DISTRIBUTION AND FORAGING BY THE LEAF-CUTTING ANT, *Atta cephalotes* L., IN COFFEE PLANTATIONS WITH DIFFERENT TYPES OF MANAGEMENT AND LANDSCAPE CONTEXTS, AND ALTERNATIVES TO INSECTICIDES FOR ITS CONTROL

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by

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AUTHORIZATION TO SUBMIT DISSERTATION

This dissertation of Edgar Herney Varón Devia, submitted for the degree of Ph.D with a major in Entomology and titled "Distribution and foraging by the leaf-cutting ant *Atta cephalotes L.* in coffee plantations with different types of management and landscape contexts and alternatives to insecticides for its control", has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies, and to the Graduate School at CATIE for approval.

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ABSTRACT

Atta cephalotes L., the predominant leaf-cutting ant species found in coffee farms in the Turrialba region of Costa Rica, is considered a pest of the crop because it removes coffee foliage. I applied agroecosystem and landscape level perspectives to study *A. cephalotes* foraging, colony distribution and dynamics in coffee agroecosystems in the Turrialba region. I also conducted field assays to assess effects of control methods on colonies of different sizes and to examine the efficacy of alternatives to insecticides.

Colony density (number of colonies/ha) and foraging of *A. cephalotes* were studied in different coffee agroecosystems, ranging from monoculture to highly diversified systems, and with either conventional or organic inputs. *A. cephalotes* colony density was higher in monocultures compared to more diversified coffee systems. The percentage of shade within the farm was directly related to *A. cephalotes* colony density. The proportion of coffee plant tissue being collected by *A. cephalotes* was highest in monocultures and lowest in farms with complex shade (more than three shade tree species present).

Number of colonies and total surface area of colonies were greater near the edges of coffee farms than closer to the interior (>30 m from edge). This effect was significantly stronger for edges adjacent to riparian forest strips than other edge types. There was only limited evidence of the influence of landscape features at greater distances from farms (up to 2000 m) on within-farm colony densities.

Sulfluramid and sodium octoborate caused the greatest mortality among 9 treatments tested in bait formulations on *A. cephalotes* colony activity and mortality. One of the alternative baits tested (active ingredient, propagules of *Paecilomyces* sp. 0484) caused significant reduction in colony activity (worker movements into and out of nest openings), but failed to cause significant colony mortality. Effects of treatments on colony activity were stronger on large (>30 m^2 of nest surface area) than on medium (1.1-30 m^2) and small colonies 0.03-1 m^2.

Coffee farmers would benefit from reduced attack *by A. cephalotes* by increasing the amount of shade, planting shade species palatable to *A. cephalotes* but economically unimportant or capable of withstanding ant attacks.

VITA

Edgar Herney Varón Devia was born in Cajamarca (Colombia) in 1973. In 1990, he began studying Agronomy at the Universidad del Tolima in Ibagué (Colombia), receiving the Agronomist Engineer degree in 1995. In 1996, he worked developing research projects in the Amazonian region of Colombia within the Corporación Colombiana de Investigación Agropecuaria (CORPOICA) Regional 10, including agroforestry projects with small farmers that included the management of soil improving systems with legumes, planning and establishment of polycultures with fruit and wood trees, management and conservation of germplasm of promissory species, germplasm breeding of Amazonian fruit trees and the processing of products derived from Amazonian fruit-tree species.

In 2001 he got involved in M.Sc. study at CATIE in Ecological Agriculture with an emphasis on Integrated Pest Management and a subspecialization in Tropical Agroforestry. His thesis dealed with the potential of ants as biological control agents of the coffee-berry borer and the mahogany shoot borer. In 2003, he started a Ph.D. study in Entomology within a Joint Program between the University of Idaho and CATIE. The current dissertation analyzed on-farm and landscape variables influencing harvest, distribution and density of *Atta cephalotes*, a polyphagous herbivore ant present in coffee plantations of Costa Rica, as a way to improve control strategies for this pest.

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DEDICATION

To God, who gave me the wisdom of taking advantage of the unique opportunity of pursuing a Ph.D.

To my eternal love, my wife, Amparo, who enjoyed and suffered with me through this academic process.

To my daughter, Jessica, who has grown up in Costa Rica and will always remember and miss this beautiful country.

To my parents, who have missed us for almost six years.

To my brother and my sister, who always supported my decision to specialize professionally.

To my friends, who made my life easier and shared the difficulties.

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INTRODUCTORY CHAPTER

Importance of coffee in Costa Rica

The area currently planted to coffee in Costa Rica is 102,888 ha. Coffee exports contribute US$ 197 million to the Costa Rican economy annually (ICAFE 2005). Coffee in the Turrialba region occupies 11,911 ha, which comprises 11.57% of the total coffee planted area in Costa Rica (Zamora and Romero 2006).

Structural types of coffee plantations

Coffee plantations vary along a continuum from very simple to very complex. Several structural types can be found:

a. Open sun monocultures with no shade canopy.
b. Plantations wih lateral shading from linear tree plantings.
c. Monolayered shade canopies: coffee plantations with one shade stratum and, typically, only one shade species.
d. Two-layered shade canopies.
e. Multistory coffee polycultures with three or more species and three or four vertical strata.
f. Rustic coffee plantations in which the understory of the natural forest is cleared to plant the coffee bushes (Somarriba *et al*. 2004).

The incorporation of trees into agroecosystems is a practice with a long history (Nair 1983). This practice has received the name “agroforestry” (Wiersum 1981; Nair 1983). Trees are capable of substantially altering the conditions of the ecosystem of which they are part (Reifsnyder and Darnhofer 1989; Farrell 1990). The sustainable productivity of agroforestry systems is due in large part to this capability of trees (Gliessman 1998). Shaded coffee plantations that have a diverse and structurally complex tree component have a high potential to retain biodiversity (Perfecto *et al*. 1996).

Biology of leaf-cutting ants

Ants in the subfamily Myrmicini, tribe Attini, have the habit of cultivating and eating fungi (Weber 1966). There are 24 species of *Acromyrmex* and 15 known species of *Atta* (Wilson 1986*),* which are considered as true leaf-cutting ants because they cultivate the fungus (*Attamyces* sp.) (Cherrett *et al*. 1989) on freshly cut plant parts and rely mainly on it for nourishment.

The main advantage of fungus culture is that it allows the ants to utilize efficiently diverse forms of fresh vegetation, including flowers, fruits, leaves, and stems. Effects of leaf-cutting ants range from selective and patchy damage on individual plants to landscape scale influence on the plant community (Wirth *et al*. 2003). They are very abundant in neotropical and subtropical forests, where they consume more plant tissue than other important herbivores, including mammals, birds, and other kinds of insects (Wilson 1986). Therefore, they are considered one of the most destructive herbivores in these forests (Mariconi 1970; Weber 1972; Cherrett and Peregrine 1976; Wilson 1986; Hanson 1995).

Each mature *Atta* colony consists of one queen and thousands of workers. Every year, each mature colony produces fertile individuals which are winged (Wirth *et. al*. 2003) and engage in a nuptial flight. Such flight may be a single annual event or may be composed of several swarming events. During the nuptial flight, the queen is fertilized and obtains enough sperm to last her lifetime (Fowler *et. al*. 1986).

Before departing on her nuptial flight, each *Atta* queen packs a small wad of mycelia of the symbiotic fungus into her infrabucal pocket (cibarium). Following the nuptial flight, the mated queen casts off her wings and excavates a nest chamber in the soil. The queen then regurgitates the mycelial wad, which serves as an inoculum to start a new fungus garden (Wirth *et al.* 2003). She also begins producing the first eggs (Autuori 1956). When the first workers eclose, they begin to feed on the fungus and they take over the fungus culture activities (Wirth *et al.* 2003). *A. cephalotes* colonies can live up to 10 years (Weber 1976).

Leaf-cutting ants as agricultural pests

Polyphagy of leaf-cutting ants allows them to cause damage not only to native forest tree species (Rockwood 1976) but also to many agricultural crops, notably citrus (*Citrus* spp.), cacao (*Theobroma* cacao), cassava (*Manihot esculenta*), coffee (*Coffea arabica*), maize (*Zea mays*) and cotton (*Gossypium hirsutum*) and forest plantations (Cherrett 1986;

Hölldobler and Wilson 1990). Moreover, in addition to direct crop damage, their impacts on agriculture include loss of land surface and destruction of farm roads because of their large colonies, leading to accidents involving machinery and livestock (Hölldobler and Wilson 1990).

Total losses caused by leaf-cutting ants are probably in the billions of dollars (Hölldobler and Wilson 1990). Vilela (1986) reported that injury by them was responsible for 75% of the expenses in reforestation in Brazil. Cherrett and Sims (1968) suggested annual losses of US $160,000 (4% of the crop value) in cocoa plantations in Trinidad. Amante (1972) estimated annual sugar cane losses in Sao Paulo State at US$6.3 million. Robinson (1979) estimated annual losses over all of Paraguay to be between US$6.3-7.9 million. In a survey of 27 countries, Cherrett and Peregrine (1976) found that 47 crops were reported as being attacked by leaf-cutting ants. Little has changed since these early reports and leaf-cutting ants remain a significant pest of important agricultural commodities (Lima 1992; Zanuncio *et al.* 1996; Melara *et al.* 1998;). Coffee is prominent among these commodities (Barreto *et al*. 1998).

Biological control of leaf-cutting ants

Several biological control approaches for leaf-cutting ants have been implemented throughout the decades. Although the ants themselves have few natural enemies and none that have a large impact on their populations, the cultured fungus appears vulnerable to biological control agents. Entomopathogenic and antagonist fungal strains have been studied for their control (Jaccoud *et al*. 1999). Some strains have proved to be successful against laboratory colonies (Jaccoud *et al*. 1999; Ortiz and Orduz 2000). However, under field conditions only a minimal number of strains have had significant effects on controlling their colonies (López and Orduz 2003).

Another possibility for control comes from botanical sources of toxins that affect the ants. Plants such as neem (*Azadirachta indica*), sesame (*Sesasum indicum*), castor bean (*Ricinus communis*) and jack bean (*Cannavalia ensiformis*) have been tested for their control (Mullenax 1979; Bueno *et al*. 1995; Hebling *et al*. 2000). As is true for fungal entomopathogenic and antagonists, however, most of the work has been done in the laboratory without corroboration under field conditions (Silagyi 2002).

Action thresholds and improved methods for managing leaf-cutting ants

Methods that have been used to control leaf-cutting ants include thermal fogging, poison baits and even explosives to blow up the nests (Vilela 1986). Although several insecticides can be effective in bait formulations against them, trade has been dominated by those containing dodecachlor, a compound now banned in several countries. New bait formulations mainly contain chlorpyriphos or sulfluramid (Zanuncio *et al*. 1996).

Currently, in Costa Rica, there are two insecticides available that are widely used by farmers: sodium octaborate and sulfluramid. The latter is used against several leaf-cutting ant species (Zanuncio *et al*. 2000), but its residual activity is not long enough to prevent the development of new colonies (Pinhão *et al.* 1993). Information on sodium octaborate activity on *Atta* spp. colonies is scanty. Both compounds are relatively expensive for small farmers, not always are highly effective at destroying colonies and have some of the limitations of conventional insecticides (Diehl-Fleig and Valim-Labres 1993).

Methods for managing leaf-cutting ants in agricultural systems could be improved by considering their ecological characteristics. For instance, the likelihood of colony formation and ant attack apparently differ depending on landscape features and vegetational composition (Zanetti *et al*. 1999). In addition, knowledge on temporal dynamics of colony formation may help guide farmers in making the most effective use of insecticides.

For example, presumably there could be an optimal time for treating new colonies after annual nuptial dispersal and colony formation. At that time farmers could simultanesouly treat older colonies and those newly established by foundresses. On the other hand, colonies have inherently high mortality rates just after establishment (Autuori 1956; Fowler *et al*. 1986). Thus, treating new nests immediately after colonization could result in insecticide waste, because only a small percentage of nests will eventually establish, while waiting too long could allow colonies to become larger and more difficult to treat successfully. With appropriate study, an optimal time for colony treatment could be devised.

In addition, ant seasonal activity levels, as well as landscape level factors, coupled with varying crop vulnerability to ant attack can help establish the level of risk of attack. As with many other pests, design of risk-weighted thresholds and optimal treatment schedules for leaf-cutting ant management could be very beneficial to farmers. No such thresholds and schedules have been ever developed for leaf-cutting ants in any crop, even though some efforts have been made for tree plantations (Zanetti *et al*. 1999).

Studies of the ecological aspects of these ants can provide the basis for innovative methods for controlling them. For instance, in Brazil, Zanetti *et al*. (2000) studied the relationship between density and size of the *Atta* spp. colonies and timber production in *Eucalyptus* plantations. Also, Oliveira *et al.* (1993) developed a monitoring control system for leaf-cutting ants in Brazil called SIMFOR (its Portuguese acronym), which was applied at Mannesmann Florestal Ltda. for *Eucalyptus* plantations. This system was based on information about plant species and origin, type and quantity of the applied insecticide, number of hours invested in control, type of surrounding vegetation, distance from the edge of the plantation to the native forest and the presence or absence of native forest strips. By using this system, Zannetti *et al.* (1999) determined that in *Eucalyptus* plots surrounded by native vegetation strips, control costs were lower than plantations devoid of strips, which may justify their use in economic terms.

A landscape perspective to address the problem of leaf-cutting ants

Zannetti *et al.* (1999) also found that costs for ant control were higher in *Eucalyptus* plots surrounded by native forest fragments, so landscape level interactions are evidently influencing both ant density and colony size. Knowledge of the spatial distribution of forest fragments within a region may help to assess the risk and level of attack by leaf-cutting ants. Spatial patterns can be scale-dependent (Crist and Wiens 1996; Schooley and Wiens 2001), with distinct ecological processes influencing patterns at different spatial scales.

Landscape ecology addresses animal and plant abundance and distribution, as well as ecological processes that are dependent on habitat mosaics at a scale larger than the individual farm or field, and how landscape heterogeneity can be managed to benefit society (Naveh and Lieberman 1984; Risser *et al*. 1984; Allen and Hoekstra 1992). An emphasis on spatial heterogeneity, human influences, and spatial-temporal dynamics distinguishes landscape ecology from other types of ecological research (Freemark *et al.* 1995).

Occurrence of species and their population densities, as well as population dynamics, trophic interactions, and community structure within a habitat, may depend on processes at larger spatial scales than included within individual farms (Kareiva and Wennergren 1995). In the case of leaf-cutting ants as agricultural pests, the specifics and intensity of these landscape level effects, if known, could contribute to develop ecologically-based management approaches to deal with them. The density, distribution and colony size of *Atta* spp. and *Acromyrmex* spp. are of importance in relation to their potential impact on the ecosystem. Likewise, colony density and distribution may respond to landscape and local scale features. Therefore, a landscape approach could provide new insights and perspectives for ecological studies (Kessler *et al.* 1992) and for leaf-cutting ants could help to identify important cues for their managing.

For instance, a methodology that includes the use of GPS, aerial photography, soil classification and predominant vegetation was developed for *Atta texana* (Kulhavy *et al*. 2001). It is geared to assess the effect of temporal and spatial distribution of *A. texana* on soil texture and organic matter within mounds and adjacent areas, to estimate the landscape area affected by this species on different sites in a forested landscape in Texas. Thus, the availability of image technology and spatial analysis at larger scales now makes feasible gaining insights into leaf-cutting ant ecology and management.

Organic coffee production and leaf-cutting ants

In Costa Rica, conventional coffee farmers rely on insecticides commercially available such as sulfluramid, sodium octaborate, foxim, malathion, clorpirifos, terbufos and metamidophos to control leaf-cutting ants (E.H. Varón, unpublished data). Some of these insecticides are considered highly toxic and are not always effective for leaf-cutting ant control. Therefore, most affected farmers face serious constraints dealing with leaf-cutting ants.

Organic coffee production, on the other hand, has increased annually between 10-30% in Costa Rica in recent years, reaching 6,487 ha by 2001 (Fredkorpset 2006) and apparently continuing to increase (G. Soto, pers. comm.). Currently, organic coffee represents 0.69% of the total coffee production in Costa Rica (ICAFE 2005).

Organic farming offers important economic opportunities for some resource-poor producers, as it provides them access to a more special and profitable niche market in the face of falling coffee prices (Nestel 1995). However, since farmers involved in this type of production are encouraged not to use either synthetic insecticides or fertilizers and rely upon natural approaches (Marín and Soto 2002), they have limited options to deal with leaf-cutting ants, which may exacerbate problems in their plantations. Therefore, in the search for new strategies to manage leaf-cutting ants, it is necessary to develop cost-effective and environmentally sound approaches and methods.

In Turrialba, Costa Rica, organic farmers are organized into APOT (Asociación de Productores Orgánicos de Turrialba = Organic Growers Association of Turrialba), which includes 130 small growers (with less than 2 ha each) producing coffee, vegetables or fruits.

Almost all of their crops are normally affected by leaf-cutting ants, so that they treat their nests with a wide array of plant extracts, among which jack bean (*Canavalia ensiformis*) and hot pepper (*Capsicum frutescens*) stand out. Unfortunately, results are rather inconsistent in controlling them. Therefore, most farmers have switched to some synthetic products that are not banned for organic production, like sodium octaborate. Despite its effectiveness, however, sodium octaborate is used on a restricted basis and relatively expensive for resource-poor farmers, as one kilogram costs about US$7; treating an old (mature) nest of some 100 m^2 requires 2 kg, amounting to US$14 per nest, which is not easily affordable for them.

Thus, both economic and environmental factors have fostered the interest of APOT members in becoming involved in research looking for alternative methods to deal with leaf-cutting ants. Therefore, for the Turrialba region it is important to explore alternatives to ensure long-term certifiability of their products as organic and also to decrease their expenditures on managing leaf-cutting ants.

Proposed work: improved methods for control of leaf-cutting ants in coffee

In the proposed work, we applied a landscape level perspective to understand leaf-cutting ant colony foraging, distribution and dynamics in coffee agroecosystems in the Turrialba region. Our effort was geared towards improving current management practices, while considering economic, environmental and human health issues.

Our project was integrated closely with a National Science Foundation (USA)-funded project for the Turrialba region, being carried out by faculty and graduate students in the Joint Doctoral Program offered by the University of Idaho and CATIE. This NSF-IGERT (Integrative Graduate Education and Research Traineeship) project addresses biodiversity conservation, while maintaining or increasing productivity in fragmented agricultural landscapes.

One team of students in the IGERT project studied coffee production in Turrialba and its members examined the social context of production and the effects of adjacent forest corridors on bird and potential insect pest communities associated with nearby coffee plantations. My dissertation research was partly collaborative with this team, and included objectives examining the effects of adjacent forest on leaf-cutting ants as coffee pests. Because the IGERT project included a social component, in which coffee farmer attitudes and knowledge of forest adjacent to coffee are investigated, I was able to build upon this and enrich it by considering farmer attitudes about leaf-cutting ants and by providing these cooperators with our information for improving control of these pests.

The objectives of this dissertation were:

GENERAL

1. To determine what local and landscape factors are influencing the spatial and temporal distribution, and harvesting by *A. cephalotes* in Turrialba region coffee agroecosystems.

2. To develop methods for *A. cephalotes* control based on natural products and biological agents.

SPECIFIC

1a. To determine the relationship between local and landscape-level variables and harvesting of coffee by *A. cephalotes.*

1b. To determine the relationship between local and landscape-level variables and *A. cephalotes* colony densities..

2a. To test antagonist and entomopathogenic microorganisms as well as promising botanical extracts for *A. cephalotes* control..

2b. To develop an *A. cephalotes* injury risk model as a basis for recommendations for coffee farmers regarding its management.

In order to address these objectives, several studies were carried out within coffee agroecosystems in the Turrialba region. The second chapter of this dissertation (following this one) focuses on how plant diversity influences harvesting by *A. cephalotes* colonies in coffee plantations. The third chapter examines landscape and on-farm variables that influence the density of *A. cephalotes* colonies in coffee plantations. The fourth chapter deals with the influence of riparian forest edges on the distribution and density of *A. cephalotes* colonies in coffee farms that are adjacent to these edges. The fifth chapter explores alternative tactics to manage *A. cephalotes*. The sixth and concluding chapter summarizes the findings of the entire dissertation research and discusses their implications..

In summary, this dissertation was aimed at studying some ecological aspects that could be relevant for *A. cephalotes* management. Its practical goal was to improve our knowledge of the response of this particular insect species to different ecological aspects within coffee agroecosystems, so that farmers from the Turrialba region can devise better strategies to deal with leaf-cutting ants.

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CHAPTER 2

Effect of farm diversity on harvesting of coffee by the leaf-cutting ant *Atta cephalotes*

Running Head: Coffee farm diversity and Atta cephalotes *harvesting*

Keywords Costa Rica, *Erythrina poeppigiana*, *Coffea arabica*, leaf-cutting ants, agroforestry systems, monocultures, vegetational diversity, agroecosystems.

Abstract 1 In Mesoamerica, shade trees are often included within coffee (*Coffea arabica*) agroforestry systems. Shade trees potentially protect the main crop by increasing vegetational diversity and reducing insect herbivory through one or more mechanisms.

2 The effect of on-farm vegetational diversity on harvesting of coffee leaves by the leaf-cutting ant, *Atta cephalotes* L.*,* was examined on 15 coffee farms varying in vegetational diversity in Turrialba, Costa Rica. The farms ranged from coffee monocultures to complex-shade coffee systems with more than three tree species present. The vegetational diversity of each farm was quantified using a leaf area index (LAI).

3 The species composition and biomass of the plant material being carried into colonies by ants was collected, identified to species, and its biomass was quantified four times during one year for at least two colonies in each of the 15 farms.

4 The proportion of plant biomass that was coffee being retrieved by *A. cephalotes* differed significantly among farm management types, and was highest (40%) in monocultures and lowest (<1%) in farms with complex shade. Coffee was always harvested in a lower proportion than predicted based upon its relative abundance on the farms.

5 In dual-choice bioassays with laboratory colonies, *A. cephalotes* significantly preferred the leaves of the predominant shade tree species on the farms, poró (*Erythrina poeppigiana*) over coffee.

6 Results indicate risk of injury by *A. cephalotes* can reduced in botanically diverse coffee agroecosystems due at least in part to foraging preference for plants other than coffee.

Introduction

In Mesoamerica and the Caribbean, it is common to observe shade trees within coffee, *Coffea arabica* L., agroforestry systems. Shade trees provide a suitable microclimate for the coffee crop, add organic matter through leaf-litter, fix nitrogen (depending on the species), enhance nutrient cycling, and decrease soil erosion (Beer *et al*., 1998). Shade trees may also protect crops by reducing densities or attack on the main crop by potential herbivores (Sileshi *et al.,* 2005). Monocultures are notoriously vulnerable to pest outbreaks (Gibson & Jones, 1977) and some authors propose that structural and floristic complexity may, by several mechanisms, reduce the probability of severe pest outbreaks in plant communities (Andow, 1991).

