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Spatio-Temporal Variation in an Ant-Plant Interaction

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Kellie M. Kuhn, Ph.D.
University of Connecticut, 2013

The role of mutualisms in structuring communities is poorly understood, in large part because potential mutualistic interactions are often identified, but rarely quantified. I tested the hypothesis, proposed in 1979, that the interaction between the ant *Myrmelachista flavocotea* (Formicidae: Formicinae) and its obligate host plants *Ocotea atirrensis* and *O. dendrodaphne* (Lauraceae) is a mutualistic interaction. Despite the high abundance of *Myrmelachista* ants in tropical forests, relatively little is known about them, because of their timid nature and their habit of living inside plants. I used a combination of observations and experiments to analyze the interaction between *M. flavocotea* and *Ocotea* and to explore the potential higher-order community effects of the interaction. *Myrmelachista flavocotea* and *Ocotea* plants form an association early in the life history of both participants. *Ocotea* seedlings were colonized by multiple *M. flavocotea* queens. Mature colonies had only a single queen, apparently as a result of secondary monogyny. Presence of multiple foundresses may be critical in ensuring the successful founding by at least one queen, thus allowing the perpetuation of the *Myrmelachista-Ocotea* interaction. I found the outcome of the interaction is highly variable. Some ant colonies readily defend their host plant, while other colonies were never observed to defend their host plant. *Ocotea* inhabited by the most aggressive ant colonies suffer the least herbivore damage. The density of ants inside *Ocotea* stems, not colony size or body size of workers, was the best predictor of colony aggression and host plant defense. *Myrmelachista flavocotea* clearly acts as
an inducible agent of biotic defense that responds to chemical cues from damaged leaves. *Myrmelachista* also influences the density of *Ocotea* seedlings by killing plants that grow in the vicinity of their host plant. Vegetative killing by *M. flavocotea* appears to be a mechanism to reduce competition with other *M. flavocotea* colonies and likely benefits host plants through decreased intraspecific competition. *M. flavocotea* and *Ocotea* receive reciprocal benefits from their partnership, which supports the hypothesis that the *Myrmelachista-Ocotea* interaction is a mutualism. The host plant defense behavior of the small, enigmatic, and relatively timid ant *M. flavocotea* has demonstrated community level effects.
Spatio-Temporal Variation in an Ant-Plant Interaction

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B.Sc., Frostburg State University, 2001

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A Dissertation

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Spatio-Temporal Variation in an Ant-Plant Interaction

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2013
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# TABLE OF CONTENTS

Acknowledgements........................................................................................................ iii

Table of Contents .............................................................................................................. v

List Of Tables .................................................................................................................. vii

List Of Figures ................................................................................................................ viii

Chapter One. Colony founding by the ant *Myrmelachista flavocotea* .................................1

Abstract ........................................................................................................................... 2

Introduction ..................................................................................................................... 3

Methods ........................................................................................................................... 4

Results ............................................................................................................................. 8

Discussion ....................................................................................................................... 10

Tables ............................................................................................................................... 14

Figures ............................................................................................................................. 19

Literature Cited ............................................................................................................... 27

Chapter Two. Ontogenetic variation in the benefits of ants to their host plants in an obligate ant-plant mutualism ........................................................................................................... 28

Abstract ........................................................................................................................... 29

Introduction ..................................................................................................................... 30

Methods ........................................................................................................................... 32

Results ............................................................................................................................. 36
Discussion .................................................................................................................................. 38
Tables......................................................................................................................................... 43
Figures......................................................................................................................................... 46
Literature Cited .............................................................................................................................. 58

Chapter Three. Vegetative pruning by the obligate plant-ant *Myrmelachista flavocotea*

determined by presence of competitors, not by host plant identity ............................................. 64

Abstract ........................................................................................................................................ 65
Introduction .................................................................................................................................... 66
Methods .......................................................................................................................................... 69
Results .......................................................................................................................................... 73
Discussion ..................................................................................................................................... 75
Figures .......................................................................................................................................... 78
Literature Cited ............................................................................................................................... 83

Chapter Four. Induced response of the ant *Myrmelachista* to simulated herbivore damage to their

host plants ....................................................................................................................................... 87

Abstract ........................................................................................................................................ 88
Introduction .................................................................................................................................... 89
Methods .......................................................................................................................................... 91
Results .......................................................................................................................................... 96
Discussion ..................................................................................................................................... 98
Tables .......................................................................................................................................... 103
Figures .......................................................................................................................................... 105
Literature Cited ............................................................................................................................... 112
LIST OF TABLES

Chapter One. Colony founding by the ant *Myrmelachista flavocotea* ........................................1

Table 1.1 .............................................................................................................................................. 14

Table 1.2.............................................................................................................................................. 15

Table 1.3.............................................................................................................................................. 18

Chapter Two. Ontogenetic variation in the benefits of ants to their host plants in an obligate ant-plant mutualism....................................................................................................................28

Table 2.1.............................................................................................................................................. 43

Chapter Three. Vegetative pruning by the obligate plant-ant *Myrmelachista flavocotea*
determined by presence of competitors, not by host plant identity .............................................64

Chapter Four. Induced response of the ant *Myrmelachista* to simulated herbivore damage to their host plants .................................................................87

Table 4.1.............................................................................................................................................. 102

Table 4.2.............................................................................................................................................. 103
LIST OF FIGURES

Chapter One. Colony founding by the ant *Myrmelachista flavocotea* ........................................1

Figures 1.1 ........................................................................................................................................20
Figures 1.2 ........................................................................................................................................21
Figures 1.3 ........................................................................................................................................22
Figures 1.4 ........................................................................................................................................23

Chapter Two. Ontogenetic variation in the benefits of ants to their host plants in an obligate ant-plant mutualism..................................................................................................................28

Figures 2.1 ........................................................................................................................................48
Figures 2.2 ........................................................................................................................................49
Figures 2.3 ........................................................................................................................................50
Figures 2.4 ........................................................................................................................................51
Figures 2.5 ........................................................................................................................................52
Figures 2.6 ........................................................................................................................................53
Figures 2.7 ........................................................................................................................................54
Figures 2.8 ........................................................................................................................................55
Figures 2.9 ........................................................................................................................................56
Figures 2.10......................................................................................................................................57

Chapter Three. Vegetative pruning by the obligate plant-ant *Myrmelachista flavocotea*
determined by presence of competitors, not by host plant identity ............................................64

Figures 3.1 ........................................................................................................................................79
Figures 3.2 ........................................................................................................................................80
Figures 3.3.............................................................................................................................................. 81
Figures 3.4............................................................................................................................................... 82

Chapter Four. Induced response of the ant *Myrmelachista* to simulated herbivore damage to their host plants ........................................................................................................................................ 87

Figures 4.1.............................................................................................................................................. 106
Figures 4.2.............................................................................................................................................. 107
Figures 4.3.............................................................................................................................................. 108
Figures 4.4.............................................................................................................................................. 109
Figures 4.5.............................................................................................................................................. 110
CHAPTER ONE

COLONY FOUNDING BY THE ANT MYRMELACHISTA FLAVOCOTEA
ABSTRACT

Identifying key life history characters is crucial to understanding the selective forces that influence species interactions and reciprocal evolution. We often know little about colony founding behavior and colony structure of ants involved in obligate interactions with plants. Here I describe colony founding behavior of *Myrmelachista flavocotea* (Formicidae: Formicinae) on its obligate host plants *Ocotea atirrensis* and *O. dendrodaphne* (Lauraceae). *Ocotea* seedlings produce specialized nodules on the mainstem that are used as domatia by founding queens. In this study, *Ocotea* seedlings were colonized by multiple *M. flavocotea* queens. Mature colonies typically had only a single queen, apparently as a result of secondary monogyny. The number of foundress queens per tree was positively correlated with seedling height and stem diameter (nesting space) at time of colony founding. The extent to which foundress queens cooperate in colony founding is not known. Nonetheless, colony establishment by multiple foundress queens may be critical in ensuring the successful founding by at least one queen, thus allowing the perpetuation of the *Myrmelachista-Ocotea* interaction.

**Keywords:** ant-plant interaction, colony founding, dependent founding, haplometrosis, secondary monogyny, Myrmelachista, Ocotea, polygyny
INTRODUCTION

Identifying life history characters that are pivotal in determining the outcome of species interactions is critical for understanding the selective forces that drive reciprocal evolution (Chesson and Huntly, 1988). A key life history trait for ants is the colony founding strategy used by newly mated queens (gynes; Wheeler, 1933; Hölldobler and Wilson, 1977; Heinze and Keller, 2000). In ant societies, the founding strategy is critical to the successful establishment and development of the colony. The two broad colony founding strategies (or syndromes) used by ants to initiate colony formation are haplometrosis (a single foundress) and pleometrosis (multiple foundresses) (Wheeler, 1933; Bartz and Hölldobler, 1982; Tschinkel and Howard, 1983). Success of a colony founding strategy depends on the environmental conditions at the time of colony establishment (Pamilo, 1991; Pamilo and Rosengren, 1984; Bourke and Franks 1995; Tchinkel, 2006). While the impacts of environmental heterogeneity and ecological contingencies in driving alternative founding strategies are not completely understood, pleometrosis is typically favored over haplometrosis when potential nest sites are limited, when physiological and dispersal costs are high, and when skew of reproductive potential is low (production of brood is similar for each queen) (Bourke and Franks, 1995). In the case of pleometrotic founding, the presence of multiple reproducing queens allows a large work-force to be rapidly assembled, greatly increasing the likelihood of successful colony establishment (Bartz and Hölldobler, 1982). Pleometrotic colonies have higher survival rates, grow faster, and are more successful in winning territorial fights compared to monogynous colonies (Tschinkel and Howard, 1983; Bourke and Franks, 1995). The disadvantage of cooperative founding is that the presence of multiple reproducing queens results in strong intraspecific competition for space and resources.
In the tropics a large number of ant species are associated with plants (Rico-Gray and Oliveira, 2007). Some associations between tropical ants and plants have become specialized, whereby the plants (myrmecophytes) form specialized structures that provide living space or food (extra-floral nectaries and Beltian bodies) for ants. Some other ant-plant associations have become so tightly linked (i.e., obligate) that neither the ants nor the plants can live without their mutualistic partner (e.g., Acacia-Pseudomyrmex, Janzen, 1966). In ant-plant protection mutualisms, ants benefit their host plant through protection from herbivores and encroaching vegetation (Janzen, 1966; Davidson et al., 1988). Mutualisms may also be nutritional, with workers depositing nutrients inside living space within plant structures (Janzen, 1974; Beattie, 1989; Solano and Dejean, 2004). For ants that have obligate associations with plants, variable success of colony founding strategies by ant partners not only impacts the fitness of the colony but also has important fitness consequences for the plant partner (Janzen, 1967; McKey, 1988; Vasconcelos, 1993).

Colony founding strategies of plant-ants are less well studied than the strategies of their ground-dwelling counterparts. The objective of this study was to investigate the colony-founding strategy of the ant Myrmelachista flavocotea (Formicidae: Formicinae) to infer how life history characters might influence the interaction between the ant and its obligate host plants Ocotea atirrensis and O. dendrodaphne (Lauraceae).

**MATERIALS AND METHODS**

**Study site**

This study was conducted at the Organization for Tropical Studies La Selva Biological Station (10°25’N, 084°04’W; hereafter referred to as La Selva) in Heredia Province, Costa Rica.
At an elevation of 35-145 m, with about 4 m of rainfall per year, La Selva is characterized as a Caribbean lowland tropical rainforest.

**Study System**

*Myrmelachista flavocotea* is a small formicine ant that lives inside the stems of its obligate host plants *O. atirrensis* and *O. dendrodaphne* (Stout, 1979; Longino, 2006). The *Myrmelachista-Ocotea* interaction has been demonstrated to be a protection mutualism (Kuhn, unpublished). In the *Myrmelachista-Ocotea* system, the plant provides living space for the ants. For their part, the ants provide numerous benefits for *Ocotea* trees, including defense against herbivores, removal of spores, lichens, epiphylls and debris from leaf surfaces, protection from encroaching plants, and beneficial nutrients from food items brought into stems (Stout, 1979; McNett et al., 2009; Kuhn, unpublished). At La Selva, *M. flavocotea* live only in *Ocotea* trees. All *O. atirrensis* and *O. dendrodaphne* that I have discovered at the site had resident *Myrmelachista* ants.

