Late-instar larva with fecal shield, (B) pupa adhering to top of a leaf and (C) adult on underside of leaf.

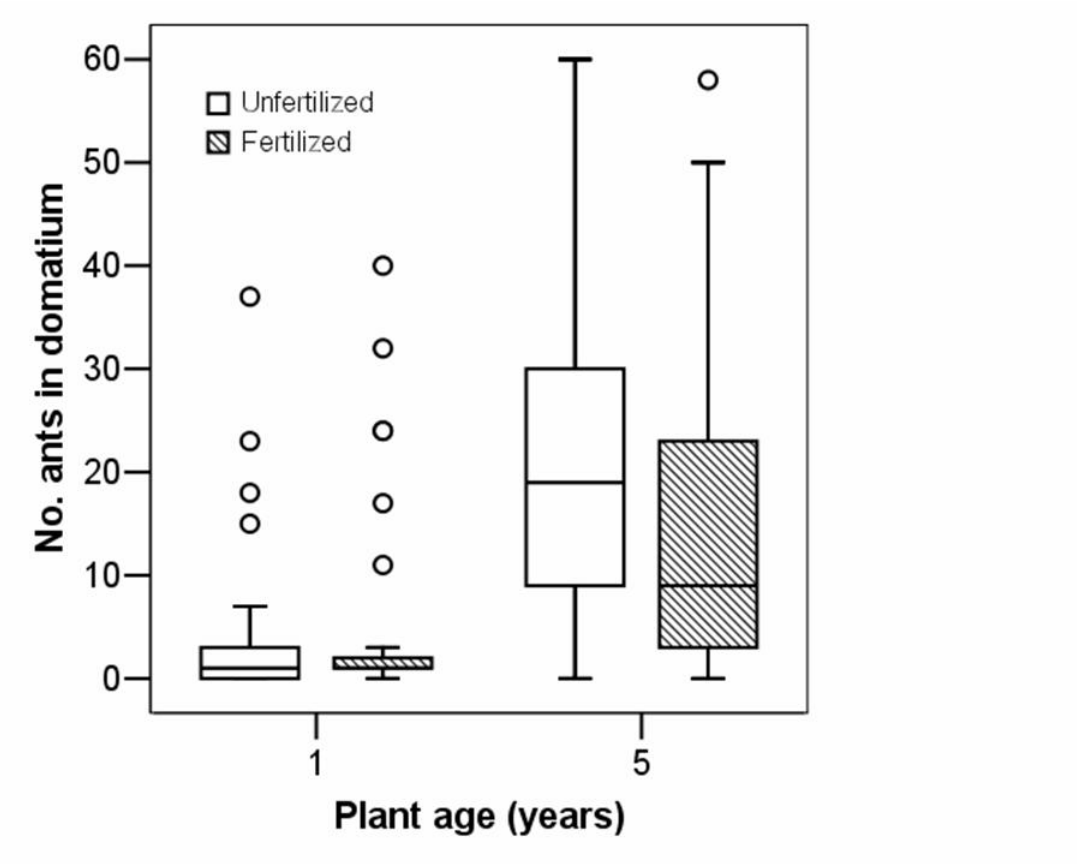

Figure 3-2. The number of worker ants present in focal domatia varied with plant age but fertilization treatment had no effect. Boxplots show inter-quartile ranges and expected minimum and maximum values, with values beyond the 95% CI indicated by open circles. All of the high outliers in the 1-yr-old plants were domatia occupied by *Crematogaster carinata*.

