

**The Diversity and Ecology of Spiders in Coffee
Agroecosystems in the Soconusco Region, Chiapas,
Mexico**

by

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DEDICATION

To Esmeralda Marín González, entrepreneur woman and valiant mother that has fought many struggles. Te quiero mamá.

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PREFACE

Agriculture and pasturelands cover up 40% of the Earth's terrestrial surface (Foley et al. 2005) and depending on their agricultural management these systems support biodiversity and function as connectors among forest fragments scattered across landscapes (Vandermeer and Carvajal 2001, Perfecto et al. 2009). In addition, the biodiversity found in agricultural fields may provide ecosystem services such as biological control and pollination (Power 2010). Thus in order to set meaningful biodiversity conservation agendas that encompass forest fragments and their surrounding agricultural matrix as well to ensure sustainable food systems, we need to understand how local agricultural management and landscape factors affect the biodiversity found in agroecosystems. The goal of this dissertation is to understand how local and landscape variables influence biodiversity in coffee agroecosystems. More specifically, I aim to understand the patterns of richness, abundance and composition of spiders, a diverse and abundant predatory taxon in agroecosystems to local and landscape factors in a coffee landscape in the Soconusco, Chiapas, Mexico. Indeed, I ask how the patterns of richness, abundance and composition of spiders change across a gradient of agricultural management in the Soconusco, as well to determine the influence of aggressive ants and distance to forest on spider diversity.

Over the last 20 years we have learned that the agricultural management of agroecosystems influences their suitability as habitat for biodiversity (Donald et al. 2001, Tscharntke et al. 2005, Donald et al. 2006, Tscharntke et al. 2012). Agroecosystems under intensified management (pesticide use, monoculture implementation and intensive pruning) have significantly less species richness (Perfecto et al. 1996, Donald et al. 2001, Donald et al. 2006, Philpott et al. 2008, Lin and Perfecto 2012). In addition to the influence of management, biodiversity within agricultural fields is also affected by keystone species, including ants (Risch and Carrol 1982, Stadler and Dixon 2005, Styrsky et al. 2010, Vandermeer et al. 2010). Finally, landscape factors, such as distance from the agricultural plot to the nearest forest fragment, can also affect biodiversity patterns in agricultural fields.

In the tropics, agroforestry systems play a crucial role in biodiversity conservation as well in supporting farmers' livelihoods (Clough et al. 2010, Lin and Perfecto 2012). Coffee agroforestry systems are based on the combination of growing coffee (*Coffea arabica* L) plants under the shade of trees that were purposely left standing or were planted for providing timber, fire wood and non-wood products such as fruits and fiber (Moguel and Toledo 1999). Coffee agroforestry systems are found over a variety of agricultural management ranging from complex coffee systems with a diverse array of trees and high canopy cover values to coffee plantations with predominance of a single tree genus and sparse shade (Moguel and Toledo 1999). In Mexico the intensification of coffee agroforestry systems started in the 1970's when the IMCAFE, a Mexican extension agency, heavily promoted the use of the tree genus *Inga* in coffee systems as a magic bullet that would solve many problems associated with coffee cultivation

(Romero-Alvarado et al. 2002). Although, the IMCAFE was dissolved in 1989, the intensification of coffee agroecosystems is still underway and continues to be propelled by a variety of economic and cultural factors. The negative effects of coffee intensification on biodiversity have been reported for a variety of taxa including ants, beetles, birds, and epiphytes (see reviews by Perfecto et al. 1996, Philpott et al. 2008 and Lin and Perfecto 2012). However, there is an empty space in the literature regarding the effect of coffee intensification on spiders, a key predatory group. Indeed, spiders are abundant generalist predators in natural ecosystems and agroecosystems (Coddington and Levi 1991, Schmitz 1997, Schmidt et al. 2003, Schmitz 2005), however, few studies about spider ecology have taken place in coffee agroecosystems (Vandermeer et al. 2002, Pinkus Rendón et al. 2006).

Furthermore, most of the biodiversity studies in coffee agroecosystems have explored the effects of local management on the diversity of a specific taxon, but have not explored interactions with other taxa (e.g. ants). Finally, most of these studies have been focused on the local effects of management but very few examine landscape factors such as distance to forest. Thus in my dissertation I attempt to fill the gap that exists in the understanding of how spider communities are associated with local factors such as coffee intensification and aggressive ants and with landscape factors such as distance to the forest.

Tropical regions are characterized by a strong seasonality reflected in the amount of pluvial precipitation that defines the dry and rainy seasons (Wolda 1988). Thus any positive effect that complex agroforestry coffee systems have on spider diversity may be better reflected in the dry season because coffee systems with high shade canopy cover

have higher humidity. In chapter 1, I explore how the local management and distance to forest influence ground spider diversity in a coffee landscape in both dry and rainy seasons. In 2011, I sampled ground dwelling spiders in 37 sites scattered in a coffee landscape in the Soconusco, Chiapas, Mexico; 27 sites were distributed in coffee farms and 10 sites in forest fragments. I characterized these sites by taking vegetation characteristics and canopy cover and gathering distance to the nearest forest fragment using a Geographic Information System. My expectations were that: 1) spider diversity would linearly and positively respond to the increase of canopy cover, 2) the positive effect of high shade coffee would be higher over the dry season, the harshest season in the tropics and 3) spider diversity in the coffee plots would be negatively and linearly affected by distance to the nearest forest fragment. Results showed that local ground dwelling spider richness and abundance differed significantly across seasons. Furthermore, results showed that local ground dwelling spider richness and local abundance did not respond positively to coffee management. Instead local spider richness and abundance tended to be higher in the low shade coffee agroecosystems. Nonetheless, in terms of accumulated spider richness the results are more intriguing. Indeed, results showed that in the dry season the high shade coffee systems harbored higher richness than any other system. On the contrary, in the rainy season the accumulated spider richness was higher in the low shade system than in the two other systems. Results on orthopteran abundance recorded for the rainy season showed that orthopterans were more abundant in the low shade system, thus suggesting that prey availability could have driven the high accumulated and local spider richness in the low shade systems in the rainy season. In addition, the response of spider species composition to management also

changed across seasons. Species composition showed its maximum dissimilarity in the dry season and across forests. Results also showed that the response of spider diversity to distance to forest was season dependent. No relationship between distance to forest and spider diversity was found for the dry season, but a mild effect of distance to forest on spider abundance was found in the rainy season. In this chapter I also document the unexpected large correlation of spider richness and abundance with the slope of the terrain.

Although predators, spiders are affected by the structure of the vegetation at a local/microhabitat scale since spiders rely on plant structure for attaching webs, hiding from predators and for nesting places. In addition, the negative effect of ants on arthropods in natural ecosystems and agroecosystems has been reported elsewhere and in coffee agroecosystems previous studies have suggested that the aggressive ants *Azteca instabilis* have a negative impact on the spiders found in the coffee layer (see Vandermeer et al 2002). In chapter 2, I address the spider diversity found in the trunks of shade trees in relation to tree characteristics, the presence of the aggressive ants *A. instabilis*, canopy cover and distance to forest. In 2012, I set up nineteen one-hectare plots across a range of coffee management and measured vegetation characteristics in each of them. In addition, I gathered distance to the forest by using a Geographic Information System. In each one-hectare plot, I selected two pairs of trees, in one pair the trees belonged to the *Inga* genus whereas in the other pair the trees belonged to another genus. Furthermore, within each pair, one of the trees had an active *A. instabilis* nest, whereas the other tree did not have one. My expectations were that local spider richness, abundance and biomass found in the trunks of shade trees would decrease in the presence

of *A. instabilis* and in trees with straight trunks. Results show that local spider richness, abundance and biomass responded positively to tree characteristics and to the presence of *A. instabilis* ants. However, the response of spider abundance and biomass was driven by *Ischothele digitata* and *Azilia guatemalensis*, the two most abundant spider species; the exclusion of these species from the dataset made the response to vanish. Results indicate that these spiders prey upon the workers and queens of *A. instabilis*. In addition, a composition analysis showed that the species composition was not strongly affected by the presence of the *A. instabilis* ants. No effects of canopy cover or distance to the forest were found.

Across a variety of systems ranging from tropical agroforestry systems to temperate grasslands a growing literature has reported positive correlations between spiders and ants. In chapter 3, I address the effect of the aggressive ants *Azteca instabilis* on the spider diversity found in coffee plants in two contrasting coffee agroecosystems. Results showed that local spider richness and abundance were higher in the most intensified coffee agroecosystem and that *A. instabilis* ants have a positive effect on the spider richness and abundance. Species composition did not change significantly in the presence of the *A. instabilis* ants; nonetheless, three spider species were significantly more abundant in the presence of the ants. In addition, in order to explore potential mechanisms that would explain the high local spider richness and abundance in the presence of *A. instabilis* ants I quantified potential prey for spiders in the presence and absence of *A. instabilis* by carrying out visual counts of the insects found in the coffee plants as well by using sticky traps. I found that overall more insects were found in the

presence of *A. instabilis* ants, thus suggesting that high levels of potential prey resources might be driving the richness and abundance of spiders.

In chapter 4, I explore the influence of coffee plant attributes and the presence of the aggressive ants *A. instabilis* on the abundance and spatial distribution of *Pocobletus* sp. nova, the most abundant spider species found in the presence of *A. instabilis* in coffee bushes. I address *Pocobletus* spatial distribution in relation to *A. instabilis* as well the relationships among *Pocobletus* abundance, coffee branchiness, and *A. instabilis* presence by setting up four plots, three 20 x 20 m and one 25 x 20 m plots and sampling *Pocobletus* and their predators in each coffee plant found inside these plots. In addition, I measured potential prey abundance by collecting insects in the presence and absence of *A. instabilis* with sticky traps. Results show that the spatial distribution of *Pocobletus* is positively associated with the presence of *A. instabilis*, however, it also suggests that other variables in addition to the presence of *A. instabilis* influence the spatial distribution of *Pocobletus*. Indeed, *Pocobletus* abundance was better explained by the presence of two ant species (*A. instabilis* and *Pheidole synanthropica*) and by coffee branchiness. Results also show that the abundance of spider predators of *Pocobletus* sp. decreased in the presence of *A. instabilis* and that the number of potential prey for *Pocobletus* increased in the presence of the ants. Potential mechanisms underlying the association between *Pocobletus* sp. and *A. instabilis* are higher resource abundance and enemy free space.

In my dissertation research I aim to document how changes in coffee management affect spiders inhabiting the ground, coffee and tree trunk strata as well to document the response of spiders inhabiting the ground and tree trunk layers to distance to the nearest forest fragment, a critical landscape factor. In addition, I document the ecological

interactions between the aggressive ant, *A. instabilis*, and spiders found in the coffee and the tree trunk layer; these interactions range from predation to the creation of enemy free space for the spiders. It is my hope that this research will contribute to a better understanding of biodiversity in agricultural landscapes and the factors that influence this agrobiodiversity.

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ABSTRACT

Agricultural landscapes are globally distributed and can function as refuges for biodiversity. My research focuses on elucidating the factors that influence predators and their ecological interactions in agricultural landscapes. I studied patterns of richness and abundance of a predator group, spiders, to understand its response to local factors (agricultural management, presence of aggressive ants) and landscape factors (distance to forest) in a coffee landscape composed of forest remnants (6%) and coffee farms (94%).

First, I examined the patterns of ground dwelling spider diversity associated with coffee management intensification as well as the response of spider diversity to canopy cover, slope of the terrain, litter biomass, and distance to forest. Findings show that local spider richness and abundance were generally higher in simplified agroecosystems. Distance to forest had a mild correlation with spider abundance; in contrast local spider richness and abundance had a strong negative correlation with slope.

Second, I examined the response of tree-trunk dwelling spider diversity and biomass to *Azteca instabilis* ants, tree characteristics, canopy cover and distance to forest. Results show that local spider richness was positively correlated with tree diameter, whereas spider abundance and biomass were positively correlated with tree diameter, and *A. instabilis*.