Prior to colonization of *Ocotea* seedlings by *Myrmelachista* queens, seedlings produce a swollen nodule (hereafter referred to simply as a *nodule*) along the apical region of the mainstem (Figure 1). Only a single nodule is produced per plant. Multiple queens may colonize a single nodule. Once a seedling is colonized the mainstem becomes woody. Seedlings grown in the absence of queens in shade houses did not become woody even after two years, suggesting that it is the presence of ants that induces woody tissue development around the nodule (Kuhn, unpublished). Development of woody tissue causes an apparent constriction of the nodule around the queens’ nesting chambers, which reveals the location of queens within stems. To distinguish fleshy from woody structures, woody structures will hereafter be referred to as *nodes* (Figure 2).
**Field Methods**

To determine at which life stage *M. flavocotea* form an association with *Ocotea*, I located and tagged newly germinated *Ocotea* seedlings and monitored them over time. To collect colonies and determine the number of *Myrmelachista* queens present in nascent and mature colonies, I harvested 38 *Ocotea* trees (34 *O. atirrensis* and 4 *O. dendrodaphne*) of various ontogenetic stages (size classes: seedling [< 0.5 m], intermediate [0.5 m – 2 m], and mature [> 2 m]). *Ocotea atirrensis* and *O. dendrodaphne* are both understory treelets (adult trees 1.5 - 3.5 m tall). Their small stature allows for easy harvest of trees using a handsaw and pruners. When harvesting trees, I cut each tree into 5 - 20 cm segments and placed each segment separately into a labeled plastic ziplock bag. Bags were sealed to prevent ants from escaping. I harvested trees only when *Myrmelachista* were not active (e.g., immediately after rain events, or during periods of light rainfall). To prevent the escape of alates (winged males and females) during harvest, I first removed all branches, and then quickly cut and bagged segments of the bole starting at the top of the tree. I am confident that virtually all individuals of each colony were collected. To kill ants, segments were placed in a freezer for at least 48 hours. Ants were then extracted from stems by cutting the stems in half, lengthwise, and removing the ants with a soft, camel-hair paintbrush. All segments were thoroughly inspected to ensure that all ants were collected. Ants were stored in 95% ethanol. The total numbers of queens, workers, reproductives (winged males and virgin queens) and total colony size (= workers + reproductives + brood) were determined for each tree.

As part of a larger project, I collected annual censuses on > 550 *Ocotea* trees between 2008 and 2013. Of the > 550 *Ocotea* plants surveyed in the larger study, 189 were seedlings. Seventy-eight seedlings censused were large enough to have nodules and were inhabited by
Myrmelachista queens. Among harvested seedlings, I found that each node contained either a single live queen or the head capsule of a single dead queen (Figure 3), indicating that the number of nodes along the mainstem was a good indicator of the number of queens that initially colonized each Ocotea. Once Ocotea stem diameter exceeds ~ 4 cm, nodes become inconspicuous and the number of founding queens could be determined only after the plant was harvested. Because colonization of Ocotea occurs only before plants became woody, colonization is restricted to young plants or on vegetative sprouts from damaged or dying mature trees.

For all trees (harvested and censused), I measured height, branch lengths, and bole diameter. I used data from censuses to determine if there was a relationship between seedling size and number of founding queens (number of nodes) present on Ocotea seedlings.

To determine the level of aggression among founding queens inhabiting the same host plant, I removed two foundress queens from each of six of the harvested Ocotea seedlings. I collected foundress queens by shaving away stem tissue with a razor blade to expand the entrance hole until I could see a queen. I then placed an aspirator over the hole and extracted the queen(s). To find nascent colonies with foundress queens, I located seedlings (height < 0.5 m) with nodules that were still green (i.e., not yet woody) and had entrance holes. In plants at the seedling stage, because ants occur only within the swollen nodules on the mainstem, the location of queens can quickly be deduced. Multiple queens were sometimes aspirated from the same entrance hole. To collect the rest of the colony (eggs, broods, nanitic workers and any additional queens), I harvested seedlings as described above. Singleton queens extracted with an aspirator were not used in behavior trials, but their colonies were collected and the queen and colony killed as described above. I put each pair of foundress queens into a 2-dram glass vial and
watched the queens interact for 30 minutes. I scored their behavior as amicable (antennation, grooming), neutral, or aggressive (biting, grappling). After behavioral trials, queens were killed and combined with their colonies in ethanol for subsequent study.

**Statistical analysis**

Seedling height and stem diameter of seedlings, with and without nodules, were compared using $t$-tests. Count data for number of nodes per seedling were not normally distributed. I determined that the distributions for number of nodes per seedling for *O. attirensis* and *O. dendrodaphne* were consistent with a Poisson distribution, using Kolmogorov-Smirnov (when $n > 30$) and Chi square (when $n < 30$) goodness of fit tests (Table 1). To determine if there was a relationship between foundress number (i.e., number of nodes) and plant size (seedling height), I used Poisson regression analysis for *O. attirensis* and *O. dendrodaphne* (O’Hara and Kotze, 2010). Seedling height and stem diameter were log-transformed to normalize data prior to analysis. I analyzed data using JMP®10. Data are presented as means ± 1 SD.

**RESULTS**

*Ocotea* seeds germinated from late June to late July. When plants were ~ 25 cm tall (at 1-2 years) they began to produce domatia in the form of a single nodule on the mainstem (Figure 1). Shortly after the formation of a nodule on a seedling, recently mated *Myrmelachista* queens (foundresses) discovered the seedling, chewed a hole through the epidermis, excavated the pith, and moved inside the *Ocotea* stem (personal observation; Figure 1). Once inside the *Ocotea* stem, *Myrmelachista flavocotea* exhibited a claustral colony founding strategy (the queen does not re-emerge to forage, but rather survives and rears her first brood on energy from fat stores and histolysis of flight muscles [Brown and Bonhoeffer, 2003]). Production of lignified tissue
(secondary xylem) caused the stem to become woody, constricting the nodule around the chamber containing the queen, thereby revealing her location (Figure 2). When multiple queens occurred in the same seedling, constriction of the mainstem narrowed the passage between queen chambers, perhaps restricting movement, but it did not entirely segregate queen chambers from one another (Figure 3).

The number of queens per tree ranged from 1-9 (Table 2). Founding by multiple queens was revealed by the presence of multiple living queens, or one living queen and multiple dead queens. Often only head capsules of dead queens remained. Mature colonies (colonies with full-sized workers) were typically monogynous, indicating that supernumerary queens had eventually been eliminated. I did find head capsules in four mature colonies. Generally, I did not find head capsules of dead queens in large trees (> 2 m) in which *Myrmelachista* colonies had obviously been established for years, allowing sufficient time for head capsules to completely decompose. At the beginning of this study I had not yet begun to look for head capsules in debris, so I may have undercounted the number of founding queens for five of the colonies.

In three of the six behavioral trials with pairs of queens, I found that queens from the same plant were overtly aggressive towards each other, biting and grappling, when placed together in a vial. In the three other trials, queens assumed aggressive postures, raising their heads and opening their mandibles, then moved to opposite ends of the vial.

*Ocotea* seedlings without nodules were significantly smaller than seedling with nodules or nodes (Table 3). *Ocotea* seedlings often had multiple nodes on the mainstem (*O. atirrensis*: $3.2 \pm 1.5$ nodes, range = 1-9, N = 71 trees; *O. dendrodaphne*: $2.4 \pm 1.3$ nodes, range = 1-5, N = 7 trees). Only 10 of the 78 seedlings (nine *O. atirrensis* and one *O. dendrodaphne*) had a single node on the mainstem. The number of nodes on the mainstem was significantly correlated with
plant height and stem diameter for *O. atirrensis* (height, GLM regression: P = 0.04 [Figure 4];

diameter, GLM regression: P = 0.001, N = 71). The relationship between the number of nodes and
tree height for *O. dendrodaphne* seedlings was positive but not significant (height, GLM

regression: P = 0.09 [Figure 4]; diameter, GLM regression: P = 0.42, N = 7).

**DISCUSSION**

At La Selva, I found that *Ocotea* seedlings are often colonized by multiple queens. Of the

78 *Ocotea* seedlings surveyed, 87% had more than one queen. It is unclear whether polygyny is

the result of haplometrosis by multiple queens or pleometrosis. Although haplometrosis is the

typical founding strategy described for ants in the subfamily Formicinae (Wheeler, 1933; Heinze

and Keller, 2000), pleometrosis has been described for a number of species in the group (Choe

and Perlman, 1997). *Camponotus macarangae*, for example, construct isolated nesting chambers

within their host plant *Macaranga lamellata* (Euphorbiaceae), where they rear their brood in

seclusion (claustral rearing) until first workers eclose (Maschwitz et al., 1996). Multiple *C.

macarangae* queens colonize a single *M. lamellata* seedling, where they excavate nesting

chambers within different internodes, resulting in spatial segregation of the queens. Independent

haplometroic founding by multiple queens within the same plant has been described for several

other ant-myrmecophyte systems (e.g., *Aztea-Cecropia*, Longino 1989, 1991b; *Tetraponera-

Barteria*, Yumoto and Maruhashi 1999). Unlike the examples given above, the nesting chambers

of *M. flavocotea* queens are connected (at least partially, Figure 3) within the nodule, so it is

likely that queens are in contact with each other during some period of colony establishment. *M.

flavocotea* queens were found to be aggressive and generally intolerant to the presence of other

queens. One caveat, however, is that nanitic workers were found in all colonies from which
queens were collected for use in behavior trials. Presence of workers is known to stimulate aggression between queens (even in known cooperative founders; e.g., *Solenopsis invicta*, Tschinkel and Howard, 1983). Therefore, I hesitate to state conclusively that *M. flavocotea* queens are always intolerant. From an evolutionary perspective, the interaction between queens need not be amicable (two cooperating queens) for polygyny to be mutually beneficial. Under some scenarios queens that establish nesting space in trees that have already been colonized by another queen may have higher rates of success compared to queens that found alone (independent of colony founding strategy). For example, presence of multiple queens might dissuade or inhibit encroachment of the nest by abundant generalist ants, such as *Pheidole*, *Crematogaster*, and *Wasmannia*.

The presence of multiple queens in mature colonies is likely the result of daughter queens reentering their natal colony (i.e., secondary polygyny, Hölldobler and Wilson, 1990; Feldhaar et al., 2000). In the single case of two living queens in a mature colony in this study, it is unclear whether the second queen had been adopted into the colony, or instead, that she had simply not yet been killed. The presence of head capsules of five other queens in the bole of the same host tree leads me to suspect the latter. Furthermore, while both queens had enlarged gasters, only one was found in proximity to eggs and brood, suggesting that the colony was functionally monogynous.

After colonization of *Ocotea* by gynes, *Ocotea* seedlings begin producing woody tissue. Development of woody tissue appears to constrict stem width, partially trapping queens in their brood chambers (Figure 3). Therefore, it unlikely that direct aggression between queens was the cause of reduction in supernumerary queens (see Figure 2). Instead, reduction in supernumerary queens was likely due to a collective action by workers to starve or kill all but a single queen
(secondary monogyny; Bartz and Hölldobler, 1982; Tschinkel and Howard, 1983). In the case of secondary monogyny, workers and brood of dead queens are often absorbed by the colony of the surviving queen (Portner and Tschinkel, 1986; Trunzer et al., 1998), allowing colonies with multiple founding queens to grow faster, thus increasing the likelihood of successful colony establishment compared to colonies with primary monogyny (Bartz and Hölldobler, 1982; Bourke and Franks, 1995; Trunzer et al., 1998). Rapid increase in the number of workers shortens the time needed for the colony to grow large enough to defend the host plant against herbivores and encroaching vegetation. Polygynous founding followed by secondary monogyny appears to be a common founding strategy for plant-ants (e.g., Janzen, 1973; Longino, 1991a, 1991b; Vasconcelos, 1993, Maschwitz, 1996; Yumoto and Maruhashi, 1999).

Pleometrotic founding is expected to be favored when habitat or resource availability is spatially or temporally variable (i.e., patchy), and competition among queens for nesting sites is high (Hölldobler and Wilson, 1977; Herbers, 1986; Bourke and Franks, 1995). Haplometrotic founding by multiple queens within the same plant might arise due to ecological constraints similar to conditions that favor pleometrotic founding. In the Myrmelachista-Ocotea system the presence of multiple founding queens in each seedling is likely the result of spatial and temporal patchiness in nesting sites. While Ocotea trees are relatively abundant at La Selva, Ocotea are found exclusively on patchy, phosphorus-rich alluvial soils (McPherson, 2006), resulting in a patchy dispersion pattern (Kuhn, unpublished data). Moreover, since 2008, I have observed newly germinated Ocotea seedlings in only two years (2009 and 2012). The presence of multiple foundress queens within Ocotea seedlings suggests that competition for newly available nesting space may be intense. Moreover, the presence of multiple M. flavocotea queens may increase probability of queen survival by deterring other species of ants from moving into nesting space.
Ocotea seedlings that fail to acquire a successful Myrmelachista colony die (personal observation). If survival of Ocotea seedlings depends on the successful founding of a colony, and if the probability of founding success increases with queen number, then seedlings that grow larger prior to the onset of domatia production (i.e., increased nesting space available to foundress queens) might be favored. I found that the number of founding queens per seedling appeared to be a function of plant size at the time of domatia production. It is unknown how the number of founding queens influences the outcome of the ant-plant interactions in this system. Future work is needed to determine if number of founding queens influences colony success and the growth, reproduction, and survival of Ocotea trees. In many ant-plant systems, the number of queens can be determined only by destructive sampling methods, which limits our ability to determine how the number of founding queens influences the fitness of the colony or plant. In the Ocotea-Myrmelachista system, however, the number of nodes along the main stem of Ocotea seedlings is a reliable indicator of the number of founding queens, allowing quantitative analysis of how founding strategies influence the outcome of this ant-plant interaction. The successful establishment of Myrmelachista colonies on Ocotea seedlings appears to be critical to the survival of the ants and the plant in this ant-plant association.
Table 1.1. Results from goodness of fit test to determine if data are from a Poisson distribution. Kolmogorov-Smirnov test (D) was used when sample size < 30. Chi-square test ($\chi^2$) was used when sample size > 30. The $H_0$ that the data are from a Poisson distribution was not rejected (P>0.05) for both species.