Ants (Formicidae) in the neotropical tribe Attini (subfamily Myrmicini) normally culture and eat fungi (Weber, 1966). The 24 known species of *Acromyrmex* and 15 species of *Atta* (Wilson, 1986) are true leaf-cutting ants in which the colonies rely on harvesting living plant tissues to grow the fungus (*Leucoprinus*: Leucocoprineae) (Chapela *et al*., 1994), which serves as larval food (Quinland & Cherrett, 1979). Although they do not directly consume plants, leaf-cutting ants are important functional herbivores in lowland neotropical forests (Cherrett, 1986); the amount of vegetation cut from tropical forests by *Atta* spp. alone has been estimated to be 12–17% of leaf production (Wirth *et al*., 2003).

Atta cephalotes and other Attini are generalists (Cherrett, 1968; *sensu* Feeny, 1976). Their polyphagy, abundance, capacity to attack both native and non-native plant species (Rockwood, 1976), and their ability to thrive using just a few species if these are the only ones available, all predispose these ants to becoming pests of agricultural commodities (Zanuncio *et al*., 1996). Crops affected include citrus, cacao, cassava, maize, cotton and coffee (Cherrett, 1986). In addition to damaging crops directly, these ants construct huge subterranean colonies that can intefere with cultivation and damage farm roads, leading to accidents involving machinery and livestock (Hölldobler & Wilson, 1990).

Coffee grown in the Western Hemisphere is notable for its relative lack of insect pests, possibly because few native species have evolved mechanisms to overcome natural defenses of this introduced crop (Frischknecht *et al*., 1986; Perfecto *et al*., 1996). Nonetheless, a few insects, including leaf-cutting ants, can become economically important coffee pests in the Americas (Cherret & Peregrine, 1976; Barreto *et al*., 1998). Injury to coffee by leaf-cutting ants occurs in low to mid-elevation coffee plantations (between 600 and 1500 m), where ant abundance is high (they do not occur above 2000 m in Costa Rica, Doerder *et al*., 2005; Longino, 2005). This includes large areas of coffee grown in Central

America. The ants injure coffee by defoliating bushes up to 13 m from the colony and by damaging the roots of the bushes adjacent to the colony (E.H. Varón, unpublished observations).

Although leaf-cutting ants are generalists, they exhibit some discrimination among potential hosts (Rockwood, 1976). For example, leaf-cutting ants caused more damage in monoculture cassava than in diversified systems that included alternative food sources (Blanton & Ewel, 1985). These authors concluded that *A. cephalotes* behaved as a “switch-forager”, capable of exploiting alternate resources when a favored resource is less abundant. A similar protective effect of diversification could be important in coffee production systems in Central America, which range from monocultures to highly diversified agroforestry systems.

To test this, we conducted a field survey to assess the effect of on-farm vegetational diversity on harvesting of coffee leaves by *A. cephalotes* in the Turrialba region of Costa Rica. We compared the proportion of biomass that was coffee, the diversity of plant types and species taken, and the total amount of biomass harvested by the ants in coffee farms differing in vegetational diversity and undergoing organic or conventional management. To examine a possible mechanism affecting their harvesting behavior, we measured *A. cephalotes* foraging preference in a laboratory bioassay for coffee vs. poró (*Erythrina poeppigiana*, Fabaceae), the prevalent shade tree in coffee systems in the Turrialba region.

Methods

Field Survey of Ant Foraging

Selecting farms for the study. The study area was located in the Turrialba region, in the vicinity of the city of Turrialba (9.91°N, 83.69°W) in the Caribbean watershed of Costa Rica, with annual average values of rainfall, temperature and relative humidity of 2479 mm, 21.7ºC air temperature and 87%, respectively. This area includes the life zones of premontane humid forest and tropical humid forest (Tosi, 1989).

We selected 15 farms differing in vegetational diversity and management practices, and representing the most common coffee agroecosystems of the region, based on a previous survey. These farms were placed into five management types: Monoculture (MC), Diversified Conventional (DC), Diversified Organic (DO), Highly Diversified Conventional (HDC) and Highly Diversified Organic (HDO) with three farms in each category (Table 2.1.). All farms selected were infested with *A. cephalotes* colonies, and all were planted with the widely cultivated coffee variety ‘Caturra’.

Among the diversified and highly diversified management types, both organic and conventional production systems occurred, whereas the farms under monoculture were all conventional. The conventional farms receive external chemical inputs, such as herbicides (glyphosate and oxyfluorfen), as well as inorganic sources of major nutrients (N, P, K). They are also sprayed with insecticides and fungicides; herbicides decrease overall vegetational diversity by reducing the abundance of understory plants. On organic farms, managers rely on manual control of weeds and chicken or other organic manure for fertilization and do not use insecticides or fungicides. Our sample of farms allowed comparisons between conventional and organic management within the two types of diversified systems, and comparisons between monoculture and diversified systems employing chemical inputs (Table 2.1).

To characterize the vegetational diversity on each farm, a leaf area index (LAI) assessment was carried out using a plumb-bob method (Ewel *et al*. 1982). At 30 randomly selected locations on each farm we recorded all plant species touching a cord suspended vertically through the vegetation from ground level to approximately 5 m, and when necessary, touching the imaginary extension of that cord above 5 m. LAI samples were taken from six points separated by 1 m in each of five locations randomly located within each farm for a total of 30 points per farm. The LAI provided a basis for comparing abundance and diversity of plant species being harvested by *A. cephalotes* with the available vegetation on the farms.

Recording Ant Foraging. Plant material harvested by *A. cephalotes* was determined on each farm by direct observations at four intervals during 2004. Sampling periods were January-March, April-June, July-September and October-December. Observations were made only on days without rain. For each sampling period, we observed at least two colonies located randomly on each farm and recorded all plant material being brought to the nest during three 10-min periods, between 8:00-11:00, when ant activity was greatest (E. Varón, unpublished observations).

The plant material being carried to the colony during the observation periods was collected from the ants, returned to the laboratory, identified to plant species, dried at 60ºC for 48 h, and weighed. Plant species were placed into six categories: coffee, broadleaf weeds, grasses (Cyperaceae and Poaceae), trees (woody plants), other crops, and other plants (those not fitting the first five categories).

Statistical analysis. An analysis of variance (ANOVA) followed by orthogonal contrasts was used to determine the effect of farm management type on the proportion of coffee in the total dry biomass tissue being harvested. Data were pooled from all four sample dates because a previous ANOVA test including sample time showed no effect of sample date on responses (*F* = 1.36, d.f. = (3,28), *P* = 0.2759). Data were transformed to the square root (% + 0.5) in order to comply with assumptions of normality. Similar analyses were carried out using the absolute dry biomass of coffee tissue and the total dry biomass of tissue of all species taken per hour by *A. cephalotes* as the response variable.

Multivariate analysis of variance (MANOVA) was used to compare *A. cephalotes* harvest profiles among the management types, using as response variable the percentage of the total dry biomass of tissue harvested comprised of the plant categories selected. As for the ANOVA, a previous MANOVA test detected no sample date effect, thus data from all four sample dates were pooled and transformed to the square root (% + 0.5) for the MANOVA. A similar MANOVA used total dry biomass of tissues in each plant category as the response variable. For each MANOVA carried out we used the Wilks-Lambda, Lawley-Hotelling, Pillai and Roy tests. The MANOVA was considered significant only if this was indicated by at least three of these four tests.

Jaccard's index of similarity (Legendre & Legendre, 1998) was used to compare *A. cephalotes* harvest profiles from the farm management types, based on the percentage of each of the plant species observed being harvested. All analyses, excepting MANOVA, which was performed using InfoStat (2005), were performed in SAS (2001). In order to test whether or not harvesting of poró and coffee by *A. cephalotes* was merely a function of the availability of these species in the coffee farms, the proportion of coffee and poró being harvested by the ants on each farm was standardized by dividing it by the proportional availability of each species based on the LAI estimate. A one-sample *t*-test was then performed to determine whether this standardized consumption of coffee or poró on all farms (*n* = 15 for coffee, *n* = 10 for poró) differed from 1, the expected value under the assumption that harvesting is determined by availability.

Laboratory Bioassay for Ant Preference

In order to test the hypothesis that the difference in proportion of poró or coffee taken in diversified farms vs. monocultures was influenced by an inherent preference by ants for either one of the species, we conducted a controlled dual-choice bioassay.

We placed five leaf disks of poró and five leaf discs of coffee Caturra variety (all disks were 3.80 cm^2) into an acrylic box (20 x20x20 cm) containing a laboratory colony *of A. cephalotes.* The leaf samples were fresh, collected from an organic shaded coffee plantation at CATIE. Colonies were established from field-collected queens and workers, together with soil and the symbiotic fungus. The colonies were deprived of food for 24 h before they were, submitted to the choice test.

The box was sealed and the leaf area removed by the ants was assessed after 24 h. To quantify leaf area removed, the percentage lost in each quarter of each disc was visually estimated. The choice test was repeated four times for each one of five different laboratory colonies.

Statistical analysis. A Student's *t*-test was used to compare the percentage of area removed from each plant species by each colony. Data were standardized to the proportion of each plant species consumed in each trial. Students *t*-test was used to determine if either species was consumed more than the other by each colony (*n* = 4). Data for each colony were pooled to obtain a single estimate, and these values were used to determine the overall preference for either poró or coffee (*n* = 5).

Results

Field Survey of Ant Foraging

Amount (g/colony/h) of coffee foliage harvested by *A. cephalotes* differed significantly between monocultures and all other management categories (Table 2.2, Fig. 2.1). Of the tissues harvested by the ants, approximately 40% was coffee in monocultures and only 10% or less was coffee in the diversified systems (Fig. 2.2). The percentage of coffee in the total harvest was significantly higher in the monoculture than in the other categories of farms (Table 2.3). None of the other comparisons were statistically significant.

The MANOVA for the effect of coffee management type on total amount harvested from all plant categories was significant (Wilks: 0.0039, *F* = 3.02, d.f. = (24, 19), *P* = 0.0083; Pillai: 2.38, *F* = 1.96, d.f. = (24, 32), *P* = 0.0373; Lawlley-Hotelling: 27.55, *F* = 4.01, d.f. = (24, 14), *P* = 0.0047; Roy: 22.84, *F* = 30.45, d.f. = (6, 8), *P* < 0.0001). Monoculture differed from all other management types (Lawley-Hotelling, d.f. = (10, 14), *P* ≤ 0.05). The individual ANOVAs were only significant for coffee and grasses and the contrast Monoculture vs. All others were significant for each plant category, except for Other crops and Other plants, but none of the other contrasts was significant (Table 2.2).

The MANOVA for the effect of coffee management type on percentage biomass harvested from all plant categories also differed significantly among management types except for Pillai's (Wilks: 0.0016, *F* = 4.18, d.f. = (24, 19), *P* = 0.0012; Pillai: 2.27, *F* = 1.75, d.f. = (24, 32), *P* = 0.0685; Lawlley-Hotelling: 95.72, *F* = 13.96, d.f. = (24,14), *P* < 0.0001; Roy, 92.55,

F = 123.40, d.f. = (6, 8), $P < 0.0001$). Monoculture differed significantly from the other systems (Lawley-Hotelling, d.f. = (10, 14), $P \leq 0.05$). The individual ANOVAs were significant for coffee, broadleaf weeds and trees and the contrast Monoculture vs. All others was significant for each plant category except for Other Crop and Other plants, but none of the other contrasts was significant (Table 2.3). Values for percent harvested from all plant categories in each management type are presented in Fig. 2.2. Patterns were similar for total amounts harvested (data not shown).

Atta cephalotes workers were observed to collect plant tissue from more than 35 plant species during this study, which is consistent with the polyphagy of this ant species. The ants collected material from different species in the different management systems (Table 2.4). The number of species collected ranged from 8 (monoculture) to 23 (highly diversified organic) (Table 2.4). Jaccard's index of similarity using harvest profiles was greater among the various diversified systems than between the monoculture and most of these other systems, except for the diversified conventional system (Table 2.5). Jaccard's index of similarity using LAI index was also generally greater among the various diversified systems than between the monoculture and most of these other systems, except for the diversified conventional system (Table 2.6).

When the proportion of coffee consumed was standardized by its availability based on proportional representation in the LAI, coffee was under-consumed (Fig. 2.3). For example, in monocultures where coffee comprises more than 85% of the available plant material (Table 2.1), it represented about 40% of harvested plant material. In contrast, poró was consumed approximately in proportion to its availability although there was large variability in its consumption (Fig. 2.3).

Total dry biomass taken by *A. cephalotes* colonies per nest per hour averaged 2.03 g/colony/hour across all the systems and did not differ among systems ($P = 0.86$).

Laboratory Bioassay for Ant Preference

In the laboratory dual choice tests, most colonies of *A. cephalotes* preferred poró over coffee ($P = 0.0302$). However, colony B significantly preferred coffee and colony D showed no significant preference for either one (Fig. 2.5).

Discussion

In the coffee monoculture systems examined in this study, *A. cephalotes* harvested primarily from broadleaf weeds and coffee plants, whereas in the more diverse systems, these ants also harvested from other available plant material, mainly trees. As a result, the proportion of coffee within plant material harvested was approximately 40% in monocultures and 10% or less in diversified systems. In the most diversified systems, coffee comprised less than 1% of the material harvested.

Therefore, in Turrialba coffee systems, as in cassava agroecosystems (Blanton & Ewel, 1985), *A. cephalotes* behaves as a facultative polyphagous herbivore that opportunistically consumes resources, depending upon their availability. From a pest management perspective, the implication of this behavior is that diversification may reduce the risk of attack from ants by distributing their depredations throughout the system and diluting their impact on coffee, which is the most economically important crop. Thus, where *A. cephalotes* is a coffee pest, diversification of the agroecosystem may help reduce its impacts.

Reduced herbivory by insects in vegetationally diverse agroecosystems is theoretically caused by either increased natural enemies in such systems (enemies hypothesis) or by impediments to host finding by the herbivore (resource concentration hypothesis) (Root, 1973). Natural enemies are relatively unimportant for leaf-cutting ants (Cherrett, 1986) and the resource concentration hypothesis concerns specialist herbivores (Kareiva, 1983). Impacts of a polyphagous insect, such as *A. cephalotes,* could be reduced through simple dilution, or some form of associational resistance (Atsatt & O'Dowd, 1976). In the case of dilution, if all potential hosts are attacked in proportion to their abundance and the amount of biomass less vulnerable to injury is greater, attack will be reduced on the vulnerable crop. In one form of associational resistance the presence of preferred alternative host actively attracts foragers away from a target species, or otherwise interferes with foragers in locating the target species.

A simple dilution mechanism appears to be inadequate to explain observed effects of diversification on coffee foraging by *A. cephalotes* in this study. Coffee always represented a lower proportion of total harvested plant material than would be expected based on its relative availability alone (Fig. 2.3). This indicates that other sources of plant material are preferred over coffee by *A. cephalotes,* regardless of the management system.

Some other plant species in Turrialba coffee farms were taken in closer proportion to their availability, such as: *Spermacoce latifolia* in monocultures; *Erythrina poeppigiana* and *Pseudoelephantopus spicatus* in diversified organic systems; *E. poeppigiana* and *Impatiens balsamina* in diversified conventional systems; *Cedrela odorata* in highly diversified organic systems and *Cordia alliodora* and *Citrus sinensis* in highly diversified conventional systems (Table 2.7, Appendix 1).

Although these species were taken on average close to its availability, there was considerable variation in harvesting among individual farms for each of them. For example, even though poró was on average taken in proportion to availability, it was ignored by ants in some farms where it comprised 25-30% of estimated available plant biomass, but harvested in greater proportion than available in other farms (Fig. 2.3). This variability could be caused by the presence of other plant species present in the farms that are more or less preferred than poró.

We did not detect differences in the total rate of biomass removal by ants among the coffee production systems. This contrasts with the result of Blanton & Ewel (1985) who reported that greater vegetational diversity of cassava production was associated with reduced total consumption (leaf area/plot) by *A. cephalotes*. One explanation for this difference is that the ants apparently prefer cassava over other plants and the under-represented cassava in diversified systems reduced overall foraging by the ants. The experiments differed in other ways (16 X 16 m plots, 33 nighttime and daytime surveys, measurement of mean area and mass of leaf tissue removed during 5 min observations).

Our laboratory assay shows that coffee is less preferred by the ants as compared with the predominant shade tree, poró. Chemical and physical characteristics of the leaf tissues probably account for this. Lower ant preference for coffee as compared with some other plants could be related to the presence of defensive chemicals, notably purine alkaloids, in the coffee leaves (Frischknecht *et al*., 1986). Coffee leaf disks were also heavier (0.020-0.022 g/ cm^2) than poró leaf disks (0.016-0.017 g/cm^2) and tend to be thicker (E.H. Varón, personal observation), the mesophyll of coffee has crystalline inclusions that could cause the leaves to be tougher (N. Vásquez, personal communication). We did not examine behavioral preferences for potential alternative hosts other than poró, but *A. cephalotes* workers consumed broadleaf weeds in equal proportion to coffee in monocultures, despite overall greater abundance of coffee in these systems (75-96% of all leaf material) (Table 2.1). This lack of preference for coffee leaves becomes apparent when the availability of the plant species is plotted against consumption (Fig. 2.3).

Poró and other plant sources may attract ants for ecological factors other than ant intrinsic preference for the plant tissues. These include physical accessibility, presence of competitors or aggressors on the plants, and indirect effects of associated plant species on foraging behavior, such as the influence on the microclimatic changes in humidity and temperature (Bach, 1993). If such factors are operative, understanding them could provide the basis for cultural practices to increase the effectiveness of the attractant-decoy effect that protects coffee plants from *A. cephalotes* in diversified coffee production systems.

Higher similarity indices between harvest profiles and LAI index for monoculture and the diversified conventional systems (Tables 2.5, 2.6) reflects their similarity of the underlying plant communities. Monocultures and Diversified Conventional (DC) systems have an equally intensive weed management. Shade trees are also intensively pruned in DC systems (at least twice a year), so that for some part of the year this system becomes a functional monoculture.

In summary, our results show that simplification of coffee agroecosystems can increase coffee leaf harvesting by *A. cephalotes,* and thus their potential as pests of this crop. This effect compounds with the tendency of density of colonies of these ants to be greater in monoculture than in coffee management systems with shade (E.H. Varón, unpublished data). Thus, diversification of the coffee agroecosystem could help reduce damage by *A. cephalotes* in regions where it can be a coffee pest.

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Table 2.1 Characteristics of the coffee management types studied in the Turrialba region, Costa Rica, 2004.

Management type*	Main tree species**	Other predominant species***	Predominant weeds†	Availability of coffee leaves (%) ± SE††	Inputs
Monoculture Conventional	none		Dc, Ef, Ib, Pn, Ps, Sl	87.3 ± 6.34	G, O, NPK
Diversified Conventional	Ep	Ma	Cd, Dc, Ef, Ib, Pn, Sl	82.2 ± 6.18	G, O, NPK
Diversified Organic	Ep	Ma	Cd, Dc, Ib, Ps	53.0 ± 5.45	OM
Highly Diversified Conventional	Ep, Ca, Co, Ed	Cs	Ib, Pn, Ps, Sl	42.4 ± 8.14	G, O, NPK
Highly Diversified Organic	Ep, Ca, Co , Ed	Me	Bp, Cd, Dc, Ib, Sl	48.5 ± 4.12	OM

*Three farms were sampled in each management type. ** Predominant shade tree species. ***Non-shade tree species present that are abundant. †Main weed species present based on the *A. cephalotes* harvest: Plant species abbreviations: *Bp: Bidens pilosa, Ca: Cordia alliodora, Cd: Commelina difusa, Co: Cedrela odorata, Cs: Citrus sinensis, Dc: Drymaria cordata, Ed: Eucalyptus deglupta, Ef: Emilia fosbergii, Ep: Erythrina poeppigiana, Ib: Impatiens balsamina, Ma: Musa acuminata, Me: Manihot esculenta, Pn: Phyllanthus niruri, Ps: Pseudoelephantopus spicatus, Sl: Spermacoce latifolia.* ††Percentage of coffee leaves availability based on LAI assessment using a plumb-bub method (Ewel *et al*. 1982) with 30 sampling points in each farm. Inputs: G: Glyphosate, O: Oxyfluorfen, NPK: N.P-K (18-5-15), OM: Organic manure.

Table 2.2 ANOVA results for the effect of coffee management type on the rate of harvest (g/colony/h) of different classes of plant material by *A. cephalotes.* Turrialba region, Costa Rica, 2004.

	Coffee	Broadleaf weeds	Grasses	Trees	Other crops	Other plants
ANOVA statistics						
F (d.f. = 4, 10)	18.76	2.37	5.89	2.22	1.52	0.64
$P > F$	0.0001	0.1228	0.0106	0.1394	0.2678	0.6459
Orthogonal contrasts (d.f. = 1, 10)			$P > F$			
Monoculture vs. All others	< 0.0001	0.0140	0.0007	0.0331	0.7655	0.4993
Diversified systems vs. Highly diversified systems	0.2611	0.5042	0.7949	0.2638	0.0824	0.9290
Organic vs. Conventional (Diversified systems)	0.1535	0.8842	0.8124	0.2823	0.5540	0.3765
Organic vs. Conventional (Highly diversified systems)	0.6903	0.7126	0.6315	0.7614	0.1979	0.2983

Table 2.3 ANOVA results for the effect of coffee management type on the percentage of biomass harvested by *A. cephalotes* for different classes of plant material. Turrialba region, Costa Rica, 2004.

	Coffee	Broadleaf weeds	Grasses	Trees	Other crops	Other plants
ANOVA statistics						
F (d.f. = 4, 10)	13.96	6.06	3.20	15.68	3.16	0.72
P > *F*	0.0004	0.0096	0.0620	0.0003	0.0640	0.5953
Orthogonal contrasts (d.f. = 1, 10)			*P* > *F*			
Monoculture vs. All others	< 0.0001	0.0015	0.0057	<0.0001	0.1244	0.9358
Diversified systems vs. Highly diversified systems	0.0830	0.0861	0.7916	0.3312	0.0631	0.5355
Organic vs. Conventional (Diversified systems)	0.2650	0.2014	0.6130	0.1010	0.5329	0.1490
Organic vs. Conventional (Highly diversified systems)	0.4825	0.7123	0.7043	0.1917	0.0489	0.8600

Table 2.4 Plant species harvested by *A. cephalotes* in different coffee management types. Turrialba region, Costa Rica, 2004.