Third, I examined spiders in coffee plants and their response to *A. instabilis* in contrasting coffee agroecosystems. Results show that spider richness and abundance were higher in the intensified sites and in the presence of *A. instabilis*. Potential mechanisms for the spider-ant association were prey availability and protection against predators.

Fourth, I examined the abundance of the most abundant spider genus in coffee plants, *Pocobletus*, in relation to coffee branchiness and ants presence. Results show that *Pocobletus* is positively associated with coffee branchiness and the presence of *A. instabilis* and *P. synanthropica* ants. Evidence suggested that the positive association *Pocobletus*-ants was due to prey abundance and enemy-free space.

In sum, ground and coffee dwelling spiders were locally more diverse and abundant in the intensified agroecosystems, whereas tree-trunk dwelling spiders were more sensitive to coffee intensification. Spiders were positively associated with aggressive ants. Distance to forest was not an important predictor of spider diversity.

CHAPTER 1

RESPONSE OF GROUND SPIDERS TO LOCAL AND LANDSCAPE FACTORS IN A COFFEE LANDSCAPE

Abstract

Biodiversity and agriculture provide ecosystem services to humanity. However, agriculture has been considered as one of the main factors driving the current biodiversity loss. In order to secure the provisioning of ecosystem services detailed analyses of the relationship between biodiversity and agriculture are required. We studied ground spider diversity in a 52 km² coffee landscape in Southern Mexico, and asked the following questions. Does coffee management affect ground spider diversity? What are the factors that influence ground spider diversity in a coffee landscape? Does ground spider diversity respond to different variables in different seasons? Over the dry and rainy seasons of 2011 we sampled ground spiders with pitfall traps in thirty-seven 20 x 20 m sites distributed in coffee plantations (27 sites) and forest fragments (10 sites). Using Principal Component Analysis we analyzed the variation in coffee sites based on tree species richness, tree density, canopy cover and proportion of *Inga* trees. In addition, for each 20 x 20 m site we measured leaf litter variables, invertebrate dry biomass, slope of the terrain, elevation and distance to the nearest forest. Data were analyzed with general and

generalized linear models. Results show that coffee sites separated in two groups, which we categorized as low-shade and high shade. Agricultural management had a strong influence on spider richness and abundance. Across seasons local spider richness and abundance had or tended to have higher values in the low shade coffee. In addition, the predictors underlying spider richness and abundance were also season dependent, with slope of the terrain being the strongest predictor in the dry season and canopy cover being the strongest predictor in the rainy season. We conclude that in this coffee landscape, both coffee and forest are needed for spider conservation but that spider diversity thrives in coffee agroecosystems.

Key words: landscape, local factors, coffee landscape, Araneae

Introduction

Biodiversity provides many ecosystem services to humans, such as pollination, pest control and food provisioning (Balvanera et al. 2006). Nonetheless, we are facing a great loss of biodiversity and, agriculture is considered as one of the main factors causing this loss (Foley et al. 2011). Agriculture and pastures cover up about 40% of the terrestrial surface of the Earth (Foley et al. 2005) and this percentage is not projected to decrease in the upcoming years, hence a more detailed analysis of the relationships between agriculture and biodiversity is needed. Indeed, over the last 20 years we have learned that the way in which agriculture is practiced affects the biodiversity present in agricultural fields, the persistence of biodiversity in the landscape (Donald et al. 2001, Tschardtke et al. 2005, Donald et al. 2006, Tschardtke et al. 2012), and the provisioning of ecosystem services that this biodiversity provides to the agricultural fields (e.g. pollination, biological control). At the field level, agricultural intensification (e.g., monoculture implementation, use of pesticides and fertilizers) negatively affects biodiversity (Holzschuh et al. 2008) and potentially affects the provisioning of ecosystem services (Gabriel and Tschardtke 2007). In addition, in simplified landscapes, the local management of the agricultural fields (organic vs conventional practices) is not as important as the landscape context in which agriculture is practiced (Purtauf et al. 2005, Roschewitz et al. 2005). For example, in temperate zones, spider richness does not respond to local management (Clough et al. 2005, Bruggisser et al. 2010, Gardiner et al. 2010, Kerzicnik et al. 2013, but see Schmidt and Tschardtke 2005b) but instead, responds to the landscape composition and diversity (Clough et al. 2005, Schmidt and Tschardtke

2005a, b, Oberg et al. 2007, Schmidt-Entling and Dobeli 2009, Gardiner et al. 2010). Thus the effects of agricultural intensification need to be addressed at several scales, including local, landscape and regional scales (Tscharntke et al. 2005, Clough et al. 2007a, Clough et al. 2007b, Tscharntke et al. 2012). Most of the studies analyzing the landscape and local effects of agriculture on biodiversity and ecosystem services have been carried out in temperate zones of the United States and Europe (Tscharntke et al. 2007, Gardiner et al. 2010, Woltz et al. 2012) and little is known about how these factors play out in tropical regions (but see Stenchly et al. 2011, Avelino et al. 2012, Stenchly et al. 2012).

Coffee agroecosystems are found in mountainous and in flat zones of the Neotropics and they play an extremely important role in biodiversity conservation in that region (reviewed by Perfecto et al. 1996, Moguel and Toledo 1999, Lin and Perfecto 2012). As with many agricultural systems, coffee agroecosystems cover a full range of management practices. The most complex coffee agroecosystems are agroforestry systems that have high shade tree canopy cover and richness and tend toward organic management, whereas the most simplified systems are coffee monocultures without shade trees. However, in most areas, especially those that are found in mountainous regions with rugged topography, the most intensive systems have a low percent of canopy cover and few species of trees, most of them in a single genus (Moguel and Toledo 1999). An extensive literature on biodiversity in coffee farms indicates that coffee systems with high shade cover and tree diversity support higher species richness of associated (or wild) biodiversity than coffee systems that are structurally less diverse (Lin and Perfecto 2012). However, spiders seem to be an exception to what has been found for

other taxa. Studies examining spider diversity on coffee plants have found that accumulated spider richness does not differ between the most and least intensified systems (Marín and Perfecto 2013), but that local spider abundance is higher in the most intensified coffee plantations (Pinkus-Rendon et al. 2006a, Marín and Perfecto 2013a).

Here we examine the effect of local coffee management, local habitat variables (leaf litter biomass, invertebrate biomass and percent of canopy cover), and one critical landscape factor (distance to the forest) on the abundance and richness of ground spiders in a coffee landscape composed of coffee farms under several management categories and small forest fragments. We studied spiders because they are a very diverse predatory taxon (Coddington and Levi 1991) whose role in controlling insect populations (Schmitz et al. 1997, Schmidt et al. 2003) and, even affecting community structure (Schmitz 2008) has been reported for both natural ecosystems and agroecosystems (Schmidt et al. 2003). Specifically, we asked the following questions: 1) Are spider richness, abundance and composition affected by coffee management? 2) How do local and landscape factors influence ground spider richness and abundance in a coffee landscape? and 3) Does spider richness and abundance respond to different variables in different seasons?

Material and Methods

Study Area

The study took place in the Soconusco region of the state of Chiapas, Mexico, a coffee growing region characterized by rugged terrain covered by coffee farms and scattered forest fragments. The coffee landscape studied here covers an area of 52 km² (coordinates 15.202 N, 92.383 W [NW corner] and 15.144 N, 92.297 W [SE corner], fig.

1.1) (Philpott et al. 2008) and is characterized by large coffee farms (~300 ha) that cover 94% of the landscape and small remnants of evergreen tropical forest (6%) (Philpott et al. 2008, De la Mora et al. 2013). The study area covers an altitudinal gradient ranging from 800 to 1450 meters above sea level, and has two well-defined seasons; a dry season from November to April and a rainy season from mid-late May to October. In this landscape thirty-seven 20 x 20 m sites were established in forest remnants (10 sites) and in coffee plantations (27 sites) that varied in their management (De la Mora et al. 2013).

Site characterization

Coffee management. For every 20 x 20 m site we collected data on percent canopy cover, tree species richness, tree density and proportion of *Inga* trees (except *Inga laurina*, a species more often found in the forest). We decided to include the proportion of *Inga* trees in the analysis as a measure of intensification because after its introduction in the 1980's, the intensive and extensive use of *Inga* trees has become a hallmark of simplified coffee farms (Romero-Alvarado et al. 2002). We used these data in a Principal Component Analysis to separate the coffee sites into high shade and low shade habitats (Table 1). Data were collected once in the dry season and once in the rainy season of 2011. Although most of the coffee sites we sampled were previously classified for another study (De la Mora et al. 2013), due to changes in agricultural management in some farms we replaced some sites and updated the management variables of the coffee sites in the dry season (Table 1). We collected leaf samples from the tree species found in the forest sites in April 2011, which we identified and deposited at the Herbarium of El Colegio de la Frontera Sur (San Cristóbal and Tapachula). We identified the tree species found in the coffee sites in the field. We measured canopy cover with a concave

densitometer at 0, 5 and 10 m from the center of the site at each of the four cardinal directions (N, S, W, E). In forest sites we measured the canopy cover in July 2011, whereas in coffee sites canopy cover was measured twice, once in March 2011 (dry season) and once in July 2011 (rainy season).

Local variables. In addition to the coffee management variables, for each site we measured a set of local variables, the distance to the nearest forest fragment and two environmental variables (elevation and slope). The local variables measured per site were leaf litter depth, leaf litter dry biomass, percent of soil covered by leaf litter, twigs and woody debris (rainy season only), invertebrate dry biomass and orthopteran abundance (rainy season only). We took forty leaf litter depth measurements per site as follows. In each 20 by 20 m site we set up two 20 m transects, transect 1 was set up in a North-South direction and transect 2 was set up in a West-East direction. Then starting at the intersection of both transects (the center of each site) we took leaf litter depth measurements with a flat ruler every meter, for a total of 10 measurements in each cardinal direction and 40 measurements in each site. To estimate leaf litter biomass, we haphazardly threw a 25 x 25 cm² square five times within each 20 x 20 m site then collected all leaf litter contained inside the 25 x 25 cm² square, labeled the sample and dried the leaf litter at 70 °C for 5-6 days. We assessed ground cover in the rainy season, by sampling 20 haphazardly selected 25 x 25 cm² quadrats in each of the 20 x 20 m sites. Within each quadrat we measured the percent of soil covered by leaf litter, twigs and woody debris. As a surrogate for invertebrate biomass we assessed invertebrate dry biomass of all arthropods (minus spiders and ants) collected with pitfall traps (see spider sampling below). Arthropods were dried at 60 °C for three days and then weighed.

Spiders and ants were not dried with other arthropods, as specimens were required for proper identification. Instead, for spiders, we measured body length and used this value in length-dry biomass equations developed for spiders (Hofer and Ott 2009). We then added estimated spider biomass values to the dry weight of other arthropods to obtain total dry biomass per site. We did not include ants in the measure of invertebrate dry biomass because this would have biased results, given that in some traps, entire ant nests were caught. In addition, since the literature (Beckerman et al. 1997) has reported that orthopterans are preyed upon by spiders we counted the number of orthopterans collected in the pitfall traps of all sites (except two low-shade and one high-shade sites) but only for the rainy season due to logistics. The distance to the nearest forest edge was calculated using a Geographic Information System (GIS) based on IKONOS® image and a digital elevation model taken on December 10, 2005 and updated in 2009 (Philpott et al. 2008, De la Mora et al. 2013). Elevation was taken with a GPS unit (Garmin GPS 60) and slope of terrain was measured in 3-5 different spots within each site using a Suunto clinometer. The number of measurements depended on the accessibility of the terrain. All variables (except distance to the forest, elevation and slope of the terrain, soil cover, and orthopteran abundance) were measured twice, once in March 2011 (dry season) and once in July 2011 (rainy season) due to seasonal and management changes. Indeed, due to changes in management between the dry season and rainy season and access feasibility to the forest, the number of sites in each habitat category changed somewhat for the rainy season. In total we sampled 33 sites, with 8 forest sites and 25 coffee sites.