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Table 1.2. The number of *Myrmelachista flavocotea* queens, workers, and total colony size found in each colony harvested from *Ocotea atirrensis* and *O. dendrodaphne* plants. Trees are arranged in order of total colony size. Nest space was total linear area (cm) of hollow living space in *Ocotea* bole and stems.

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*Ocotea dendrodaphne*

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*Pheidole* spp. present

* Did not search for head capsules

† *Pseudomyrmex* spp. present
Table 1.3. Comparison of seedling height and stem diameter of *Ocotea atirrensis* (N = 178) and *O. dendrodaphne* (N = 11) that have ant-occupied nodules or nodes and on the mainstem versus seedlings that lack nodules or nodes. Means were compared using t-tests. Height and diameter were log transformed prior to analysis to conform to assumptions of normality.

<table>
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<tr>
<th></th>
<th>No nodules or nodes</th>
<th>With nodules or nodes</th>
<th>Range</th>
<th>Range</th>
<th>Test Statistic</th>
<th>DF</th>
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<tr>
<td>Height</td>
<td>9.5 ± 7.2</td>
<td>27.6 ± 12.3</td>
<td>4.4 - 17.7</td>
<td>10.2 - 88.8</td>
<td>7.65</td>
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<td>&lt; 0.0001</td>
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<td>Diameter</td>
<td>2.5 ± 0.8</td>
<td>4.3 ± 1.6</td>
<td>1.5 - 3.1</td>
<td>2.7 - 9.6</td>
<td>8.08</td>
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<tr>
<td><strong>O. dendrodaphne</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Height</td>
<td>19.1 ± 3.6</td>
<td>27.7 ± 8.8</td>
<td>22.5</td>
<td>19.0 - 43.8</td>
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<tr>
<td>Diameter</td>
<td>3.2 ± 0.9</td>
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<td>2.7 - 4.6</td>
<td>1.19</td>
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</table>
FIGURE LEGENDS

Figure 1.1. Nodule on the mainstem of an *Ocotea atirrensis* seedling. Arrows indicate openings excavated by *Myrmelachista flavocotea*.

Figure 1.2. Photograph of the exterior of the mainstem of an *Ocotea atirrensis* seedling, showing three distinct nodes. Each node is (or was) occupied by a single queen. Therefore, the number of nodules can be used as a proxy for the number of foundress queens.

Figure 1.3. Cross section of an *Ocotea* mainstem showing domatia space in the stem occupied by founding queens. I found two living queens with their brood and the head capsule of a third queen in this stem. Nanitic workers fill the chamber once occupied by foundress queen #2.

Figure 1.4. Relationship between height (log-transformed) and the number of nodes growing along the mainstem of seedlings of (a) *O. atirrensis* and (b) *O. dendrodaphne*. 
Figure 1.1.
Figure 1.2.
Figure 1.3.
Figure 1.4.
REFERENCES


Herbers, J.M. 1986 Nest site limitation and facultative polygyny in the ant *Leptothorax*


CHAPTER 2

ONTOGENETIC VARIATION IN THE BENEFITS OF ANTS TO THEIR
HOST PLANTS IN AN OBLIGATE ANT-PLANT MUTUALISM
ABSTRACT

Species interactions can be highly conditional. For species involved in obligate associations, the ontogenetic stages of each participant will likely have important consequences for the outcome of the interaction. The role of ontogeny in influencing the outcome of mutualistic species interactions is poorly understood. Here I show how the benefits that the ant *Myrmelachista flavocotea* (Formicidae: Formicinae) confers to its obligate host plants *Ocotea atirrensis* and *O. dendrodaphne* (Lauraceae) are influenced by the ontogenetic stage of both the ants and the plants. To do this, I examined the outcome of the *Myrmelachista-Ocotea* interaction across an ontogenetic chronosequence of plant and ant colony development. *Myrmelachista* ants form an association with *Ocotea* seedlings and maintain that association with the same host plant until the plant or the queen dies. Contrary to predictions in the literature, I found that neither large colonies nor colonies with the largest workers offered the best defense to their host plants against herbivores. Instead, ant colonies of intermediate size, occupying trees of medium stature, were the most aggressive. Trees occupied by aggressive ants benefited from decreased levels of herbivory. The density of ants inside *Ocotea* stems, not colony size, was the best predictor of colony aggression and host plant defense. Density of ants in large trees likely decreases due to reproductive constraints on aging queens, which cannot produce enough workers to fill nesting space. The ontogenetically conditional outcome in the mutualistic interaction between *Myrmelachista* and *Ocotea* highlights the need to consider ontogeny as an important factor influencing the outcomes of mutualistic species interactions, and therefore, their ecological and evolutionary dynamics through time and space.

Keywords: ant-plant mutualism, conditionality, Myrmelachista, Ocotea, ontogeny
INTRODUCTION

The outcomes of species interactions are often conditional (e.g., Setälä et al. 1997, Palmer et al. 2008, Zamora 1999, van Ommeren et al. 2002, Kersch and Fonseca 2005). The direction (parasitic, mutualistic, commensal) and strength (obligate, facultative) of the interactions depend on biotic and abiotic factors, which vary in time and space (Bronstein 1994, Thompson 1994). In addition to temporal variation in environmental effects, developmental changes in the structure and physiology (ontogeny) of an organism as a function of age (or size-class) will directly affect differential mortality, growth and maintenance costs, thus influencing trophic position, functional role, and the outcome of species interactions (Gould 1977, Werner and Gilliam 1984). The influence of ontogeny on altering the outcome of species interactions has been fairly well studied for negative species interaction (e.g., Brown 1971, Dunham 1980, Fuiman 1994, Relyea and Werner 2000, Miriti 2006, Valienta-Banuet and Verdú 2008, Soliveres et al. 2010). The role of ontogeny in determining the outcome of mutualisms, however, remains poorly understood (Cushman and Whitham 1989, Bronstein 1998, Stachowicz 2001). In this paper, I will analyze the extent to which the observed variation in an obligate ant-plant mutualism is determined by the ontogenic stage of the participants.

Increasing evidence suggests that age- and size-specific life history traits can alter the outcome of apparent mutualistic interactions (Cushman and Whitham 1989, Pringle et al. 2012). Ant-plant interactions are particularly well suited to the study of the role of ontogeny in determining outcomes in potentially mutualistic interactions (Bronstein 1998, Heil and McKey 2003). Myrmecophytes, plants with specialized structures that feed (e.g., Beltian bodies, nectaries) or house (domatia) their ant partners, often form associations with ants early in the life histories of both participants (Longino 1989, Blatrix et al. 2012, Kuhn 2013, Chapter 1).
Production of structures that attract ants generally begins at the seedling stage (Brout and McKey 2008, Blatrix et al. 2012). Newly formed domatia are colonized by newly mated queen(s). The establishment of a nascent colony on myrmecophyte seedlings binds the participants and couples their life-histories. As plants grow, potential nesting space for the ants expands, allowing for the expansion of the ant colony. The ant-plant provides food and living space, and the plant-ants defend their host plant against herbivores and encroaching vegetation, delivering nutrients to host plants and increasing plant growth and fitness (reviewed by Rico-Gray and Oliveira 2007). Timing of onset in the association between ants and plants should have important consequences for the strength (obligate, facultative) and direction (parasitic, commensal, mutualistic) of plant-ant interactions (Blatrix et al. 2012).

A number of studies have examined ontogenic succession of ant-plant interactions through the serial replacement of colonies of one ant species by another species as plants grow (Fonseca 1993, Fonseca and Benson 2003, Dejean et al. 2008, Palmer et al. 2010). Fewer studies have considered how ontogeny influences the outcome of interactions across the onotogeneic life stages of the same two participants (e.g., Val and Dirzo 2003, Palmer et al. 2010, Pringle et al. 2012). Given that nesting space can limit colony size for ants, growth rates of myrmecophytes may constrain population expansion of resident ants (Fonseca 1993). Rocha and Bergallo (1992) determined that colony size of the ant *Azteca muelleri* was positively correlated with the size of its *Cecropia* host plant. Increased numbers of workers was beneficial to the plant because it decreased the residence time of herbivores and decreased overall levels of herbivory. Such observations have led to the prediction that the benefits of hosting ants should be positively correlated with colony size, so that large trees will be better protected than small trees (Fonseca 1993, but see Pringle et al. 2012). Here I quantify the levels of host plant protection offered by
the plant-ant *Myrmelachista flavocotea* to its obligate host plants *Ocotea atirrensis* and *O. dendrodaphne* (Lauraceae) across an ontogenic chronosequence, to test the prediction that colony protection increases with colony size and size-stage of host plant.

**MATERIALS AND METHODS**

**Study Site**

This study was conducted at the Organization for Tropical Studies (OTS) La Selva Biological field station (hereafter referred to as La Selva) in Heredia Province, Costa Rica (10°26′N, 83°59′W). La Selva is characterized as lowland Caribbean rainforest with a mean annual rainfall of about 4 m (Organization for Tropical Studies, unpublished data, http://www.ots.ac.cr/meteoro). Data from this study were collected between 2009 and 2011 as part of an ongoing study initiated in 2008.

**Study System**

*Myrmelachista flavocotea* is a small, yellow formicine ant that, at La Selva, is an obligate inhabitant of *Ocotea atirrensis* and *O. dendrodaphne* (Stout 1979, Longino, 2006). The interaction between *Myrmelachista* and *Ocotea* is hypothesized to be a protection mutualism (Stout 1979). In the *Myrmelachista-Ocotea* system the plant provides living space for the ants. In return, the ants provide numerous benefits for *Ocotea* trees, including defense against herbivores (Kuhn 2013, Chapter 3), removal of spores, lichens, epiphylls and debris from leaf surfaces (Kuhn, unpublished data), and protection from encroaching plants (Kuhn 2013, Chapter 4). *Ocotea* trees also benefit from nutrient additions from dietary items brought by ants into stems (McNett et al., 2010).
*Myrmelachista flavocotea* and *Octoea* trees have an intimate association through all life-history stages. *Ocotea* seedlings produce a specialized structure on the mainstem that is colonized by newly-mated *Myrmelachista* queens (Kuhn 2013, Chapter 1). At La Selva, multiple queens arrive on young plants, but only one queen successfully establishes a colony on the plant (Kuhn 2013, Chapter 1). In the beginning of the association, the number of workers per colony is necessarily low. As the colony grows and expands into all available living spaces, ants begin to actively expand their living space by excavating pith in new plant segments.

**Host plant traits**

Here I present tree data from 2009, the first year in which a complete census of 250 *Ocotea* plants was conducted. The following measurements were made for each tree: tree height, branch lengths, stem diameter, and levels of herbivory. *Ocotea* < 0.5 m were characterized as seedlings, while *Ocotea* > 2 m were typically mature, fruit-bearing trees. To determine herbivore damage, I randomly selected five leaves per tree and measured them *in situ*. I photographed each leaf on a white background and later determined leaf area and percent leaf area removed by herbivores using the software package ImageJ© (Sheffield, 2007).

**Colony structure**

To determine colony size, I collected 38 *M. flavocotea* colonies from *Ocotea* trees, (34 *O. atirrensis* and 4 *O. dendrodaphne*). I cut each tree into 5 - 20 cm segments and placed each segment separately into a labeled plastic ziplock bag. Bags were sealed to prevent ants from escaping. To kill ants, segments were placed in a freezer for at least 48 hours. I am confident that virtually all individuals of each colony were collected. To collect ants, I cut stems in half, lengthwise, and removed ants with a soft camel-hair paintbrush. Length and width of domatia
space was measured for each segment. I inspected each segment to ensure that all ants were collected. Ants were stored in 95% ethanol.

The number of ants of each life stage was determined for each tree segment. Life stages scored were: queen, eggs, larvae, pupae, young and old workers (worker age was determined using color of cuticular pigmentation, light = young, dark = old; Weir 1954), and alates (males and gynes). Ants were counted under a Wild Heerbrugg M8 Stereoscope (15x magnification). I estimated the density of ants within the stems of *Ocotea* plants by dividing the total number of workers in the colony by total living space, measured as the linear area (cm) of hollow space in *Ocotea* boles and stems used as domatia by *M. flavocotea*.

I harvested trees in 2009, 2010 and 2011. To draw inferences about the relationship between colony size and nesting space (linear domatia space within stems) and other plant and colony attributes, I used behavior and tree trait data from the year the plant was harvested. Typically trees were harvested just a few days to two weeks after tree size and colony aggression data were collected; however a few trees were harvested opportunistically before being measured.