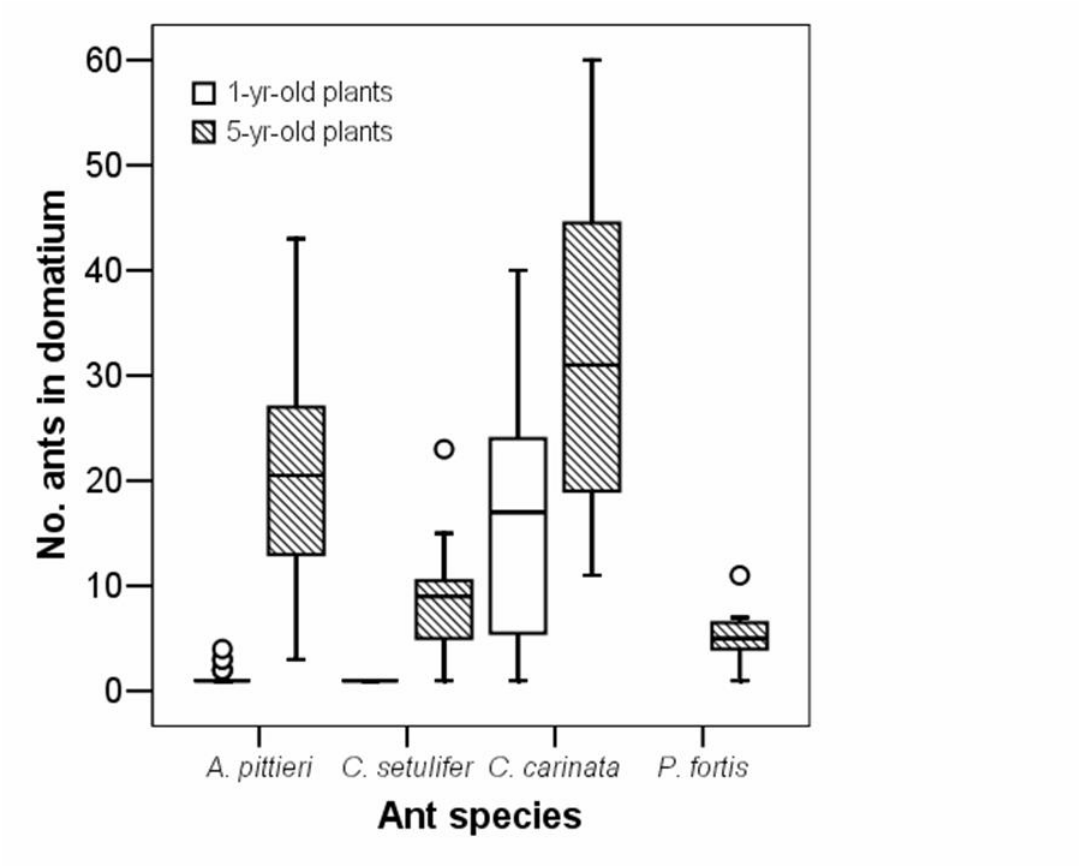

Figure 3-3. The number of worker ants present in focal domatia varied according to plant age and the identity of the ant species. Boxplots show inter-quartile ranges and expected minimum and maximum values, with values beyond the 95% CI indicated by open circles. *Pseudomyrmex fortis* was not present in any domatia from 1-yr-old plants.