Species	Common name	Plant :ategory*	System**				
			MC	DC	DO	HDC	HDO
Averrhoa carambola	Star fruit	T				X	
Bidens pilosa	Hairy beggarticks	BL					X
Bombacopsis quinata	Pochote	T					X
Byrsomina crassifolia	Nance	T				X	
Carica papaya	Papaya	OC				X	
Cecropia peltata	Trum tree	T					X
Cedrela odorata	Spanish cedar	T				X	X
Citrus limetta	Sweet lemon	T					X
Citrus sinensis	Orange	T				X	X
Coffea arabica	Coffee	C	X	X	X	X	X
Commelina diffusa	Wandering jew	BW		X	X		X
Cordia alliodora	Laurel	T				X	X
Drymaria cordata	Chickweed	BW	X	X	X	X	X
Emilia fosbergii	Cupid's shaving brush	BW	X	X			
Erythrina poeppigiana	Poró	T		X	X	X	X
Eucalyptus sp.	Eucaliptus	T		X		X	X
Impatiens balsamina	Impatiens	BW	X	X	X	X	X
Inga sp.	Guaba	T			X		X
Licania arborea	Canilla de mula	OP			X		
Loranthus sp.	Mistletoe	OP	X			X	
Manihot esculenta	Cassava	OC			X	X	X
Miconia sp.	Velvetleaf	OP			X		
Musa acuminate	Banana	OC		X	X	X	X
Phyllanthus niruri	Gale of the wind	BW	X	X		X	X
Pseudoelephantopus spicatus	Iron weed	BW	X		X	X	X
Psidium friedrichsthalianum	Cas Guava	T					X
Psidium guajava	Guava	T			X	X	X
Spermacoce latifolia	Buttonweed	BW	X	X		X	X
Spondias dulcis	Jewish plum	T				X	
Spondias purpurea	Plum	T					X
Swietenia macrophylla	Mahogany	T		X			
Vernonia brachiata	Vernonia	OP					X
Xanthosoma sp.	Yautia	OP			X		
Cyperaceae		G	X				
Poaceae		G	X	X	X	X	X

* Coffee (C); Tree (T); Broad-leaf weed (BL); Grass (G); Other crop (OC); Other plant (OP).
** MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified Conventional; HDO: Highly Diversified Organic.

Table 2.5 Jaccard similarity indices for plant species collected by *A. cephalotes* among coffee management types differing in diversity and management. Turrialba region, Costa Rica, 2004.

	MC	DC	DO	HDC
DC	0.46	-	-	-
DO	0.21	0.35	-	-
HDC	0.31	0.36	0.34	
HDO	0.24	0.36	0.40	0.46

MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC Highly Diversified Conventional; HDO: Highly Diversified Organic..

Table 2.6 Jaccard similarity indices for plant species among coffee management types differing in diversity and management, based on LAI measurements. Turrialba region, Costa Rica, 2004.

	MC	DC	DO	HDC
DC	0.60	-		-
DO	0.20	0.27	-	-
HDC	0.13	0.18	0.27	
HDO	0.20	0.40	0.20	0.47

MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified Conventional; HDO: Highly Diversified Organic.

Table 2.7 Availability vs. harvest values for the main plant species taken by *A. cephalotes* in the different coffee management types. Turrialba region, Costa Rica, 2004.

Plant species	Management type	Availability (%)	Consumption (%)
Spermacoce latifolia	MC	6.25	3.09
Impatiens balsamina	DC	8.16	12.86
Erythrina poeepigiana	DC	12.73	48.21
Erythrina poeepigiana	DO	30.41	39.54
Pseudoelephantopus spicatus	DO	2.56	20.97
Cedrela odorata	HDO	3.61	15.38
Citrus sinensis	HDC	39.01	47.43
Cordia alliodora	HDC	25.58	55.38

MC: Monoculture Conventional: DC: Diversified Conventional; DO: Diversified Organic; HDO: Highly Diversified Organic; HDC: Highly Diversified Conventional.

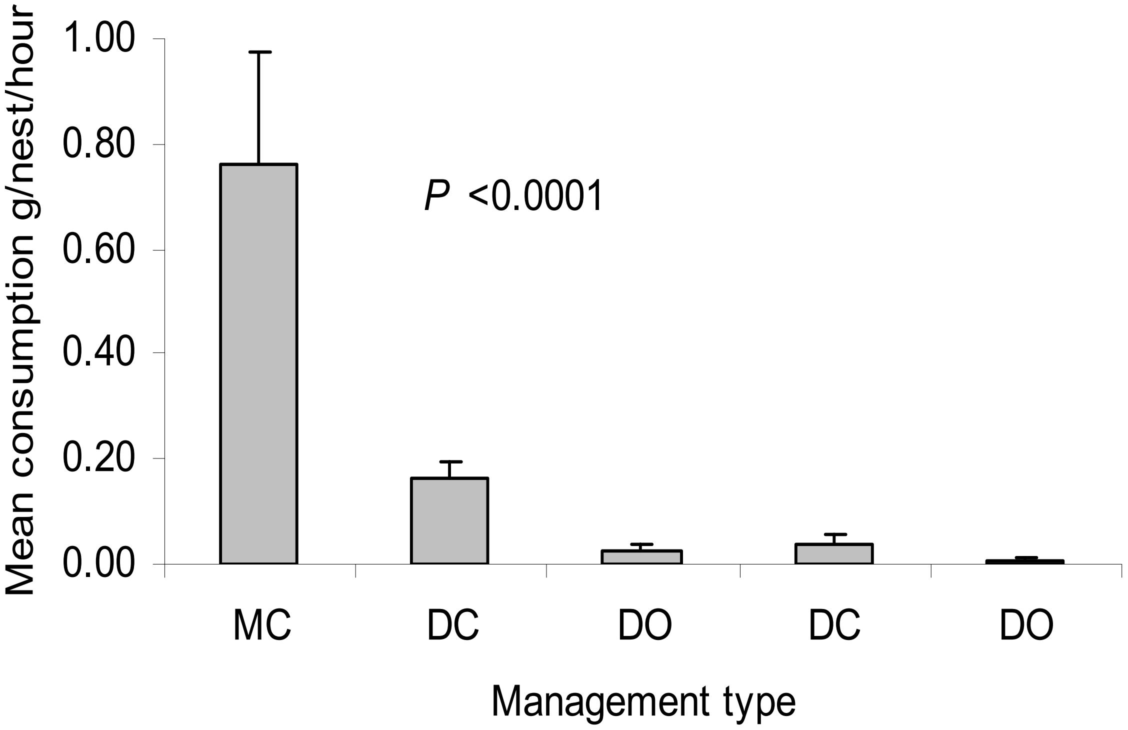

Figure 2.1 Rate of harvest (g/colony/h) (means ± SE) of coffee leaves by *A. cephalotes* in different coffee management types.Turrialba region, Costa Rica, 2004. MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified onventional; HDO: Highly Diversified Organic.

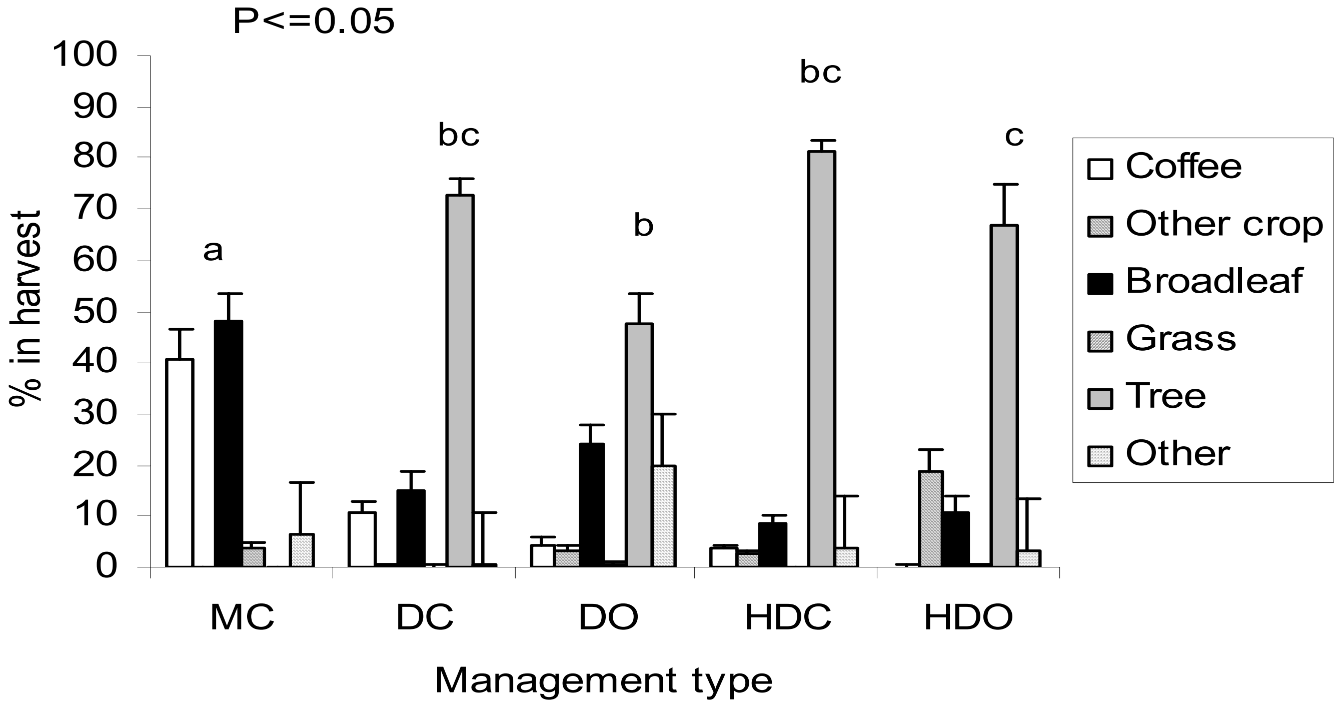

Figure 2.2 Percentage of coffee and four other plant categories comprising the biomass harvested (means ± SE) by *A. cephalotes* in different coffee management types. Turrialba region, Costa Rica, 2004. MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified Conventional; HDO: Highly Diversified Organic.

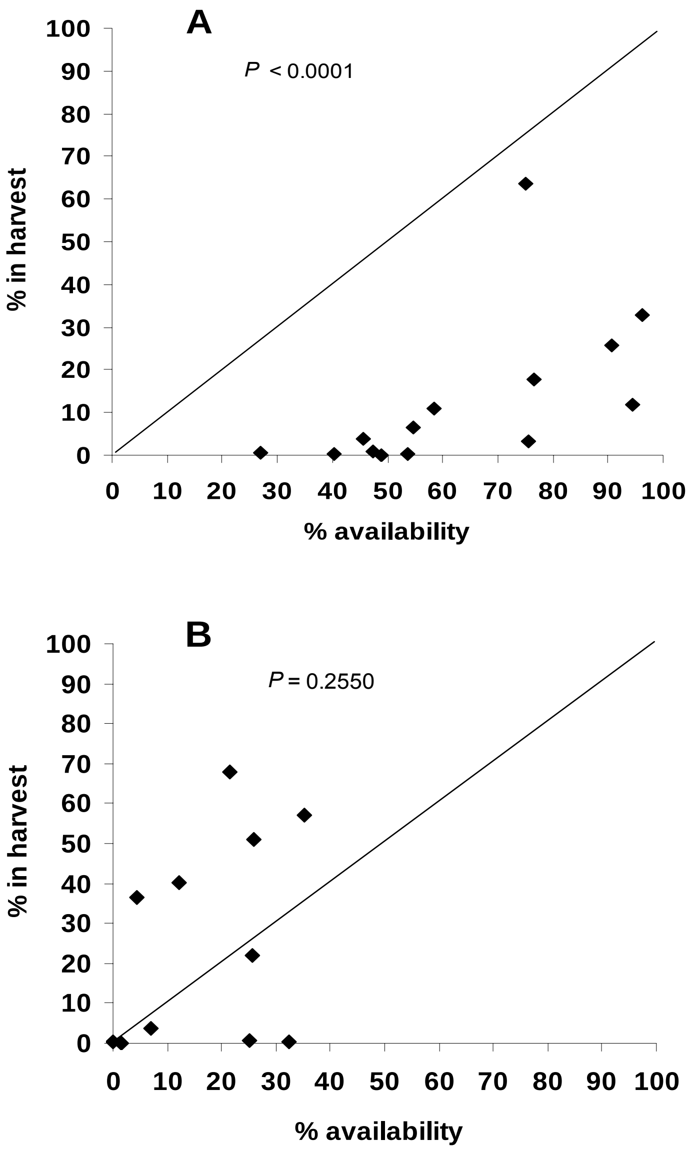

Figure 2.3 Percentage of coffee and poró leaves in coffee systems based on LAI (Availability) vs. the percentage of these plants in material harvested by *A. cephalotes* in coffee farms. Turialba region, Costa Rica, 2004. The *P* values are for a *t*-test to determine whether the points are located off of the diagonal representing consumption = availability (one-sample test for null hypothesis x = 1, in which x = proportion consumed/proportion available). A) Coffee; B) Poró.

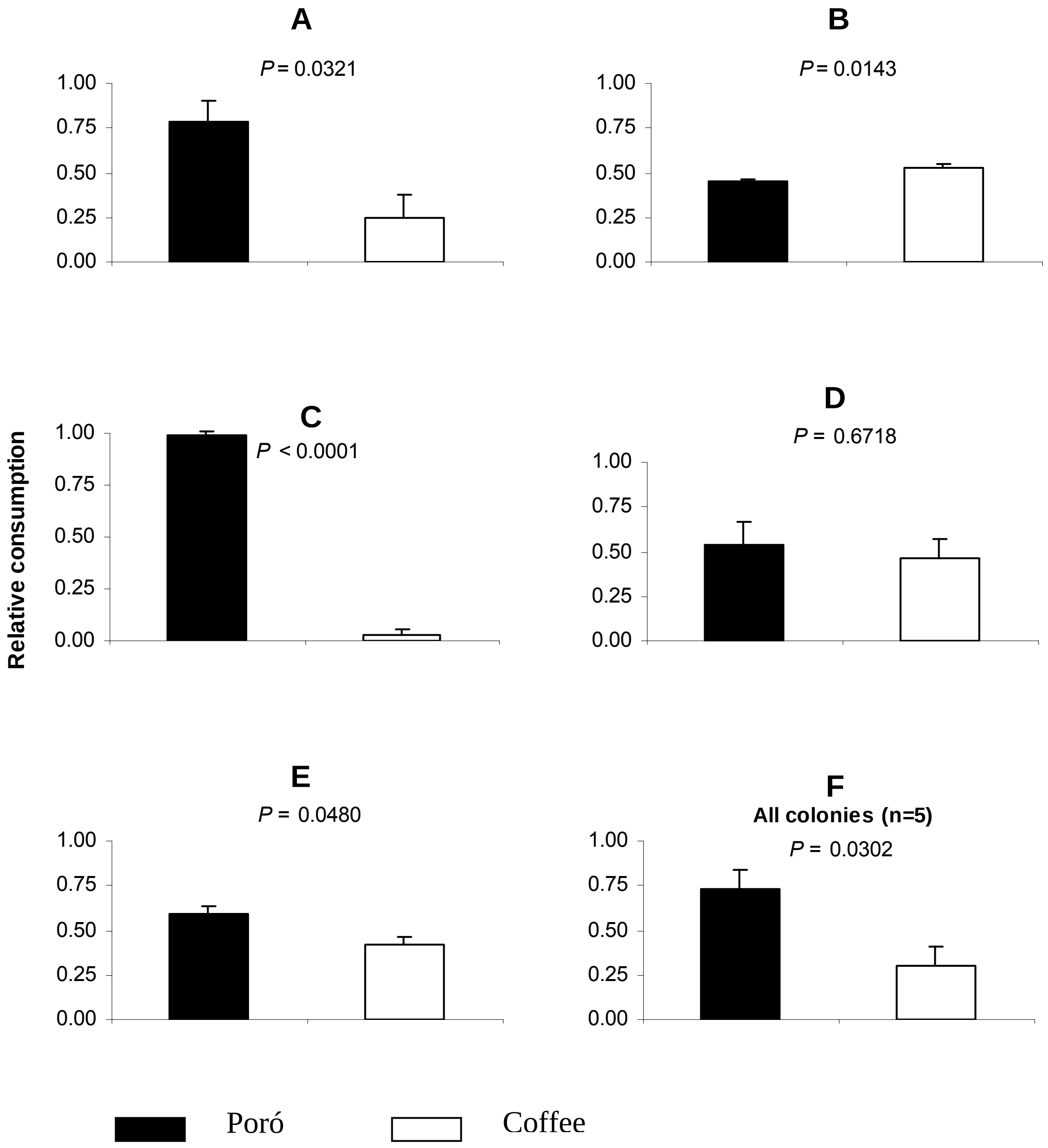

Figure 2.4 Harvest (means ± SE) by *A. cephalotes* colonies in choice tests, under laboratory conditions. *E. poeppigiana* vs. coffee var. Caturra. Turrialba region, Costa Rica, 2004. Five individual colonies (panels A-E) and overall mean for all colonies (panel F).

CHAPTER 3

Effect of coffee farm diversity and landscape on density of colonies of the leaf-cutting ant *Atta cephalotes*

Running Head: Coffee farm diversity and A. cephalotes *density*

Keywords *Coffea arabica*, leaf-cutting ants, agroforestry systems, monocultures, percentage of shade, vegetational diversity.

Abstract 1 In Mesoamerica and the Caribbean region it is common to observe shade trees within coffee agroforestry systems (*Coffea arabica*). Shade trees may potentially protect the main crop in the system by decreasing the density of herbivores.

2 In order to test the protective effect of vegetational diversity for reducing densities of the leaf-cutting ant *Atta cephalotes* L. in coffee plantations, 24 farms were selected representing five categories of vegetational diversity and management, from coffee monoculture to highly diversified shade coffee, as follows: Coffee Conventional, Diversified Conventional, Diversified Organic, Highly Diversified Conventional, Highly Diversified Organic.

3 Other on-farm variables that could influence ant colony density were measured, including percentage of shade and bare soil, soil pH, texture and organic matter, elevation and the closest distance to a forest patch for each farm. The effect of different land use coverage on density of colonies (number of colonies/ha) at different buffer radii was analyzed.

4 The density of *A. cephalotes* colonies was significantly higher in monocultures, compared to the other systems. Also, colony density was significantly lower in the diversified organic system compared to the diversified conventional system. Colony density was only significantly related to on-farm percentage of shade based on simple linear regressions. In a multiple non-linear regression analysis the percentage of shade, (% of shade)^2, and forest proximity were significantly related to colony density. There was an inverse relationship between colony density and percentage of shade and forest proximity. No other variables were significantly related to colony density.

5 The density of new *A. cephalotes* colonies (those established within one year) was significantly higher in monocultures compared to other systems. No

othersignificant effects were found. Neither the density of old colonies nor total surface area of colonies were affected by farm type. Density of new colonies was inversely related to percentage of shade based on simple linear regression analysis and a nonlinear regression including percentage of shade and (% of shade)^2. No other variables were significantly related to new colony density.

6 ANCOVA showed that density of new colonies was directly related to the fallow land use coverage within the 2000 m buffer radius and to the forest coverage within the 500 m buffer radius.

7 Rate of colonization by *A. cephalotes* queens (represented by the number of new colonies) was higher in monocultures probably due to the lower level of shade in these systems compared to diversified systems. Forest and fallow presence and forest proximity also seem to play a role on the density of *A. cephalotes* colonies found on coffee farms.

8 Increasing shade by agroecosystem diversification may decrease *A. cephalotes* pressure on coffee farms.

Introduction

In Mesoamerica and the Caribbean it is common to observe shade trees within coffee, *Coffea arabica* L*.,* agroforestry systems. The trees provide a suitable microclimate for the crop, add organic matter from the leaf-litter, fix nitrogen (depending on tree species), enhance nutrient cycling, and decrease soil erosion (Beer *et al*., 1998). Shade trees, by increasing vegetational diversity might also protect crops by reducing densities or attack on the crop by insect pests. Monocultures are notoriously vulnerable to pest outbreaks (Gibson & Jones, 1977) and some agricultural ecologists propose that structural and floristic complexity of polycultures can, by several mechanisms, reduce the probability of severe pest outbreaks in plant communities (Pimentel, 1961; Tahvanainen & Root, 1972; Root, 1973; Altieri & Letourneau, 1982; Altieri, 2002).

Coffee grown in the Western Hemisphere has relatively few insect pests, possibly because few native species have evolved mechanisms to overcome natural defenses of this introduced crop (Frischknecht *et al*., 1986; Perfecto *et al*., 1996). Nonetheless, a few species, including leaf-cutting ants (Formicidae: Myrmicini: Attini: *Atta*), can be economically important coffee pests in the Americas (Cherret & Peregrine, 1976; Barreto *et al*., 1998). These ant species occur from sea level to approximately 2000 m in the neotropics (Doerder *et al*., 2005; Longino, 2005), a distribution that overlaps with much of the coffee grown in

Central America (600-2000 m in elevation). In Costa Rica, the dominant leaf-cutting ant species is *Atta cephalotes.* These ants can injure coffee by defoliating bushes up to 13 m away from the colony, and by damaging the roots of the bushes adjacent to the colony (E. H. Varón, pers. obs.).

The effect of coffee plantation diversification on *A. cephalotes* abundance is unknown, and there are no clear predictions from theory. These ants have few natural enemies, so the "enemies hypothesis", which predicts greater predator impact in diversified systems (Root, 1973), is likely irrelevant. In addition, they are polyphagous, and the "resource concentration hypothesis", which considers the effects of vegetational diversity on host finding by insects (Root, 1973), is equivocal concerning polyphagous herbivores. In 29 studies of the effects of agroecosystem diversification on polyphagous insect herbivores summarized by Andow (1991), polyphagous insects were less abundant in diversified systems in 13 cases and more abundant in 15 cases. Variability in response depends on the relative attractiveness of different species in the system to the herbivores and the effects of other factors, such as microclimate in individual species (Risch 1981).

We examined the effects of on-farm vegetational diversity on the density of *A. cephalotes* colonies in Turrialba, an important coffee-growing region in Costa Rica. We also gathered data about farm variables such as percentage of shade and bare soil, and a number of soil characteristics (pH, texture and organic matter), to examine their association with density of *A. cephalotes* colonies. Our objective was to determine whether diversification or other characteristics of coffee farms were associated with reduced or increased *A. cephalotes* populations and could provide a basis for cultural control of these ants as pests. The specifics and intensity of these responses, if known, could contribute to developing ecologically-based management approaches for *Atta* species as pests.

In addition to within-farm habitat characteristics, recent studies have shown that insect diversity, or abundance of particular taxa within a site is influenced by landscape level factors such as the proportion of the nearby landscape under cultivation or in forest (Tscharntke & Brandl, 2004). Such effects can be scale-dependent (Crist & Wiens, 1996) with distinct processes influencing patterns at different spatial scales. A landscape ecological perspective (Kessler *et al.,* 1992; Freemark *et al.,* 1995) for leaf-cutting ants could help to discover factors outside of the individual farmer's control, but important for estimating the risks of reaching damaging populations of *A. cephalotes.* Therefore, we included landscape variables such as elevation, forest proximity and the proportion of different land use coverages at different scales present in our study area as correlates with *A. cephalotes* abundance.