**Worker body size**

The average body size of *M. flavocotea* workers was determined for nine colonies collected from *O. atirrensis*. Total body length was measured from clypeus to the tip of the gaster under a Wild Heerbrugg M8 Stereoscope (15x magnification) fitted with a 10 mm micrometer. Twenty-five ants per tree segment were measured. When fewer than 25 ants were present all ants in that segment were measured. Average body size was calculated for each of the nine colonies.
**Ant aggression**

I determined the level of colony aggression of the *M. flavocotea* colony inhabiting each *Ocotea* tree measured in the yearly census. These ants readily attack foreign objects (biotic and abiotic) placed on their host plant. Ants attack objects by curling their abdomens and spraying objects with formic acid from the venom gland in their gasters. I took advantage of *Myrmelachista* ants’ propensity to attack foreign objects as a way to quantify colony aggression. I measured colony level aggression by wrapping a 10-cm segment of 24-gauge galvanized steel wire around the mainstem of the tree ~4 cm from the top of the plant. I counted the number of ants attacking the wire each minute for 15 minutes. The median number of ants attacking the wire was used as an index of colony aggression (this number is hereafter referred to as colony aggression). I repeated aggression trials at least three times for each colony. Aggression trials were conducted within a two-week period.

**Statistics**

I used general linear models fitted to a Poisson distribution to analyze count data (Zuur et al., 2009; O’Hara and Kotze, 2010). Data for *O. atirrensis* and *O. dendrodaphne* were pooled prior to analyses. I used Poisson regression to examine the effect of ontogenetic stage of *Ocotea* had on colony aggression by regressing tree height and colony aggression. Small *Ocotea* branches are too small to house ants, particularly on small trees, so I used only tree height in analyses where external plant measurements were used. Tree height was a highly reliable proxy for internal domatia space $R^2 = 0.97$; Figure 1). I used linear regression to examine the relationships between average worker body size and nesting space and body size and colony aggression. I used Poisson regression to examine the relationship between (1) colony aggression and colony size, (2) colony aggression and tree height, and (3) total number of brood and nest
space. Tree height and nest space were log-transformed prior to analyses. I used quadratic regression to examine the relationship between mean percent herbivore damage and tree height (log-transformed). Finally, I used Poisson regression to examine the relationship between density of ant in domatia space and colony aggression. Density was log-transformed prior to analysis. I used Akaike information criterion (AIC) model selection criteria to select between linear and quadratic models (Zuur et al. 2009).

**RESULTS**

**Colony Structure**

Of the 38 *Ocotea* trees harvested, 35 contained a viable *M. flavocotea* colony with at least one reproducing queen (Table 1). Colony size of *M. flavocotea*, which ranged from 1 to 5912 workers (Table 1), was positively correlated with tree height (GLM: N = 38, P < 0.0001). Mean body size was significantly correlated with total nesting space (F<sub>1,8</sub> = 26.93, P = 0.001, $R^2 = 0.79$; Figure 3).

Incipient colonies had very few workers and brood (Table 1). The first workers produced were nanitic workers (miniature ants reared on limited resources [Porter and Tschinkel, 1986]). Nanitic workers were observed foraging on leaves and carrying food items into *Ocotea* stems. Nanitic workers did not defend their host plant. Full-sized workers were found in colonies with > 50 workers. Full-sized workers were observed foraging around their host plant and defending the plant against herbivores and encroaching vegetation. Reproductive castes (alate males and gynes) were observed in 11 colonies (Table 3). Alates were observed in small colonies with as few as 179 workers.
Ants expanded their nest space within *Ocotea* stems by excavating the pith from inside new growth. In seedlings, all ants (a queen, eggs, brood, and nanitic workers) were located in the hollowed-out queen chamber (Figure 4). In larger plants, eggs and brood were located in segments near the queen. It was not uncommon, however, to find brood and pupae in branches. The relationship between the number of brood per colony and plant size was curvilinear, steeper for smaller plants and declining for large plants (GLM: N = 38, P < 0.0001; Figure 5). Young workers (identified by their light color) were found in the mainstem in the vicinity of the queen and brood, while older workers were found throughout the hollowed-out branches and bole of the tree, particularly at the top of the mainstem and in branch tips (Figure 4). Alates were typically found in the top segments of *Ocotea* main stems and in branch tips.

Five *Ocotea* trees that looked unhealthy prior to harvest were found to be inhabited by *Psuedomyrmex* spp. (N = 2) and *Pheidole* spp (N = 3). Despite their large size these *Ocotea* were occupied by few *M. flavocotea*. Apparently, *Psuedomyrmex* and *Pheidole* were living in domatia space that was initially excavated by *M. flavocotea*.

**Myrmelachista-Ocotea interaction**

Colony aggression varied strongly both within and among *M. flavocotea* colonies (Figure 6). In some colonies ants were rarely observed outside of their nesting space, while in other colonies they were aggressive towards any foreign object (natural or synthetic) placed on their host plant. Aggression was not positively correlated with colony size; rather aggression was slightly higher for colonies of intermediate size (GLM: N = 38, P < 0.001; Figure 7), nor was there any relationship between colony aggression and average body size of workers (F(_1,7_) = 0.518, P = 0.504, R^2 = 0.09).
Colonies living in trees of intermediate size were more aggressive than colonies occupying small or large trees (GLM: N = 136, P < 0.001; Figure 8). Moreover, plants in the intermediate size class had less herbivore damage (F_{2,51} = 4.88, R^2 = 0.17, P = 0.012, Figure 9). This pattern of ant aggression and tree protection was related to ant density. I found a significant modal relationship between colony aggression and tree size, such that trees of intermediate size had the highest ant densities and the most aggressive colonies compared to large and small plants (GLM: N = 33, P < 0.0001; Figure 10).

**DISCUSSION**

Within the regional population of *Myrmelachista flavocotea* at La Selva, colonies were found to vary greatly in their propensity to defend their host. Some colonies readily attacked foreign objects placed on their host plants, while ants of other colonies were rarely observed outside domatia. While the cause of the observed variation is not readily apparent, ontogenetic stage of the host plant seems to be important in mediating the outcome of the species interaction. I found that colony size (number of workers) and body size of workers increased with tree size. These results are consistent with results from other studies that have demonstrated that colony size and worker size increases with domatia space (Rocha and Bergallo 1992, Fonseca 1993, 1999). Contrary to predictions that larger colonies better protect their host plants, however, I found that trees occupied by the largest colonies were not the best protected against herbivores. Rather, *Ocotea* trees of intermediate size, occupied by moderate sized *Myrmelachista* colonies had the lowest levels of herbivore damage.

The highest levels of colony aggression were found in colonies with high densities of ants, as measured by the number of ants divided by the total linear living space inside stems. I
hypothesize that the rate and frequency of contact between workers is proportional to colony density and that increased contact between workers at higher densities increases the efficiency with which ants detect and respond to the experimental foreign stimulus, including the wire attached to their host plant in my experiment. Within social groups, per capita rates of interactions among group members are known to increase with group size (Pacala et al. 1996). Members of a colony coordinate their actions to find and exploit resources or respond to a threat by directly or indirectly (e.g., by means of chemical trails) exchanging information about the environment (Goss et al. 1989, Beekman et al. 2001). Workers in large colonies that are spread over large areas potentially have more information available to them. The number of individuals participating in this process, which is a function of colony size, should have a positive effect on efficiency of collective action (Beckers et al. 1989, Dornhaus et al. 2012). Chemical signals such as pheromone trails, which are effective at mobilizing a large number of recruits, are easier to maintain in large groups due to positive feedback in signal strength and number of ants using the trails (Dorigo et al. 1999). Individuals in larger groups are also likely to interact with nestmates more frequently, possibly leading to higher rates of information flow (Adler and Grodon 1992, Pacala et al. 1996, Burkhardt 1998, Gordon and Mehdiabadi 1999). This is likely, however, only if information brought to the colony is available to most individuals. I found that large Ocotea trees occupied by large Myrmelachista colonies did not always find and attack pieces of wire attached to the mainstem of their host plant. It appears that recruitment to wires on large plants was slow because potential recruits become spatially separated, thus interrupting the flow of information among colony mates. On large Ocotea tree ants, low ant densities mean that ants are less likely to encounter another ant or a pheromone trial and are thus less likely to recruit to the presence of herbivores, resulting in higher levels of herbivory. Constraints on nest space arising
from plant architecture might be a primary contributor to the wide spatial dispersion of workers on larger plants.

Both ant colonies and plants are modular organisms that grow by addition of workers and shoots, respectively. The relative rates of production of workers and shoot tissue determine the density of ants per host plant and therefore whether the ant colony continues to provide the same level of defense as the plant grows. I found that reproductive output by queens did not increase linearly with plant size, indicating that colony growth did not keep pace with tree growth. At La Selva, *M. flavocotea* colonies are typically monogynous (Kuhn 2013, Chapter 1). While queen replacement might be possible, it does not appear to be typical in *M. flavocotea* colonies at La Selva. When a queen depletes her egg or sperm reserves, the colony enters a state of senescence. Over time, as plants continue to grow, density is further reduced, eventually resulting in a breakdown in the *Myrmelachista-Ocotea* interaction.

Mortality rates for plants and ant colonies are high during early life stages (plants: Fenner, 1987; ants: Janzen, 1967; Perfecta and Vandermeer, 1993; Bernasconi and Keller, 1999). High levels of mortality early in the cycle of renewal is expected to impose selection for life history traits that promote establishment and survival of both participants during these early ontogenetic stages (McKey, 1988). The most common agents of mortality for *Ocotea* are tree falls and branch falls (Kuhn, unpublished data). Large *Ocotea* occur in or near light gaps created by tree falls, and appear less likely to be crushed from above. High tree morality and damage rates due to tree falls are common in tropical forests (e.g., Matelson et al. 1995). I found that *M. flavocotea* colonies produced reproductives in colonies with as few as 179 workers, suggesting that *M. flavocotea* colonies reach reproductive maturity rapidly. Early reproduction may be a bet-hedging strategy that increases fitness when nesting sites are unreliable (i.e., host plants die).
Decreased worker density reduces the colony’s ability to defend its host plant. As worker density decreases, the ants are not able to prevent other ant species from moving into *Ocotea* stems. Three of the large trees I harvested were found to house *Pheidole* and *Psudeomyrmex* ants in addition to a very few *Myrmelachista* ants. *Myrmelachista flavocotea* are tidy ants; workers gather debris and refuse and drop it off their host tree or accumulate waste and debris in a refuse pile in the stem segment closest to the ground. In contrast, I found nesting space occupied by *Pheidole* and *Psudeomyrmex* often contained debris, soil, and frass. It was common to see fungus and rot associated with these debris piles inside stems. Once *Ocotea* lose their *Myrmelachista* ants it appears that plants succumb to herbivore damage and disease.

Species interactions (including mutualism, parasitism, and commensalism) are defined by the *net* outcome of the interaction. Snapshot characterization of an interaction at a given place and a specific time can yield a misleading and incomplete understanding of the interactions (Bronstein 1998). For example, in east Africa, *Acacia drepanolobium* can be occupied by one of four ant species that live inside swollen thorns of *Acacia* plants (Palmer et al. 2010). Over time, competitively dominant ants replace the less competitive ant species, so that during its lifetime the plant will have interacted with multiple ant partners. Depending on the identity of the ant species, the short-term effects of *Acacia*-ant interactions range from strongly mutualistic to parasitic. By examining ontogenetic series, Palmer et al. (2010) found that *Acacia* interacting with multiple ant partners (including parasites) had a higher lifetime fitness compared to trees that interacted with fewer ant species over time. Inferences about the net effects of species interactions made at any one life-history stage may be insufficient for determining the lifetime benefits (Palmer et al. 2010). I found that the outcome of the *Myrmelachista*-*Ocotea* association is highly variable across ontogenetic life stages. The life history stage of the ant and the plant
clearly affected the density of ants per tree, thus influencing the ability of ants to defend their host plant. If I had focused my study on only mature trees (>2 m), I would have concluded that the interaction between *Myrmelachista* and *Ocotea* was not mutually beneficial, as the large plants are not well protected against herbivores. My study highlights the need to consider the temporal variation due to ontogeny in the study of mutualistic interactions.
Table 2.1. The number of *Myrmelachista flavocotea* workers, brood, and alates (winged gynes and males) in each colony harvested from *Ocotea* spp. Trees are arranged in order of total colony size. Nest space was measured as the total linear area (cm) of hollow space in *Ocotea* boles and stems used as domatia by *M. flavocotea*.

<table>
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<th>Nest space (cm)</th>
<th>Workers</th>
<th>Reproductive Virgin</th>
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<tr>
<td></td>
<td>Queen</td>
<td>Eggs</td>
<td>Old</td>
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*O. dendrodaphne*

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|    | †Pheidole spp. present |
|    | *Did not search for head capsules |
|    | †Pseudomyrmex spp. present |
FIGURE LEGENDS

Figure 2.1. Relationship between tree height and internal linear domatia (living) space.

Figure 2.2. Relationship between total nesting space (length of mainstem and branches; log-transformed) and total colony size for Myrmelachista flavocotea colonies living in (a) Ocotea atirrensis and (b) O. dendrodaphne.

Figure 2.3. Relationship between mean body length of Myrmelachista flavocotea workers and available nesting space.

Figure 2.4. Composition of Myrmelachista flavocotea colonies from (a) a small (seedling, Ati003), (b) an intermediate size (Ati013), and (c) a large (Ati297) Ocotea tree. Data are the proportion of ants from the following life-history stages: eggs, brood (larvae + pupae), young workers, mature workers, alates (mature and immature), and queen. The location of ants of different life stages are presented as the proportions of each type within each segment. Segments along the mainstem are labeled A1 (top position) to An (most basal position). Branches are numbered from top (B1) to most basal (Bn) position along the mainstem. White circles indicate the segment in which the queen was located.