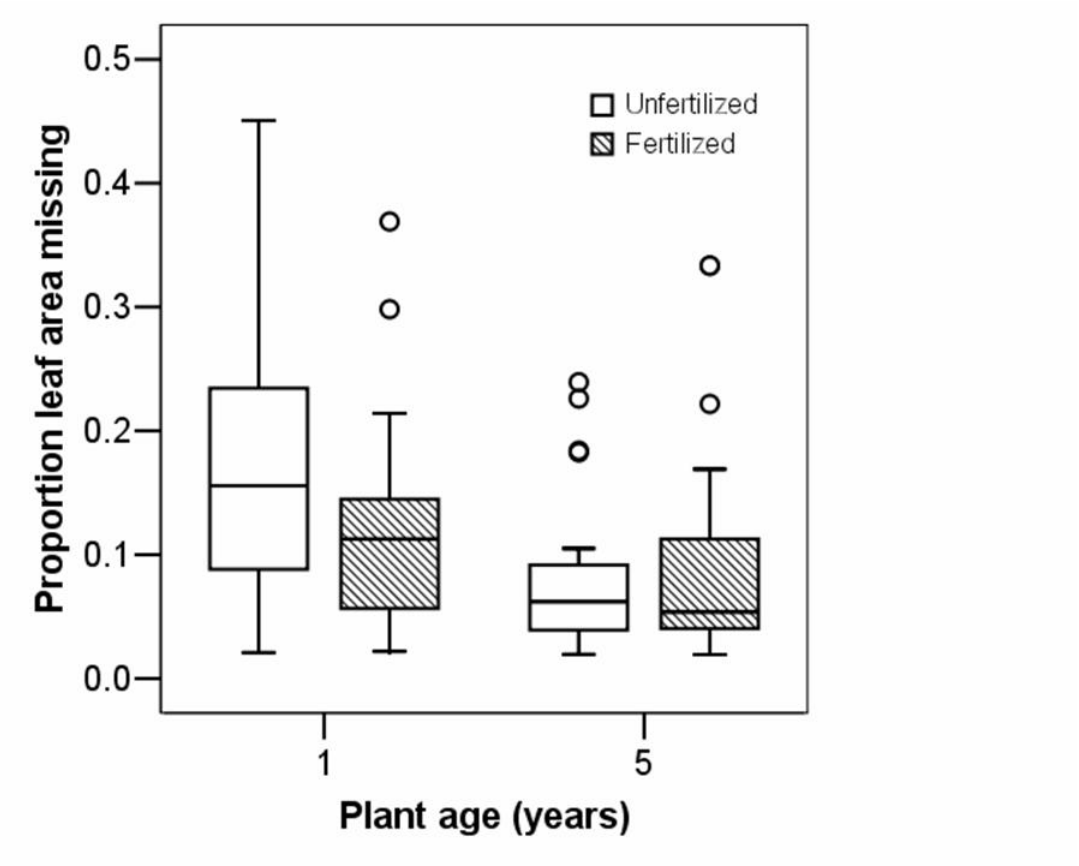

Figure 3-4. The proportion of leaf area missing was affected at $P < 0.10$ by plant age and fertilization treatment. In general 1-yr-old plants experienced more proportional leaf damage than 5-yr-old plants, and for the 1-yr-old plants fertilization reduced herbivore damage. Boxplots show inter-quartile ranges and expected minimum and maximum values, with values beyond the 95% CI indicated by open circles.