Methods

Selection and classification of farms

The study area was the Turrialba region, located at the Caribbean watershed of Costa Rica, with annual average values of rainfall, temperature and relative humidity of 2479 mm, 21.7ºC and 87%, respectively. This area comprises the life zones of premountain humid forest and tropical humid forest (Tosi, 1989). Historically, such a region has been largely devoted to agriculture (mainly coffee and sugar cane), but retains patches of riparian forest and contains some areas of conserved secondary forest and transitions into more continuous forest at higher elevations. This area is important from a biodiversity conservation perspective as the Turrialba-Jiménez Biological Corridor (TJBC), which is a component of the Central American Biological Corridor.

From a group of 131 farms (86 organic and 45 conventional) visited prior to the study, we selected 24 farms (less than 4 ha each) differing in vegetation diversity and management, representative of the most common coffee agroecosystems in the region. These farms were grouped into five management types: Monoculture Conventional (MC), Diversified Conventional (DC), Diversified Organic (DO), Highly Diversified Conventional (HDC) and Highly Diversified Organic (HDO) and, with five farms in each category excepting MC, where it was only possible to sample four farms (Table 3.1).

Sampling

Sampling was carried out two months after the main nuptial flight of 2005, which was recorded on July 1st, 2005. Thus, sampling occurred from September through November. This procedure allowed us to include and distinguish between new colonies that were established after the 2005 nuptial flight, from the surviving colonies from previous years. New colonies have a single tower-like mound of small size < 200 cm^2 in area, and with a small entrance hole, whereas older colonies are flattened, with larger entrance holes and a colony surface area > 200 cm^2.

In each farm a total area of 1000 m^2 was sampled. Four 125-m^2 plots were established, one at each edge of the farm (North, West, East and South), and a single 500-m^2 area was established in the center of the farm. In each plot, all *A. cephalotes* colonies were recorded, located by a GPS unit (Garmin 12XL, Garmin International Inc., Olathe, KS, USA), and measured using a metric tape to determine the maximum length (l) and width (w) of the colony mound. The colony surface area was estimated as ($\pi(l/2)(w/2)$) (Hernández *et al.*, 1999).

Other variables measured in each plot were: the percentage of shade, determined with a spherical crown densiometer (Model 102167, Forestry Suppliers Inc., Jackson, MS, USA); the approximate percentage of bare ground in each plot, assessed visually; and the soil pH, percentage of organic matter and clay. Soil property measurements were based on subsamples from the edge and center of each plot, combined and air-dried prior to their analysis in the laboratory. Organic matter was measured by multiplying the percentage of organic carbon obtained by a 1.724 factor. The organic carbon was obtained by combustion at the Thermo Finnigan analyzer with a previous digestion with HCl+1, in order to eliminate carbonates (Nelson & Sommer, 1996). The pH was measured in water (Díaz Romeu & Hunter, 1978). The percentage of clay was obtained by the Bouyoucos method (Forsythe, 1985).

Statistical analysis

An analysis of variance (ANOVA) followed by planned orthogonal contrasts was used to determine the effect of management type on the overall colony density of *A. cephalotes* (number of colonies/ha). The analysis was performed for the total number of colonies, old colonies and for new colonies detected in 2005, to determine if colonization rates differed among the systems. Data were transformed to square root (x+ 0.5) to comply with assumptions of normality. Colony surface areas were also subject to ANOVA followed by planned orthogonal contrasts. Data were subject to log (x+0.5) transformation in order to comply with assumptions of normality. Simple regressions were carried out both between shade and colony density and shade and new colony density. Since shade percentage was the only variable directly related to densities of total colonies and new colonies by simple regression, it was subjected to quadratic and cubic power transformation $(\% \text{ shade})^2$, $(\% \text{ shade})^3$ in regression analyses, in order to determine if the relationship with colony density was better fitted on that way. The logics of this procedure is the possibility that the relationships between biological events are related to outside factors in a non-linear way.

Multiple regressions including all the variables measured for each farm were performed. Because the variables (farm elevation and soil organic matter, pH and percentage of clay) exhibited colinearity, it was necessary to eliminate all but the most significant in each analysis (InfoStat, 2005).

For the landscape variables (elevation and forest proximity), a land-use map (Appendix 2) and an elevation model of the Turrialba-Jiménez Biological Corridor was used to measure the shortest distance of each farm from a forest patch and the elevation of each farm, as well; such a planning resource was built in 2005 using the program ArcView GIS 3.3 (ESRI, 2002).

In addition, the influence of land use at different spatial scales was assessed by establishing a central point on each farm around which a series of buffers of three different radii (100, 500, 2000 m) was established. The first radius was based on a previously established forest edge effect on *A. cephalotes* (E.H. Varón, unpublished), the second was the highest observed distance to forest from coffee farms in the study area and the third radius was the greatest distance available in the mapped land use areas used in the analysis.

The analysis uses land cover areas at each buffer radius as covariates of the management types as main effects in an analysis of covariance (ANCOVA). Response variables were total colony density and new colony density. The land use classification types for this analysis were: forest, pasture, pasture with trees, coffee, sugarcane, fallow, and agroforestry systems. Unless otherwise noted, all analyses were carried out in SAS (SAS, 2001).

Results

The density of *A. cephalotes* colonies was significantly different among the management types. It was higher in monocultures, compared to the other systems (Table 3.2, Fig. 3.1). Also, a significant difference was found between organic and conventional systems within diversified systems, with conventional systems having higher density of colonies (Table 3.2). Density of *A. cephalotes* new colonies was also significantly different among the management types (Fig. 3.2). It was higher in monocultures compared to the other management types, whereas densities of old colonies did not differ among management types (Table 3.2, Fig. 3.3). No difference was found due to farm management type in the total colony surface area on each farm (Table 3.3), but monocultures tended to have higher total colony surface areas (Fig. 3.4).

The simple regression between shade and colony density was highly significant (F = 14.70, d.f. = (1, 22), P = 0.0009, R^2 = 0.40) (Table 3.4, Fig. 3.5). Multiple regression between colony density and the different local and landscape variables measured, showed that the variables percentage of shade (F = 12.11, d.f. = (1, 20), P = 0.0024), (percentage of shade)^2 (F = 6.35, d.f. = (1, 20), P = 0.0204) and forest proximity (F = 6.64, d.f. = (1, 20), P =

0.0180) were inversely related to density of colonies and together explained 60% of the variation of that variable (F= 10.13, d.f. = (3, 20), *P* = 0.0003) (Table 3.4).

The simple regression between shade and density of new colonies was also highly significant (*F* = 9.35, d.f. = (1, 22), *P* = 0.0058, R^2 = 0.29) (Table 3.4, Fig. 3.6). Multiple regression between density of new colonies and different local and landscape variables showed that shade (*F* = 37.02, d.f. = (1,21), *P* < 0.0001) and $(\text{shade})^2$ (*F* = 26.76, d.f. = (1, 21), (*P* < 0.0001) together explained 69% of the variation of new colony density (*F* = 23.53, d.f. = (2, 21), *P* <0.0001) (Table 3.4).

ANCOVA for land use coverage at different radii did not detect significant relationships for any of the land use coverages and total colony density (Table 3.5). Density of new colonies was directly related to percent forest cover within the 500 m buffer (*F* = 5.61, d.f. = (1, 18), *P* = 0.0292) and fallow coverage (*F* = 9.84, d.f. = (1, 18), *P* = 0.0057) within thee 2,000m buffer (Table 3.6).

Discussion

In our study, densities of *A. cephalotes* colonies were significantly lower in polycultures (diversified coffee plantations of all types) compared to coffee in monoculture. A distinguishing feature of polycultures is the presence of overstory trees that provide shade. Both total and new *A. cephalotes* colony densities decreased significantly with increasing percentage of shade across all farm types.

The significant effect of shade level on *A. cephalotes* colony densities could reflect an underlying causal relationship, as *A. cephalotes* is considered a forest gap specialist (Jaffé & Vilela, 1989). After the nuptial flight, newly inseminated queens search for nesting sites (Fowler *et al*., 1984) and may favor areas with few or no trees and with large areas of bare soil. Two other leaf-cutting ant species, *A. capiguara* and *A. bisphaerica* seem to favor areas with bare soil; they are known to make more colonization attempts in areas with high densities of mature colonies. In both of these cases, queens may have mistaken large colony mounds for disturbed areas (Fowler *et al*., 1984).

A. cephalotes colonies density was also greater in diversified conventionally managed farms than in diversified organic farms. This effect is evidently not due to differences in insecticide use because both use chemical control for the ants. Organic farmers in Costa Rica are allowed to use a chemical bait (sodium octaborate) although on a restricted basis. Conventional farmers also use sodium octaborate, as well as sulfluramid (E.H. Varón, unpublished data). Therefore, the confounding effect of insecticidal inputs between the two systems was likely minimal. Consistent with this presumption, although more insecticides are probably used on conventional farms, colony densities remained higher on these farms, indicating that insecticides are either ineffective or not applied on new colonies during colony establishment.

The significant difference in density of colonies between organic and conventional diversified farms could be explained by differences in shade between these management systems. This is suggested because when shade is included as a covariate in an ANCOVA, the management type treatment effect is lost (P = 0.5831). Moreover, there was a greater than two-fold difference in the average shade percentage between Conventional Diversified Systems (25.50%), and Organic Diversified Systems (63.66%) (Table 3.1).

Organic growers strive to keep a relatively high amount of shade in their systems in order to decrease weed problems, since otherwise they must control weeds manually (G. Soto, personal communication). On the other hand, conventional growers manage for moderate shade in order to increase the productivity of the farm while controlling weeds chemically. That shade difference between both management types appears to be playing a role in the significantly different *A. cephalotes* colonies density found on the farms.

No differences were found among systems for total colony surface area or for older colonies and these variables were not related to the level of shade on the farms. It is likely that despite the higher colonization rates (represented by more new colonies) on conventional farms (which had also the highest expenditures trying to control *A. cephalotes,* E.H. Varón, unpublished data), use of insecticides eliminates some of these new colonies, resulting in the similar densities of older colonies and total colony surface areas in the two systems. Elevation was not significantly related with the density of *A. cephalotes* colonies on the farms in this study*,* despite the fact that this and other *Atta* species are limited to altitudes below 2000 m (Longino, 2005). *A cephalotes* prevalence is evidently not constrained within the range of elevations we studied (735-1146 masl).

Forest proximity influenced the densities of total colonies on the farms and coffee farms closer to forest fragments had greater densities of colonies (Table 3.4). Nearby forests areas may be sources for *A. cephalotes* colonization, since this species is a normal forest inhabitant (Rockwood, 1973) and colonies appear to be abundant near edges within the forest (E.H. Varón & S. Eigenbrode, pers. obs.), whether because of the biotic and abiotic conditions, the lack of insecticide treatments there, or some combination of these factors.

Evidence for the influence of land use types at different spatial scales across the landscape on ant colonies densities within farms is weak. We found significant correlations between densities of new colonies and fallow coverage at the buffer radius within 2,000 m and for forest coverage within 500 m (Table 3.6), but not for other land use types and distances. This may indicate that those land use areas could be playing a role as sources of mature colonies producing mated queens that later produce new colonies on coffee farms.

The response by leaf-cutting ant densities to diversification in coffee systems may be explained by either the natural enemy or the resource concentration hypothesis. Natural enemies are relatively unimportant for leaf-cutting ants (Cherrett, 1986). In the case of the resource concentration hypothesis, if important for explaining the outcome of *A. cephalotes* densities, the mechanisms would be less straightforward for this polyphagous insect than for monophagous insects (Andow, 1991).

In addition to these often-proposed mechanisms, other factors associated with agroecosystem diversification can influence insect herbivore density. In the present case in coffee, the percentage of shade was inversely related to densities of *A. cephalotes* colonies. This could indicate that microclimatic factors associated with shade influence the ants. In natural settings these ants appear to favor forest gaps (Jaffé & Vilela 1989) so complex shade in the system could mimic contiguous forest canopy and reduce colonization by the ants. The percentage of shade, however, is roughly correlated with vegetational diversity, so the vegetational and microclimatic effects cannot be separated.

Density of *A. cephalotes* colonies could also be influenced by landscape patterns outside the farm and ranging up to kilometers of distance (Cherrett, 1969). In the present study, forest adjacent to farms (proximity) and the amount of forest and fallow areas were related to *A. cephalotes* colonies on Turrialba coffee farms (within 500 and 2,000 m, respectively).

Although the mechanisms are not clear, it appears that increasing the percentage of shade in Turrialba coffee farms during the nuptial flight (beginning of rainy season) could decrease the number of new colonies and hence decrease the pest pressure by *A. cephalotes* on the farm. At the landscape level, area-wide management of colonies within riparian forests and fallow areas could provide an additional means for decreasing *A. cephalotes* colonies on farms.

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Table 3.1 Characteristics of coffee management types compared for density of *A. cephalotes* colonies, Turrialba region, Costa Rica, 2005.

Management type*	Main tree species present**	Other important species present***	% shade ± SE	Inputs
Monoculture Conventional†	None		18.28 ± 6.10	G, O N-P-K
Diversified Organic	Ep	None	63.66 ± 5.01	OM
Diversified Conventional	Ep	None	25.50 ± 7.94	G,O N-P-K
Highly Diversified Organic	Ep Ca	Ma	71.35 ± 5.81	OM
Highly Diversified Conventional	Ep Ca	Ma	59.86 ± 6.31	G, O N-P-K

*Five farms were sampled in each management type. ** Predominant shade tree species, Ep: *Erythrina poeppigiana*, Ca: *Cordia alliodora*. ***Non-shade tree species present that were abundant, Ma: *Manihot sculenta*. † Only four farms sampled. G: Glyphosate; O: Oxyfluorfen; N-P-K (18-5-15): Major elements; OM: Organic Manure.

Table 3.2 Partial ANOVA results for densities of *A. cephalotes* total colonies, new colonies and old colonies in different coffee management types of Turrialba region, Costa Rica, 2005.

Comparison	Total colonies density			New colonies density			Old colonies density		
	Model			Model			Model		
	df	*F*	Pr > F	df	*F*	Pr > *F*	df	*F*	Pr > F
	4,19	3.81	0.0193	4,19		0.0501	4, 19	0.72	0.5911
Monoculture vs. All others (Diversified and Highly diversified systems)	1,19	7.44	0. 0133	1,19	6.19	0.0223	1,19	14.4	0.2006
Diversified systems vs. Highly diversified systems (organic and conventional)	1,19	0.89	0.3571	1,19	0.47	0.5012	1,19	0.01	0.9683
Organic vs. Conventional (Diversified systems)	1,19	6.85	0.0169	1,19	3.90	0.0629	1,19	5.48	0.4239
Organic vs. Conventional (Highly diversified systems)	1,19	0.07	0.7969	1,19	1.01	0.3271	1, 19	3.59	0.5160

Table 3.3 Partial ANOVA results for *A. cephalotes* total colony surface areas (m^2) in different coffee management types of Turrialba region, Costa Rica, 2005.

Comparison*	d.f.	*F*	Pr > *F*
Model	4,19	0.55	0.6995
Monoculture vs. All others (Diversified and Highly diversified systems)	1,19	0.59	0. 4528
Diversified systems vs. Highly diversified systems (organic and conventional)	1,19	0.00	0.9770
Organic vs. Conventional (Diversified systems only)	1,19	1.52	0.2329
Organic vs. Conventional (Highly diversified systems only)	1,19	0.10	0.7509

Table 3.4 Multiple and simple regressions of densities of *A. cephalotes* colonies and new colonies vs. the most relevant landscape and local variables found in Turrialba region, Costa Rica, 2005.

		Pr>F value			
	R^2	Model	% Shade	(% Shade)^2	Forest proximity
Total colonies/ha	0.40	0.0009*	0.0009	.	.
	0.60	0.0003**	0.0024	0.0204	0.0180
New colonies/ha	0.29	0.0058***	0.0058	.	.
	0.69	<0.0001†	< 0.0001	< 0.0001	-

* *Total colonies/ha= 108.716 –1.19(%shade).* Simple regression.

** *Total colonies/ha= 176.64 – 4.11(%shade)+0.03(%shade*^{2}*) - 0.14(forest proximity).* Multiple regression.

*** *New colonies/ha= 54.59 – 0.774(%shade).* Simple regression.

† *New colonies/ha= 101.42 – 3.66(%shade) + 0.03(%shade*^{2}*)* Multiple regression.

Table 3.5 ANCOVA results for the land use coverage relationship with the variable densities of *A. cephalotes* total colonies in coffee management types of Turrialba region, Costa Rica, 2005.

Land use coverage	Buffer radius											
	100 m				500 m				2000 m			
	Model Pr>F	R^2	Pr >F Type III SS		Model	R^2	Pr >F Type III SS		Model	R^2	Pr >F Type III SS	
			Treatment*	Covariate			Treatment	Covariate			Treatment	Covariate
Forest	0.0648	0.41	0.0449	0.9851	0.0526	0.43	0.0485	0.4819	0.0643	0.41	0.0390	0.8871
Pasture	0.0496	0.43	0.0736	0.4256	0.0455	0.44	0.0794	0.3611	0.0586	0.42	0.0350	0.6250
Coffee	0.0492	0.43	0.0302	0.4190	0.0642	0.41	0.0381	0.8749	0.0647	0.41	0.0502	0.9528
Sugarcane	0.0647	0.41	0.0582	0.9408	0.0619	0.41	0.0436	0.7391	0.0636	0.41	0.0407	0.8313
Fallow	0.0606	0.42	0.0360	0.6890	0.0538	0.42	0.1062	0.5066	0.0575	0.42	0.0388	0.5930
Pasture with trees	-	-	-	-	-	-	-	-	0.0269	0.48	0.0172	0.1527
Agroforestry Systems	-	-	-	-	-	-	-	-	0.0546	0.42	0.0327	0.5226

*Management types: MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified Conventional; HDO: Highly Diversified Organic.

Table 3.6 ANCOVA results for the land use coverage relationship with the variable density of *A. cephalotes* new colonies in coffee management types of Turrialba region, Costa Rica, 2005.

Land use coverage	Buffer radius											
	100 m				500 m				2000 m			
	Model	R^2	Pr >F Type III SS		Model	R^2	Pr >F Type III SS		Model	R^2	Pr >F Type III SS	
			Treatment*	Covariate			Treatment	Covariate			Treatment	Covariate
Forest	0.1463	0.34	0.0940	0.5849	0.0292	0.49	0.1342	0.0292	0.0955	0.38	0.1291	0.2432
Pasture	0.1636	0.33	0.1536	0.9134	0.0761	0.40	0.2343	0.1666	0.1139	0.36	0.1601	0.3353
Coffee	0.0952	0.38	0.0777	0.2417	0.1613	0.33	0.1030	0.8252	0.1249	0.36	0.0778	0.4039
Sugarcane	0.0959	0.38	0.0581	0.2448	0.1414	0.34	0.0889	0.5356	0.1614	0.33	0.1032	0.8290
Fallow	0.1286	0.35	0.0861	0.4293	0.1605	0.33	0.1863	0.8051	0.0061	0.57	0.0272	0.0057
Pasture with trees	-	-	-	-	-	-	-	-	0.1557	0.34	0.1282	0.7088
Agroforestry Systems	-	-	-	-	-	-	-	-	0.1342	0.35	0.0851	0.4721

*Management types: MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified Conventional; HDO: Highly Diversified Organic.

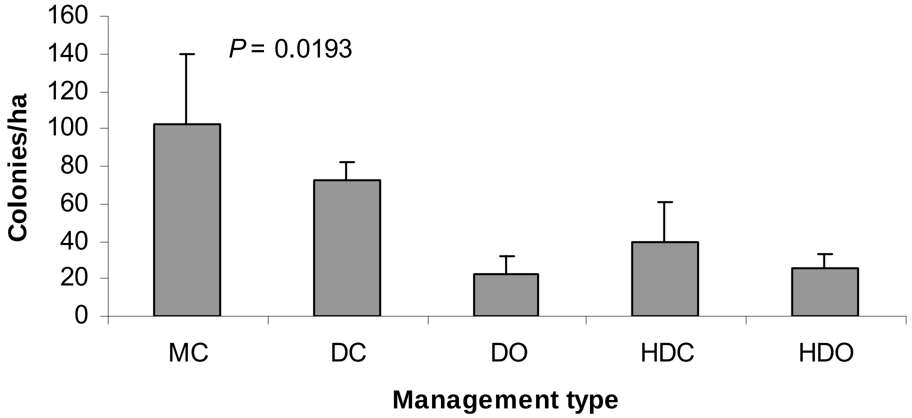

Figure 3.1 *A. cephalotes* total colony density in different coffee management types in the Turrialba region, Costa Rica, 2005. MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified Conventional; HDO: Highly Diversified Organic.

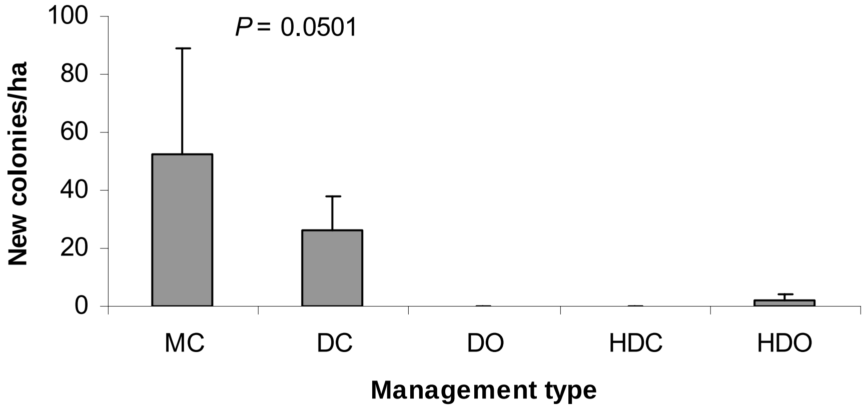

Figure 3.2 Density of *A. cephalotes* new colonies in different coffee management types in the Turrialba region, Costa Rica, 2005. MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified Conventional; HDO: Highly Diversified Organic.

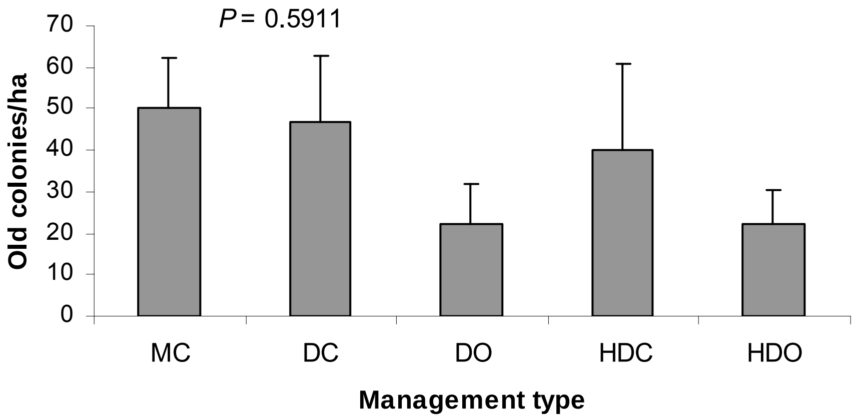

Figure 3.3 Density of *A. cephalotes* old colonies in different coffee management types in the Turrialba region, Costa Rica, 2005. MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified Conventional; HDO: Highly Diversified Organic.