Spider sampling

We sampled spiders by placing five pitfall traps (diameter = 7cm, height = 5 cm) within 1m² at the center of each site. Traps were filled with 125 ml of a soapy solution of 25% propylene glycol (a non toxic preservative) and buried into the ground with their rim at the level of the ground. We set up traps between 7 a.m. to 1 p.m. and left them open for 5 days. At the end of the fifth day we collected and transported the traps to the laboratory and placed all collected specimens in 95% ethanol. We identified spiders to family by following the spiders of North America (Ubick et al. 2005) and to species and morphospecies following selected literature (Platnick 2013). Voucher specimens were deposited in the Arachnological Collection at El Colegio de la Frontera Sur in Tapachula and at the Department of Natural and Applied Sciences at the University of Wisconsin Green Bay.

Statistical Analyses

Habitat characteristics

We analyzed the variation in management variables in the coffee sites with Principal Component Analysis. More specifically, in the Principal Component Analysis we included percent canopy cover, tree density, tree richness and proportion of *Inga* trees. In addition, we tested differences among forest and coffee habitats with respect to percent canopy cover, tree density, tree richness, proportion of *Inga* trees, leaf litter depth, dry leaf litter biomass, invertebrate dry biomass and orthopteran abundance with univariate analysis of variance followed with Tukey HSD tests. When variables were not normally distributed they were square root transformed.

Coffee management and spider diversity

Richness and abundance. To test whether total species richness was significantly different among the three habitats (forest, high shade and low shade), we constructed individual-based rarefaction curves and their respective standard deviations with R software following Gotelli and Ellison (2012). To evaluate whether spider diversity differed in forest, high-shade coffee, and low-shade coffee, we compared mean spider richness and abundance with analysis of variance and subsequent Tukey HSD tests. In addition, we compared mean spider richness and abundance in forest and coffee sites with t tests. We tested for normality in the distribution of the errors with qqnorm plots. When errors were not normally distributed, we applied the square root transformation.

Guilds. We calculated the change in guild composition across seasons and habitats by sorting spiders into four guild categories (cursorial hunters, web builders, kleptoparasites, and sit and wait hunters) and calculating their percentages in each habitat and season. Following Ubick et al. (2005) and field observations we included as cursorial hunters those spiders that do not build a web but that rather actively pursue prey; web builders, those spiders that rely on weaving a web for catching prey; kleptoparasites, those spiders that invade webs of other spiders for obtaining food; and as sit and wait hunters, those spiders with limited movement —such as trap door spiders.

Dominant families and species composition. We identified the dominant families and their richness and abundance in forest and coffee sites for each season and we compared mean spider richness and abundance in forest and coffee sites with t tests and Wilcoxon tests when normality criteria were not met even after transforming the variable. We analyzed the change in species composition across habitats using Non-metric multidimensional scaling (NMDS), a technique widely used for comparing

changes in species composition across habitats (Winfree et al. 2008, Borcard et al. 2011, Hogg and Daane 2013). This technique creates a species composition distance matrix between sites based on a similarity index, which measures how close are two points in regards to species composition. Then sites are ranked according to their differences and ranked differences are considered for evaluating the similarity between sites through an iterative processes. We used the Bray-Curtis similarity index. To assess the significance of the difference in species composition among high shade, low shade and forest habitats we used Analysis of Similarities (ANOSIM), which provides an R-value. R values close to 1 mean that habitats are greatly dissimilar whereas values close to 0 mean that habitats are very similar.

Effect of the local and landscape variables on spider diversity

In order to elucidate which factors have the strongest association with ground spider richness and abundance, we used general linear models and generalized linear models.

In all cases, spider abundance or spider richness were the response variables and the local and landscape variables were the predictor variables. We tested the error distribution of the response variables by using Cook's distance. When errors were normally distributed, we analyzed data with a general linear model in the mode of a multiple linear regression. When errors were not normally distributed we used generalized linear models with a negative binomial distribution for count data (Faraway 2006). We applied backward model selection and variables were retained based on their probability value. We calculated correlation among predictor variables using variance inflation factors (vif) and variables that had a value greater than 2.6 were discarded from the model. We chose

between different models by using AIC values. All analyses were carried out with R software (R Core Team 2012) using the packages carr, lmtest, MASS, vegan.

Results

Site characteristics

The Principal Component Analysis (PCA) using percent canopy cover, tree species richness, tree density and proportion of *Inga* showed that coffee sites separated into two groups (Fig. 1.2a & b). In the dry season, PCA axis 1 explained 64 % of the variance and was positively correlated with tree species richness, tree density and canopy cover and negatively correlated with the proportion of *Inga* trees; PCA axis 2 explained 18% of the variance and was positively related to canopy cover (Fig. 1.2a). Based on those results we classified sites into high-shade (H: with high percent canopy cover, low proportion of *Inga* and high tree species richness and density) and low shade (L: with low percent canopy cover, high proportion of *Inga* and low tree species richness and density), thus during the dry season the PCA resulted in 14 low-shade sites, and 13 high-shade sites. Three sites had high tree richness but had very low canopy cover in the dry season, thus we decided to place them in the low-shade category. In the rainy season PCA axis 1 explained 65 % of the variance and was negatively correlated with tree species richness, tree density and canopy cover and positively correlated with the proportion of *Inga* trees; PCA axis 2 explained 15% of the variance and was positively related to canopy cover and proportion of *Inga* trees (Fig. 1.2b). During the wet season due to changes in management the number of coffee sites decreased thus the PCA resulted in 14 low-shade sites and 11 high-shade sites. Classifications were corroborated with analysis of variance

that tested for differences in management variables among the three habitat types. Across both seasons, forest sites, high-shade coffee and low-shade coffee habitats differed in percent canopy cover, tree species richness and tree density (Table 1.1). In addition, high-shade and low-shade coffee sites differed in the proportion of *Inga* trees, with the high shade sites having significantly lower proportion of *Inga* trees (Table 1.1).

Local variables differed with habitat type and with season. In the dry season, leaf litter depth was higher in forest than in high- or low-shade coffee (Table 1.1). However, no differences were found among the three habitats in the mean leaf litter dry biomass. In the rainy season, percent soil covered by leaf litter and wood debris was lower in forest than in coffee sites (Table 1.1). Invertebrate dry biomass was marginally lower in the low-shade sites than in the high-shade and forest sites in the dry season, but no differences were identified in the rainy season. Orthopteran abundance differed with habitat in the rainy season with higher abundance in the low-shade coffee sites. Due to the way in which sites were classified in the two seasons, distance to forest differed for high- and low-shade sites in the dry season with high-shade sites closer to forest than low-shade sites. Elevation did not differ with habitat, but slope of the terrain was steeper in forests compared with coffee sites (Table 1.1).

Coffee management and spider diversity

Overall, we collected 1299 spiders belonging to 25 families, 56 genera, and 88 species or morphospecies. A large fraction (50%) of species was composed of singletons (39%) and doubletons (11%). The most diverse families were Theridiidae (24% of species) and Linyphiidae (18%), whereas the most abundant families were Linyphiidae (38.5%), Lycosidae (20%) and Corinnidae (13%). The species overlap between the dry

and rainy season was 43 %. In the dry season we collected 678 individuals and 59 species or morphospecies distributed among 40 genera and 17 families. In the rainy season we collected 621 individuals, 23 families, 51 genera, and 68 species or morphospecies.

Richness. According to individual-based rarefaction curves, total species richness differed with habitat in the dry season, with the high shade habitat harboring more spider species than the low shade habitat. Nonetheless, the species accumulation curve for the forest does not plateau indicating that more sampling was required. In the rainy season, individual based rarefaction curves showed that low-shade habitats had more species than high-shade coffee and forest habitat (Fig. 1.3b). However, once again the rarefaction curve for the forest habitat does not plateau, suggesting that the forest sampling was incomplete.

Local mean spider richness per site was lower in the forest sites (5.90 species) than in either coffee habitat (H = 8 species and L = 8.42 species), but this difference was not statistically significant (Fig. 1.4a; F-statistic = 2.92, $p = 0.067$, d. f. = 2, 35). However, when the two coffee habitats were pooled together this difference became significant (Fig. 1.4a; coffee = 8.22 species; forest = 5.9 species; t-test = 2.49, $p = 0.002$, d. f. = 17.21). In contrast to the dry season, mean richness did not differ in forest (9.24 ± 0.051) and pooled coffee sites (8.26 ± 0.05) (t-test = -1.01, $p = 0.3$, Fig. 1.4b, d. f. = 14.64). However, the average species richness per site was significantly higher in forest (9.24 ± 0.051) and low-shade (9.44 ± 0.06) coffee than in the high-shade coffee (6.88 ± 0.041) (Fig. 1.4b, F-statistic = 4.059, $p = 0.027$ d. f. = 2, 23).

Abundance. In the dry season, local mean spider abundance per site was significantly lower in the forest sites (9.49 individuals) than in either coffee habitat (H = 20.5 individuals and L = 20.33 individuals), (Fig. 1.4c; F-statistic = 7.98, $p = 0.001$). Furthermore, when the two coffee habitats were pooled together this difference was still significant (Fig. 1.4c; forest = 9.49 individuals, coffee = 20.4 individuals, t-test = 5.22, $p < 0.0001$). In the rainy season, mean abundance was significantly higher in the low-shade coffee (24.29 individuals ± 0.33) than in the high shade (12.34 individuals ± 0.25) or forest (14.89 individuals ± 0.095) sites (F-statistic = 7.81, $p = 0.001$). However, when the two coffee habitats were pooled together this difference disappeared (Fig. 1.4d; coffee = 18.53 individuals; forest = 14.89 individuals; t-test = 1.45, $p = 0.16$, Fig. 1.4d).

Guilds. In the dry season, guild composition across species was relatively even with 52% classified as cursorial hunters and 48% classified as web builders; we did not find kleptoparasite or sit and wait spiders in the dry season. Spider abundance, however, was strongly biased towards web builders (64.3% of individuals) with lower abundance of cursorial hunters (35.7% of individuals). Abundance of guilds was very biased regarding habitat type. Fifty percent of all hunter spiders were recorded in the low-shade coffee habitat, 38% were recorded in the high-shade coffee habitats and 12% were recorded in the forest habitats. Web builders did not show strong preference for low-shade habitats (40%), high-shade habitats (44%), or forest habitats (16%). The most abundant species was *Walckenaeria crocea* (Linyphiidae), a web builder spider that represented 20% of all individuals collected, followed by *Meioneta* sp. 1 (Linyphiidae), a web builder that represented 15% of all individuals. Thus, just two species added up to 35% of the total abundance.

In the rainy season, guild composition across species was strongly biased towards web builders (59.2% of species) with only 33.3% of the species in the cursorial hunter guild, 4.4% kleptoparasites and 2.9% sit and wait predators. However, in term of individuals, the most abundant guild was cursorial hunters (57.3% of individuals), followed by web builders (40.9%), sit and wait predators (1.28%) and kleptoparasites (0.48%). Abundance of guilds was very biased regarding habitat type. Fifty-nine percent of all hunter spiders were recorded in the low-shade coffee habitat, 24% were recorded in the high-shade coffee habitats and 17% were recorded in the forest habitats. Likewise, web builders showed a strong preference for low-shade habitats (57%), high-shade habitats (22%), or forest habitats (21%). The two most abundant species in the rainy season were *Pirata pagicola* (Lycosidae), a cursorial spider that represented 20% of the individuals collected, followed by *Walckenaeria crocea* (Linyphiidae) a web builder spider (10%).