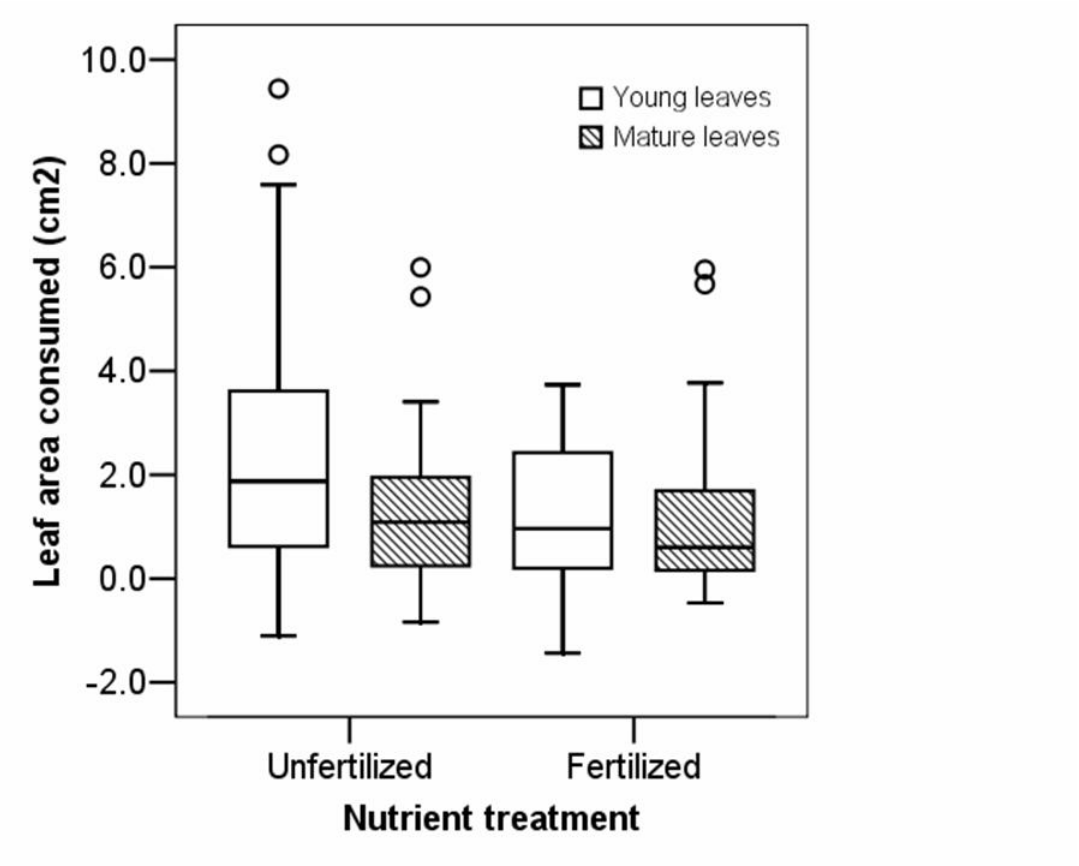

Figure 3-5. Fertilization significantly reduced the leaf area consumed by individual *Coptocycla leprosa* beetles in the leaf palatability trials. This was particularly true for young leaves, as indicated by the marginally significant (P = 0.056) interactive effect between fertilization and leaf age (Table 3-6).

CHAPTER 4
CONCLUSIONS

The overall objective of this study was to document the patterns of ant occupation in *C. alliodora* and to determine the effects of this variation on the amount of herbivore damage as the host plants aged and grew. Addressing this question first required a detailed examination of patterns of coexistence among the ant species that inhabit *C. alliodora* and discussion of the mechanisms that could account for these patterns. I then tested the effects of ant presence and abundance, in addition to the influence of plant age and fertilization, on the proportional damage of leaves surrounding focal domatia. I complemented the survey of herbivory with a palatability trial using a specialist herbivore of *C. alliodora* to determine ant-free preferences for leaves from plants differing in age and fertilization.

The two most abundant ant species I found inhabiting *C. alliodora*, *A. pittieri* and *Cr. carinata*, were also present in the highest numbers in the individual domatia sampled in the field survey of herbivory. Because the number of worker ants had a significant negative effect on proportional leaf damage, I suggest that both of these species act as mutualists and benefit the host plant by reducing herbivory. The other ant species found within *C. alliodora* domatia were generally subdominant at the whole-tree level and had fewer workers per domatium. However, because *A. pittieri* and closely related species are ubiquitous inhabitants of *C. alliodora* throughout its range (Longino 1996), at the population level it is unlikely that that less common species are important for the maintenance of the mutualism.

LIST OF REFERENCES

Alonso, L.E. 1998. Spatial and temporal variation in the ant occupants of a facultative ant-plant. Biotropica 30:201-213.

Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: a synthesis. Ecology Letters 6:1109-1122.

Beattie, A.J. 1985. The evolutionary ecology of ant-plant mutualisms. Cambridge University Press, Cambridge, UK.

Bluthgen, N., N.E. Stork and K. Fiedler. 2004. Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. Oikos 106:344-358.

Bowers, M.D. and N.E. Stamp. 1993. Effects of plant age, genotype, and herbivory on *Plantago* performance and chemistry. Ecology 74:1778-1791.

Bronstein, J.L. 1998. The contribution of ant-plant protection studies to our understanding of mutualism. Biotropica 30:150-161.

Brouat, C. and D. McKey. 2000. Origin of caulinary ant-domatia and timing of their onset in plant ontogeny: evolution of a key trait in horizontally-transmitted ant-plant symbioses. Biological Journal of the Linnean Society 71:809-819.

Brown, B.J. and T.F.H. Allen. 1989. The importance of scale in evaluating herbivory impacts. Oikos 54:189-194.

Brues, C.T. 1922. *Conoaxima*, a new genus of the hymenopteran family Eurytomidae, with a description of its larva and pupa. Psyche 29:153-158.

Bruna, E. M., D. M. Lapola and H. L. Vasconcelos. 2004. Interspecific variation in defensive responses of obligate ant-plant inhabitants: are there consequences for host plants? Oecologia 238:558-565

Bruna, E.M., H.L. Vasconcelos and S. Heredia. 2005. The effect of habitat fragmentations on communities of mutualists: Amazon ants and their host plants. Biological Conservation 124:209-216.

Bryant, J.P., P.B. Reichardt and T.B. Clausen. 1992. Chemically mediated interactions between woody plants and browsing mammals. Journal of Range Management 45:18-24.