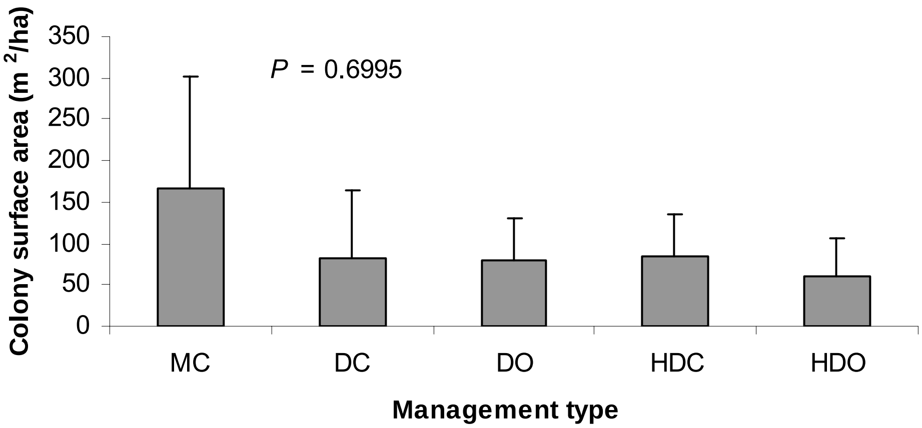

Figure 3.4 *A. cephalotes* colonies surface area (m^2/ha) in different coffee management types in the Turrialba region, Costa Rica, 2005. MC: Monoculture Conventional; DC: Diversified Conventional; DO: Diversified Organic; HDC: Highly Diversified Conventional; HDO: Highly Diversified Organic.

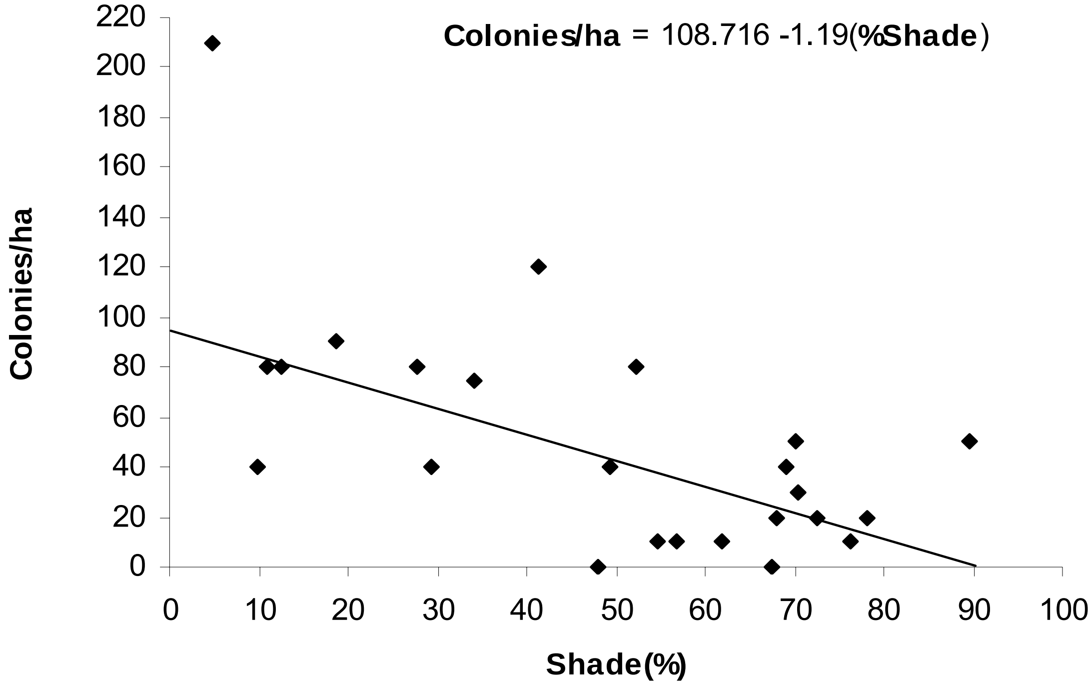

Figure 3.5. Relationship between total colony density and percentage of shade in different coffee management types in the Turrialba region, Costa Rica, 2005.

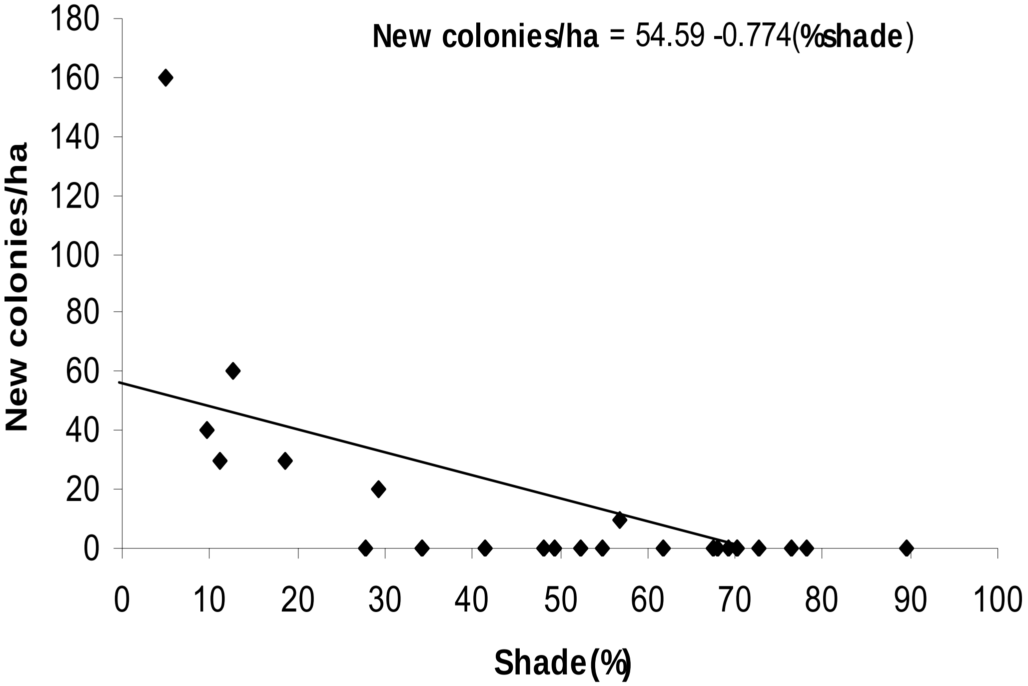

Figure 3.6. Relationship between density of new colonies and percentage of shade in different coffee management types in the Turrialba region, Costa Rica, 2005.

CHAPTER 4

Effect of riparian forest edges on the distribution, abundance and survival of *Atta cephalotes* colonies in coffee farms in the Turrialba region, Costa Rica

Running Head: Riparian forest edge and Atta cephalotes *density*

Abstract. 1 Riparian forests are commonly preserved in agricultural landscapes either due to legal protection or unsuitability for cultivation. They may affect the dynamics of insects present near cultivated areas.

2 In order to examine the effect of the presence of riparian forests near coffee farms on the distribution, abundance and survival of colonies of leaf-cutting ants, *Atta cephalotes*, three coffee farms partly surrounded by riparian forests were selected. Within each farm, three sets of three 50 m x 30 m plots were established adjacent to the riparian forest edge and extending towards the farm's interior. Three similar sets of plots were established near a non-forest edge in two of the farms. All *A. cephalotes* colonies inside the plots were counted, located using GPS technology two months after the 2004 nuptial flight for all the farms and two months before the 2005 nuptial flight for two of the farms, their mound surface areas were measured and the mound surface area growth was calculated.

3 Regardless of edge type, plots closest to either edge had significantly higher densities of colonies (no. colonies/ha) than plots further from the edge. Plots near the riparian forest edge had significantly higher densities of colonies than those at the non-forest edge. There was no interaction between edge type and distance from the edge.

4 Densities of new colonies (identified based on mound surface area and morphology in 2004) were not significantly higher at riparian forest edges than on non-riparian edges. Nest mortality between the sample dates did not differ between the riparian forest edge and the non-forest edge. Colonies in plots near the riparian forest edge had significantly greater mound surface areas than those near the non-forest edge. Colonies in plots adjacent to edges had greater mound surface areas than colonies in plots further from edges, regardless of edge type. Mound surface area growth was higher at riparian edges for small colonies (< 0.5 m^2) than for larger colonies.

5 The cause for the higher densities of colonies near riparian edges, was not determined, but the data suggest that a higher rate of establishment rather than a lower rate of attrition near forest edges is responsible for the pattern.

Key words. *Coffea arabica*, leaf-cutting ants, riparian forest, distance effect.

Introduction

Leaf-cutting ants (Formicidae: Myrmicini: Attini) are an important component in neotropical natural ecosystems, acting as agents for nutrient cycling and plant community structure in tropical moist forests (Haines, 1975). In areas where agricultural systems and natural preserved areas coexist, however, leaf-cutting ants can become agricultural pests. With the aid of their symbiotic fungi the ants are effectively polyphagous (Wirth *et al*., 2003), and are capable of causing serious injury to several valuable crop species.

In some regions of South America, leaf-cutting ants are pests of coffee (Barreto *et al*., 1998). In the Turrialba region, *Atta cephalotes* L. is the predominant leaf-cutting ant species and its colonies are commonly encountered within coffee plantations, on other agricultural land, and within riparian forests of the region.

Riparian forests are sometimes preserved in agricultural landscapes either due to legal protection or the unsuitability of riparian zones for cultivation (steepness, flooding, etc.) (Lima & Gascon, 1999). They serve important ecological functions, including dispersal corridors for forest-inhabiting animals and plants (Forman & Godron, 1986; Malanson, 1993). Riparian strips may also serve as permanent habitats for animals (Spence, 1979) and consequently as a source for individuals to disperse and colonize surrounding habitats (Malanson, 1993).

Riparian forests also can affect the abundance and diversity of insects in adjacent cultivated areas. They are known to increase species richness and abundance of beneficial insects such as ground beetles near wheat fields (French & Elliot, 1999) and pollinators near coffee fields (Ricketts, 2004), but they could also influence insect pest populations (Foley *et al*., 2002). The effect of riparian forests on coffee pests, including leaf-cutting ants, is unknown, but could be important where these forests are common near coffee farms. This occurs in the Turrialba region where preservation of these forests is part of the conservation effort of the proposed Turrialba-Jiménez Biological Corridor.

As forest gap specialists (Jaffé & Vilela, 1989), *A. cephalotes* might thrive in areas with low shade but adjacent to the forest and the resources available there. A preliminary survey revealed higher *A. cephalotes* colony densities near forest edges in coffee farms in Turrialba (Appendix 3). The present study was designed to verify and quantify this pattern, and the spatial scale over which it occurs.

Riparian forest edges could also affect the temporal dynamics of *A. cephalotes* colonies, leading to differences in net colonies densities. Colony turnover rates could differ with location in the production landscape. In the case of *A. cephalotes,* colony turnover has only been documented in secondary lowland forest (Perfecto & Vandermeer, 1993) and the effect of the forest edge was not determined in that study. In the present study, we estimated colonization and attrition of *A. cephalotes* colonies in coffee plantations at different distances from riparian forest and non-forest edges. We hypothesized that densities of leaf-cutting ant colonies are higher close to riparian forest strips than other edge types on coffee farms and that this pattern will be explicable in terms of observed colony turnover rates.

Materials and methods

Study area

The study was carried out in Santa Teresita, Turrialba, in the Caribbean watershed of Costa Rica, with annual average values of rainfall, temperature and relative humidity of 2,479 mm, 21.7ºC and 87%, respectively. The study area is located within the tropical humid forest life zone (Tosi, 1989).

Farm and riparian forest characteristics

Three large coffee farms with riparian forests at their edges were selected. Farms 1 and 3 were edged by 400-500 m-wide riparian forests, while farm 2 was edged by a 300 m-wide riparian forest. Farms 2 and 3 had non forest edges located >500m away from the riparian forest edge, while farm 1 had no non-forest edge (Table 4.1).

Sampling method

Three sets of three 50 m wide x 30 m deep plots were arranged from the riparian forest edge into the center of each farm. In each set, the first plot was located with its longest dimension immediately adjacent and parallel to the edge. The second plot extended from 40-70 m from the edge and the third extended from 80-110 m from the edge (Appendix 4). In farms 2 and 3 there were three similar sets of plots arranged near the non-forest edges on those farms. Thus, there were a total of 18 plots in farms 2 and 3 and 9 plots in farm 1.

All *A. cephalotes* colonies inside the plots were located using GPS and aerial photography in August of 2004, two months after the 2004 nuptial flight, and counted. The surface area of each colony mound was determined based on its length (l) and width (w), in which l is the greatest dimension along the mound and w is the greatest dimension across the mound and perpendicular to l. Mound surface area was calculated as ($\pi(l/2)(w/2)$) (Hernández *et al*. 1999). Colonies with a single tower-like monticule less than 0.2 m^2 and a small entrance hole were considered new colonies and all others were considered older colonies. A second sampling following the same procedures occurred in April-May, 2005, just before the nuptial flight, in order to detect changes in the distribution, surface area and survival of the colonies inside the plots. This second sampling was only possible on farms 2 and 3, because farm 1 was converted to pasture in the intervening period. Growth was calculated by the difference in percentage in surface area between the two years.

Statistical analysis

An analysis of variance (ANOVA) with a factorial design was performed to assess the effect of distance, edge type (riparian vs. non-riparian) and edge x distance on total colony density, new colonies, and mound surface area (SAS, 2001). Although there were three sets of plots from each edge in the farms, the data from each distance within farm were pooled to avoid pseudo replication. For total colony density and mound surface area, samples for the two sampling periods (2004-2005) were then combined to provide 27 observations that could be subjected to analysis, since there was not interaction between treatment and year for total colony density ($F_{1,17}$ =.1.79, P = 0.1980) nor for mound surface area ($F_{1,17}$ = 0.05, P = 0.8202). Thus, yearly results are not shown. The data for total colony density, mound surface area and new colony density were normalized by transforming them to the square root of (x+0.5). Data for mound surface area growth were normalized by using log (x).

Results

Total colony density

Using the pooled data from 2004 and 2005, there was a significant effect of edge type (forest and non-forest) ($F_{1,18}$ = 7.26, P = 0.0148) and distance from edge ($F_{2,18}$ = 5.60, P = 0.0128) on *A. cephalotes* total colony density, with the plots near riparian forest edge having higher densities than those near non-forest edge, and the plots closest to edge (0-30 m) having greater colonies density than those furthest from the edge (80-110 m) (P = 0.0039) (Table 4.2, Fig. 4.1). There was no interaction between edge and distance from edge on density of colonies ($F_{2,18}$ = 1.96, P = 0.1699) (Table 4.2).

New colony density

There were no significant effects of edge type ($F_{1,7}$ = 3.66, P = 0.0972) or distance from edge ($F_{2,7}$ = 1.21, P = 0.3529) on the densities of new colonies. There was no interaction between edge and distance from edge on densities of new colonies ($F_{2,7}$ = 0.51, P = 0.6236) (Fig. 4.2 and Table 4.2).

Total colony surface area/plot

There were significant effects of edge ($F_{1,18}$ = 4.48, P = 0.0484) and distance from edge ($F_{2,18}$ = 6.61 P = 0.0070) on *A. cephalotes* mound surface areas, with the plots near riparian forest edge having greater mound surface areas than those near the non-forest edge and plots closer to the edge (0-30 m) having greater surface areas than those from plots 40-70m (P = 0.0101) and 80-110 m (P = 0.0034) from the edge (Table 4.2, Fig. 4.3).

Colony mortality

The percentage mortality between the first and second sampling averaged 69% for new colonies and 49% for older colonies, but these did not differ significantly (t_{50}= -1.64, P = 0.1081) (Fig. 4.4). mortality was 62% near riparian forest edges, whereas near non-forest edges mortality was 42%, but these did not differ significantly ($F_{1,5}$ = 1.05, P = 0.2606) (Fig. 4.5). There was not effect of distance from edge on mortality ($F_{2,5}$ = 0.28, P = 0.7690).

Mound surface area growth

Mound surface area growth during the intersample interval was 2,297% for small (0.1-0.5 m^2), 161% for medium (0.5-5 m^2) and 53% for large (5-150m^2) colonies. There was an interaction between edge and distance from the edge ($F_{2,14} = 8.35$, $P = 0.0041$), with the small colonies having greater growth in the plots adjacent (0-30 m) to the riparian forest edge than in plots further from the edge (Fig. 4.6).

Discussion

A. cephalotes is a forest gap specialist (Jaffé & Vilela, 1989), which may cause it to colonize forest edges preferentially in artificially fragmented agricultural landscapes. In preparation for this study, E.H. Varón mapped the locations of >100 *A. cephalotes* colonies on 13 coffee farms in Turrialba and found that colony abundances and surface areas appeared to be substantially higher near forest edges or riparian strips (Appendix 3). The present replicated study validated this observation, detecting significantly higher densities of colonies near the edges than in the interiors of coffee farms and higher densities near riparian forest edges than near non-forest edges.

Melkonian *et al.* (2003) found that *A. cephalotes* foraging activity was greater for colonies near forest edges than those in the interior of the forest. They attributed this to easier access to food sources and the decrease in natural enemies, especially phorid flies, at forest edges compared to the interior of the forest. *A. cephalotes* colonies near riparian edges of coffee plantations may also benefit from abundant resources within the nearby forest and from favorable microclimatic and biotic conditions near the edge. Contrary to this expectation, colonies near riparian edges appear to harvest mostly from the available material within the coffee farm rather than in the forest (E.H. Varón, unpublished data). In addition, densities of colonies are higher near non-forest edges than farm interiors, suggesting environmental factors are as important as available resources.

Colony mortality did not differ significantly among the treatments in our study, leaving uncertain the role of attrition in producing the observed differences in colony density. The trends in mortality were not consistent with such a mechanism. Rather, colony mortality tended to be higher near forest edges than near non-forest ones. This study measured only colony mortality during a single season, so the strength and direction of longer-term patterns remain to be determined. Although the colony mortality we observed was high (around

50%), this is consistent with previous reports of colony mortality rates of leaf-cutting ants (Autuori, 1941; Jacoby, 1944; Hernández *et al*., 1999). For example, Hernández *et al*., (1999) reported a 45% rate of mortality for *A. laevigata* from the ninth to the tenth month of colony establishment in pine tree plantations in Venezuela. Perfecto & Vandermeer (1993) found an annual colony turnover rate of 22%, lower than we observed but nonetheless substantial considering their study was conducted within the La Selva forest preserve near Sarapiquí, Costa Rica.

The rate of establishment of new *A. cephalotes* colonies also did not differ among the treatments, but the trends were consistent with observed densities (cf. Figs. 4.1 and 4.2). This suggests that greater rates of establishment of colonies explains greater densities of colonies near edges of coffee plantations, especially near riparian forest edges.

The greater mound surface area growth found for small colonies at riparian edges suggests that the surviving incipient colonies find better conditions at those edges, an effect possibly related to better access to more resources. The percentage of growth was higher for new colonies than for older colonies, although large colonies grew faster in terms of absolute mound surface area (0.23 m^2 for small colonies, 1.45 m^2 for medium colonies and 10.04 m^2 for large colonies in average). In addition, if larger colonies are increasing in subterranean volume in proportion to the observed increases in mound surface area, the growth of these colonies could be even greater.

Riparian strips could affect *A. cephalotes* colonies densities at larger spatial scales than we examined in this study. For example, farms or parcels with no adjacent forest remnants or farther from these remnants might support lower average colony densities than farms that contain or are immediately adjacent to remnants.

Conclusions

Riparian forest edges frequently occur in coffee farms throughout the Turrialba region. Densities of *A. cephalotes* colonies are higher near these edges, contributing to within-farm variation and possibly overall abundance of the ant colonies on the farms. Riparian strips therefore potentially negatively impact agriculture by exacerbating an insect pest problem (Foley, 2002). Considering the the established benefits of riparian strips for preserving water quality, providing habitat for wildlife, and controlling erosion (Forman & Godron, 1986; Malanson, 1993; Lima & Gascon, 1999), this negative effect of riparian strips may need to be addressed as part of sustainable landscape level management. Once the causes of *A.*

cephalotes responses to forest edges are better understood, it might become possible to develop cultural practices to reduce or harness the response of the ants to landscape level features in order to protect vulnerable crops. Buffers strips of alternative crops or pasture grasses are examples of strategies that might be effective.

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Table 4.1 Characteristics of the studied farms. Turrialba region, Costa Rica, 2004-2005.

Farm	Riparian forest width (m)	Area (ha)	Typology	Location (CRTM X-Y coordinates)*	Non forest edge characteristics
1	400-500	90	Coffee-Poró**	538941-1105304	_
2	300	123	Coffee	536957-1106411	Grassland and peach palm plantation***
3	400-500	190	Coffee-Poró	539406-1103617	Grassland and fallow

*** Costa Rica Traverse Mercator: Projection system used for the Turrialba region.**

****** *Coffea arabica-Erythrina poeppigiana*

*** Grassland and *Bactris gasipaes* plantation

Table 4.2 Summary ANOVA results for densities of total colonies, new colonies and mound surface areas for 2004-2005 pooled samplings. Turrialba region, Costa Rica, 2004-2005.

Variable	**Model**	**Edge**	**Distance**	**Edge x Distance**	**P values for LS means comparisons**		
					0-30 vs 40-70m	**0-30 vs 80-110m**	**40-70 vs. 80-110 m**
Total colony density	0.0006	0.0148	0.0128	0.1699	0.0508	0.0034	0.2396
New colony density	0.3260	0.0972	0.3529	0.6236	0.2829	0.1827	0.7613
Colony surface area	0.0400	0.0484	0.0070	0.4605	0.0101	0.0034	0.6301

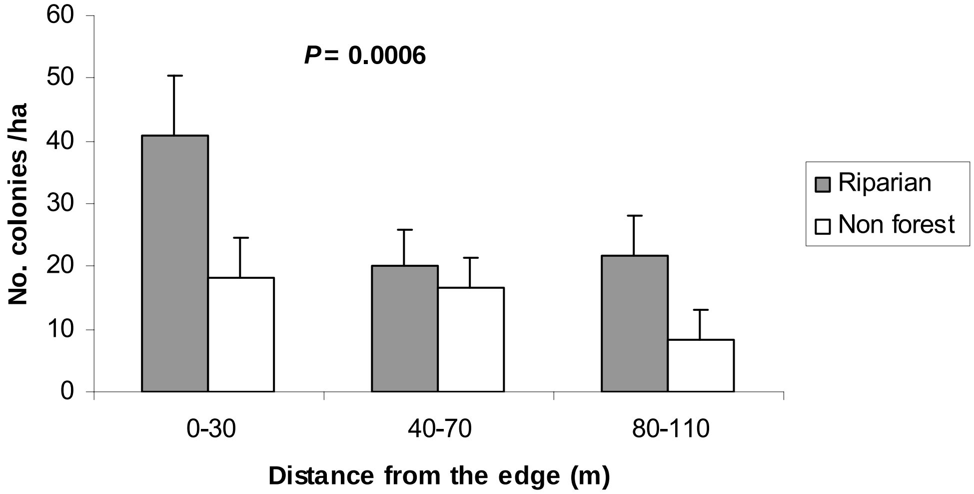

Figure 4.1 Total colony density of *A. cephalotes* at two types of edges and three distances from the edge**,** in three coffee farms in the Turrialba region, Costa Rica, 2004-2005.