Dominant families. In the dry season, the most species rich families were Linyphiidae (22% of species) and Theridiidae (20%). Interestingly these two families showed an opposing pattern, with 75% of the Theridiidae species recorded in the forest sites, (15% of the total spider richness recorded in the landscape over the dry season) and only 35% of the species found in the coffee sites whereas 92% of the Linyphiidae species were found in the coffee sites and only 35% were found in the forest sites. Indeed there was a significant difference between the average number of Theridiidae species in the forest (1.30 ± 0.40) and the coffee sites (0.44 ± 0.13) ($W = 82.5$, $p = 0.044$). On the other hand, Linyphiidae species richness was higher in coffee (3.0 ± 0.21) than in forest ($1.8 \pm$

0.25) ($W=216$, $p = 0.004$). Regarding abundance, Linyphiidae represented 49% of the total spider abundance in the landscape followed by Corinnidae (13%) and Lycosidae (11%). In the rainy season, the two most species rich families were Theridiidae (25% of species) and Linyphiidae (22%). In contrast to the dry season, the number of Theridiidae species did not differ in forest and coffee sites ($W = 97.5$, $p = 0.93$). Likewise, Linyphiidae richness did not differ between forest and coffee sites ($W=78.5$, $p=0.37$). The most abundant families were Lycosidae (28% of individuals) and Linyphiidae (27%).

Species composition. In the dry season, species composition between forest, high and low-shade coffee sites was very similar ($R = 0.17$, $p = 0.01$). However, the NMDS plot (Fig. 1.5a) shows that the forests sites tended to be more distinct from coffee sites, and also that high-shade coffee sites tended to be more similar to forest than to low-shade coffee sites. In the rainy season, species composition did not differ between forest, high-shade, or low-shade coffee sites (Fig. 1.5b, $R = 0.099$, $p = 0.04$). Indeed, the NMDS plot (Fig. 1.5b) shows that the overlap among the tree habitat types is even larger for the rainy season.

Local and landscape influence on spider richness and abundance.

In the dry season, spider richness in the coffee landscape was correlated only with slope ($\beta = -0.13$, $p < 0.0001$, $R^2 = 0.20$, Table 1.2). Spider richness decreased by 0.13 units per degree of slope of the terrain. Spider abundance in the coffee landscape in the dry season was negatively correlated with slope ($p < 0.0001$) and leaf litter biomass ($p = 0.03$) (Table 1.3).

In the rainy season, spider richness was negatively associated with canopy cover and marginally with distance to the forest fragment (Table 1.2). Species richness decreased by 0.57% for each unit increment in the percent of canopy cover.

In the dry season, spider abundance was negatively associated with the slope of the terrain (4.45 % decrease in abundance for each degree) and with leaf litter dry biomass (0.69 % decrease in abundance by each gr) (Table 1.3). However, the exclusion of *Walckenaeria crocea* from the dataset changed the response of spider abundance, in this case spider abundance was negatively associated with the slope of terrain (4.4% percent of change) but not with the leaf litter dry biomass ($p < 0.0001$), suggesting that only the abundance of *Walckenaeria crocea* was negatively associated with leaf litter dry biomass.

In the rainy season, spider abundance was negatively correlated with canopy cover, distance to the forest and positively correlated with elevation (Table 1.3). Spider abundance decreased by 1.28% per percentage increment in canopy cover. Likewise spider abundance decreased by 0.06% per meter increment in distance to the forest; whereas spider abundance increased by 0.13% per meter increment in elevation (Table 1.3). The exclusion of *Pirata pagicola*, the most abundant species in the dry season, did not change the previous pattern. In other words, other members of the spider community were associated with the same factors as this common species.

Discussion

In this study we found that the pattern of accumulated spider richness changes across seasons, with the high shade habitat harboring more species in the dry season but less species over the rainy season. In contrast, we also found that the pattern of local

spider abundance was very consistent across seasons with the low shade habitats generally having more individuals. In addition, we also found that species composition, guild composition, and families showed a clear seasonal pattern. Indeed, previous studies in the tropics have pointed out the strong effect that seasonality has on biodiversity (Janzen 1973, Wolda 1988). In tropical ecosystems, the dry season is characterized by having low species richness and abundance probably because of low resource availability and low humidity levels (Janzen 1973, Lubin 1978, Wolda 1988, Wolda and Wong 1988, Philpott et al. 2006, Pinkus-Rendon et al. 2006b, Teodoro et al. 2010). However, few studies have reported positive associations between agricultural intensification and biodiversity. On the contrary, a comprehensive literature shows that the agricultural intensification negatively affects biodiversity (Perfecto et al. 1996, Donald et al. 2001, Donald et al. 2006, Moorhead et al. 2010, Lin and Perfecto 2012) including spiders (Prieto-Benítez and Méndez 2011).

Nonetheless, our study and other literature show that spiders do not respond negatively to agricultural intensification in coffee agroforestry systems. For example, Marín and Perfecto (2013) found that accumulated spider richness in coffee plants did not differ between the intensified and non-intensified coffee sites. However, these authors also reported that the local spider richness and abundance on coffee plants were higher in the low shade coffee sites than in the high shade coffee sites. Likewise, Pinkus-Rendon et al. (2006) found a similar trend, with higher spider abundance in the low shade coffee sites than in the high shade coffee sites. This trend of no differences in spider diversity between complex (high canopy cover) and simplified (low canopy) habitats or high diversity in the most simplified systems it is not restricted to agroforestry systems, it has

been also reported in natural ecosystems. For example, Hurd and Fagan (1992) reported that cursorial spiders preferred herbaceous habitats rather than forested habitats probably because herbaceous habitats had more prey availability. Furthermore, spider diversity may not vary along a successional habitat gradient (Mallis and Hurd 2005, Pinkus-Rendon et al. 2006b). This suggests that ground spiders may prefer more open habitats either due to higher prey availability (Hurd and Fagan 1992) or that they show no preferences among habitats (Mallis and Hurd 2005). Light backgrounds allow better conspecific recognition in hunter spiders (lycosids) (Uetz et al. 2011), thus it is possible that light backgrounds such as those recorded in the low-shade coffee may have contributed the pattern. Finally, another factor that may have contributed to the response of spiders to local intensification is dispersal. Spiders are well known for their dispersal capabilities thorough ballooning and walking (Bell et al. 2005, Gardiner et al. 2010). Spiders rely on wind conditions and warmer temperatures to balloon, however, they do not have control of where they will arrive (Bell et al. 2005). Thus, it seems plausible that spiders may have more success landing in sites with low canopy cover than in sites with high canopy cover.

Although, in our study spider richness and abundance did not conform in any season to the expected pattern of higher richness and abundance in the forest sites followed by the high-shade sites, it is obvious from the rarefaction curves that the forest sites were not appropriately sampled. Therefore we advise caution when drawing conclusions about the forests sites in terms of accumulated species richness and their value for spider conservation. Furthermore, in the dry season, the NMDS analysis for

species composition (Fig. 1.5a) showed that most of the coffee sites were more similar in species composition to one another than to the forest sites, thus suggesting that the spider species that inhabit the forest over the dry season tended to be somewhat different than those that inhabit the coffee. This last observation is consistent with the fact that in the dry season the forest sites harbored more of the Theridiidae species.

In addition, the linear models that evaluated the associations between spider richness and abundance as response variables and local variables and distance to the forest as predictors showed that spider richness and abundance were associated differentially to the same predictor variables depending on the season. Indeed, spider diversity was negatively associated to the slope of the terrain (dry season) and to canopy cover (rainy season). In the dry season, both spider richness and abundance had a very strong negative correlation with slope of the terrain. Indeed, sites with steep slopes show low leaf litter retention and high lixiviation rates that negatively affect the soil nutrient content, which consequently affects the abundance of potential prey for spiders (Melguizo-Ruiz et al. 2012). Although in this study, forest sites had more pronounced slopes; we did not find significant differences in invertebrate dry biomass among the three studied habitats across seasons. Nonetheless, we found that the forest sites also had more bare soil, thus perhaps offering fewer attaching surfaces for spider webs and fewer prey for spiders. In the rainy season, canopy cover was the variable most closely associated with spider richness, whereas distance to the forest had a marginal and subtle correlation. We propose that a decrease in canopy cover means a lower proportion of bare soil because all the leaves, branches and overall debris are deposited on the ground during pruning, thus decreasing the amount of exposed soil and increasing structural

complexity. Leaf litter and woody debris create structural complexity that provides sites for web attachment as well hiding places for cursorial predators thus decreasing intraguild predation in spiders and other predators (Bultman and Uetz 1982, Finke and Denno 2002, 2006, Castro and Wise 2009). Although a decrease in canopy cover also could be interpreted as fewer trees and therefore less biomass deposition on the ground, *Inga* trees, which were more abundant in low-shade coffee sites, have been reported as excellent contributors of biomass to the ground (Szott et al. 1994). Furthermore, some lycosids prefer habitats that receive plenty of sunlight (Uetz et al. 2011), so spiders can perceive their conspecifics and start courtship. Notably, across seasons most of the hunters, many of them lycosids, were more abundant in low-shade coffee habitats. Thus is possible that the decrease in bare soil due to the deposition of leaves, branches and debris via pruning combined with open canopies favored spider diversity.

Similar to other studies with other organisms (Perfecto and Vandermeer 2002, Klein 2009, Moorhead et al. 2010), we found that spider abundance was negatively associated with distance to the nearest forest. However, this association was only present in the rainy season and was quite weak. Our results also show that coffee plantations have a diverse spider community and that over the dry season their species composition tends to be different from the forest sites. Remarkably, over the rainy season, the subtle separation in species composition between forest and coffee sites disappeared, thus suggesting that at this time of the year spider species are moving more between the forest and coffee plantations probably because of the greater species pool present in the rainy season.

The effect of elevation on species richness has been studied extensively because both productivity and species richness tend to decline with elevation (Rahbek 2005). However, the relationship between elevation and abundance has been studied less often. Consistent with our results two other studies in the tropics have found that spider abundance increases with elevation (Russellsmith and Stork 1994, Stenchly et al. 2011). Stenchly et al. (2011) reported that the abundance of spider webs in cacao trees increased with elevation. Likewise, Russellsmith and Stork (1994) found that arboreal spider density went up as elevation increased. It is possible that these results are due in part to the relatively short elevational range covered by these studies (Russellsmith and Stork [0-1350 masl], Stenchly et al. [400-950 masl], this study [800-1450 masl]).