Figure 2.5. Relationship between total number of brood per colony and nest space (log-transformed).
Figure 2.6. Colony aggression of individual *Myrmelachista flavocotea* colonies. Colony aggression was measured as average median number of ants attacking foreign object (10 cm wire) attached to their host plant, counted every minute for 15 minutes. Behavior trials were repeated at least three times per colony.

Figure 2.7. Relationship between colony aggression and colony size.

Figure 2.8. Relationship between the mean herbivory rate and tree height (log-transformed). Small (> 0.5 m), intermediate (0.5 – 2.0 m) and large (> 2.0 m) *Ocotea* trees are indicated.

Figure 2.9. Relationship between colony aggression and available nesting space (total linear domatia space).

Figure 2.10. Relationship between colony aggression and ant (log-transformed) density.
Figure 2.1.

$R^2 = 0.97$
Figure 2.2.
Figure 2.3.

\[ y = 0.002x + 4.02 \]
\[ R^2 = 0.79 \]
\[ P = 0.001 \]
Figure 2.4.
Figure 2.5.
Figure 2.6.
Figure 2.7.
Figure 2.8.
Figure 2.9.

\[ y = 5.92 - 1.70x + 15.04x^2 \]

\[ R^2 = 0.17 \]

\[ P = 0.012 \]
Figure 2.10.
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abiotic factors modulates multiple ontogenetic shifts between competition and


CHAPTER THREE

VEGETATIVE PRUNING BY THE OBLIGATE PLANT-ANT

*MYRMELACHISTA FLAVOCOTE* DETERMINED BY PRESENCE OF

COMPETITORS, NOT BY HOST PLANT IDENTITY
ABSTRACT

Many specialized plant-ants protect host plants from encroaching vegetation by pruning or killing plants that grow near their host plant. Previous studies have demonstrated that ants can discriminate between plants of their host species and plants of non-host species. Recently it has been suggested that ants might be able to discriminate between their own, individual host plant and conspecific plants. To investigate this hypothesis, I used a propagation technique known as air layering to produce clones of Ocotea atirrensia trees, which host beneficial Myrmelachista flavocotea ants. Cuttings from host plants (autoclones) and conspecific plants (alloclones) were planted under each host plant and the fate of clones determined. I also examined the role of occupancy by foreign M. flavocotea colonies on plant-killing behavior by M. flavocotea with three different experiments: (1) I placed ant-occupied clones in the presence of other occupied clones from the parent tree, (2) I placed ant-occupied clones in the presence of alloclones occupied by foreign M. flavocotea colonies; (3) I placed ant-occupied clones with unoccupied alloclones. When planted clones were unoccupied, ants did not discriminate between autoclones and alloclones; ants moved onto all unoccupied clones in the field and the shade house. Planted clones inhabited by foreign M. flavocotea colonies were killed by the ants from neighboring alloclones, which chewed holes into the epidermis of Ocotea cuttings and sprayed formic acid into the wound. Necrosis of leaf and stem tissue was conspicuous within two days of poisoning. Vegetative killing by M. flavocotea appears to be a mechanism to reduce competition with other M. flavocotea colonies. Vegetative pruning likely benefits host plants through decreased intraspecific competition.

**Keywords:** competition, host-plant recognition, Myrmelachista, Ocotea, plant-killing, pruning
INTRODUCTION

Interactions between species unite and define ecological communities (Whitaker, 1975), yet it is often difficult to observe and understand how pair-wise species interactions impact community-level dynamics. Killing of competing vegetation by ants involved in ant-plant protection mutualisms is an excellent example of a species interaction that can impact local community structure (Morawetz et al., 1992; Renner and Ricklefs, 1998; Frederickson and Gordon, 2007). In protection mutualisms, direct defense by ants of food (extra-floral nectaries, Beltian bodies) or living space produced by the host plant results in indirect protection of the plant against herbivores (reviewed by Rico-Gray and Olivera, 2007). Likewise, some plant-ants prune vegetation around their host plant, preventing invasion by and competition with other ant species (Davidson et al., 1988; Federle et al., 2002), which simultaneously benefits their host plant through reduced competition with other plants for light, nutrients, and space (Janzen, 1966).

Ants kill vegetation by biting and stinging (Janzen, 1967; Davidson et al., 1988) or, when the stinger is absent, by spraying the contents of the venom gland into an epidermal wound made by the ant (Morawetz et al., 1992). Pruning by ants can modify the diversity, abundance and spatial pattern of vegetation in proximity to host plants (Larrea-Alcázar and Simonetti, 2007). For example, ants in the genus *Pseudomyrmex* (Pseudomyrmecinae) clear vegetation from beneath their host plant, producing bare patches within the otherwise dense forest understory matrix (*P. flavicornis-Acacia collinsii* (Fabaceae), Belt, 1874; *P. ferruginea-A. cornigera*, Janzen, 1967). *Myrmelachista* (Formicinae) kill all non-host plant species in proximity to host plants, creating monocultures of its obligate host plant that can extend for nearly a hectare (*M.

Understanding the mechanisms by which ants discriminate among plants and the factors they use to determine which plants are to be killed have long been of interest to ecologists. Thomas Belt (1874) was the first to write about vegetative pruning behavior of *Psuedomyrmex* ants around their *Acacia* host plant. Since that time, numerous studies have demonstrated that ants can discriminate between host and non-host plant species transplanted in proximity to their host plant. Ants typically respond by killing non-host plant individuals (e.g., Janzen, 1967; Janzen, 1969; Morawetz et al., 1992; Renner and Ricklef, 1998; Frederickson and Gordon, 2007; Larrea-Alcázar and Simonetti, 2007; Amador-Vargas, 2011). Ants also seem to be able to discriminate between closely related plant species. Weir et al. (2012) determined that *Pseudomyrmex triplarinus* responds to chemical cues produced by plants to discriminate between it host plant, *Triplaris americana* (Polygonaceae), and its congener (*T. poeppigiana*).

But, the question remains whether ants discriminate between their own, individual host plant and other individuals of the same host plant species.

The motivation for this study stems from the observation that the ants *Myrmelachista flavocotea* (Formicinae) sometimes kill seedlings of their obligate host plant species (*Ocotea atirrensis* and *O. dendrodaphne*, Lauraceae) that establish near their own, individual host plants, but readily move onto vegetative sprouts produced by their own host plant without harming them. This observation suggests that *M. flavocotea* might discriminate among individual *Ocotea* plants. To test the hypothesis that *Myrmelachista* can discriminate between their own host plant and conspecific plants, I used a propagation technique known as *air layering* to produce clones of *Ocotea atirrensis* trees. Clones from individual host plants (*autoclones*) and from conspecific
plants (*alloclones*) were planted under each host plant and the fate of clones determined. I predicted that if *M. flavocotea* discriminate among potential host plants based on plant genotype, then clones from their host plants should survive, while alloclones would be killed. I also examined the effects of occupancy by foreign *M. flavocotea* colonies on the vegetation-killing behavior of *M. flavocotea*.

Analysis of the molecular components of ant cuticles has revealed that plant-ants share hydrocarbon and non-hydrocarbon signals (e.g., signal proteins) in common with the leaves of their host plants (Weir et al., 2012). Cuticular hydrocarbons, surface lipids that protect insects from desiccated, are used by ants in nestmate recognition (Hölldobler and Michener, 1980). The diversity of hydrocarbons found in ants is extraordinary. To date, 1000 individual hydrocarbons have been isolated from just 78 species of ants (Martin and Drijfhout, 2009). Hydrocarbons are synthesized by oenocytes, which are associated with fat bodies beneath the epidermis (Martins and Ramalho-Ortigão, 2012). Not surprisingly, given the association of oenocytes with fat bodies, hydrocarbon profiles can be altered by diet (Liang and Silverman, 2000). Among ants, hydrocarbon profiles differ among species, with considerable intraspecific differences even among colonies (Singer, 1998). Food-sharing through trophallaxis, grooming, and physical contact result in colony mates sharing similar hydrocarbon profiles, allowing ants to recognize colony mates versus non-colony mates (Singer, 1998). Presence of shared hydrocarbons between ants and their host plants is likely mediated through ants eating the honeydew produced by coccid insects that feed on the host plant (Weir et al., 2012). Additionally, experiments have demonstrated that ants recognize and prefer nest material (soil) taken from their own colony’s nest compared to soil taken from other colonies (Hangarther et al, 1970).
Given the intimate association between plant-ants and their host plants, and the acute discriminatory capabilities of ants, it seems plausible to predict that ants are able to recognize their own, individual host plants. A map of the olfactory system in ants revealed that they have 400 distinct odorant receptors, compared to only 52 in Silk moths, 61 in fruit flies, and 174 in honeybees (Zhou et al., 2012). Here I use clones of host plants (autoclones) to investigate (1) if ants prefer their host plant to intraspecific clones (alloclones), and (2) if identity of ant occupants (self or foreign) influences plant-killing behavior of *M. flavocotea*.

**Materials and Methods**

*Study site.* — This study was conducted at the Organization for Tropical Studies (OTS) La Selva Biological Station (10°26’N, 83°59’W) between May 2010 and January 2013. La Selva is located in Heredia Province, Costa Rica, at the confluence of the Río Sarapiquí and Río Puerto Viejo. La Selva is characterized as lowland Caribbean rainforest, with mean annual rainfall of approximately 4 m.

*Myrmelachista flavocotea* is a small yellow ant that lives inside the stems of *Ocotea atirrensis* and *O. dendrodaphne* (Stout, 1979; Longino, 2006), which are understory treelets (max height ≈3.5 m). Each colony consists of hundreds to thousands of workers and generally one queen (Longino, 2006; Kuhn, 2013, Chapter 1). *Ocotea* do not produce food rewards (such as Beltian bodies or extrafloral nectar) for the resident ants. *Myrmelachista* obtain food from honeydew-producing mealy bugs and by scavenging dead insects (Stout, 1979; McNett et al., 2010). The *Myrmelachista-Ocotea* interaction has been reported to be a food-for-protection mutualism (Stout, 1979). *Ocotea* provides the ant with shelter, and the ant provides the host plant with nutrient additions (McNett et al., 2010), and with protection against herbivores and
encroaching vegetation (Kuhn, 2013, Chapter 2). I have also observed that ants prune or kill plants that touch their host plant, whereas they readily move onto vegetative sprouts produced by their own host plant without harming them.

**Propagation.** — I used a propagation technique known as *air-layering* (also called *marcotting*) to make vegetative clones of *Ocotea* plants (hereafter referred to as *clones*). To propagate *Ocotea*, I girdled branches with a razor blade approximately 10-15 cm (depending on location of leaves) from branch tip. Once the bark was removed, I wrapped a Jiffy® peat pellet, cut down the center, around the wounded portion of the stem. I then wrapped the pellet with aluminum foil and secured the foil with twine (Fig. 1). I applied between 2-20 pellets on each tree (4 ± 2 pellets per tree; mean ± 1 SD), but only one pellet per branch. The total number of pellets per trees depended on the degree of branching of each tree. In all cases, I deployed pellets on fewer than half the branches on any given tree. At La Selva, *M. flavocotea* inhabit *O. atirrensis* and *O. dendrodaphne*. For this study I used exclusively *O. atirrensis* plants for two reasons. First, at La Selva, *O. atirrensis* is more abundant (density: 5 ± 3 trees/20 m², range = 0 - 11 trees/20 m², N = 20 20 x 20 m² density plots) than *O. dendrodaphne* (density: 1 ± 0.05 trees/20 m², range = 0 - 4 trees/20 m²). Second, *Ocotea atirrensis* has many more branches per tree than *O. dendrodaphne* (which typically had only 0-4 branches). I deployed pellets on 86 *O. atirrensis* trees in 2010 and on an additional 40 trees in 2011.

Branches were periodically inspected for root development. Root hormone was not required to induce root formation in *O. atirrensis*. After approximately four months (beginning in June 2010), some plants had produced what I thought would be enough root material to allow successful transplanting (Fig. 2). I harvested branches by cutting the branch proximal to the root ball. Clones were planted in 15 cm plastic grow bags with a sterile peat substrate.
Effects of foreign ants on ant-occupied clones. — In mid-June 2010, I brought 47 root-propagated clones of *O. atirrensis* (from 18 trees), including any resident ants, into the shade house (1% full sun). Clones were potted in grow bags as described above and grouped by parent tree in 10x18x4 cm plastic baskets. Baskets were evenly spaced across shade house tables with a minimum distance of ~25.5 cm to prevent leaves of different host plants from touching. Soon after transplanting (within a few hours), I noticed *M. flavocotea* workers moving among trays.