Chen, T.K., D.C. Ales, N.C. Baenziger and D.F. Wiemer. 1983. Ant-repellent triterpenoids from *Cordia alliodora*. Journal of Organic Chemistry 48:3525-3531.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343-366.

Cole, B.J. 1983. Assembly of mangrove and communities: colonization abilities. Journal of Animal Ecology 52:349-355.

Coley, P.D. and T.M. Aide. 1991. Comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. Pages 25-49 in P.W. Price, T.M. Lewinsohn, W.W. Fernandes and W.W. Benson, eds. Plant-animal interactions: evolutionary ecology in tropical and temperate regions. John Wiley and Sons, New York.

Coley, P.D. and J.A. Barone. 1996. Herbivory and plant defenses in tropical forests. Annual Review of Ecology and Systematics 27:305-335.

Coley, P.D., J.P. Bryant and F.S. Chapin, III. 1985. Resource availability and plant antiherbivore defense. Science 230:895-899.

Coley, P.D., J. Lokvam, K. Rudolph, K. Bromberg, T.E. Sackett, L. Wright, T. Brenes-Arguedas, D. Dvorett, S. Ring, A. Clark, C. Baptiste, R.T. Pennington and T.A. Kursar. 2005. Divergent defensive strategies of young leaves in two species of *Inga*. Ecology 86:2633-2643.

Davidson, D.W. 1980. Some consequences of diffuse competition in a desert ant community. American Naturalist 116:92-105.

Davidson, D.W. 1998. Resource discovery versus resource domination in ants: a functional mechanism for breaking the trade-off. Ecological Entomology 23:484-490.

Davidson, D.W. and D. McKey. 1993. The evolutionary ecology of symbiotic ant-plant relationships. Journal of Hymenoptera Research 2:13-83.

Davidson, D.W., R.R. Snelling and J.T. Longino. 1989. Competition among ants for myrmecophytes and the significance of plant trichomes. Biotropica 21:64-73.

Dejean, A., A. Quilichini, J.H.C. Delabie, J. Orivel, B. Corbara and M. Gibernau. 2004. Influence of its associated ant species on the life history of the myrmecophyte *Cordia nodosa* in French Guiana. Journal of Tropical Ecology 20:701-704.

Del Val, E. and R. Dirzo. 2003. Does ontogeny cause changes in the defensive strategies of the myrmecophyte *Cecropia peltata*? Plant Ecology 169:35-41.

Doak, D.F. 1992. Lifetime impacts of herbivory for a perennial plant. Ecology 73:2086-2099.

Dyer, L.A., C.D. Dodson, J. Beihoffer and D.K. Letourneau. 2001. Trade-offs in antiherbivore defenses in *Piper cenocladum*: ant mutualists versus plant secondary metabolites. Journal of Chemical Ecology 27:581-592.

Dyer, L.A., D.K. Letourneau, C.D. Dodson, M.A. Tobler, J.O. Stireman III and A. Hsu. 2004. Ecological causes and consequences of variation in defensive chemistry of a neotropical shrub. Ecology 85:2795-2803.

Ernest, K.A. 1989. Insect herbivory on a tropical understory tree: effects of leaf age and habitat. Biotropica 21:194-199.

Fáveri, S.B. and H.L. Vasconcelos. 2004. The *Azteca-Cecropia* association: are ants always necessary for their host plants? Biotropica 36:641-646.

Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51:565-581.

Feldhaar, H., B. Fiala, R.b. Hashim and U. Maschwitz. 2003. Patterns of the *Crematogaster-Macaranga* association: the ant partner makes the difference. Insectes Sociaux 50:9-19.

Fellers, J. 1987. Interference and exploitation in a guild of woodland ants. Ecology 68:1466-1478.

Flowers, R.W. and D.H. Janzen. 1997. Feeding records of Costa Rican leaf beetles (Coleoptera: Chrysomelidae). Florida Entomologist 80:334-366.

Folgarait, P.J. and D.W. Davidson. 1994. Antiherbivore defenses of myrmecophytic *Cecropia* under different light regimes. Oikos 71:305-320.

Folgarait, P.J. and D.W. Davidson. 1995. Myrmecophytic *Cecropia*: antiherbivore defenses under different nutrient treatments. Oecologia 104:189-206.