In conclusion, we found that the pattern of accumulated spider richness across habitat changed over seasons, thus suggesting that spiders tended to favor the high shade coffee sites in the dry season, but not in the rainy season. Simultaneously, mean local spider richness and abundance are or tend to be higher in the low-shade coffee than in the two other habitats. A final caution must be added; although local spider richness and abundance tended to be higher in the low shade system, these are not the most intensive of all sun coffee management regimes. We suggest that it is the strategy of keeping trees in the coffee plantations and pruning them constantly that may support this increase in spider diversity

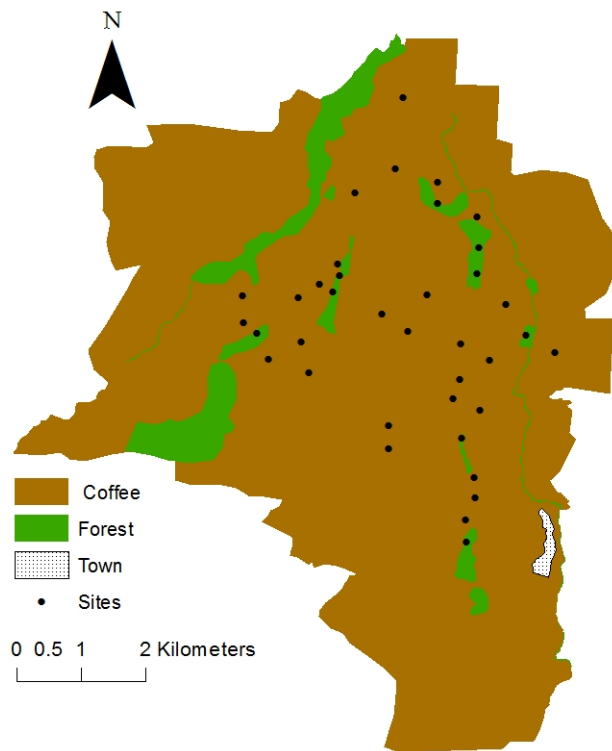


Figure 1.1. Coffee landscape in the Soconusco region, in Chiapas, Mexico. Dots represent sampled sites

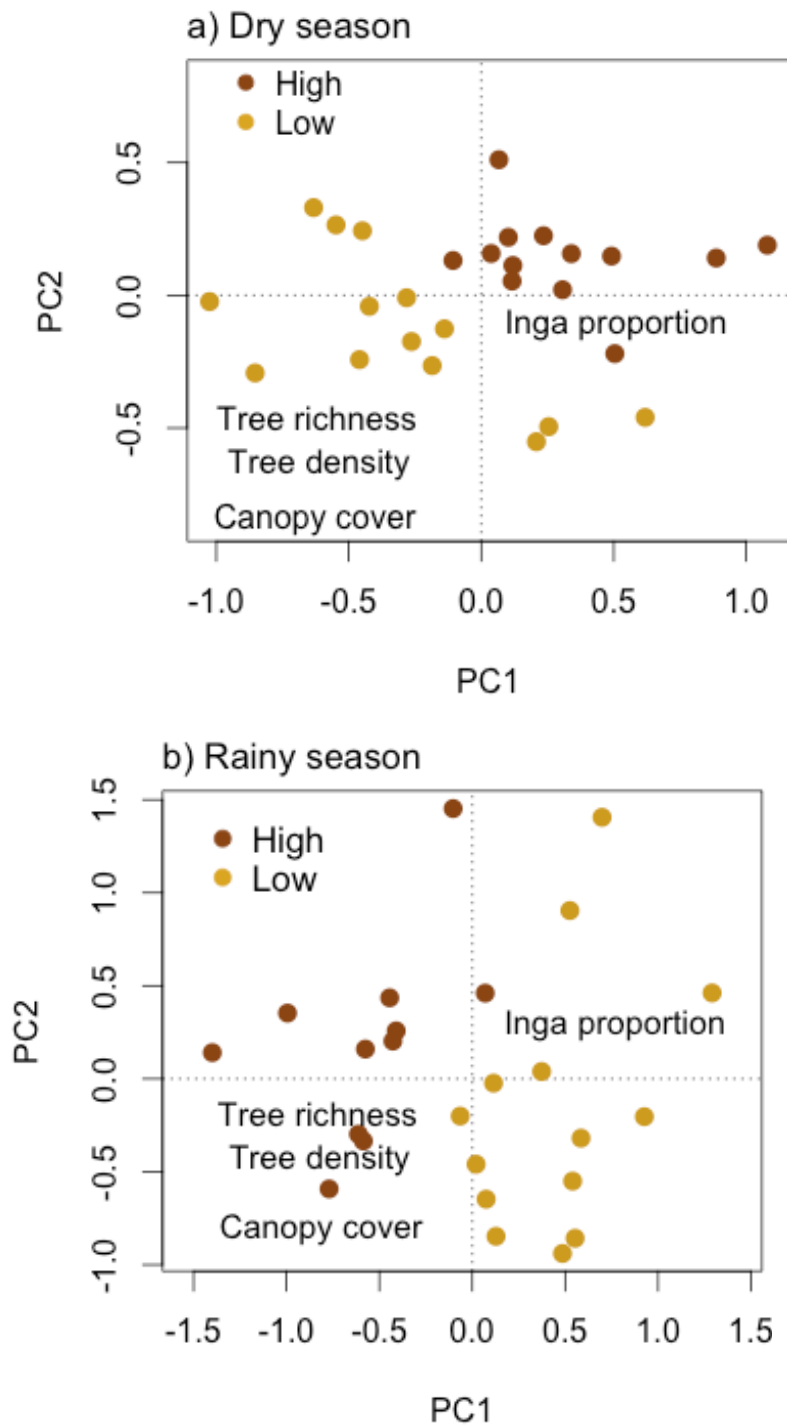


Figure 1.2. Coffee sites separated in low-shade (yellow) and high-shade (dark brown) according to the PCA based on tree richness, tree density, canopy cover and proportion of *Inga* trees (axis 1) and canopy cover (axis 2)

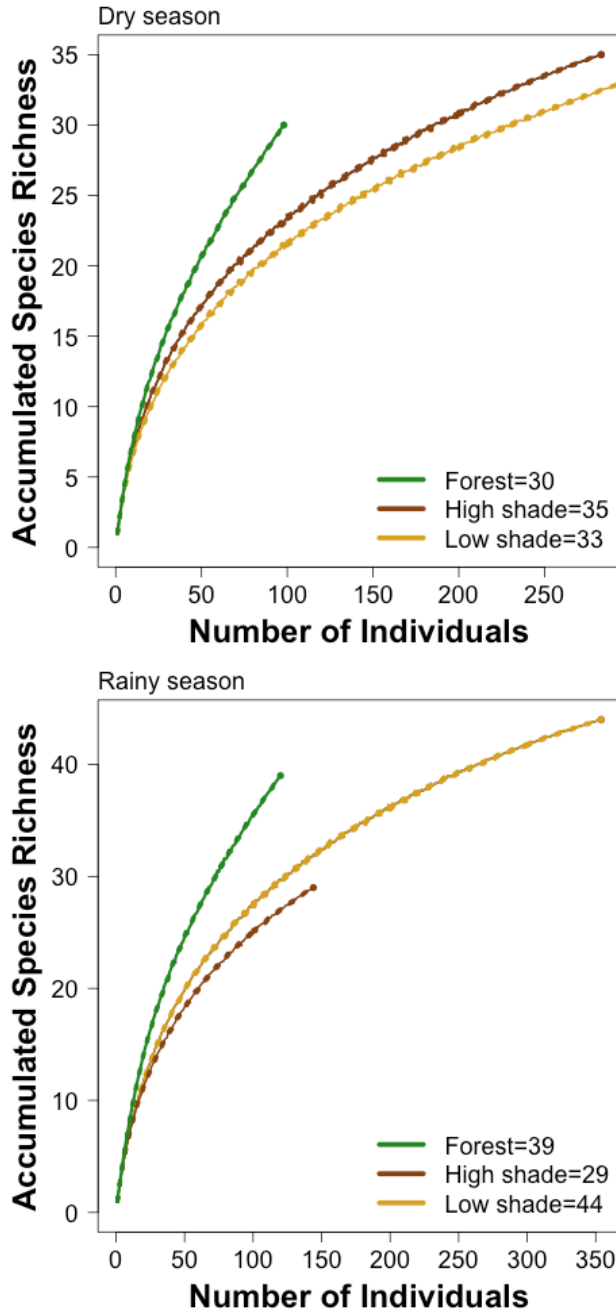


Figure 1.3. Individual based rarefaction curves for high-shade, low-shade and forest habitats in both the dry and rainy seasons

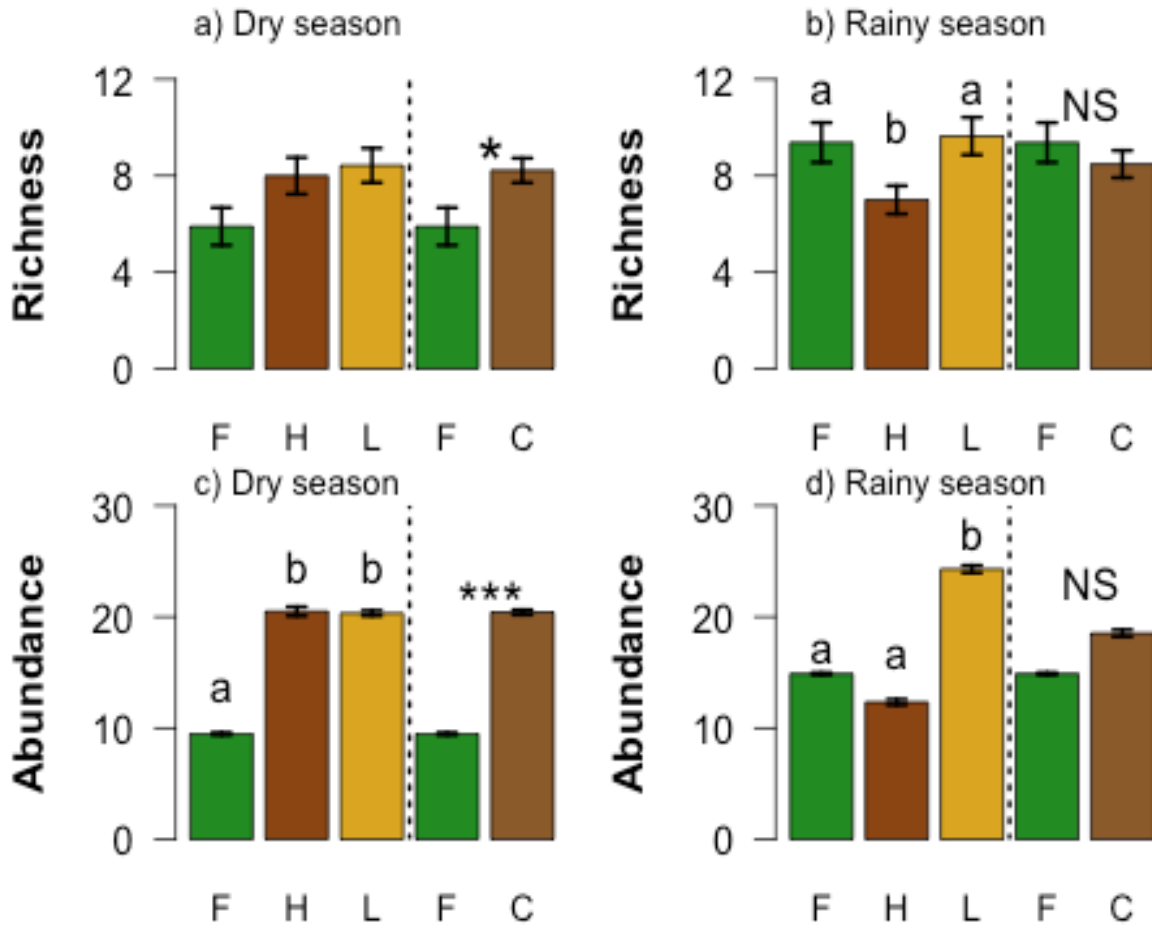


Figure 1. 4. Average spider richness and abundance in high-shade coffee (H), low-shade coffee (L) and forest (F) sites in both dry (a) and rainy (b) seasons. C stands for both high- and low-shade-coffee sites pooled together