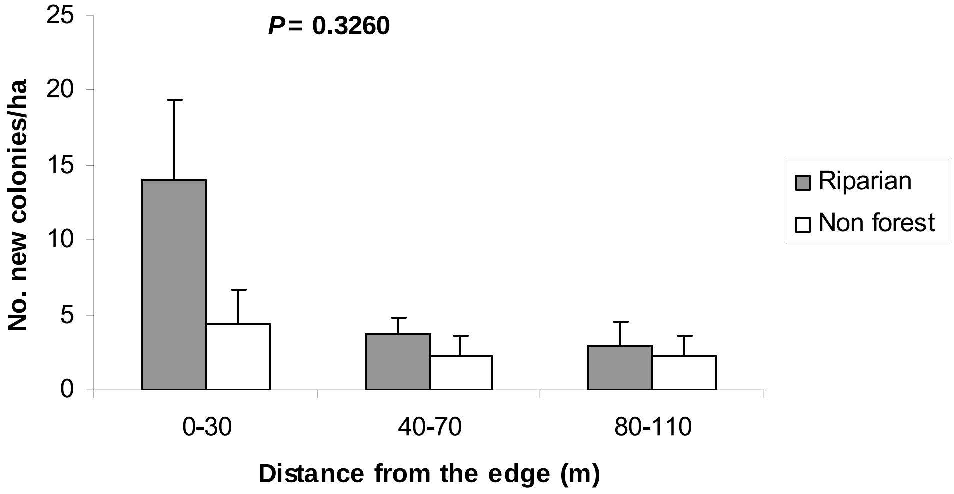

Figure 4.2 Density of new colonies of *A. cephalotes* at two types of edges and three distances from the edge**,** in three coffee farms in the Turrialba region, Costa Rica, 2004.

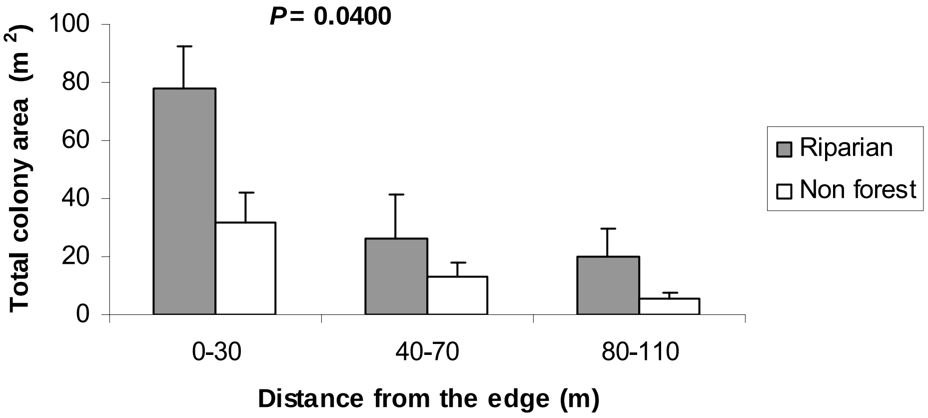

Figure 4.3 Total mound surface area of *A. cephalotes* colonies at two types of edges and three distances from the edge, in three coffee farms in the Turrialba region, Costa Rica, 2004-2005.

Figure 4.4 Mortality rates (%) in the sampling interval for new colonies (established after 2004 nuptial flight) and older colonies in two coffee farms in the Turrialba region, Costa Rica, 2005.

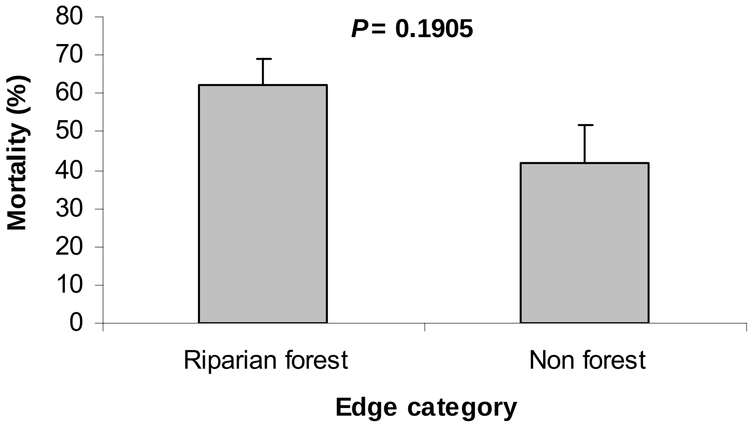

Figure 4.5 Mortality rates (%) in the sampling interval for all colonies, at two types of edges, in two coffee farms in the Turrialba region, Costa Rica, 2005.

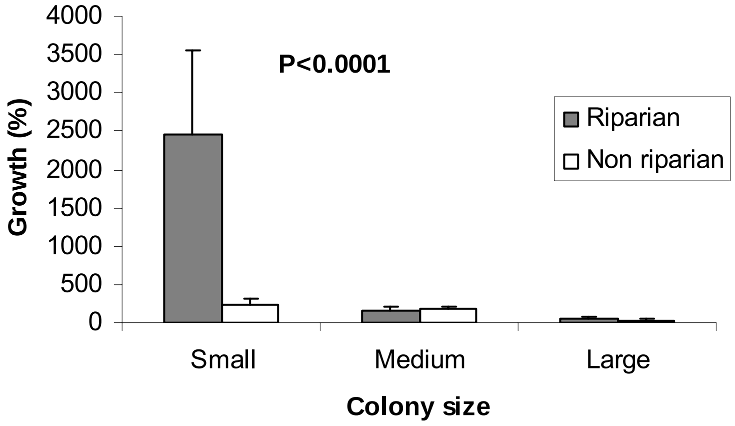

Figure 4.6 Surface area growth rate (% in sampling interval) for colonies classified at different mound surface areas: small (<0.5m^2), medium (0.5-5m^2) and large (>5 m^2), at two types of edges in two coffee farms in the Turrialba region, Costa Rica, 2005.

CHAPTER 5

Effect of baits based on fungal strains or botanical extracts on *Atta cephalotes* colonies

Running Head: Alternative baits and Atta cephalotes *control*

Keywords Leaf-cutting ants, botanical extracts, fungal control, entomopathogens, antagonists.

Abstract. *Atta cephalotes* is the predominant leaf-cutting ant and a pest of coffee in the Turrialba region, Costa Rica. Strains of the entomopathogenic fungi, *Paecilomyces* sp. and *Metarhizium anisopliae*, the antagonistic fungus (*Thrichoderma hammatum*) that affects the fungus cultured by the ants, and botanical extracts (*Hura crepitans* and *Canavalia ensiformis*) formulated as baits, were compared with two commercial insecticides (sulfluramid and sodium octoborate) for their effects on *A. cephalotes* colonies in a field experiment. Prior to the field experiment, a laboratory trial was conducted to confirm efficacy of candidate entomopathogenic fungi against *A. cephalotes*. Colonies were grouped in three surface-area categories: small (<1 m^2), medium (1.1-30 m^2) and large (>30 m^2). *A. cephalotes* colony activity (worker movements near the colony entrance in a 1 min observation) significantly decreased after treatment with both commercial insecticides. The treatment *Paecilomyces* sp. *(*isolate 0484) significantly reduced ant activity than the control, but it was less effective than the commercial treatments. All of the other fungal and botanical treatments were ineffective at reducing ant activity. Across treatments, reduction of ant activity was stronger for large colonies than for small and medium-sized ones. There was no interaction between treatment and size in the effect on colony activity. None of the alternative treatments explored was as effective as commercial insecticides for controlling the ants. Sodium octaborate caused the highest colony mortality (four consecutive weeks with no activity) (100%), followed by sulfluramid (80%), while the other treatments caused mortality rates of less than 30%.

Introduction

True leaf-cutting ants (*Atta* spp. and *Acromyrmex* spp.) (Hymenoptera: Formicidae) are important pests throughout the Neotropics (Hebling et al. 2000). A widely distributed group, *Atta* spp. ranges from southern United States to northern Argentina (López and Orduz 2003). In Costa Rica, this genus is represented by *A. cephalotes* and *A. colombica* (Longino

2005), of which the former predominates in the Turrialba region (E.H. Varón, unpublished data), an important coffee-growing region.

Historically, leaf-cutting ants were successfully controlled by means of baits containing dodecachlor (Echols 1965; Lofgren et al. 1967), but in recent years it was shown that this compound can seriously pollute the environment (Hebling et al. 2000) and cause cancer in mice (ATSDR 2005), so it has been banned in many countries (Antunes et. al. 2000). An alternative to dodecachlor, sulfluramid, has been shown to be successful in forest plantations (Zanuncio et al. 1996; 2000) and has generally replaced dodecachlor where that material has been banned.

Currently, in Costa Rican coffee plantations, including those in the Turrialba region, farmers are using sufluramid and the inorganic compound sodium octaborate for ant control. Unfortunately sulfluramid and sodium octaborate both are rather expensive for resource-poor farmers, with a typical price of US $7/K. Among potentially cost-effective and environmental friendly potential alternatives to these materials are strains of entomopathogenic or antagonistic fungi (López et al. 1999; Ortiz and Orduz 2000; López and Orduz 2003), or botanical extracts (Mullenax 1979; Chen et al. 1983; Hebling et al. 1985, 2000; Bueno et al. 1995). For instance, López and Orduz (2003) found that under field conditions, baits containing a combination of the entomopathogenic fungus *Metarhizium anisopliae* to control the ants and the antagonistic fungus *Trichoderma viride,* applied to supress *Attamyces* spp. (*A. cephalotes* symbiotic fungus) caused 100% colony mortality. Colony mortality of 80% was achieved with *T. viride*. The potential of botanical extracts for leaf-cutting ant control has been explored by Hebling et al. (2000).

In order to search for potential biorational materials and products for leaf-cutting ant control, several entomopathogenic fungi, a fungal antagonist and two botanical extracts were identified and tested against *A. cephalotes* colonies in the field. Nine entomopathogenic fungi were pre-tested for activity against *A. cephalotes* in the laboratory and a subset was then selected for the field test. The fungal antagonist and botanical extracts were selected based on prior evidence of potential efficacy. The materials were applied to *A. cephalotes* colonies in the field in baits and then activity and mortality of the colonies was monitored after treatment.

Materials and Methods

Nine strains of entomopathogenic fungi available at the Microbial Control Unit at the Tropical Agricultural Research and Higher Education Center (CATIE) were chosen for testing. The strains were selected based on their reported pathogenicity to some other insects: whiteflies (*Bemisia tabaci*), the cotton bollweevil (*Anthonomus grandis*) and the coffee berry borer (*Hypothenemus hampei*) (Herrera et al. 1999). These were compared for efficacy against the ants in the laboratory and a subset was selected for testing in the field. In addition, *Trichoderma hammatum* strain 0585 was selected for field testing because it was isolated by one of us (E.H. Varón) from within *A. cephalotes* colonies at CATIE, and other *T. hammatum* isolates have been reported antagonistic to *Attamyces* in *in vitro* evaluations in Colombia (Ortiz and Orduz 2000). Botanical extracts for field testing were selected based on prior reports of efficacy and accessibility of the plants for making extracts on farm. Farmers in Central America often mention that jack bean (*Canavalia ensiformis*) foliage has insecticidal properties on leaf-cutting ants (Silagyi 2002) and there are also formal reports of such activity (Mullenax 1979; Hebling et al. 2000). There is anecdotal evidence suggesting that “jabillo” (*Hura crepitans*) was used by Costa Rica aborigines to control leaf-cutting ants in order to protect young trees (Hilje et al. 1989) and extracts of this plant have known insecticidal activity against other insects (Adedire and Ajayi 2003).

Study area

Laboratory bioassays were carried out at the Plant Protection laboratories at CATIE, in Turrialba. Field experiments were conducted at CATIE`s field station (La Montaña) located at 650 m, in the Caribbean watershed of Costa Rica, where annual average values of rainfall, temperature and relative humidity are 2479 mm, 21.7ºC and 87%, respectively. CATIE´s facilities are located within the tropical humid forest life zone (Tosi 1989) between 9° 55’ 21’’ N and 83°39’ 40’’ O.

Laboratory assays for efficacy of entomopathogenic fungi

The nine strains of fungi were selected and compared for their pathogenicity to *A. cephalotes* workers in a laboratory bioassay (Table 1).

Individual 20-ml solutions of each strain were prepared, each consisting of distilled sterilized water (DSW), the fungal strain at a standard concentration ($5X10^7$ conidia/g), and one drop/vial of the spreader polyoxyethylensorbitan mono-oleate (Tween 80, J.T. Baker, Phillipsberg, NJ). For each treatment, four groups of five worker ants were immersed in each solution for 5 sec and each individual was then isolated in a 28.62 cm^3 cylindrical vial with a perforated cap, wetted filter paper and a leaf disk of "almendro" (*Terminalia cattapa*), a preferred food source. Leaf disks were replaced daily. The control treatment was 20 ml of DSW and one drop/vial of the spreader. Both ant mortality and the appearance of fungal mycelia and fruiting bodies on the body of the cadaver (sporulation) were monitored daily for 11 days. After 11 days, mortality had reached 100% in all treatments with fungi (Figure 5.1) and sporulation had reached 100% in one of the treatments with fungi and the others had reached a rather stable state (Figure 5.2).

Statistical analysis. An analysis of variance (ANOVA) was carried out to test for differences in worker ant mortality and percentage of individuals sporulated. Means were compared using a Duncan's multiple range test (SAS 2001). Mortality was compared on the third day, since some of the strains achieved 100% mortality by that time. The percentage of individuals that had sporulated on the fifth day was compared. This was the first day on which sporulation was detected and before it became uniformly high among all the treatments. Data were transformed using the square root (% + 0.5) method in order to comply with assumptions of normality.

Field assay

Botanical extract preparation. Plant material from *Canavalia ensiformis* was collected once from a patch on the grounds at CATIE. *Hura crepitans* plant material was collected once from CATIE`s La Lola Experiment Station in Limón, Costa Rica. Hydroalcoholic extracts (70:30) of both plants were then concentrated by means of rotary evaporation (Arciniegas 2002). The final percentage of total solids for the extracts were 42.3% for *C. ensiformis* and 33.7% for *H. crepitans.*

Bait preparation. Baits were prepared by mixing each active ingredient (either fungus or extract) in proportion to its weight as follows: active ingredient (1%), powdered orange skin (80%), wheat germ (10%), clay (5%), glycerin (1.6%), cellulose (1.5%), and starch (0.9%). The mixture was placed into a modified mill to obtain pellets. Each material was put on disinfected plastic trays and then into a drier for 3-4 days, depending on the active ingredient, to remove moisture. Once the pellet reached 10%, baits were packed into plastic bags (Ziploc) and refrigerated following the advice of E. Hidalgo (pers. comm.).

For fungal treatments, in order to determine the pelleted bait concentration, five petri dishes with soil were filled with 10 pellets containing either one of the selected entomopathogen or the fungal antagonist *T. hammatum* at increasing tenfold concentrations ($1X10^2$ conidia/g through $1X10^9$ conidia/g). They were monitored for two days, at four-hour intervals. The lowest fungal concentration causing a 100% bait sporulation was $1X10^7$ conidia/g, and that concentration was used for the field tests (E. Hidalgo, pers. comm.). Thus, the 1% of active ingredient (treatment) to be added to the bait mixture was calculated based on this concentration for fungal strains. For the blend *Paecilomyces* sp. and *T. hammatum*, half of the selected concentration was used for each fungal strain.

Additionally, pellets with either entomopathogens or antagonists were put into individual laboratory colonies of *A. cephalotes* established in acrylic boxes (20 X 20 X 20 cm), in order to determine their capacity to infect workers and the queen. These laboratory *A. cephalotes* colonies were established from field-collected queens and workers, supplied with both soil and their symbiotic fungus and maintained on ‘almendro’ (*Terminalia cattapa*) and ‘lemon’ (*Citrus limon*) leaves previously immersed in distilled water. Ant infection was only detected for baits containing *Paecilomyces* sp. 0484 and the mutualistic fungus contamination by baits containing *T. hammatum* strain 0585 was also detected.

Experimental methods. A factorial design was used, with three mound sizes and nine treatments as factors. The three size classes were based on colony surface area: small (0.03-1 m^2), medium (1.1-30 m^2) and large (30.1-1000 m^2). Colony surface area was estimated as ($\pi\ (l/2)(w/2)$), in which l and w are the greatest dimensions along and across the mound, respectively (Hernández *et al*. 1999).

The nine treatments were *Paecilomyces* sp. 0484, *Metarhizium* strain RCP-2 , *T. hammatum* 0585, a blend of the *T. hammatum* strain 0585 + *Paecilomyces* sp. RCP-2, *C. ensiformis,* and *H. crepitans*, and three controls: sodium octaborate (Omitox 3 GB, Indagro S.A., 3% a.i.), sulfluramid (Mirex-S 0.3 GB, Romagro S.A, 0.3% a.i.), and an untreated bait as an absolute control.

Bait quantity was equivalent for all treatments (6 g/m^2 = 0.06g ai/m^2), except for sodium octaborate, for which the bait was applied at a higher rate (20 g/m^2) in order to follow the recommendation of the manufacturer. For sulfluramid, the manufacturer recommendation is 6 g/m^2. The blend of the *Paecilomyces* sp. 0484 + *T. hammatum* 0585 strains was tested because in other studies blends of antagonist and entomopathogenic fungal strains gave good results controlling *A. cephalotes* colonies (López and Orduz 2003). The blend *M.*

anisopliae RCP-2 + *T. hammatum* 0585 was not used because the *Metarhizium* strain RCP-2 was not considered aggressive enough to withstand the mixture with the antagonist *Trichoderma* (E. Hidalgo, pers. comm.).

Only two fungal strains were used in field trials due to budgetary and logistical constraints. They were selected based on performance in the laboratory assay, information from the literature and other considerations presented in the results section of this paper.

Baits containing each test material were deployed on ant foraging trails 15 cm away from colony entrances showing ant foraging activity. The baits were applied between 8:00-16:00 h. A previous trial, with two colonies, showed that *A. cephalotes* workers readily accepted the used bait formulation.

There were three size classes x nine treatments x three replications for a total of 81 colonies used in this study. The colonies were selected from naturally occurring ones in the La Montaña station at CATIE property in Turrialba, Costa Rica. Colonies were first assigned to size classes and then the treatments were allocated randomly within size class. The entire array of colonies occurred within a 10-ha section of the farm.

Bait effectiveness was appraised by counting the number of ants entering and leaving each treated colony during one minute. Observations were carried out between 8:00-16:00 h. Colony activity was monitored in each colony weekly for three weeks before being treated, and for eight weeks after being treated. Assessing the activity of all 81 colonies in the study required seven hours and necessitated walking between the colonies on a prescribed route. Each week, the direction along the route and hence the order in which colonies were evaluated was reversed, to prevent any time-of-day variation in ant activity from biasing the results. Randomization each week was not feasible because the travel time between the nests assessed randomly would have been prohibitively long.

A colony was considered dead when no activity was detected over four consecutive weeks (López and Orduz 2003), after which the small surface area colonies were excavated and samples (symbiotic fungus, dead workers and queen, if possible) were taken to verify if mortality was due to the specific treatment applied on it. Excavating the medium and large colonies was impractical because of the difficulty in locating queens and fungal gardens in the tremendous volume of these colonies.

Statistical analysis. Field data were analyzed with a two-way ANOVA with a factorial design (SAS 2001). Response variables were the proportional change in mean activity after application (ants/colony/min) (obtained by dividing the mean activity for the eight weeks prior to application by the mean activity for the three weeks prior to application). Since there was no interaction between treatment and colony surface area, means for each factor were separated using Duncan's test. Colony mortality after treatment was also assessed but no statistical analysis was possible for this response because there was no replication for this variable.

Results

Laboratory assays

Ant mortality rates induced by the fungal strains tested in this study were similar during the experiment (Fig. 5.1). At the third day after treatment, mortality was already high (60-100%) and similar among all the fungal strains tested, all of which differed from untreated control (F = 4.72, d.f. = (9,30) P = 0.0006) (Table 5.2). The sporulation rates were also similar among fungal strains (Fig. 5.2). On the fifth day, when sporulation was first observed, however, its frequency ranged from 5-65% of the individual ants in each treatment and differed significantly among the fungal strains tested (F = 3.18, d.f. = 8,27, P = 0.0113) (Table 5.3).

Based on these results the *Paecilomyces* strain 0484 was selected for further study in the field assay because of its relatively high sporulation and because the mortality associated with it was similar to other strains (unlike strain 0485). *M. anisopliae* strain RCP-2 was selected for testing in the field because the fungal species has been found naturally infecting *A. cephalotes* queens (López et al. 1999) and its performance in our assay was as good as the other fungi, except the *Paecilomyce*s strains. Even though *Beauveria* strains performed well in this laboratory bioassay, they were not included in the field trial because of budgetary and logistical constraints.

Field assay

Bait effectiveness. The commercial treatments sulfluramid and sodium octaborate caused the greatest differences in proportion of ant activity before and after treatment application, and the change in ant activity was statistically different from the untreated control. The only alternative treatment that differed significantly from the control was *Paecilomyces* sp. 0484, and the effect of this treatment did not differ significantly from the commercial treatments. The remaining treatments did not differ from the absolute control (Table 5.4). The treatments in general had a stronger effect on large colonies than on small and medium colonies (Table 5.5), but there was no interaction between colony surface area and treatment (Table 5.4).

Colony mortality (defined as four consecutive weeks with no acitivity) was greatest for sodium octaborate (100%), followed by sulfluramid (80%). Some mortalitiy above controls was evident for some of the alternative treatments (Fig. 5.3). Excavation of the small colonies that were considered dead at the end of the experiment detected *Paecilomyces* 0484 on the dead queen and workers in only one of these colonies. The other excavated small colonies were found to be empty.

Discussion

None of the non-conventional materials performed as well as sulfuramid and sodium octaborate, the two commercial products currently available in Costa Rica for reducing the activity or exterminating *A. cephalotes* colonies. The alternatives to the commercial products we tested are evidently not good candidates for controlling *A. cephalotes* colonies under field conditions. The lack of effectiveness of these materials in this experiment could be specific to the formulations we used and some of the limitations of our methods.