Fonseca, C.R. 1999. Amazonian ant-plant interactions and the nesting space limitation hypothesis. Journal of Tropical Ecology 15:807-825.

Fonseca, C.R. and G. Ganade. 1996. Asymmetries, compartments and null interactions in an Amazonian ant-plant community. Journal of Animal Ecology 65:339-347.

Gaume, L., D. McKey and S. Terrin. 1998. Ant-plant-homopteran mutualism: how the third partner affects the interaction between a plant-specialist ant and its myrmecophytic host. Proceedings of the Royal Society of London Series B-Biological Sciences 265:569-575.

Haggar, J.P. and J.J. Ewel. 1995. Establishment, resource acquisition, and early productivity as determined by biomass allocation patterns of three tropical tree species. Forest Science 41:689-708.

Heil, M., B. Fiala, U. Maschwitz and K.E. Linsenmair. 2001a. On benefits of indirect defence: short- and long-term studies of antiherbivore protection via mutualistic ants. Oecologia 126:395-403.

Heil, M., B. Fiala, K.E. Linsenmair, G. Zotz, P. Menke and U. Maschwitz. 1997. Food body production in *Macaranga triloba* (Euphorbiaceae): a plant investment in anti-herbivore defence via symbiotic ant partners. Journal of Ecology 85:847-861.

Heil, M., A. Hilpert, B. Fiala and K.E. Linsenmair. 2001b. Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant. Oecologia 126:404-408.

Heil, M. and D. McKey. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. Annual Review of Ecology, Evolution and Systematics 34:425-453.

Herms, D.A. and W.J. Mattson. 1992. The dilemma of plants: to grow or defend. Quarterly Review of Biology 67:283-335.

Hölldobler, B. and C.J. Lumsden. 1980. Territorial strategies in ants. Science 210:732-739.

Hölldobler, B. and E.O. Wilson. 1990. The ants. Belknap Press, Cambridge.

Holway, D.A. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. Ecology 80:238-251.

Itino, T. and T. Itioka. 2001. Interspecific variation and ontogenetic change in antiherbivore defense in myrmecophytic *Macaranga* species. Ecology Research 16:765-774.

Ioset, J.R., A. Marston, M.P. Gupta and K. Hostettmann. 2000. Antifungal and larvicidal compounds from the root bark of *Cordia alliodora*. Journal of Natural Products 63:424-426.

Itioka, T., M. Nomura, Y. Inui, T. Itino and T. Inoue. 2000. Difference in intensity of ant defense among three species of *Macaranga* myrmecophytes in a Southeast Asian dipterocarp forest. Biotropica 32:318-326.

Janzen, D.H. 1966. Coevolution of mutualism between ants and acacias in Central America. Evolution 20:249-275.

Janzen, D.H. 1975. *Pseudomyrmex nigripilosa*: a parasite of a mutualism. Science 188:936-937.

Koptur, S. 1985. Alternative defenses against herbivores in *Inga* (Fabaceae: Mimosideae) over an elevational gradient. Ecology 66:1639-1650.

Lapola, D.M., E.M. Bruna, C. Granara de Willink and H.L. Vasconcelos. 2005. Ant-tended Hemiptera in Amazonian myrmecophytes: patterns of abundance and implications for mutualism function (Hymenoptera: Formicidae). Sociobiology 46:433-442.

Longino, J.T. 1989. Geographic variation and community structure in an ant-plant mutualism: *Azteca* and *Cecropia* in Costa Rica. Biotropica 21:126-132.

Longino, J.T. 1991. Azteca ants in *Cecropia* trees: taxonomy, colony structure, and behavior. In: C.R. Huxley and D.F. Cutter eds. Ant-plant interactions. Oxford University Press, Oxford. Pp 271-288.

Longino, J.T. 1996. Taxonomic characterization of some live-stem inhabiting *Azteca* (Hymenoptera: Formicidae) in Costa Rica, with special reference to the ants of *Cordia* (Boraginaceae) and *Triplaris* (Polygonaceae). Journal of Hymenoptera Research 5:131-156.

Lowman, M.D. 1984. An assessment of techniques for measuring herbivory: is rainforest defoliation more intense than we thought? Biotropica 16:264-268.