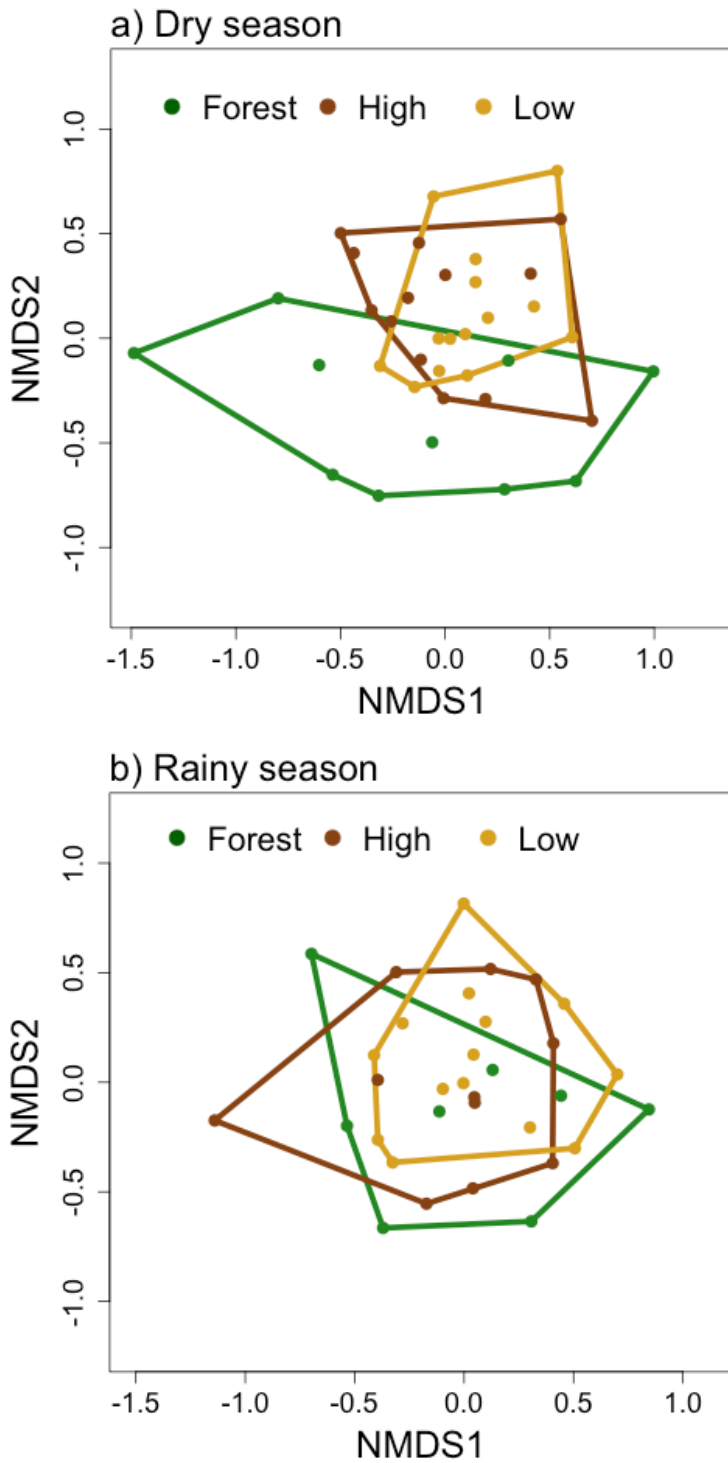


Figure 1.5. Non-metric multidimensional scaling plots for the ground spider community found in the coffee landscape over the dry (a) and rainy (b) seasons

Table 1.1. Habitat characteristics in 2011 for both dry and rainy seasons in forest and coffee sites in a coffee landscape in Southern Mexico

	Forest	High shade	Low shade	F/t statistic	P value
<i>Management variables</i>					
Canopy cover (%) dry season	96.45 ^a ± 1.42	77.04 ^b ± 3.11	41.37 ^c ± 3.91	72.15	<0.0001
Canopy cover (%) rainy season	95.83 ^a ± 1.83	74.97 ^b ± 2.91	31.16 ^c ± 4.81	68.52	<0.0001
Tree species dry season	21.5 ^a ± 1.61	6.073 ^b ± 0.52	4.42 ^b ± 0.71	73.86	<0.0001
Tree species rainy season	20.7 ^a ± 1.95	6.90 ^b ± 0.65	3.85 ^c ± 0.44	77.15	<0.0001
Tree density dry season	46 ^a ± 2.97	13.69 ^b ± 1.49	8.21 ^c ± 0.85	112	<0.0001
Tree density rainy season	45.75 ^a ± 3.89	14.82 ^b ± 1.66	8.43 ^c ± 0.94	80.3	<0.0001
Proportion of Inga trees dry season		0.47 ^a ± 0.04	0.65 ^b ± 0.07	2.05	0.051
Proportion of Inga trees rainy season		0.46 ^a ± 0.04	0.69 ^b ± 0.07	3.18	0.04
<i>Local variables</i>					
Leaf litter depth (cm) dry season	4.91 ^a ± 0.38	4.04 ^b ± 0.29	3.26 ^b ± 0.28	5.83	0.006
Leaf litter depth (cm) rainy season	3.67 ± 0.41	4.05 ± 0.40	4.17 ± 0.29	0.22	0.66
Leaf litter dry biomass (gr) dry season	59.33 ± 5.42	52.5 ± 4.73	46.49 ± 2.94	2.13	0.13
Leaf litter dry biomass (gr) rainy season	41.19 ± 4.40	37.85 ± 3.49	45.30 ± 5.87	0.53	0.6
Soil cover (%) rainy season	75.59 ^a ± 4.74	87.18 ^b ± 2.85	88.41 ^b ± 2.99	3.81	0.03
Invertebrate dry biomass (gr/m ²) dry season	0.31 ± 0.04	0.351 ± 0.04	0.23 ± 0.03	2.62	0.08
Invertebrate dry biomass (gr/m ²) rainy season	0.37 ± 0.13	0.32 ± 0.04	0.44 ± 0.07	0.69	0.51
Orthopteran abundance rainy season	28.87 ^a ± 1.28	33.7 ^a ± 0.8	66.09 ^b ± 6.1	*	0.02
<i>Landscape variable</i>					
Distance to forest (m) dry season		294.8 ^a ± 67.6	604.14 ^b ± 75.4	-3.05	0.005
Distance to forest (m) rainy season		387.8 ± 90.1	488.21 ± 68.2	0.88	0.385
<i>Environmental variables</i>					
Elevation (masl) dry season	1005 ± 64.7	944.5 ± 39.3	983.4 ± 37.3	0.42	0.66
Elevation (masl) rainy season	1049 ± 76.1	904.2 ± 25.7	1004 ± 42.7	2.3	0.12
Slope of the terrain (°) dry season	30.31 ^a ± 2.5	18.70 ^b ± 2.6	15.99 ^b ± 1.7	9.9	<0.0001
Slope of the terrain (°) rainy season	28.80 ^a ± 2.8	15.7 ^b ± 2.7	16.51 ^b ± 1.7	8.6	0.001

* GLM with negative binomial distribution does not provide an F statistic

Table 1.2. Potential drivers of spider richness in a coffee landscape during the dry and rainy seasons of 2011

Season	Variable	Estimate	Standard error	P-value	R ²	% change
Dry	Intercept	10.24	0.97	<0.0001	0.20	
	Slope (°)	-0.13	0.042	0.005		
Rainy	Intercept	2.65	0.210	<0.0001		0.57
	Canopy cover (%)	-0.0057	0.0024	0.017		
	Distance to forest (m)	-0.00046	0.0002	0.069		

Table 1.3. Potential drivers of spider abundance in a coffee landscape during the dry and rainy season of 2011

Season	Variable	Estimate	Standard error	P-value	% of change
Dry	Intercept	4.11	0.176	< 0.0001	
	Slope (°)	-0.05	0.005	< 0.0001	-4.45
	leaf litter biomass (gr)	-0.01	0.003	0.03	-0.69
Rainy	Intercept	2.21	0.42	< 0.0001	
	Canopy cover (%)	-0.013	0.0026	< 0.0001	-1.28
	Elevation (m)	0.0013	0.0004	< 0.0001	0.13
	Distance to forest (m)	-0.0006	0.0003	0.028 *	-0.06

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CHAPTER 2

SPIDERS RESPOND TO TREE CHARACTERISTICS AND AGGRESSIVE ANTS IN A COFFEE LANDSCAPE

Abstract

Spiders are a diverse predatory taxon in agroecosystems. However, knowledge about how local management and distance to forest influence spider diversity in tropical agroecosystems is limited. Intensification in coffee agroecosystems includes the elimination of a diverse canopy and its substitution with *Inga* trees, which tend to have smaller tree diameters and straight trunks with few indentations. Thus *Inga* trees may offer fewer nesting sites for web building spiders and also less hiding spaces for hunting spiders. Here we ask the following questions. What are the relationships between tree characteristics and the spider diversity associated with the trunks of shade trees? Do aggressive ants influence the spider community and biomass? Does the species richness and abundance of spiders vary with percent of canopy cover and distance to forest? In a coffee landscape in Southern Mexico we studied spider richness, abundance and biomass associated with trunks of shade trees with and without the aggressive ants *Azteca instabilis* in 2011 and 2012. We first located trees that had an *A. instabilis* nest in the trunk and then paired them with another tree from the same species with no *A. instabilis*

nest. For each tree, we counted the number of concavities (defined as the number of indentations and spaces between buttresses) found in the trunk, measured the diameter at breast height, and recorded tree identity. Results show that local spider richness, abundance and biomass were positively correlated with the number of concavities and tree diameter. In addition, spider abundance and biomass were higher in the presence of *A. instabilis*. Furthermore, *Inga* trees had significantly fewer concavities and smaller diameters at breast height than did non- *Inga* trees and supported significantly lower local spider richness, abundance, and biomass. Spiders showed no relationships with canopy cover, and distance to forest. Results show that *Inga* trees, a key component of coffee agricultural intensification, do not support a diverse spider community probably because these trees are smaller than other shade trees. This study suggests that diverse agroforests, with their consequent diversity in tree characteristics would positively affect the spider community by including variation in tree characteristics and size.

Key words: tree trunk spiders, landscape, local factors.

Introduction

Spiders are an abundant and species rich taxon of generalist predators that can affect herbivore populations and ecosystem function in natural ecosystems (Schmitz 2008, Hawlena et al. 2012) and agroecosystems (Wise et al. 1999, Schmidt et al. 2003). Because herbivore population control is a key ecosystem service in agroecosystems, it is important to know how agricultural management influences the distribution, richness and abundance of one of the most diverse predatory groups in agroecosystems (Denys and Tscharrntke 2002, Merfield et al. 2004, Teodoro et al. 2011).

Studies on the influence of agricultural management on spider diversity are contradictory. Some studies show that spider diversity is negatively affected by management intensification (Bisseleua et al. 2013) whereas others show that spiders are not negatively affected (Chen et al. 2011). For example, in agroforestry systems, where the percent of canopy cover or shade tree density is a handy tool for measuring intensification, some studies have reported that spider richness decreased in the most intensified systems (i.e. less canopy cover) whereas other have reported the opposite. Stenchly et al. (2011) found that local spider webs in cacao trees increased with shade tree density (see also Stenchly et al. 2012), whereas Pinkus-Rendon et al. (2006a) and Marin and Perfecto (2013) reported that local spider richness and abundance increased in the most intensive system (i.e. less percent of shade canopy cover).

Other studies have found significant correlations between spiders and other predators in the agroecosystems, specifically aggressive arboreal ants. Stenchly et al. (2011) reported that the abundance of spider webs on cacao trees increased with the

presence of *Philidris* ants and Marin and Perfecto (2013) found that local spider richness and abundance in coffee plants in coffee agroecosystems from Southern Mexico increased in the presence of the arboreal ant *Azteca instabilis*.

Most studies in agroforestry systems have focused upon spiders in the crop (coffee or cacao) layer and the leaf-litter layer, but none has explored the tree component of the agroforestry system, although this is an important component to examine because intensification of agroforestry systems usually involves a reduction in tree species richness and density. For example, in coffee agroforestry systems the trend is to increase the proportion of *Inga* trees at the expense of other shade tree species (Romero-Alvarado et al. 2002). Furthermore, our field observations in the Soconusco region suggested that *Inga* trees have smaller tree diameter and straight trunks with fewer concavities (number of indentations and spaces between buttresses) than other trees, thus suggesting that potentially *Inga* trees offer fewer nesting and hiding sites for web building and hunting spiders respectively.

Here we ask the following questions. What are the relationships between tree characteristics and spider diversity? Do *A. instabilis* ants influence the spider community and biomass associated with the trunks of shade trees? Does local species richness or abundance vary with percent of canopy cover, distance to the nearest forest fragment, or elevation? We predict that local spider richness; abundance and biomass increase with diameter at breast height and number of concavities on the trunk because trees with a complex trunk structure and larger diameters may offer more nesting and hiding sites. Although other studies have reported a positive association between spiders and ants in agroforestry systems, we predict that spiders would be negatively associated with *A.*

instabilis ants. We expect a negative association because these aggressive ants build their nests on the trunks of the trees and in close proximity with trunk dwelling spiders, thus the ants might chase away the spiders. We also predict that spider richness and abundance increases with increases in canopy cover, an indirect indicator of tree density and richness, to which tree trunk dwelling spiders must be sensitive. Finally, we predict that spider richness and abundance is negatively related with distance to the forest.