First, the rustic mixing procedure we used (in the interest of mimicking what would be practical on-farm) did not achieve perfect homogeneity in the mixture. Second, the stability of these materials under ambient light, temperature, humidity and moisture is not known. The relatively high temperatures, light levels and precipitation near Turrialba could have degraded them. Annual rainfall averages 2480 mm in Turrialba and the monthly rainfall averages during this study were 263 mm for September, 269 mm for October and 364 mm for November. Third, the rates we used were chosen based on prior information from our laboratory bioassays, but may have been too low for field conditions in our region. In the field, only one application of each material was made, at 6 g/m^2 and a low conidia concentration (1X10^7 conidia/g). Although López and Orduz (2003) were able to obtain higher mortality rates by applying three times and using a higher fungal concentration (1X10^9 conidia/g) and a bait dose of 20 g/m^2, commercial baits available in Costa Rica, particularly sulfluramid, are typically applied at much lower rates and our goal was to assess efficacy at these typical rates.

The bait containing *Paecilomyces* sp. 0484 was the only alternative treatment that was significantly different from the control, regarding its suppressive effect on ant activity. The potential of this fungal strain for ant control in our region merits further study.

Regarding botanical extracts, neither *C. ensiformis* nor *H. crepitans* extracts proved effective for reducing *A. cephalotes* colony activity or causing increased colony mortality. The lack of effect of *C. ensiformis* extract is contrary to a prior report that leaves of this plant caused mortality of *Atta* spp. colonies under field conditions (Mullenax 1979). The difference could be due to the application method and to the high amount of leaves that were used in that experiment (5-15 kg/colony). Hebling et al. (2000) also obtained good results controlling *A. sexdens* colonies using *C. ensiformis* leaves. The leaves, however, were applied to rather old colonies (21-year-old colonies) and the experiment was carried out under laboratory conditions, which often do not fully represent results of field conditions.

Although leaf-cutting ant colonies should be ideal environments for entomopathogenic and mycopathogenic fungi (Currie 2001), our work and others (Kermarrec et al. 1986) indicates that effectiveness of these fungi in laboratory tests does not necessarily indicate they can cause colony mortality under field conditions. Because they are social insects adapted to survive best in the colony, *in vitro* conditions in the laboratory with single workers might introduce additional stress that causes artificially high mortality in laboratory bioassays (e.g. Diehl-Fleig 1988; López et al. 1999). Also, the mutual cleaning process that the ants carry out within the colony is prevented in laboratory bioassays. Bioassay methods that better mimic conditions within the colony could improve the accuracy of tests of fungi.

Although it is laborious to establish replicate laboratory colonies, that effort could be justified as a basis for rapid and effective screening of fungi and other agents for control of leaf-cutting ants in the field.

Sulfuramid and sodium octaborate are effective and easy to use by producers for control of leaf-cutting ants, as this study corroborates, at least for the time span of this study. Their drawbacks are relatively high cost and potential environmental impacts. It therefore remains important to continue seeking for alternatives to these materials. Although previous reports indicate that combinations of antagonistic and entomopathogenic fungi can be effective for controlling the ants (López and Orduz 2003), our result suggests that effectiveness will be variable. Further work is needed to discover how to obtain reliable results with such materials.

Regardless of the material applied, our results show that effectiveness is greater for larger colonies (surface area >30 m^2). We are not aware of previous work that demonstrates this. We standardized our bait application rates on the basis of colony area, but it may be necessary to adjust application rates further based on some other function such as the estimated volume of colonies, which is likely proportional to the number of insects and size of fungal gardens in the colonies. The potential advantage of the fungal agents is that they are self-propagating so that larger colonies should not necessarily require greater concentrations of inoculum. Our result indicates, however, that colony surface area will need to be considered in developing methods for controlling ants with fungal agents.

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Table 5.1 Strains of entomopathogenic fungi tested under laboratory conditions.

Fungus species	**Strain**	**Source insect**	**Place collected**	**Date collected**[a]
B. bassiana	0084	*Phyllophaga obsoleta*	Tierra Blanca, Cartago	-[b]
B. bassiana	9205	*Diatraea saccharalis*	Unknown	-
B. bassiana	447	*Ecdytolopha torticornis*	Unknown	-
M. anisopliae	RCP-2	*Phyllophaga menetriesi*	Rio Cañas, Guanacaste	23-VII-94
M. anisopliae	ARE-2	*Galleria melonella*	Arenal, Alajuela	-
M. anisopliae	5/89	Unknown	Nicaragua	17-III-95
M. anisopliae	340	*Phyllophaga vicina*	Puntarenas	28-III-95
Paecilomyces sp.	0484	*Bemisia tabaci*	Liberia, Guanacaste	22-VIII-03
Paecilomyces sp.	0485	*Bemisia tabaci*	Turrialba, Cartago	14-X-03

[a] **Source of collection data:** Herrera *et a*l., (1999)

[b] - = No data available

Table 5.2 Percentage mortality of five individual *A. cephalotes* workers three days after treatment with entomopathogenic fungal strains in a laboratory bioassay.

Fungus spp. or strain	**Strain**	**Mean**[a]
Paecilomyces sp.	0484	100 a
B. bassiana	0084	100 a
M. anisopliae	RCP-2	100 a
B. bassiana	9205	100 a
M. anisopliae	340	100 a
B. bassiana	447	95 a
M. anisopliae	5/89	94 a
Paecilomyces sp.	0485	94 a
M. anisopliae	ARE-2	90 a
Control	-	60 b

[a] Means with the same letter are not significantly different based on a Duncan's multiple range test ($P<0.05$). Means were back-transformed to percentages from sqrt (x+0.5) after analysis.

Table 5.3 Percentage of five individual *A. cephalotes* workers showing signs of sporulation five days after being treated with entomopathogenic fungus strains in a laboratory bioassay.

Fungus spp.	**Strain**	**Mean**[a]
Paecilomyces sp.	0485	65 a
Paecilomyces sp.	0484	53 a
B. bassiana	447	30 ab
B. bassiana	0084	30 ab
M. anisopliae	RCP-2	15 b
B. bassiana	9205	10 b
M. anisopliae	5/89	10 b
M. anisopliae	340	5 b
M. anisopliae	ARE-2	5 b

[a] Means with the same letter are not significantly different based on Duncan's multiple range test ($P<0.05$); means were back-transformed to percentages from sqrt (x + 0.5) after analysis.

Table 5.4 ANOVA and means comparisons for the bait treatments using the difference in proportion between the mean activity before and after treatment application as the response variable. Turrialba, Costa Rica. 2004.

Treatment[a]	Activity before treatment[b]	Activity after treatment[b]	Proportion change[c,d]
Sulfluramid	11.79	0.30	0.9745 a
Sodium octaborate	7.97	0.30	0.9618 a
Paecilomyces sp. 0484	9.56	4.07	0.5742 b
Paecilomyces sp. 0484+ *T. hammatum* 0585	10.37	5.70	0.4499 bc
M. anisopliae RCP-2	7.60	4.51	0.4064 bc
T. hammatum 0585	10.32	6.39	0.3809 bc
H. crepitans	8.56	6.10	0.2879 bc
C. ensiformis	7.94	5.74	0.2771 c
Control	9.51	7.38	0.2254 c
F statistics			9.44
df			8,54
P			<0.0001

[a] See text for rates and application methods.

[b] Activity = number of workers entering or leaving the colony in a 1-min observation. This was measured for 3 weeks prior to treatment and 8 weeks after treatment.

[c] Means with the same letter within a column are not significantly different based on Duncan's multiple range test (P<0.05).

[d] Activity change as a proportion from the activity before the treatment.

Model F = 3.71, d.f. = (26,54), P < 0.001; Treatment x Size F= 0.79, d.f. = (16,54), P= 0.6949.

Table 5.5 ANOVA and means comparisons for colony surface area using the difference in proportion between the mean activity before and after treatment application as the response variable. Turrialba, Costa Rica. 2004.

Colony suface area[a]	Activity before Treatment[b]	Activity after treatment[b]	Proportion change[c,d]
Small	4.99	2.88	0.42 b
Medium	9.22	4.96	0.46 b
Large	13.67	5.07	0.62 a
F statistics			4.27
df			2,54
P			0.0190

[a] Size categories: Small = (0.03-1 m^2), Medium = (1.1-30 m^2), Large = (30.1-1000 m^2).
[b] Activity = number of workers entering or leaving the colony in a 1-min observation. This was measured for 3 weeks prior to treatment and 8 weeks after treatment.
[c] Means with the same letter within a column are not significantly different based on Duncan's multiple range test (P<0.05).
[d] Activity change as a proportion from the activity before the treatment.
Model F = 3.71, d.f. = (26,54), P < 0.001; Treatment x Size F= 0.79, d.f. = (16,54), P= 0.6949.

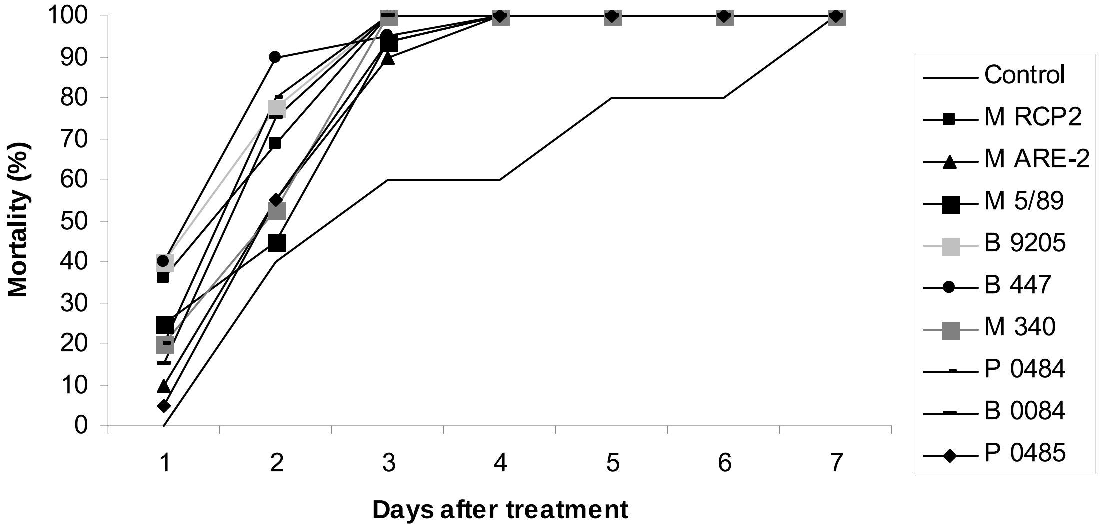

Figure 5.1 Percentage mortality of *A. cephalotes* workers during 7 days after treatment application with entomopathogenic fungus strains in laboratory assays. P 0485: *Paecilomyces* sp. strain 0485; P 0484: *Paecilomyces* sp. strain 0485; B 447: *Beauveria bassiana* strain 447; B 0084: *B. bassiana* strain 0084; B 9205: *B. bassiana* strain 9205; M RCP-2 *Metarhizum anisopliae* strain RCP-2; M 340: *M. anisopliae* strain 340; M 5/89: *M. anisopliae* strain 5/89; M ARE-2: *M. anisopliae* strain ARE-2. CATIE's Microbial Control Unit.Turrialba, Costa Rica, 2004.

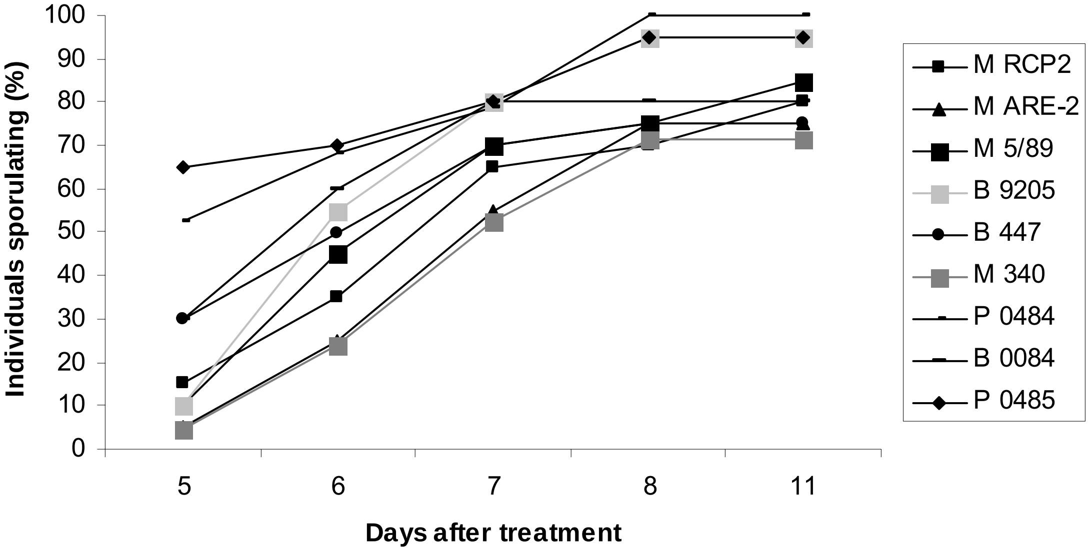

Figure 5.2 Percentage of *A. cephalotes* workers sporulating during 11 observation days after treatment application with entomopathogenic fungal strains. P 0485: *Paecilomyces* sp. strain 0485; P 0484: *Paecilomyces* sp. strain 0485; B 447: *Beauveria bassiana* strain 447; B 0084: *B. bassiana* strain 0084; B 9205: *B. bassiana* strain 9205; M RCP-2 *Metarhizum anisopliae* strain RCP-2; M 340: *M. anisopliae* strain 340; M 5/89: *M. anisopliae* strain 5/89; M ARE-2: *M. anisopliae* strain ARE-2. CATIE’s Microbial Control Unit.Turrialba, Costa Rica, 2004.CATIE’s Microbial Control Unit.Turrialba, Costa Rica, 2004.

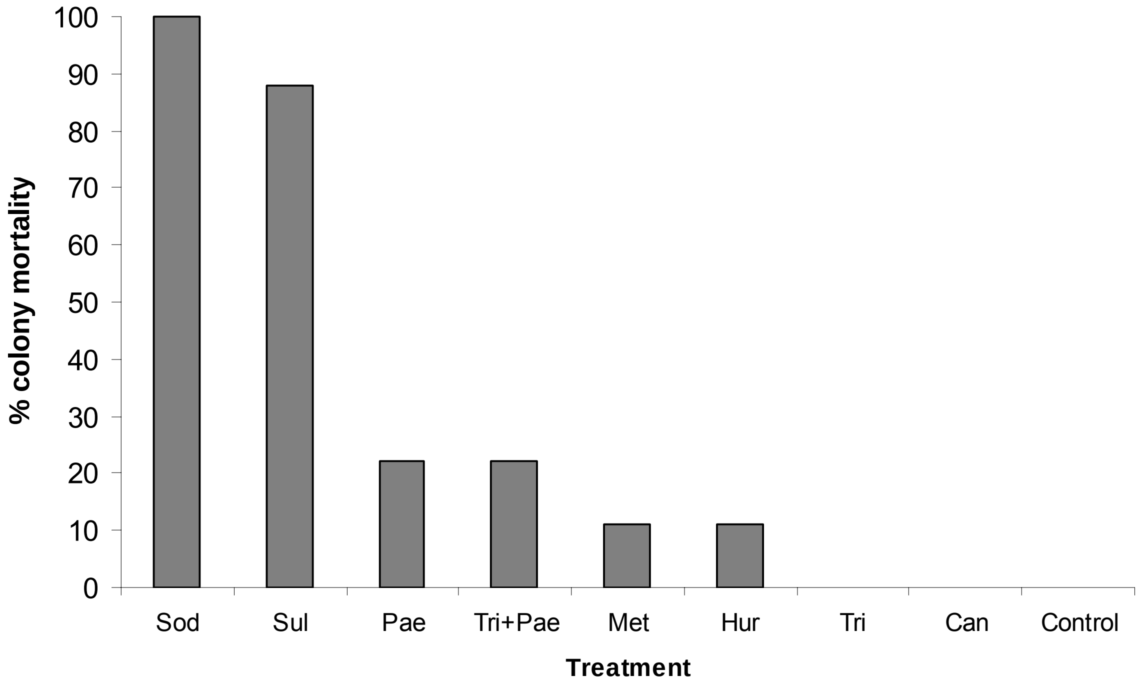

Figure 5.3 Mortality of *A. cephalotes* colonies after being subject to a single treatment application of different fungal and botanical baits during the field bioassay. Pae= *Paecilomyces* sp. 0484; Tri= *Trichoderma hammatum* 0585; Tri+Pae = *Trichoderma hammatum* 0585 + *Paecilomyces* sp. 0484; Met= *Metarhizium anisopliae* RCP-2; Hur= *Hura crepitans*; Can= *Canavalia ensiformis*; Sod= Sodium octaborate; Sul = Sulfluramid; Con=Control. Turrialba, Costa Rica, 2005. Means with the same letter are not significantly different based on Duncan's multiple range test (P<0.05).

CHAPTER 6

CONCLUDING CHAPTER

Introduction

Two *Atta* species*, A. cephalotes* and *A. colombica,* occur in Costa Rica (Longino 2005)*.* In preparation for my dissertation research*,* I conducted a survey and found that *A. cephalotes* was by far the predominant leaf-cutting ant species found in coffee farms in the Turrialba region, although some colonies of *A. colombica* and *Acromyrmex* spp. were also found (E.H. Varón, unpublished data). I also conducted informal surveys that indicated 77% of the coffee farmers of the region apply insecticides to control leaf-cutting ants (Appendix 5). Many of these farmers complained about the low efficiency and high cost of insecticides currently available for ant control, as compared with dodechachlor, the former product of choice now banned in Costa Rica and other countries.

There are several types of coffee systems in the Turrialba region, and leaf-cutting ants are problematic in most of them. In monocultures *A. cephalotes* attacks to coffee plants can be severe. In more diversified systems, the polyphagous *A. cephalotes* attack other plant species as well as coffee. Thus, for one or another reason, coffee farmers in the region try to control *A. cephalotes* colonies.

Given the importance of *A. cephalotes* as a pest of coffee in the Turrialba region, this dissertation research was designed to examine some biological and ecological factors related to improving its management. My aim was to provide information useful in practical terms, and consistent with the economic constraints and environmental and human health concerns of the region. The approach I took was two-fold: 1) to study effects of within-farm and landscape-level factors on colonies density and *A. cephalotes* foraging, as a basis for assessing risk of attack and developing cultural methods to reduce it, and 2) to explore alternatives to current insecticides and improved ways of applying these materials, to help producers with the immediate problems of intervention to control the ants.

The research on these two approaches has been presented in the preceding chapters of this dissertation. In this final chapter, I will summarize the specific objectives of the work and the principal findings. I will then provide a synthesis of these results and summarize the implications for farmers seeking to manage *A. cephalotes.* Finally, I will highlight the new information and questions raised during my research pertaining to the management and ecology of *A. cephalotes* in studied landscapes.

Objectives and Principal Findings

General Objective 1. To determine what local and landscape factors are influencing the spatial and temporal distribution, and harvesting by *A. cephalotes* in Turrialba region coffee agroecosystems.

Specific Objectives

1a. To determine the relationship between local and landscape-level variables and leaf-cutting ant foraging

Foraging by *A. cephalotes* in coffee agroecosystems was influenced by vegetational diversity within coffee farms (Chapter 2). In monocultures, *A. cephalotes* colony foraging was restricted to a few plant species, principally coffee and broadleaf weeds. In more diversified systems with shade trees, the ants foraged primarily from the trees, and coffee and broadleaf weeds became minor components of their diet.

Reduced attack by the ants on coffee plants in more diversified production systems appears to be due to an attractant-decoy mechanism. Although coffee comprises approximately 40% of the vegetation in more diversified systems, the ants in these systems virtually ignore coffee, which comprises <1% of their diet, foraging instead from trees and other plants on these farms. A laboratory bioassay showed that leaves from the predominant shade tree, poró (*Erythrina poeppigiana*), was preferred over coffee, providing support for an attractant-decoy mechanism based on differences in plant characteristics.

From an applied perspective, these results indicate that coffee on farms with low vegetational diversity is at greater risk of attack by *A. cephalotes* than is coffee on more diversified farms. Farmers should therefore obtain some protection from the effects of the ants by planting shade species palatable to *A. cephalotes*, and either tolerant to ant injury or relatively expendable for the farmer. For example, poró (*E. poeppigiana)* is acceptable to *A. cephalotes* and provides effective shade but is not a traded commodity.

Other examples are three native timber species that occur in coffee: laurel (*Cordia alliodora*), mahogany (*Swietenia macrophylla*), and Spanish cedar (*Cedrela odorata*). Each is readily attacked by the ants (Appendix 1), but could withstand periodic *A. cephalotes* attacks once the trees have past the critical establishment period (Cherrett 1986; Vilela 1986). In contrast to these timber species, fruit trees, especially citrus trees, are not suitable as ant decoys because these trees are highly susceptible to injury from defoliation by the ants, even at adult stages (Chapter 2; Cherrett and Sims 1968; Cherrett and Jutsum 1983).

Another source of food for *A. cephalotes*, mainly in non-shade coffee plantations, was broadleaf weeds, especially *Spermacoce latifolia,* which represented approximately an 8% of the total biomass consumption. These plants also could serve to reduce attack on coffee monocultures and appear to do so, based on my results that show a significant harvest on this plant category in monocultures (Chapter 2). Nonetheless, relative preference for these species as compared with coffee by *A. cephalotes* workers was not assessed, nor was their effect on coffee foraging by the ants rigorously tested in this thesis.

1b. To determine the relationship between local and landscape-level variables and *A. cephalotes* colony densities.

Effects of within-farm vegetational diversity on colony density

To address this, I examined *A. cephalotes* colonies density (colonies/ha) in farms with different levels of vegetational diversity. Colonies density was higher in monocultures than in four more-diversified systems (Chapter 3). Higher colonies densities in monocultures could be due to the lower shade levels in these systems. Based on regression analysis, the level of shade in coffee systems was negatively related to both total colonies density and new colonies density. Lower new colonies density in systems with more shade could occur if shade trees interfere with the behavior of new queens seeking colony sites after the nuptial flight. Other factors associated with shade that could potentially reduce the establishment and survival of leaf-cutting ant colonies could be a decreasing activity due to lower temperatures that could put such colonies at disadvantage. Also, higher shade levels could increase the capability of entomopathogens and antagonists to attack colonies, due to the potential higher humidity levels at those systems.

There was also a significantly lower colonies density in the organic diversified systems than in the conventional diversified systems (Chapter 3). It is not possible to determine if differences in inputs influenced this pattern. Organic farms also differed from conventional ones in vegetational diversity and shade levels. It could, however, also have been influenced by the potential lower temperatures and high humidity levels mentioned before, causing a lower colony activity and making the colonies more prone to diseases.

Together, the results of Chapters 2 and 3 show that both *A. cephalotes* colonies density and propensity of the colonies to attack coffee are reduced in diversified coffee systems. These two benefits of vegetational diversity for protection from the ants are complementary and synergistic.

Of course farmers' decisions on how much shade to incorporate and how to manage the shade trees will be influenced by considerations other than leaf-cutting ants. The factors differ depending on whether the farm is managed for organic or conventional production and what incentives there are for reducing inputs, controlling weeds, obtaining income from shade species, and personal preferences. Nonetheless the potentially higher costs of intervention for ant control in less diversified systems should be a consideration when making decisions about farm management.