Manners, G.D. and L. Jurd. 1977. The hydroquinone terpenoids of *Cordia alliodora*. Journal of the Chemical Society, Perkins Transactions I 4:405-410.

Marquis, R.J. 1984. Leaf herbivores decrease fitness of a tropical plant. Science 226:537-539.

McGlynn, T.M. 2000. Do Lanchester's laws of combat describe competition in ants? Behavioral Ecology 11:686-690.

McKey, D. 1974. Adaptive patterns in alkaloid physiology. American Naturalist 108:305-320.

McKey, D. 1984. Interaction of the ant-plant *Leonardoxa africana* (Caesalpiniaceae) with its obligate inhabitants in a rainforest in Cameroon. Biotropica 16:81-99.

Michelangeli, F.A. 2003. Ant protection against herbivory in three species of *Tococa* (Melastomataceae) occupying different environments. Biotropica 35:181-188.

Mihaliak, C.A. and D.E. Lincoln. 1989. Plant biomass partitioning and chemical defense: response to defoliation and nitrate limitation. Oecologia 80:122-126.

Moir, M. and R.H. Thomson. 1973. Naturally occurring quinines. Part XXII. Terpenoid in *Cordia* spp. Journal of the Chemical Society, Perkins Transactions I 13:1352-1356.

Möser, J. 2000. Interaktionen zwischen Herbivoren, Ameisen und Parasitoiden auf *Cordia alliodora* (Boraginacea). Diplomarbeit Universität Hannover.

Nichols-Orians, C.M. 1991. Environmentally induced differences in plant traits: consequences for susceptibility to a leaf-cutter ant. Ecology 72:1609-1623.

Nomura, M., T. Itioka and T. Itino. 2000. Variations in abiotic defense within myrmecophytic and non-myrmecophytic species of *Macaranga* in a Bornean dipterocarp forest. Ecological Research 15:1-11.

Nomura, M., T. Itioka and K. Murase. 2001. Non-ant antiherbivore defenses before plant-ant colonization in *Macaranga* myrmecophytes. Population Ecology 43:207-212.

O'Neal, M.E., D.A. Landis and R. Isaacs. 2002. An inexpensive, accurate method for measuring leaf area and defoliation through digital image analysis. Journal of Economic Entomology 95:1190-1194.

Pacala, S.W. and M.J. Crawley. Herbivores and plant diversity. American Naturalist 140:243-260.

Palmer, T.M. 2003. Spatial heterogeneity influences competition and coexistence in an African acacia ant guild. Ecology 84:2843-2855.

Palmer, T.M. 2004. Wars of attrition: colony size determines competitive outcomes in a guild of Acacia ants. Animal Behavior 68:993-1004.

Palmer, T.M., M.L. Stanton and T.P. Young. 2003. Competition and coexistence: exploring mechanisms that restrict diversity within mutualist guilds. American Naturalist 162:563-579.

Palmer, T.M., T.P. Young and M.L. Stanton. 2002. Burning bridges: priority effects and the persistence of a competitively subordinant acacia-ant in Laikipia, Kenya. Oecologia 133:372-379.

Palmer, T.M., T.P. Young, M.L. Stanton and E. Wenk. 2000. Short-term dynamics of an acacia ant community in Laikipia, Kenya. Oecologia 123:425-435.

Pinhiero, J.C. and D.M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer-Verlag, New York.

Rojas, L., C. Godoy, P. Hanson and L. Hilje. 2001. A survey of homopteran species (Auchenorrhyncha) from coffee shrubs and poro and laurel trees in shaded coffee plantations, in Turrialba, Costa Rica. Revista de Biologia Tropical 49:1057-1065.

Sagers, C.L. and P.D. Coley. 1995. Benefits and costs of defense in a neotropical shrub. Ecology 76:1835-1843.

Schupp, E.W. 1986. *Azteca* protection of *Cecropia*: ant occupation benefits juvenile trees. Oecologia 70:379-385.

Stamp, N. 2003. Out of the quagmire of plant defense hypotheses. Quarterly Review of Biology 78:23-55.

Stanton, M.L., T.M. Palmer and T.P. Young. 2002. Competition-colonization trade-offs in a guild of African acacia-ants. Ecological Monographs 72:347-363.