To determine the effects of foreign *Myrmelachista* ants (non-colony mates) on clones, I divided clones into two groups. In the first group (*contact treatment*), I allowed ants to range freely move between baskets (N = 9 baskets). In the second group (N = 9 baskets), I placed clones from individual trees, in their baskets, inside unsealed plastic Ziplock® Big Bags (3-gallon) that were suspended from ropes to prevent ants from moving between bags (*isolation treatment*). I monitored health of the clones and determined the number of clones surviving from each host plant daily for eight weeks. I compared the proportion of clones surviving from the isolation treatment and from the contact treatment groups using a chi-squared test. At the end of this experiment, I removed all ants (see below) from all surviving clones and continued to grow the clones in the shade houses for use in subsequent experiments.

Ant removal. — In a first attempt to eliminate ants from clones in the shadehouse, I poisoned ants with carbon dioxide (CO\textsubscript{2}) by placing *Ocotea* clones in large Ziplock® bags and filling the bags with CO\textsubscript{2}. After 12 hours, I replaced the gas and exposed ants to CO\textsubscript{2} for an additional 18 hours. Carbon dioxide poisoning killed ants that were outside plant stems, but was ineffective at killing ants inside stems. In a second attempt to kill ants, I submerged plants in water for one hour. I was not completely successful at eliminating ants using either poisoning or drowning techniques, so finally, I prevented ants from moving between plants by placing clones
inside Ziplock® bags that were partially closed. When ants were observed on plants they were aspirated off and killed. When I was sure all ants had died (~2.5 months after harvesting), plants were removed from their bags and spread out on tables in the shade house. Ants were successfully removed from 115 clones in 2010. Clones were grown in the shade house at La Selva, at ambient temperature, for one year. Clones were watered daily, and fertilized with liquid fertilizer monthly or as needed. No Myrmelachista ants discovered and colonized these plants during this period.

**Host-discrimination experiment.** — To test the hypothesis that ants can discriminate between their own, individual host plant and foreign conspecific plants, I transplanted clones from parent plants (autoclones) and clones from foreign plants (alloclones) under naturally occurring *O. atirrensis* host plants in the field. These *Ocotea* clones had been grown in shade houses, as described above, in the absence of *Myrmelachista* for more than 1 year, presumably enough time for all colony-specific cues to have faded. Of the 115 clones from which ants were removed, 20 died while in a shade house, of unknown causes.

In June 2012, I planted four clones (2 autoclones and 2 alloclones), in a circular arrangement, approximately 10 cm from the base of each experimental *Ocotea* tree in the field, alternating autoclone and alloclone. Because the experimental design called for two clones for each host plant, I was restricted to 17 host plants (34 autoclones and 34 alloclones). Alloclones were taken from two different trees. To prevent transplant stress, clones were planted in their plastic grow bags. I applied Tanglefoot® (a sticky resin) to the base of one of the two clones of each type to exclude ants. Plant health and colonization of each clone by ants was monitored every day for the first week and then every week thereafter for 8 weeks. At time of inspection, I recorded the presence and activities (patrolling, excavating, and formic acid poisoning) of ants.
on each clone. After one month, I removed the Tanglefoot® from clones, and measured plant health and colonization as before. A final assessment of plant health was made in January 2013. I predicted that Myrmelachista would discriminate between autoclones and alloclones and would kill all alloclones; therefore I expected a mortality rate for this experiment of 50% concentrated on the alloclones.

**Effects of ants on unoccupied clones.** — To determine if ants would move onto ant-free alloclones in the shade house, in June 2013, I harvested 20 clones from 10 *O. atirrensis* (2 clones per plant) and transplanted them as described above. I haphazardly selected 40 alloclones from a pool of plants I was growing in the shade house. I divided these 40 alloclones into 10 groups. I placed four clones from four different trees into 10x18x4 cm plastic baskets in the shade house and added two fresh autoclones taken from a single host plant to each of the baskets (i.e., two fresh cuttings with ants and four alloclones from which the ants had been removed). To isolate plants with ants, I placed each group of plants, in their baskets, inside plastic bags and suspended them to prevent movement of ants between bags. Approximately every other day for 30 d, I monitored the health of clones and recorded the number of clones surviving. Ants were removed from all surviving clones, and the clones were retained for future experiments.

All statistical analyzes were performed using JMP® 10. Data are presented as means ± 1 SD.

**RESULTS**

**Effects of contact with foreign clones.** — Ants on autoclones, isolated from non-nestmates, were observed to move among clones of their host plants, entering domatia, foraging, and grooming leaves. The ants in the contact treatment group were observed leaving their
autoclones and attacking alloclones. The proportion of clones surviving until the end of 60 d was significantly higher for plants kept isolated from foreign *M. flavocotea* colonies (0.92 ± 0.13 survivorship, range = 0.75 – 1, N = 9) compared to clones kept in the presence of foreign ants (0.04 ± 0.09 survivorship, range = 0 - 0.2, N = 9; \(X^2 = 14.19\), d.f. = 1, \(P = 0.0002\); Figure 3). Ninety-six percent of the clones exposed to ants from foreign colonies were killed (Figure 3). Plants were killed with formic acid in a manner previously described for *Myrmelachista* (Morawetz et al., 1992; Renner and Ricklefs, 1998; Frederickson and Gordon, 2007): ants chewed a hole in the epidermis of stems and sprayed formic acid directly into the wound resulting in a systemic spread of herbicide (Figure 4). Interspecific aggression between ants was low. Invading ants detected by resident ants while damaging a clone were sprayed with formic acid, but generally ants moving between plants avoided contact with other ants. Necrosis of the leaves and stems were observed within two days of being exposed to workers of foreign ant colonies.

**Transplant experiment.** — Four clones died after being transplanted; three clones (2 autoclones and 1 alloclone) were killed by the rooting behavior of white-collared peccaries (*Pecari tajacu*, Tayassuidae), and one other alloclone died of an unknown cause. All other clones were alive in July 2012 and January 2013. There was no difference in the percentage of autoclones (94%) and alloclones (94%) surviving to until January 2013 (\(X^2 = 0.82\), d.f. = 1, \(P = 0.670\)). In all cases, the first time *M. flavocotea* were observed on clones, ants were observed simultaneously on both autoclones and alloclones. Ants colonized the stems of autoclones and alloclones.

**Effects of ants on unoccupied clones.** — When clones occupied with ants were introduced into the baskets of alloclones (non-host) with no ants, I found that *M. flavocotea*
began to excavate pith and moved into the stems of unoccupied clones within hours of the introduction. In total, ninety-five percent of the clones survived to the end of 30 d. Three of the recently harvested clones died, likely due to insufficient root development. All alloclones survived to the end of the experiment.

**DISCUSSION**

I predicted that, if *M. flavocotea* preferred their own, individual host plant over conspecifics, the ants would kill clones from foreign plants but not clones from their own host plant. I also predicted that ants should discriminate between clones based on the identity of ant occupants and should kill clones occupied by foreign ants. By using clones of the host plants, I controlled for genotypic differences in cues produced by plants, and I was able separate ant and host plant cues. Previous studies have not controlled for host plant identity when determining the response of plant-ants to foreign ants. Contrary to my predictions, *M. flavocotea* did not prefer clones from its host plant to clones from non-host *O. atirrensis* trees. Rather, *M. flavocotea* expanded its colony into the unoccupied clones in the shade house and in the field, regardless of clone origin. Moreover, plant identity did not influence the rate of colonization onto *Ocotea* clones. I conclude that *M. flavocotea* does not have a preference for its own individual host plant, as ants were equally as likely to adopt any unoccupied conspecific plants as suitable nest space. The observation that *Myrmelachista* did not kill conspecific host plants is in line with ant responses to conspecifics in other ant-plant systems (Morowetz et al., 1992; Renner and Ricklefs, 1998; Amador-Vargas, 2011). Amador-Vargas (2011) observed that *Psuedomyrmex spinicola* did not prune seedlings of their host plant species, *Acacia collinsii*, but readily killed heterospecifics planted in proximity of the host plant.
Many myrmecophytes readily produce vegetative sprouts in response to damage (e.g. *Acacia, Piper, Cecropia, Tococa, Clidemia*) and can potentially be cloned by air layering. Propagation of plants through air layering has two advantages. First, air layering allows for the production of plants with identical genotypes. Controlling for plant genotype is often desirable, and could be useful in teasing apart the mechanisms responsible for the extensive temporal and spatial variation observed in the outcome of ant-plant interactions. Second, air layering is a way of producing plants even in years with low seed set and fruit production.

*Myrmelachista flavocotea* did kill alloclones, but only if those clones were occupied by ants from foreign *M. flavocotea* colonies. My data support the hypothesis that pruning behavior observed by plant-ants, while benefitting their host plant, is a mechanism to reduce competition with other ants (Davidson et al., 1988; Yumoto and Maruhashi, 1999). Specifically, it appears the *M. flavocotea* reduces intraspecific competition by eliminating the nesting space of competing colonies. Morawetz et al. (1992) determined that another species of *Myrmelachista, M. nigella*, kills branches from conspecific plants moved from one patch of *Tococa* (patch = a single *Myrmelachista* colony) to a foreign *Tococa* patch. Branches moved to a different location within the same *Tococa* patch survived. They interpreted these results to indicate that *Myrmelachista* could discriminate between *Tococa* plants in their home patch and those of foreign patches, based on the territorial marks of the foreign ants. It appears that like *M. nigella*, *M. flavocotea* is sensitive to the presence of intraspecific competitors, and discriminate between plants based on the identity of resident ants.

Pruning behavior of *Myrmelachista* may likely be an important mechanism influencing the dispersion pattern of adult *O. atirrensis* trees. Vegetative pruning by ants has the ability to decrease density of plants surrounding their host plant (Janzen, 1966; Renner and Ricklefs, 1998;
Amador-Vargas, 2011). *Ocotea* fruits are eaten by birds and the seeds are later regurgitated under song perches (Wenny and Levey, 1998). As a result of seed dispersal by birds, *Ocotea* seedlings often tend to be spatially clumped (Gibson and Wheelwright, 1995; Wenny and Levey, 1998). *Myrmelachista flavocotea* likely encounter neighboring *Ocotea* plants while they are scavenging for insects on the ground around their host plants. My shade house experiments revealed that foraging ants kill plants occupied by foreign colonies. Small colonies are unlikely to be able to successfully defend against workers from a larger neighboring colony (Gordon and Kulig, 1996). Presence of colonized seedlings within the foraging territory of an established *M. flavocotea* colony will likely be eliminated through the killing of those seedlings. Pruning rates increase with ant density (Federle et al., 2002), so large colonies are likely to be more effective at eliminating competitors compared to smaller colonies. The number of *M. flavocotea* workers per colony increases with plant size (Kuhn, 2013, Chapter 2) and seedlings, in particular, have very few workers. Indeed, I have often observed that seedlings that establish under larger *Ocotea* trees have necrotic leaf damage consistent with formic acid poisoning. Reduction in density of *Ocotea* seedlings might benefit the plants through decreased competition while likely reducing foraging competition for ants (Janzen, 1967).
FIGURE LEGENDS

Figure 3.1. Illustration of the steps required for propagation using the air layering technique. To propagate *Ocotea atirrensis* plants (a) the stem was girded with a razor blade, (b) the wound was covered with a Jiffy peat pellet, and (c) the pellet was covered with aluminum foil. Illustration modified from www.fao.org.

Figure 3.2. Roots growing from a branch of *Ocotea atirrensis* after propagation by air layering.

Figure 3.3. Proportion of *Ocotea atirrensis* clones surviving over 8 weeks in the presence of *Myrmelachista flavocotea* workers isolated to clones occupied by colony mates (open circles) and free-ranging ants open to clones occupied by ants from foreign colonies (solid circles).

Figure 3.4. Necrosis on leaves caused by formic acid poisoning injected into the leaves by *Myrmelachista flavocotea*.
Figure 3.1.

a.)

b.)

c.)
Figure 3.2.
Figure 3.3.

![Graph showing the proportion of clones surviving over time for isolated and free-ranging conditions. The graph plots time in weeks on the x-axis and proportion of clones surviving on the y-axis. The isolated condition shows a sharper decline compared to the free-ranging condition, which remains relatively stable.]
Figure 3.4.
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Chapter Four

Induced response of the ant *Myrmelachista* to simulated herbivore damage to their host plants
ABSTRACT

To reduce herbivore pressure, some plant species respond to herbivore damage by producing structures or volatile chemicals that attract natural predators of herbivores, such as ants. Predators act as inducible agents of biotic defense, analogous to chemicals, such as secondary metabolites, that act to reduce further plant damage. Here I test the hypothesis that in highly evolved ant-plant systems ants can act as inducible agents of biotic defense. I test this hypothesis by examining the role of volatile leaf compounds in mediating the interaction between the ant *Myrmelachista flavocotea* (Formicinae) and its obligate host plants *Ocotea atirrensis* and *O. dendrodaphne* (Lauraceae). Artificial damage to leaves of host plants induced a rapid aggressive response by *Myrmelachista flavocotea*. Colony aggression remained elevated for 7 d and 60 d for colonies inhabiting damaged *O. atirrensis* and *O. dendrodaphne*, respectively. When pieces of leaf tissue from *Ocotea* and *Welfia* palms (a control) were placed on leaves of undamaged *Ocotea* plants, *Myrmelachista* showed a strong response to the *Ocotea* stimulus by not to *Welfia* stimulus. Ants also showed an increased response to the presence of volatile leaf extracts from *Ocotea* leaves. Significantly more ants recruited to paper disks soaked with extracts from *Ocotea* leaves compared to controls. There was no difference in the response of ants to leaf extracts from new, medium or old aged leaves. *Myrmelachista* showed a significant increase in the proportions of ants attacking paper disks soaked in extract from herbivore-damaged leaves compared to intact leaves. *Myrmelachista flavocotea* clearly acts as an inducible agent of biotic defense that responds to volatile leaf compounds produced by their host plant, as would be predicted in an obligate ant-plant mutualism.