Material and Methods

Study site

Our study took place in a coffee landscape located in the Soconusco region in the Southern state of Chiapas, Mexico. This coffee landscape covers a surface of 62 km² (coordinates 15.202 N, 92.383 W [NW corner], 15.144 N, 92.297 W [SE corner]) of which 94 % are covered by large coffee farms (~300 ha) and only 6 % is covered by small remnants of evergreen tropical forest (Philpott et al. 2008). This landscape shows a marked seasonality characterized by a dry season from December through May and a rainy season from May to November. The studied landscape covers an elevation gradient that ranges from 672 to 1189 meters above sea level and receives ca. 4500 mm annual precipitation. The distance to the nearest forest fragment ranged from 68 to 996 m.

Tree and plot characteristics

In 2011 we carried out a preliminary study and sampled spiders from sixteen pairs of shade trees for a total of thirty-two shade trees distributed in three coffee farms. We first located trees that had an *A. instabilis* nest in the trunk and then paired this tree with another tree from the same species located at least 6 m away and with no *A. instabilis* nest. In total we sampled 8 shade tree species with *Alchornea latifolia* being represented

in 4 tree pairs, *Syzygium jambos* in 3 tree pairs, *Cordia* sp, *Ocotea* sp., and *Inga rodrigueziana* in 2 tree pairs each, and *Cedrella odorata*, *Zanthoxylum* sp., and *Inga micheliana* in 1 tree pair each. In 2011, the distance between trees belonging to same pair was between 6m and 30 m, whereas the distance between trees with *A. instabilis* nests was approximately 50 m.

In 2012, we sampled 19 one-hectare circular plots spatially distributed in the landscape and selected 35 pairs of trees, with and without *A. instabilis* nests, for a total of 70 trees. Eighteen of the tree pairs belonged to the genus *Inga*, the most common tree planted in coffee farms in Mexico, and 17 were non-*Inga* pairs. We sampled 3 *Inga* species, with *I. rodrigueziana* in 12 tree pairs, *I. paterna* in 5 tree pairs and *I. micheliana* in 1 tree pair. The non-*Inga* species were distributed in 7 species. *S. jambos* was in 4 tree pairs, *Tabebuia aurea* and *Yucca guatemalensis* were in 3 tree pairs each; *Alchornea latifolia* and *Ficus* sp. were in 2 tree pairs each; and *Ocotea* sp. and *Persea* sp. were in 1 tree pair each. We identified active nests of *A. instabilis* by hitting trees with a stick and eliciting the swarming behavior of the ants. All but three of the one-hectare circular plots contained two pairs of trees, an *Inga* pair and a non-*Inga* pair. Three plots contained only one pair of trees. In 2012 the distance between trees belonging to same pair was between 10-15 m and the distance between trees with *A. instabilis* was approximately 25 m.

In both years for each sampled tree we recorded its species, diameter at breast height (cm) and number of concavities (including indentations and the space between tree buttresses) in the trunk starting at 0 m and ending at 2.5 m above ground. In 2012, for each 1-ha plot we measured percent of canopy cover at three points established 15 m

away from the center of the plot and at 0°, 120° and 240°. At each point, canopy cover readings were taken in the four cardinal directions with a hand held densiometer. In addition, for each plot we measured the distance to the nearest forest edge using a Geographic Information System (GIS) (Philpott et al. 2008). Elevation was taken with a GPS unit (Garmin GPS 60).

Spider sampling

In 2011, we sampled the tree trunks in search of spiders and their webs from zero up to 2.5 meters height. Spider individuals were collected by hand except the individuals of *Ischnothele digitata*, which we were able to identify in the field. Individuals of *I. digitata* (adult average body size ~ 14 mm) although concealed inside of their webs, have whitish funnel webs that are easy to count and identify in the field (Coyle 1995); we collected a few specimens and their webs to verify identity and thereafter counted webs. Whenever possible, we ensured that *I. digitata* webs were occupied by disturbing the spider with one aluminum wire that was inserted into the funnel web. Spider collection was conducted from July 25 to July 30 and from August 8 to 9.

In 2012, we modified our sampling method because we suspected that the visual sampling of trees in 2011 could have biased our results by omitting the very small spiders, which represent the majority of spider individuals. We initially decided to sample all trees using a beating sheet and a paintbrush. We swept the trunk with the paintbrush and falling spiders were collected in the beating sheet. However, samples from the trees with *A. instabilis* returned primarily ants and some spiders that were damaged. Therefore, we decided to discard these samples and modify the sampling method. Trees without an *A. instabilis* nest were sampled using the paintbrush and beating sheet. For trees with an

A. instabilis nest, we collected spiders by carefully looking in crevices and bark irregularities and hand collected the spiders. Before and during collections we carried out field observations on the spiders found in the presence/absence of *A. instabilis* and in some selected cases we collected their webs. Although this difference in methods could have affected our results, we think that instead it allowed us to get a good grasp of the interactions that take place in the tree trunks since in order to try to make both methods comparable we spent a good amount of time sampling the both trees with and without *A. instabilis*.

We preserved all spiders in 95% alcohol and identified all spiders to family using The spiders from North America (Ubick et al. 2005) and to species and morphospecies following selected literature (Platnick 2013). Voucher specimens were deposited in the Arachnological Collection at El Colegio de la Frontera Sur in Tapachula. For each collected spider we measured its body length in mm and estimated dry biomass (mg) using the body length-dry biomass equations reported elsewhere for spiders (Hofer and Ott 2009). In the case of *I. digitata* and *Azilia guatemalensis* for which we did not collect all individuals, we used the modal body length (*I. digitata* = 5 mm) and average body length (*A. guatemalensis* = 3.8 mm) obtained from 10 individuals collected for identification. For *I. digitata*, we used the modal instead of the average body length because two collected adults biased the average value. Both the modal and average values were smaller than the values reported in the literature (Coyle 1995, Alvarez-Padilla 2008) and thus our biomass estimates are conservative.

Data analysis

To evaluate whether tree characteristics (diameter at the breast height and number of concavities) varied between *Inga* and non-*Inga* trees we used t-tests. More specifically, we tested differences in diameter at breast height and number of concavities on the trunk on each shade tree between the *Inga* and the non-*Inga* trees. To evaluate whether spider richness, abundance and biomass varied with tree characteristics, presence of *A. instabilis*, percent of canopy cover, and distance to the forest we analyzed data with generalized linear models. The response variables were spider richness, spider abundance (or its surrogate, webs, in the case of the whitish funnel web), and spider biomass and the predictor variables were number of concavities in the trunk of the sampled tree, diameter at breast height (cm), elevation (m), presence/absence of *A. instabilis*, percent of canopy cover (only for 2012) and distance to the nearest forest fragment (only for 2012). For both years we tested differences in the average spider body length in the presence and absence of *A. instabilis* with t-tests.

We checked the distribution of the residuals of the response variables by two means. First we plotted the standardized residuals vs. the leverage. Secondly, we used the Cook's distance as a mean for identifying any influential points, with a cutoff value of 0.5. Error distribution for richness data was normal and therefore we used multiple linear regressions. However, residual distribution of abundance and biomass data were not normally distributed, therefore we used a generalized linear model with a negative binomial distribution for abundance data, whereas for biomass we used two types of transformations. For 2011, we used the Box-Cox transformation and for 2012 we used the natural logarithm. We applied different transformations to the biomass data because the same transformation did not normalize it across years.

Finally, for analyzing whether the spider community associated with tree trunks differed with the presence of *A. instabilis* we used non-metric multidimensional scaling (NMDS) followed by Analysis of Similarities (ANOSIM). NMDS is a technique widely used for comparing species composition across habitats/conditions (Winfree et al. 2008, Borcard et al. 2011, Hogg and Daane 2013). This technique creates a species composition distance matrix between sites based on a similarity index (in this case the Bray-Curtis similarity index), which measures how close two points are in regards to species composition. Then sites are ranked among them according to their differences and these ranked differences are considered for evaluating the similarity between sites through iterative processes. Whereas ANOSIM is a test that assess the significance of the difference in species composition between habitats/conditions. ANOSIM provides an R-value that indicates the degree of similarity; values close to 1 indicate low similarity among habitats/conditions whereas low values of R indicate high similarity. All analyses were carried out with R software (R Core Team 2012) using the packages MASS and Vegan.

Results

Tree characteristics

Tree characteristics were significantly different between *Inga* and non-*Inga* trees. Non-*Inga* trees had more concavities in the trunk (19.53 ± 0.56) than *Inga* trees (6.79 ± 0.33) (t-test = -6.42, $p < 0.0001$, Fig. 2.1a). Likewise non-*Inga* trees had larger diameter at breast height (66 ± 3.26) than *Inga* trees (39.99 ± 2.73) (t-test = -4.83, $p < 0.0001$, Fig. 2.1b). Furthermore, spider richness, abundance and biomass were significantly higher in the non-*Inga* trees than in the *Inga* trees (Fig. 2.2).

Spider diversity

Overall, considering both years we had 1786 abundance records (1216 individuals and 570 *I. digitata* funnel webs) distributed across 74 spider species associated with the trunks of the shade trees in coffee farms. In 2011 we had 673 abundance records (490 individuals and 183 *I. digitata* funnel webs) across 40 species whereas in 2012 we had 1113 records (726 individuals and 387 *I. digitata* funnel webs) across 64 species. Over the two years the most abundant species were *Ischnothele digitata* (570 webs, 33 % of all individuals/webs), *Azilia guatemalensis* (305 individuals, 17 %), *Tidarren* sp. (106 individuals, 6 %), *Philoponella saginella* (73 individuals, 4 %) and *Mysmenopsis palpalis* (73 individuals, 4%).

Spider richness

In 2011, spider richness on the trunks of the shade trees was positively correlated with the number of concavities on the tree trunks and negatively related to elevation (Table 2.1).

In 2012, spider richness was positively related to the number of concavities but not to any other variable. In both years the relationship between spider richness and *A. instabilis* was marginally significant but in different directions. While in 2011 spider species richness showed a trend toward a positive association with *A. instabilis*, in 2012 the trend was toward a negative association.

Spider abundance

In 2011, spider abundance was strongly and positively associated with the presence of an *A. instabilis* nest on the shade trees as well to the number of concavities, but negatively related to elevation (Table 2.2). With the presence of an *A. instabilis* nest on the trunk of a shade tree, spider abundance was 74%, higher than in the absence of a nest. Spider

abundance increased by 7.87% with each concavity in the trunks. In 2012, spider abundance was again strongly positively related to the presence of an *A. instabilis* nest in the trunk of shade trees, as well to the number of concavities and tree diameter at breast height. With the presence of an *A. instabilis* nest on the trunk of a shade tree, spider abundance was 54% higher than in the absence of a nest. Spider abundance increased by 3.05% with each concavity in the trunks and by 1.5% with each cm in the diameter at breast height (Table 2.2). However, the exclusion of *I. digitata* and *A. guatemalensis*, the two most abundant spiders changed the previous pattern. In 2011 the exclusion of *I. digitata* and *A. guatemalensis* from the data set eliminated the positive correlation of spider abundance and *A. instabilis* (Table 2.2), whereas in 2012, the exclusion of *I. digitata* made the correlation to vanish.

Spider biomass

In 2011 and 2012, spider biomass was positively related to the presence of an *A. instabilis* nest on the shade trees and to the number of concavities on the trunks (Table 2.3). As with spider abundance, the positive relationship between spider biomass and *A. instabilis* was strongly dependent upon *I. digitata* and *A. guatemalensis*. In both years, when only other species of spiders were considered, the effect of *A. instabilis* disappeared (Table 2.3).