Stevens, K.L., L. Jurd and G. Manners. 1973. Alliodorin, a phenolic terpenoid from *Cordia alliodora*. Tetrahedron Letters 31:2955-2958.

Strauss, S.Y. and A.A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends in Ecology and Evolution 14:179-185.

Tillberg, C.V. 2003. The evolution and ecology of an ant-plant symbiosis. Ph.D. Dissertation, University of Colorado, Boulder, CO.

Tillberg, C.V. 2004. Friend or foe? A behavioral and stable isotopic investigation of an ant-plant symbiosis. Oecologia 140:506-515.

Tokeshi, M. 1999. Species coexistence: ecological and evolutionary perspectives. Blackwell Scientific, Oxford, UK.

Vasconcelos, H.L. 1993. Ant colonization of *Maieta guianensis* seedlings, an Amazonian ant-plant. Oecologia 95:439-443.

Vasconcelos, H.C. and A.B. Casimiro. 1997. Influence of *Azteca alfari* ants on the exploitation of *Cecropia* trees by a leaf-cutting ant. Biotropica 29:84-92.

Vasconcelos, H.L. and D.W. Davidson. 2000. Relationship between plant size and ant associates in two Amazonian ant-plants. Biotropica 32:100-111.

Vanisree, M., J. Kavitha and G.V. Subbaraju. 2002. Synthesis of methyl 3-(2,4,5-trimethoxyphenyl)-propionate, an antifungal and larvicidal constituent of *Cordia alliodora*. Asian Journal of Chemistry 14:534-536.

Wheeler, W.M. 1942. Studies of Neotropical ant-plants and their ants. Bulletin of the Museum of Comparative Zoology 90:1-262.

Wilkens, R.T., J.M. Spoerke and N.E. Stamp. 1996. Differential responses of growth and two soluble phenolics of tomato to resource availability. Ecology 77:247-258.

Worthen, W.B. 1989. Predator-mediated coexistence in laboratory communities of mycophagous *Drosophila* (Diptera: Drosophilidae). Ecological Entomology 14:117-126.

Young, T.P., C.H. Stubblefield and L.A. Isbell. 1997. Ants on swollen-thorn acacias: species coexistence in a simple system. Oecologia 109:98-107.

Yu, D.W. and H.B. Wilson. 2001. The competition-colonization trade-off is dead; long live the competition-colonization trade-off. American Naturalist 49-63.

Yu, D.W., H.B. Wilson, M.E. Frederickson, W. Palomino, R. de la Colina, D.P. Edwards and A.A. Balareso. 2004. Experimental demonstration of species coexistence enabled by dispersal limitation. Journal of Animal Ecology 73:1102-1114.

Yu, D. W., H. B. Wilson, N. E. Pierce. 2001. An empirical model of species coexistence in a spatially structured environment. Ecology 82: 1761–1771.

Yu, D.W. and D.W. Davidson. 1997. Experimental studies of species-specificity in *Cecropia*-ant relationships. Ecological Monographs 67:273-294.

Zangerl, A.R. and C.E. Rutledge. 1996. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. American Naturalist 147:599-608.

BIOGRAPHICAL SKETCH

Matthew David Trager was born in Gainesville, Florida, on April 9th, 1980, to Kim A. Trager and James C. Trager. While attending Westridge Elementary School in Ballwin, Missouri, he won third place in his school science fair with an insect collection comprising specimens he caught and curated. Matthew attended Grinnell College in Grinnell, Iowa, where he earned a Bachelor of Arts degree in anthropology in 2002. While in college he spent his summers restoring prairies for The Nature Conservancy in Iowa, lobbying for The Wilderness Society's public policy department in Washington, DC, and conducting research on grassland plant diversity at Kansas State University's Konza Prairie. He also spent the fall of 2000 in Tanzania, taking classes at the University of Dar es Salaam and conducting research in Serengeti National Park. Following his undergraduate education, Matthew worked in the plant ecology lab at Archbold Biological Station in south-central Florida for a year where he participated in long- and short term ecological studies of several federally listed plant species. In the fall of 2003, Matthew joined Dr. Emilio Bruna's lab at the University of Florida to pursue graduate studies through the School of Natural Resources and Environment. He received his Master of Science degree in Interdisciplinary Ecology in December, 2005.