Effects of riparian forest edges on colony density

Atta cephalotes colonies densities (colonies/ha) were higher near riparian forest edges on three farms studied, and the total surface area covered by the nests of these colonies was also higher at these ecotones than elsewhere on the farms (Chapter 4). The cause of this pattern is uncertain but could be related to colonization behavior by founding queens, early stage survival of colonies, or both. Open areas near contiguous forest may offer better access to resources and environmental conditions for colony growth and defense. If so, it would be adaptive for founding queens to colonize such areas preferentially.

This result could be important in the Turrialba region, because riparian forest strips occur throughout the landscape, including near many coffee farms. These strips are likely to be important components of the proposed Turrialba-Jiménez Biological Corridor. Management of the corridor for biodiversity conservation will likely include maintaining or increasing riparian forest area and increasing the number of coffee farms with edges adjoining this forest. If farms adjoining forest are at greater risk of *A. cephalotes* attack, as the work in this dissertation suggests, then this could represent a potential conflict between conservation and production objectives.

A possible approach to this conflict suggested by my results and the known biology of leaf-cutting ants would be establishment of buffers between forest and farm edges. These buffers could be planted to coffee with intermediate shade levels. They would serve to diminish the attractiveness to the ants of the coffee/forest boundary. The effectiveness and feasibility of such buffers would need to be examined experimentally.

Effects of larger landscape level patterns on colony density

I found only weak evidence for larger-spatial-scale land-use patterns on *A. cephalotes* colonies (Chapter 3). Forest coverage area within a 500-m radius and fallow coverage area within a 2000-m radius from the farm were directly related to the density of *A. cephalotes* new colonies in the coffee farms studied. Forest proximity (meters) was inversely related to total colony density in coffee farms, although there was an interaction with farm shade levels that suggests that its actual effect will depend on the shade levels present on the farm.

A. cephalotes are abundant in natural forests and fallow areas and colonies in these land use types could act as sources of ants for coffee farms. If so, the presence of forest or fallow lands could be important for determining *A. cephalotes* population dynamics on farms throughout the region. The relative importance of these longer-distance immigrants versus local colonies for establishment of new colonies of ants on farms is unknown. The behavior of nuptial queens can not be readily observed. Genetic techniques could in the future help to discover these regional patterns of ant dispersal and colonization. If long-distance immigration by the ants into coffee is important, it is possible that the control of *A. cephalotes* in some forested areas could be advisable. Long-distance movements of the ants from forests to coffee could also represent another potential conflict between coffee production and conservation in the landscape.

General Objective 2. To develop methods for *A. cephalotes* control based on natural products and biological agents.

2a. Testing of antagonist and entomopathogenic microorganisms as well as promising botanical extracts for *A. cephalotes* control.

This objective was addressed in Chapter 5. Although several alternatives to commercially available insecticides were tested, all performed poorly compared with the commercial products sodium octaborate and sulfuramid. My results confirmed that these two insecticides, deployed in baits, are effective at reducing ant activity and causing colony mortality at least for the time span studied here (8 weeks). Two botanical extracts and four fungal preparations were ineffective or only marginally effective.

Detected treatment effects were more pronounced for larger colonies (30.1-1000 m^2) than for small and medium-sized colonies (nest areas from 0.03-1 m^2, and 1.1-30 m^2 respectively), even though the applied materials were adjusted based on area of the colony (larger colonies received more active ingredient or treatment). For some reason, larger colonies are more susceptible so rates should not necessarily be determined on a linear basis with colony area. The necessary rate fucntion could be determined experimentally.

Among the alternatives to octaborate and sulfuramid only the treatment including the entomopathogenic fungus (*Paecilomyces* sp. 0484) was significantly different to the control in reducing *A. cephalotes* colony activity. The treatment did not, however, cause significant ant colony mortality. Due to logistical constraints only a few potential alternatives to commercial insecticides were tested. Other materials could be included in future studies.

The mortality of individual workers was high in the untreated controls in the laboratory bioassays designed to assess the effectiveness of fungal treatments (Chapter 5), as has been reported by others (Diehl-Fleig 1988; López *et al*. 1999). Future laboratory screening of materials for *A. cephalotes* control should use captive colonies instead of individual workers. Although costly, results would be more meaningful because they would avoid confusing mortality of individual workers due to other factors than treatments. Colonies can be maintained without mortality during a long time in the lab (Weber 1976; Hebling et al. 2000; E. H. Varón, pers. obs.) and testing whole colonies would target this important biological unit. There is a need to improve the bait production process to produce a more homogeneous final product. Finally, it would be important to test deployment alternatives to treated baits for applying alternative products, such as deploying them on either laden workers or trails, in order to make sure the compound used to control is in close contact with the workers.

2b. To develop an *A. cephalotes* injury risk model as a basis for recommendations for coffee farmers, regarding its management.

Only a few of the parameters and variables needed to construct a comprehensive and quantitative risk model for *A. cephalotes* colony density and herbivory are known after this study. My dissertation research provided estimates on the effects of shade levels on *A. cephalotes* colonies density and the propensity of the colonies to attack coffee are reduced in diversified coffee systems. Considering a multiplicative effect of these two factors, overall risk of attack by leaf-cutting ants on coffee plants could be approximately 25-fold lower in diversified systems as compared with monocultures in the Turrialba region. This number comes up after having found that total colonies densities was 2.5-fold higher in monocultures than in diversified systems and that the percentage of coffee in harvest was approximately 10-fold higher in monocultures than in diversified systems.

Nonetheless, a sound risk model would require the inclusion of a more representative number of coffee farms, overall conventional farms, which are much more widespread across the region. Additional estimates of differential risk of coffee varieties will be required, since only the Caturra variety was studied here. It is also important to understand better the actual role that protected or abandoned areas are playing on colony densities found at coffee farms. Additional research, especially in the field, is required to obtain these estimates.

Recommendations to farmers

Although a precise risk model could not be constructed, results of my research can establish the basis of a set of recommendations for *A. cephalotes* control in coffee farms of the Turrialba region:

- Include shade trees in the farm, in order to increase shade levels and therefore decrease colonization by *A. cephalotes*.

- Include shade trees that are palatable to *A. cephalotes*, but that should be either not commercially valuable or they should be capable of tolerating ant attack, in order to divert ants from harvesting coffee.

Indicated additional research to improve *A. cephalotes* management and understand its ecology in studied landscapes.

We do not know the best time within the year to control leaf-cutting ants. We already know that the nuptial flight is a crucial event within the *A. cephalotes* life cycle and according to surveys, farmers do not have a fixed period for controlling *A. cephalotes* colonies (Appendix 5). A fixed period for control should take into account the nuptial flight event.

Possible approaches that consider the nuptial cycle are 1) farmers employ some control action on mature colonies at some time before the nuptial flight, which could disrupt the production of reproductive individual ants and reduce the establishment of new colonies, 2) farmers focus the control two months after the nuptial flight and treat any new colonies observed then, because these colonies are more susceptible to treatments, 3) some combination of these two approaches. Controlled studies could be designed to examine the effectiveness of pretreatments to reduce nuptial flights and establishment of new colonies. Controlled studies could be designed to determine the long-term effectiveness of treating colonies at a prescribed time after the nuptial flight in order to focus on newly established colonies.

Improved methods for using ant control could reduce insecticide costs to farmers and increase effectiveness of alternatives to insecticides if any are discovered. Moreover, it will be worth determining if the decreasing activity efficiency on larger colonies of the current and alternative control treatments is improved by basing amount of insecticide or fungicide applied on an estimate of the colony volume, instead of the current product quantity estimation approach proposed by manufacturers based on surface colony area. By using this estimation, the proportion of insecticide or fungicide will be better adjusted to the real colony size. The challenge of such an estimation is that sometimes the costs of controlling large nests could be prohibitive for farmers.

Additional laboratory and field choice tests with shade trees other than poró and other broad-leaf species could help identify which are likely effective for reducing *A. cephalotes* foraging on coffee.

Although forest and fallow areas in the landscape may be related to colonies density in coffee agroecosystems, we do not know if the colonies from these areas are the sources of colonies at coffee systems. In order to pursue these questions it would be necessary to set up mark and recapture experiments or genetic studies.

We also have learned that *A. cephalotes* tend to prefer colonizing near riparian forest edges. Therefore, it would be useful to know about the effect of including buffer areas with intercropped coffee having medium shade levels at the riparian forest edge, as a way to decrease further colonization events.

Finally, we have learned that control treatments tended to have a higher effect on larger colonies and that the tested alternative treatments were not as efficient as the current available treatments. In order to determine if other compounds not tested here would be promising, it would be necessary to carry out appropriate laboratory and field tests, including the surface area as a factor.

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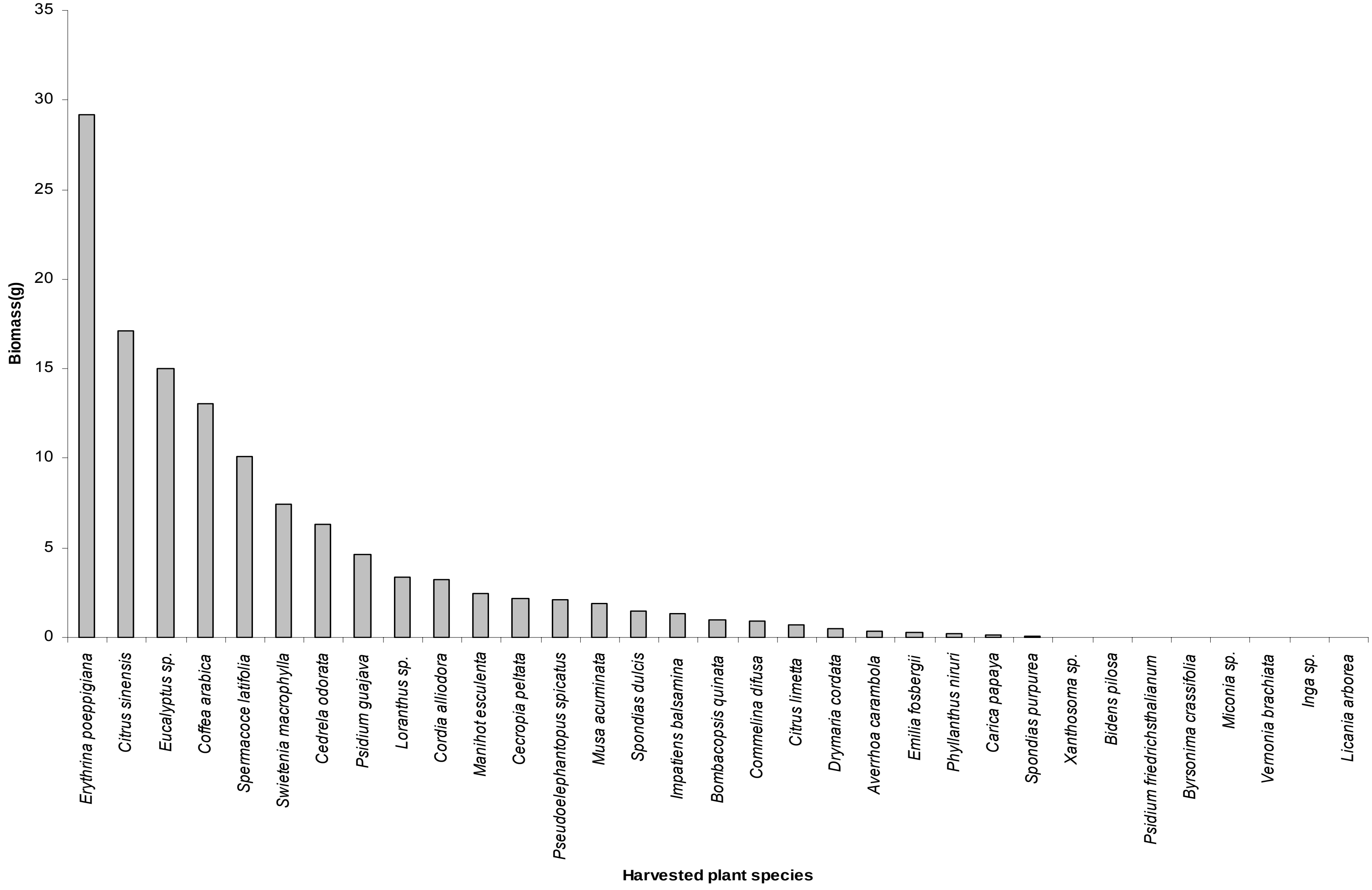
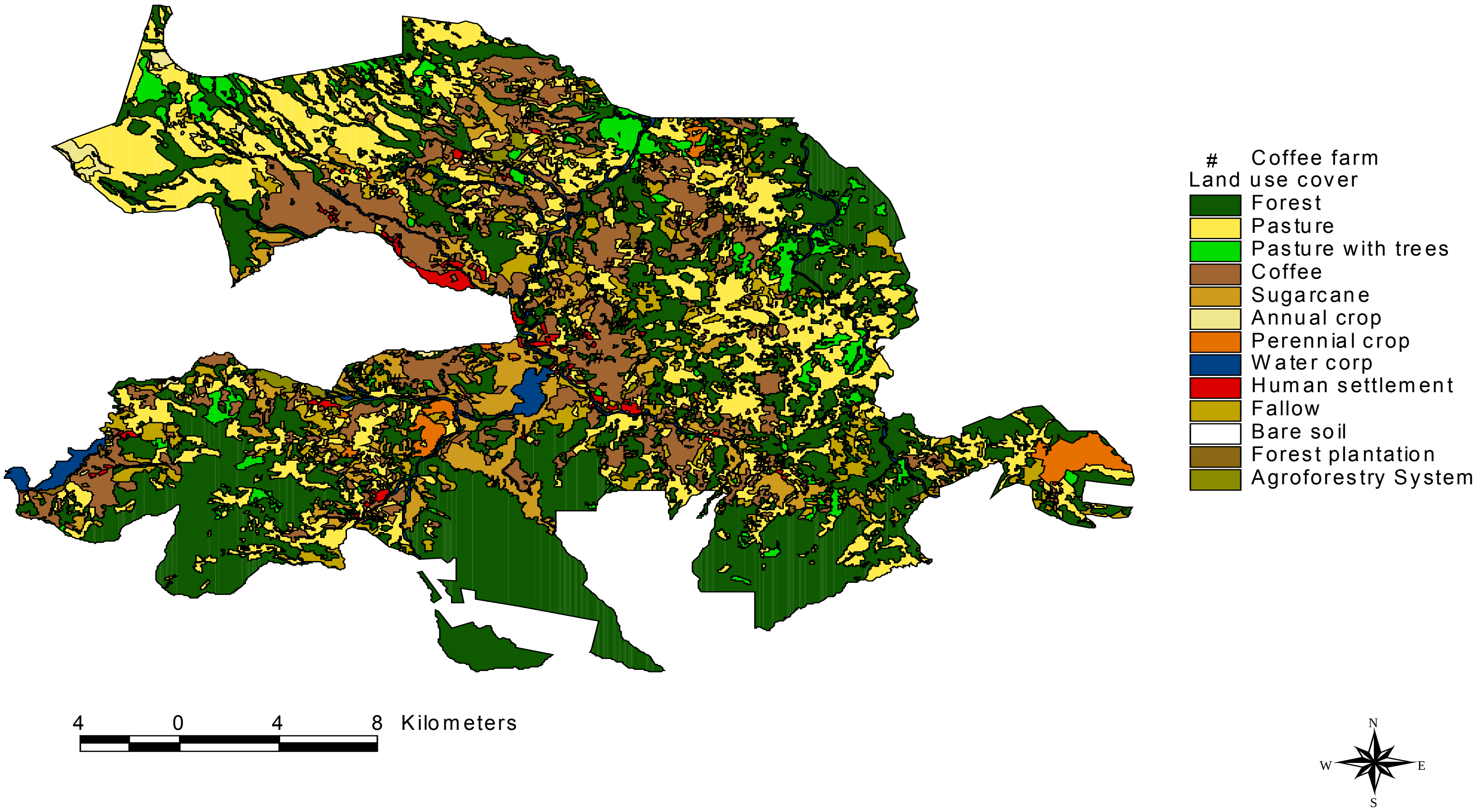
APPENDICES

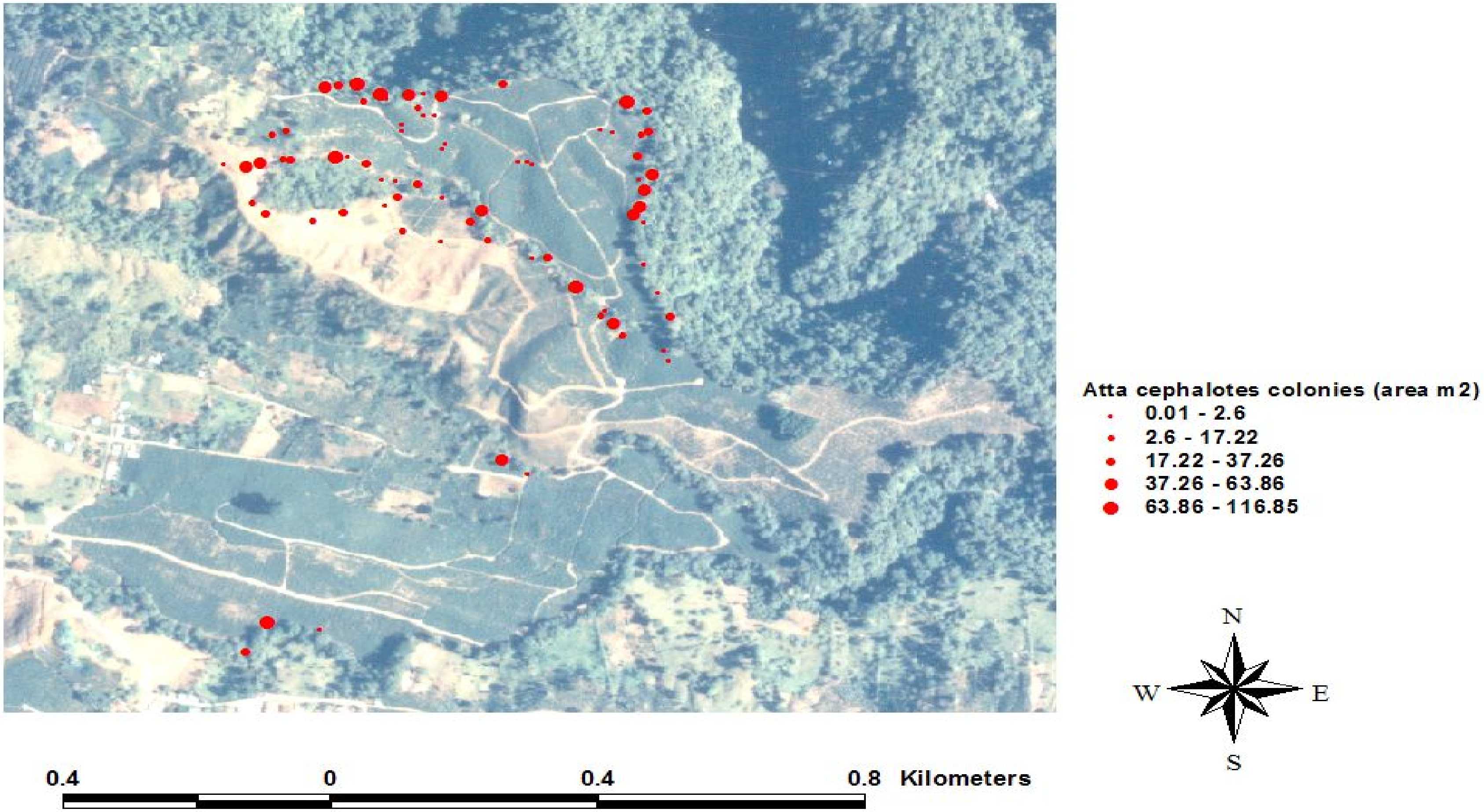
Appendix 1 Plant species harvested by *A. cephalotes* colonies in coffee farms in Turrialba region, Costa Rica. 2004.



Appendix 2 Distribution of research farms in the proposed Turriallba-Jiménez biological corridor.



Appendix 3 *A. cephalotes* colony distribution at El Sauce coffee farm. Turrialba region, Costa Rica. 2003.



Appendix 4 Plot arrangement at coffee farms with riparian and non-riparian forest edges.

Appendix 5 Main results of surveys of coffee farmers about *Atta cephalotes* control in the Turrialba region during different stages of the research. 2004-2005.

Farm	Control	Product*	Product quantity/year	Reason to control	Time to apply
1	Yes	SO	25 kg	Timber trees injury	.
2	No	None	None	None	.
3	Yes	SO, SU, FO	1.5 kg each	Undetermined injury	.
4	No	None	None	None	.
5	Yes	SU	1 kg	Fruit trees injury	.
6	Yes	SU	1 kg	Coffee and timber trees injury	.
7	Yes	BO	60 gal	Undetermined injury	.
8	Yes	SU	2.5 kg.	Crops injury	.
9	Yes	SU, FO	6 kg. each	Undetermined injury	.
10	No	None	None	None	.
11	Yes	SU	1 kg	.	Any
12	Yes	SO	1 kg	.	Any
13	Yes	SU	2 kg	Coffee, timber and fruit trees injury	Any
14	Yes	MA, FO	40 kg each	Undetermined injury	.
15	Yes	SU	4 kg	.	.
16	Yes	SU	0.5 kg	.	Any
17	Yes	Y	.	Citrus trees injury	.
18	No	None	None	Fruit trees injury	.
19	Yes	SU	5 kg	Citrus trees injury	.
20	Yes	SU	.	Crops injury	.
21	Yes	SU, MA	1.5 kg each	.	Any
22	Yes	SO	1 kg	.	Early rainy season
23	Yes	FO,SU	2 kg each	Trees injury	.
24	No	None	None	None	.
25	Yes	SU	3 kg	.	Any
26	Yes	SU	Unknown	Undetermined injury	.
27	Yes	SU	2.5 kg	.	Any
28	Yes	SU, FO	125 kg, 30 kg, (respectively.)	Timber trees injury	.
29	Yes	SU, FO, DI	10 kg	Undetermined injury	.
30	Yes	SU	1 kg	Trees injury	.
31	Yes	.	.	.	Any
32	Yes	SO	1.5 kg	.	Any
33	Yes	SU	0.5 kg	.	Any
34	Yes	SU	5 kg	Coffee and timber trees injury	.
35	Yes	SU	20 kg	.	Any
36	Yes	XA	4.5 kg, 1 kg	Coffee and citrus trees injury	.
37	Yes	SU, FO	(respectively)	Timber and fruit trees injury	.
38	Yes	FO	1.5 kg	Undetermined injury	.
39	Yes	SU	2 kg	Undetermined injury	.
40	Yes	FO,MA	1.5 kg	Undetermined injury	.

*SU:Sulfluramid, SO: Sodium octaborate, FO: Foxim, MA: Malathion, DI:Diclorovinil, BO: Burned Oil, Y: Yeast, XA: *Xanthosoma* plants.