**Keywords:** ant-plant interaction, biotic defense, induced defense, Myrmelachista, Ocotea

88
INTRODUCTION

Herbivores are strong agents of selection on plants. Defense against herbivores is achieved through increased production of structures (e.g., thorns, spines, trichomes) and chemicals (allochemicals) and can be either persistent (i.e., constituent) or induced (Berryman 1988, Karban and Myers 1989, Stamp 2003). Unlike constituent defenses, which are always present, inducible defenses require specific biotic cues (such as feeding by herbivores) for activation (Harvell 1986). Some plant species respond to herbivore damage by producing structures (extrafloral nectaries) or volatile chemicals that attract natural predators of herbivores, such as predatory ants (Dicke et al. 1990a, 1990b; Takabayashi and Dicke 1996; Agrawal and Rutter 1998). Production of volatile organic compounds through plant damage causes a rapid and localized buildup of volatile chemicals in the vicinity of leaf damage (Heil 2008), allowing chemical cues to be used by predators of herbivores to find the site of damage (DeMoraes et al. 1998, Dicke et al. 1999, Paré and Tumlinson 1998) and to synchronize their response to herbivore presence (Bruin et al. 1992, Vittecoq et al. 2011). When a leaf is attacked by an herbivore, mechanical damage in combination with molecules in the saliva of herbivores causes the formation and release of de novo biochemical substances within the plant that convey information about plant status (so called infochemicals, Dicke and Sabelis 1988). Over evolutionary time, predators have associated volatile leaf chemicals induced by herbivore feeding with the presence of herbivores on which they can prey (Heil 2008, 2009).

Some plant species, particularly in the tropics, rely on ants for defense against herbivores (myrmecophytes). In specialized ant-plant mutualisms, plants produce structures that provide food and nesting sites (domatia) to ants that directly defend these resources. Direct defense of resources results in indirect defense of their host plant. In these systems, mutualist ants are
analogous to induced defensive secondary compounds (Janzen 1966; Rehr et al. 1973; McKey 1984, 1988). Defensive ants are ideal inducible defenses because they are mobile and rapidly deployable (Agrawal 1998). For example, the myrmecophyte *Macaranga tanarius* (Euphorbiaceae) significantly increases its production of nectar for the first two hours after dusk, the time of day when herbivore damage to the plant is highest (Heil et al. 2000). Nectar is an important food item for *Macaranga’s* ant partners, so presumably increased nectar production will result in increased visitation by ants and thus increased protection against herbivores during a time when its host plant is most vulnerable to herbivore attack (Heil et al. 2000). In the *Petalomyrmex phylax* (Formicinae) - *Leonardoxa africana* (Caesalpiniaceae) system, *Petalomyrmex* ants respond rapidly and intensely to volatile compounds released by *Leonardoxa*, resulting in high levels of recruitment to sites of plant damage (Vittecoq *et al.* 2011). In tightly evolved ant-plant systems like these, reciprocal selection on ants and plants is expected to result in a well-developed, rapidly induced response syndrome (Agrawal 1998).

Numerous studies have shown that ants respond rapidly to volatile leaf chemicals released when leaves are damaged (Agrawal 1998). Less is known about other aspects of induced defense behavior by ants, such as whether ants respond more effectively to volatile organic compounds released by their own host plants compared to compounds released by other plant species. Here I test the hypothesis of Agrawal (1998) that, in highly evolved ant-plant systems, ants should act as inducible agents of biotic defense. I test this hypothesis by examining the role of volatile leaf compounds in mediating the interaction between *Ocotea* and *Myrmelachista*. *Myrmelachista flavocotea* are obligate inhabitants of the hollowed-out stems of the trees *Ocotea atirrensis* and *O. dendrodaphne* ([Lauraceae] Longino 2006). In ant-plant systems in which plants offer hollow structures to nesting ants, the partners form a constant and
intimate association, which can lead to extreme specialization. Housing specialized ants can be a highly efficient defense strategy (Heil and McKey 2003). While it is has been discovered that *M. flavocotea* patrols its host plant and defends its host plant against herbivores (Kuhn 2013, Chapter 2, this study), whether *M. flavocotea* exhibits inducible defense of its host plant is unknown. In this study, I examined the response of *M. flavocotea* as an agent of biotic defense for its obligate host plants *O. atirrensis* and *O. dendrodaphne*. Specifically, I determined (1) if aggressive behavior in *Myrmelachista* is an inducible response to simulated herbivory, (2) if induced defense is persistent (and for how long). I also examined the response of ants to organic compounds extracted from *Ocotea* leaves to test if *Myrmelachista* exhibit a response to volatile organic compounds (1) specific to their host plant, (2) from leaves of different ages, and (3) from leaves damaged by a natural herbivore.

**MATERIALS AND METHODS**

*Study system.* — This study was conducted at the Organization for Tropical Studies La Selva Biological field station (longitude: 84°00'12.922" W, latitude: 10°25'52.610" N; hereafter referred to as La Selva) in Heredia Province, Costa Rica. La Selva is classified as a lowland tropical wet forest (elevation 30-150 m), with an annual rainfall total of 4 m (sensu Holdridge 1947). Data were collected from May – August 2009, 17 June - 23 July 2012, and 2 January – 17 January 2012.

*Myrmelachista flavocotea* is a small, yellow formicine ant that, at La Selva, is an obligate inhabitant of *Ocotea atirrensis* and *O. dendrodaphne* (Longino, 2006). The interaction between *Myrmelachista* and *Ocotea* is hypothesized to be a protection mutualism (Stout 1979). In the *Myrmelachista-Ocotea* system the plant provides living space for the ants. In exchange for
nesting space, the ants have been shown to provide numerous benefits for *Ocotea* trees, including defense against herbivores, removal of spores, lichens, epiphylls, and debris from leaf surface, and protection from encroaching plants (Kuhn unpublished). *Ocotea* trees also possibly benefit from nutrient additions from dietary items brought by ants into stems (McNett et al. 2010).

**Colony aggression.** — *Myrmelachista* ants readily attack foreign objects (biotic and abiotic) placed on their host plant. I took advantage of *Myrmelachista* ants’ propensity to attack foreign objects as a way to quantify colony aggression. I measured colony level aggression by wrapping a 10-cm segment of 24-gauge galvanized steel wire around the main stem of the tree ~4 cm from the top of the plant. I counted the number of ants attacking the wire each minute for the 15 minutes. I repeated aggression trials at least three times for each colony within a two-week period. The average median number of ants attacking the wire was used as an index of colony aggression (this number is hereafter referred to as colony aggression).

**Host plant traits.** — To determine herbivore damage, I randomly selected five leaves per tree and measured them *in situ*. I photographed each leaf on a white background and later determined leaf area and percent leaf area removed by herbivores using the software package ImageJ© (Sheffield, 2007). I used Poisson regression to examine the relationship between herbivory and colony aggression using a General Linear Model (GLM) fitted to a Poisson distribution.

After photographing leaves, I measured leaf toughness and leaf thickness. Leaf thickness was measured using a 0.001 mm digital micrometer. Leaf toughness was measured using a penetrometer constructed from a 10 g Medio-Line Pesola scale modified with a pressure set. I built a “punch board” out of stiff cardboard covered with waterproofing material to hold leaves
in place while measurements were taken. Three locations on each leaf were haphazardly selected and toughness and thickness measurements were measured at each location.

Response to simulated herbivory. — To test the hypothesis that ants increase defense of their host tree with increasing levels of herbivory (i.e., plant defense is inducible), I conducted an experiment in which I measured the behavioral response of Myrmelachista to artificial herbivory. For 48 O. atirrensis and 18 O. dendrodaphne, I measured the response of ants to the presence of a foreign object placed on the main stem of their host plant (as described above in “Colony Aggression”). On half of the trees of each species I used a standard paper hole punch to remove ~5% of the total leaf area (treatment group). The remaining 14 O. atirrensis and 9 O. dendrodaphne were not damaged (control plants). For the control plants, I mimicked the actions of punching holes without actually damaging leaves. I measured colony aggression 1 hr, 1 d, 7 d, 30 d, and 60 d after the initial treatment. I compared colony aggression across time and between treatments using a two-way, repeated-measures analysis of variance. Data for O. atirrensis did not meet the assumption of sphericity regarding the overall variance–covariance data matrix, so I used the Greenhouse–Geisser epsilon correction to adjust the degrees-of-freedom allowed in the statistical model (Quinn and Keough, 2002). Data for O. dendrodaphne did meet assumptions for sphericity, so I used a univariate unadjusted F test for these data. Sample size for O. dendrodaphne was too small to calculate time x treatment effects, so responses of ants in the control and treatment groups at each time were compared using univariate analysis.

Response to plant identity. — To determine if Myrmelachista were more excited by volatile leaf compounds produced by their host plant species or by volatiles of green leaves in general, I presented Myrmelachista ants with leaf circles punched from the leaves of O. atirrensis and O. dendrodaphne as well from Welfia regia (Arecaceae). Welfia regia (hereafter
referred to as palm) was selected as a control because it is the second most common woody plant at La Selva and is easily identified (Matlock and Hartshorn 1999). Leaf circles were punched using a standard hole punch. Leaf circles were stored in separate, sealed 2-dram glass containers. The hole punch was rinsed with ethanol before switching leaf types. Circles were deployed within an hour of being punched and new leaf circles collected as needed. Plants from which leaf circles were obtained were not used in the experiment.

A single leaf circle from each of the three plant species (*O. atirrensis*, *O. dendrodaphne* and palm) was placed on the surface of an undamaged *O. atirrensis* or *O. dendrodaphne* leaf equidistant from the petiole. I deployed leaf circles on an intact leaf near the top of the *Ocotea* main stem. The order of the leaf circles on the leaves was determined using a random number generator, with each treatment assigned a specific number. The number of ants recruiting to each of the leaf circles was recorded every minute for ten minutes. The activity levels and levels of recruitment between colonies were variable. To allow comparison between colonies I converted frequency data to proportion of ants that attacked each leaf-circle type. Only trials in which at least five ants recruited to leaf circles during the behavior trial were used in the analyses. Behavioral trials were conducted for 29 *Myrmelachista* colonies (16 inhabiting *O. atirrensis* and 13 inhabiting *O. dendrodaphne*). The proportions of ants attacking different stimulus types were arcsine transformed and compared using a one-way ANOVA, with Tukey’s post hoc test.

**Extractions of Volatiles.** — I conducted experiments in August 2009 and 2010 to work out an experimental protocol for extracting volatile leaf compounds from *Ocotea* leaves. I modified a methanol extraction protocol designed by Dyer et al. (2003) to suit the *Myrmelachista-Ocotea* system. *Myrmelachista* were found to have an adverse reaction to a high concentration of methanol. I made a serial dilution of methanol until I found a concentration that
would not repel ants.

To make extractions, 20 g (wet weight) of leaf material was soaked in 75 mL of 1% methanol solution for 12 hours. At the end of this period, leaf material was filtered from the solution, and the solution put into an opaque glass jar for storage in a refrigerator at 4 °C.

Using a 1-ml disposable pipette, small paper disks were soaked with the appropriate extract. The disks were punched out of filter paper with a standard hole punch. Control paper disks were soaked with a 1% methanol solution. Paper disks were allowed to air dry for a few minutes to allow some of the methanol to dissipate, then placed in a sealed glass vials until needed. Additional extract was applied as needed to keep paper disks moist.

**Response to leaf age.** — To determine if *M. flavocotea* respond differently to leaf compounds from *Ocotea* leaves of different ages, organic leaf compounds were extracted from new, medium, and old-aged leaves. Care was taken to select leaves with no signs of herbivory. Volatile-soaked paper disks were placed on the leaf surface on intact *O. atirrensis* and *O. dendrodaphne* leaves. Paper disks were arranged in a row, equidistant from the petiole in randomly determined order. Colony aggression was measured on 30 *Myrmelachista* colonies (17 inhabiting *O. atirrensis* and 13 inhabiting *O. dendrodaphne*) as described above. I compared differences in the proportion of ants responding to extracts from new, medium, and old leaves using a one-way ANOVA, with Tukey ad-hoc post tests. Data were arcsine transformed prior to analysis.

**Response to leaf damage.** — To test the hypothesis that *Myrmelachista flavocotea* shows an increased response to volatile compounds released by their host plants when damaged by an herbivore, I compared ants’ response to extracts made from damaged and undamaged leaves. To induce herbivory, we collected *Adhemarius ypsilon* (Sphingidae) and *Isanthrene championi*
(Arctiidae) caterpillars (both of which are *Ocotea* specialists, Janzen and Hallwachs 2009), placed caterpillars on leaves of *Ocotea* spp., and allowed them to feed until they consumed approximately 25% of leaf area on each leaf. We then harvested the herbivore-damaged leaves as well as intact leaves from a different *Ocotea* plant, performed leaf extractions, and deployed extract-soaked paper disks onto leaf surfaces, as described above. A total of 24 *Myrmelachista* colonies (11 inhabiting *O. atirrensis* and 13 inhabiting *O. dendrodaphne*) were tested. I compared differences in the ants’ response to extracts made from herbivore-damaged leaves, intact leaves, and control paper disks using Wilcoxon each-pair comparisons. All data were analyzed using JMP® 10

**RESULTS**

*Response to simulated herbivory.* — There was a significant negative relationship between colony aggression and herbivory (average percent herbivory) for colonies inhabiting both *O. atirrensis* and *O. dendrodaphne* (GLM regression: P < 0.0001, N = 73, and P < 0.0001, N = 22; Figure 1). When I damaged leaves to simulate herbivore damage, the median number of ants attacking wires increased significantly, compared to pre-treatment levels (repeated measures output: Table 1, Figure 2). Damage to leaves elicited an immediate response from *M. flavocotea*. Ants poured out of stem domatia and rapidly moved to specific leaves with damage. Ants approached damaged areas with gaster curved in an aggressive posture, prepared to spray formic acid. Ants ran around the wounded area with gasters curved in what appeared to be “anticipation” of an encounter with an herbivore or other threat. Levels of colony aggression were persistently higher for damaged trees compared to control trees for 7 d, for colonies inhabiting *O. atirrensis* and 60 d for colonies occupying *O. dendrodaphne* (Figure 2).
**Host plant traits.** — Leaves from *O. dendrodaphne* were significantly thicker ($t = 11.02$ d.f. = 208, $P < 0.0001$) and tougher ($t = 16.4$, d.f. = 208, $p < 0.0001$) than leaves of *O. atirrensis* (Table 2).

**Response to plant identity.** — There were significant differences in the proportion of ants recruiting to leaf circles from *Ocotea* versus palm leaves placed on *O. atirrensis* ($F_{2,33} = 8.21$, $P = 0.001$) and *O. dendrodaphne* ($F_{2,20} = 10.52$, $P < 0.001$). However, there was no difference in the proportion of ants recruiting to leaf circles from *O. atirrensis* versus *O. dendrodaphne* (Figure 3).

**Response to leaf age.** — Significantly more ants recruited to paper disks soaked with extracts from *Ocotea* leaves compared to controls (*O. atirrensis*: $F_{3,67} = 6.46$, $P < 0.001$, *O. dendrodaphne*: $F_{3,51} = 10.91$, $P < 0.001$). There was no difference, however, in the response of ants to paper disks soaked with extracts from new, medium, or old-aged leaves for colonies inhabiting *O. atirrensis* and *O. dendrodaphne* (Figure 4). There was no significant effect of species $x$ treatment interaction in the response of ants to leaf age.

**Response to leaf damage.** — The proportion of ants attacking paper disks soaked with extract differed between herbivore-damaged leaves, intact leaves, and methanol (controls) (Figure 5). For colonies inhabiting *O. atirrensis*, there was a significant difference between the proportions of ants attacking paper disks soaked with extract from herbivore-damaged vs. intact leaves, but no difference in the proportion of ants attacking paper disks soaked with extract from intact leaves vs. controls (Figure 5). For ants inhabiting *O. dendrodaphne*, there was a significant difference between the proportion of ants attacking paper disks soaked in extract from herbivore-damaged leaves compared to intact leaves and controls (Figure 6). There was no difference in the proportion of ants attacking paper disks coated with extract from intact leaves vs. controls.
(Figure 6). Failure to detect a difference between the number of ants attacking extract from intact leaves and control leaves was likely due to small sample size. There was no significant species \( x \) treatment effect in the response of ants to leaf damage.

**DISCUSSION**

*Myrmelachista flavocotea* aggression was significantly negatively correlated with herbivore damage suffered by *Ocotea* plants. I concluded that *M. flavocotea* is an inducible biotic defense for their host plants. When *Ocotea* leaves were damaged, the number of ants recruiting to a foreign object placed on the main stem of their host plant increased significantly. In other studies, induced aggression in ants has been shown to result in the immediate removal of herbivores by ants, protecting the host plant to further herbivore damage (Agrawal and Rutter 1996). Working with *Azteca* ants on *Cecropia*, Agrawal and Dubin-Thaler (1999) found that, after inflicting small-scale, localized damage on leaves, recruitment of ants to damaged leaves increased, as did the time spent patrolling damaged areas. I observed that a large number of *M. flavocotea* workers rapidly recruited to the sites of leaf damage. When an herbivore was actually present, ants attacked the herbivore (personal observation). Large herbivores escaped attack by jumping off *Ocotea* plants. Smaller herbivores were overpowered, killed, cut into pieces, and carried into *Ocotea* stems. The ability of *Ocotea* to induce aggressive behavior in ants with volatile compounds likely contributes to the documented pattern that *Ocotea* occupied by the most aggressive *Myrmelachista* ants suffered the lowest levels of herbivory. Aggressive colonies can quickly locate herbivores and prevent further damage.

One of the most surprising results from this study was that the induction of biotic defense by *Myrmelachista* inhabiting *O. atirrensis* and *O. dendrodaphne* persisted for 7 days and 60 d,
respectively. Most studies have described short-term increases in predator defense of plants following leaf damage (e.g., a few hours, Dicke et al. 1990a). Presumably, the persistence of biotic defense by ants is the result of continued chemical production by the host plant, which may be associated with the level of investment in leaf material by plants. Ocotea have evergreen leaves. Leaves I marked in 2008 could still be found on plants of both species in 2013. Leaves of O. dendrodaphne are significantly thicker than leaves of O. atirrensis. In a recent study on plant defense strategies, Read et al. (2009) found that, contrary to previous predictions, leaf toughness is often positively correlated with the presence of tannins and phenolic leaf chemicals. Ocotea trees employ multiple defenses through mechanical (thick, tough leaves) and chemical (induced biotic defense) means to protect their costly leaves.

*Myrmelachista flavocotea* responded rapidly to leaf circles from *Ocotea* plants. The ants did not show a higher degree of response to *Ocotea* leaf circles from different *Ocotea* species. Ants did, however, show a significantly lower level of response to leaf circles from palm leaves compared to *Ocotea* leaves. Therefore, it appears that *Myrmelachista* does not have a general response to volatile compounds produced by green leaves. Similar response rates of ants to leaf circles from *O. atirrensis* and *O. dendrodaphne* suggest that *Myrmelachista* is responding to compounds that are shared between the two *Ocotea* species. Previous work has demonstrated that all *Ocotea* species share ten major foliar compounds (Takaku *et al.* 2007). Monoterpenes, specifically α- and β-pinene, are the most abundant volatiles released from *Ocotea* leaves (Takaku *et al.* 2007, Kuhn and McGlynn unpublished data). Terpenes have biosynthetic origins from acetyl-coA or glycolytic intermediates produced by plants as secondary metabolites (Gershenzon and Croteau 1991). Monoterpenes are one class of herbivore-induced terpenoid volatiles (Lerdau *et al.* 1994, Dicke and van Loon 2000). *Welfia* leaves emit isoprene, but
undetectable amount of monoterpene (Geron et al. 2002). Isoprene is not found in the leaves of *Ocotea* trees (Takaku et al. 2007). Therefore, I can conclude that *Myrmelachista* behavior is not sensitive to isoprene. Future efforts should aim to isolate the particular molecules that elicit defense response by *M. flavocotea*.

New leaves often sustain the most herbivory (Coley and Barone 1996) and have the highest potential value for the plant (Harper 1989). I found no significant difference in the proportion of ants recruiting to extracts taken from *Ocotea* leaves of different ages. This result differs from the findings of most other studies examining leaf damage and age. For example, Romero and Izzo (2004) found that damage to both young and old leaves of *Hirtella myrmecophila* induced ant recruitment, but that recruitment to new leaves was higher. Differences between the findings of those studies and this study are likely due to differences in experimental protocol. Most studies measuring the response of ants to damage of new leaves measure the response *in situ*. In my experiments, I placed paper disks on mature leaves near the top of naturally occurring *Ocotea* trees; this position did not always correspond to the presence of new leaves. *Ocotea*, as with other plants with modular growth, flush new leaves at branch tips. In the *Myrmelachista-Ocotea* system, I have found that colony aggression is strongly correlated with the number of worker ants in branch tips (Kuhn, unpublished data). Ant occupancy and activity appear to be higher at branch tips, because this is where ants are busy excavating nest space in new plant growth, which allows for colony expansion. Presence of ants in branch tips might allow for better defense of new leaves compared to medium and old-aged leaves, because new leaves occur in areas where ants are active. Observation of increased recruitment of ants to new leaves may have more to do with the location of new leaves on plants, rather than an
increase in the production of leaf volatiles. Indeed, studies have found that the blend and proportions of monoterpenes are similar for leaves of different ages (Staudt et al. 2001).

Type of leaf damage is known to have profound effects on the identity and quantity of volatiles produced by plants (Agrawal 1998). Turlings et al. (1990) found that corn seedlings with artificial damage did not release large amounts of terpenoid volatiles. When oral secretions of caterpillars were applied to the site of artificial damage, however, the amount of terpenoids produced rapidly increased. Because leaf volatiles are induced in the presence of oral secretions of chewing arthropods, I predicted that leaves consumed by herbivores would elicit a strong response in the defense behavior of M. flavocotea. In fact, M. flavocotea was shown to have a graded response to the presence of plant extracts, with the highest recruitment to disks soaked in extract from eaten leaves. Taken together, the evidence from this study shows that M. flavocotea is clearly an inducible biotic defense agent that responds to volatile leaf compounds produced by their host plant, as would be predicted in an ant-plant mutualism.
Table 4.1. Results from repeated measures analysis. The Greenhouse–Geisser epsilon correction was used to adjust the degrees-of-freedom for *Ocotea atirrensis*.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Epsilon</th>
<th>F Stat</th>
<th>DF nom</th>
<th>DF dem</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. atirrensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>0.61</td>
<td>8.23</td>
<td>3.1</td>
<td>83.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>0.62</td>
<td>3.65</td>
<td>3.1</td>
<td>83.3</td>
<td>0.015</td>
</tr>
<tr>
<td><em>O. dendrodaphne</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>4.64</td>
<td>5</td>
<td>60</td>
<td>0.028</td>
</tr>
</tbody>
</table>
Table 4.2. Average (± 1 SD) thickness and toughness measurements of *Ocotea atirrensis* and *O. dendrodaphne* leaves.

<table>
<thead>
<tr>
<th>Species</th>
<th>Thickness (mm)</th>
<th>Toughness (g/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. atirrensis</em></td>
<td>0.14 ± 0.001</td>
<td>80.5 ± 18.5</td>
</tr>
<tr>
<td><em>O. dendrodaphne</em></td>
<td>0.17 ± 0.001</td>
<td>124.9 ± 16.1</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 4.1. Relationship between average median number of *Myrmelachista flavocotea* attacking a foreign object attached to their host plant and percent herbivory damage sustained by their host plants, *Ocotea atirrensis* and *O. dendrodaphne*.

Figure 4.2. Average median number of ants attacking a piece of wire attached to the host plant 1 hr, 1 d, 7 d, 30 d, and 60 d after 5 percent of leaf area of leaves was removed from their host plants *Ocotea atirrensis* and *O. dendrodaphne* using a hole punch to simulate herbivory. Asterisks indicate where p < 0.05.

Figure 4.3. Average proportion of ants attacking pieces of leaves (leaf circles) from *Ocotea atirrensis* (white bars), *O. dendrodaphne* (dappled bars), and Welfia palm (gray bars) placed on the surface of an undamaged *O. atirrensis* or *O. dendrodaphne* leaf. Different letters represent significant differences (Tukey's HSD, p < 0.05).

Figure 4.4. Average proportion of ants attacking paper disks soaked with leaf extracts from new (white bar), medium (striped bar) and old-aged (gray bar) leaves. Controls (dappled bar) were paper disks treated with methanol. Different letters represent significant differences (Tukey's HSD, p < 0.05).

Figure 4.5. Average proportion of ants attacking paper disks soaked with leaf extracts from herbivore-damaged (gray bar) and intact (dappled) leaves. Disks were placed on intact leaves on
*Ocotea atirrensis* and *O. dendrodaphne* trees. Controls (white bar) were paper disks treated with methanol. Herbivore damage on leaves was caused by *Adhemarius ypsilon* (Sphingidae) and *Isanthrene championi* (Arctiidae) caterpillars. Different letters represent significant differences (Wilcoxon each pair comparison, $p < 0.05$).
Figure 4.1.

O. atirrensis

O. dendrodaphne

Colony aggression

Percent herbivore damage

Colony aggression

Percent herbivore damage
Figure 4.2.

**O. atirrensis**

- Control
- Punched

**O. dendrodaphne**

- Control
- Punched

Average median number of ants attacking

Time:
- Pre-trt
- 1 hr
- 1 d
- 7 d
- 30 d
- 60 d
Figure 4.3.
Figure 4.4.
Figure 4.5.
LITERATURE CITED


Holdridge, L.R. 1964. Life Zone Ecology. Tropical Science Center, San Jose.