Species composition and body length in relation to A. instabilis

Species composition varied across trees that had an *A. instabilis* nest and those that did not have a nest. For 2011, the NMDS plot and the R ANOSIM value show that the spider assemblage were significantly different in those trees that had an *A. instabilis* nest and those that did not have one ($R = 0.53, p = 0.001$, Fig. 2.3a). For 2012, the two

assemblages were more similar but still showed some level of segregation ($R=0.24$, $p = 0.001$, Fig. 2.3b).

In 2011, average body length (mm) did not vary significantly between spiders collected in the presence of *A. instabilis* (5.59) and its absence (5.18) (t-test = -0.38, $p = 0.7$). However, in 2012, average body length (mm) was significantly higher for spiders collected in the presence of *A. instabilis* (3.5 ± 0.13) than in its absence (2.57 ± 0.15) (t-test = -4.53, $p < 0.0001$).

Discussion

Our study shows that in coffee agroforestry systems, the species richness of spiders found on the trunk of shade trees increases with the number of concavities found on those trees. In addition, we found that spider abundance and biomass are positively associated with tree characteristics, such as the number of concavities and diameter at breast height, and to the presence of *A. instabilis* ants. Surprisingly, spider richness, abundance and biomass were unrelated to canopy cover or distance to forest.

Tree trunks are a poorly explored section of trees in both natural ecosystems (Michel et al. 2011) and agroecosystems, and this is the first study to examine the spiders that inhabit the trunks of trees in tropical agroforestry systems. The result that tree concavities can contribute to spider richness, abundance and biomass (Fig. 2.2) is an important one because it suggests that tree characteristics, and not just tree density, are important for maintaining a diverse and abundant tree spider assemblage in forestry and agroforestry systems (Bisseleua et al. 2013). Indeed, a well-established literature has reported that spiders are sensitive to plant structure (Wise 1993, de Souza and Martins

2005, Sanders et al. 2008, Pearson 2009, Podgaiski et al. 2013), and our results indicate that larger trees with more concavities harbored more species richness, abundance and biomass, probably because trees with these characteristics offer a more complex surface upon which spiders may attach their webs, find prey or hide.

Our expectations about a potential negative effect of *A. instabilis* on spider richness did not hold. Over the two years of study, we found no significant relationship between local spider richness and *A. instabilis*. This result differs to that which we found in the coffee layer, where local spider richness and abundance increased in the presence of *A. instabilis*. Probably, this is because trees with *A. instabilis* were dominated by just a few species. In regards to *A. instabilis* and spider abundance and biomass, our expectations did not hold either. Rather, we found a dramatic increase in spider abundance and biomass in the presence of *A. instabilis* in both years. This result suggests that these ants are a key element for spiders associated with the trunks of shade trees.

The relationships between *A. instabilis* and spider abundance and biomass seem to be driven by *I. digitata* and *A. guatemalensis* as statistical relationships were eliminated when one or both of these species were removed from the dataset (Table 2.2). A possible explanation for the higher abundance of these two spider species in the presence of *A. instabilis* ants is that these spiders significantly prey upon the ants. *Ischnothele digita* is a subsocial funnel web builder that likes to build its web in crevices, such as road banks but also on the trunks on trees (Coyle 1995). Coyle (1995) also reported that *I. digitata* preys mainly upon ants and beetles, which we corroborated in field observations and by inspecting some webs (online supplementary materials Video 1). In our study site, we found *I. digitata* almost exclusively on tree trunks that have *A. instabilis* nests, and

infrequently in road banks. *Azilia guatemalensis* was the second most abundant spider on the trunks of shade trees and was also strongly associated with *A. instabilis*. *A. guatemalensis* (adult average body size 6 mm) builds horizontal orb webs in trees (Alvarez-Padilla 2008). *A. guatemalensis* was also found on trees without *A. instabilis*, but at lower numbers. However, most of the times that we found *A. guatemalensis* in trees without *A. instabilis*, there were other ants nesting on the trunks, such as *Crematogaster*, suggesting that *A. guatemalensis* has a preference for tree trunks with ants.

Spider assemblages in the presence of *A. instabilis* were not dramatically different from those in its absence. Nonetheless, there were statistical differences between the spider communities in both years of study (Figure 2.3). Even more, for 2012, the year for which we have the most complete set of spiders the NMDS plot suggest that trees that did not have an *A. instabilis* nest showed a more diverse spider community than trees with *A. instabilis*. Indeed, most of the *A. instabilis* points clutter among them and seem to be a subset of the trees without *A. instabilis*, except some points that reach far to the right. We think that these points are in part due to presence of some spiders, such as *A. guatemalensis*, that although reached high abundances in the presence of *A. instabilis*, they were also found in the absence of *A. instabilis*, probably because in those trees other ants were present.

We found no relationships between canopy cover or distance to forest, and spider richness, abundance and biomass; however we found a negative relationship between the response variables and elevation. Previous studies in the same coffee landscape have shown that the local richness and abundance of ground and coffee dwelling spider was

higher in low shade coffee systems. We suggest that these differences in spider response to canopy cover are probably because the tree trunk spiders studied here depend strongly on trees, so a decrease in shade, which is related to tree density, would be disadvantageous. Distance to the nearest forest fragment ranged from 68 to 996 m but was unrelated to spider community structure. Although some studies have reported a negative relationship between distance to the forest and richness and abundance of a variety of taxa (Klein et al. 2003, Baldissera et al. 2004, Klein 2009), the available information for spiders seems to suggest that in agroforestry systems distance to the forest it is not an important variable. For example, Stenchly et al. (2012) found that spider diversity in cacao agroforests did not vary with distance to nearest forest (see also Stenchly et al. 2011). Likewise Marín et al. (Chapter 1) did not find a correlation between distance to forest and the ground dwelling spider diversity in coffee agroforests over the dry season. Nonetheless, Baldissera et al. (2004) reported a very strong effect of distance to the forest on spiders at distances as short as 50m in a pasture system. Although, more studies are needed in order to clearly elucidate the role of distance to the forest on spider communities, the available literature suggest that in agroforests, that have relatively high matrix quality compared to pasture systems, distance to forest might not be a very significant predictor of spider diversity.

The relationship between elevation and spider diversity changed across years. In 2011, we found a negative relationship between elevation and spider richness and abundance, whereas neither relationship was significant in 2012. We suggest that this difference is because in 2011 we found fewer species, and for those spiders elevation was a key factor.

Overall, this study shows that in coffee agroforests, local tree trunk spider richness, abundance and biomass are positively associated with tree diameter, number of concavities on the trunk, and the presence of *A. instabilis* ants and negatively associated with *Inga* trees. These results suggest that in order to keep a diverse community of spiders associated with the shade trees the conservation of diverse agroforestry systems, which tend to have larger trees and fewer *Inga* individuals, is required.

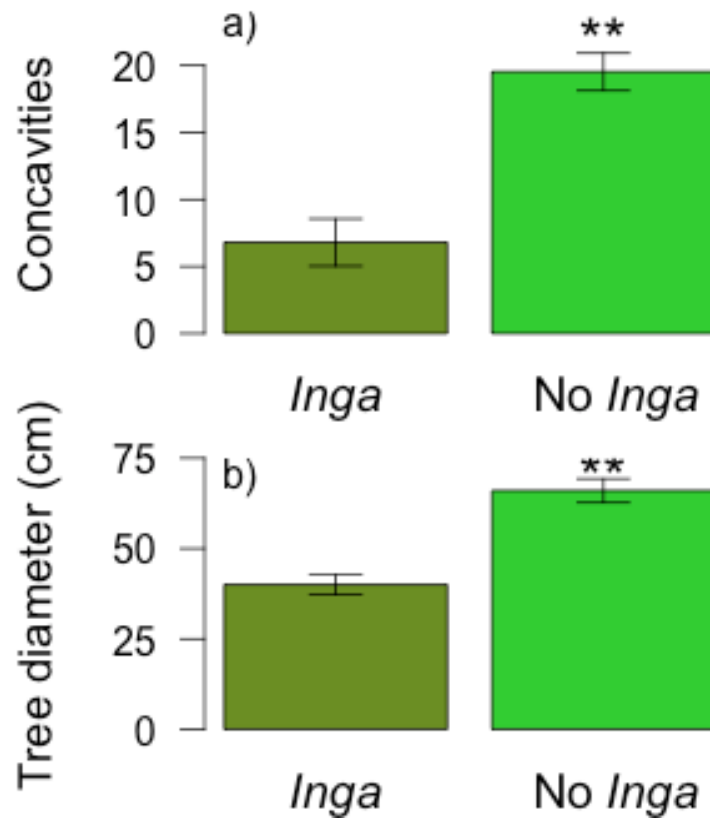


Figure 2.1. Differences in tree characteristics between *Inga* and non-*Inga* trees

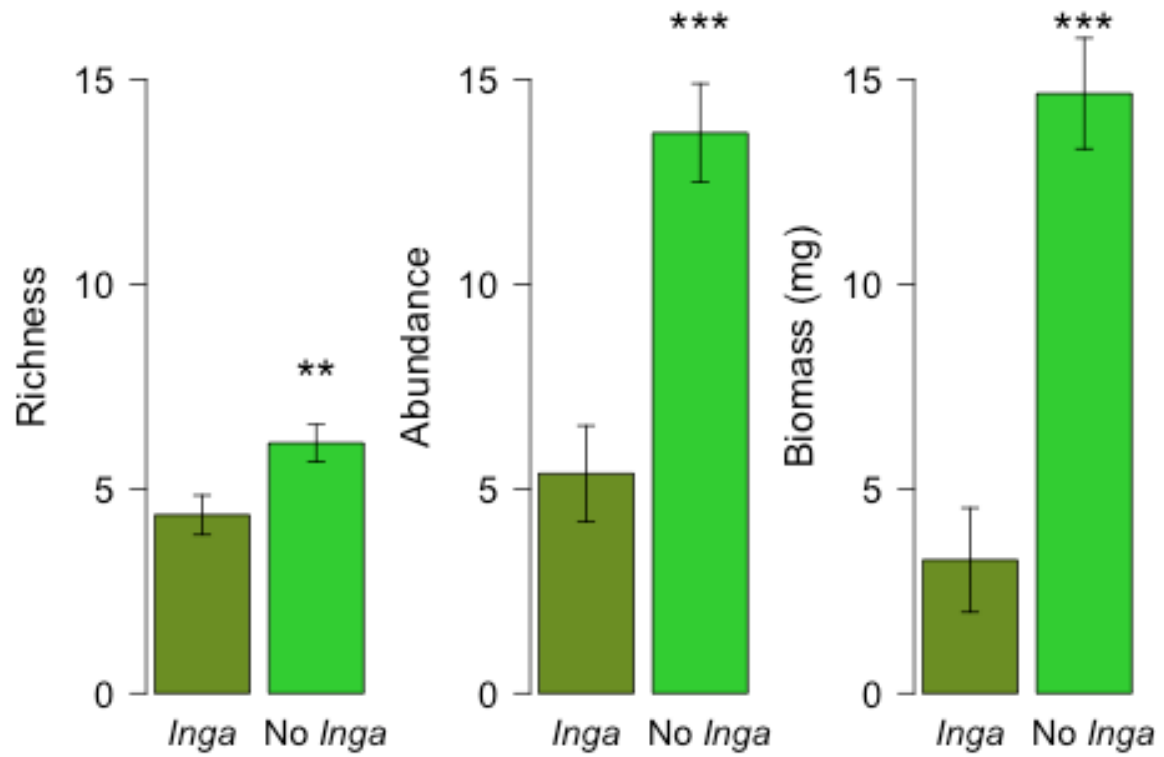


Figure 2.2. Spider richness, abundance and biomass found in *Inga* and non-*Inga* trees

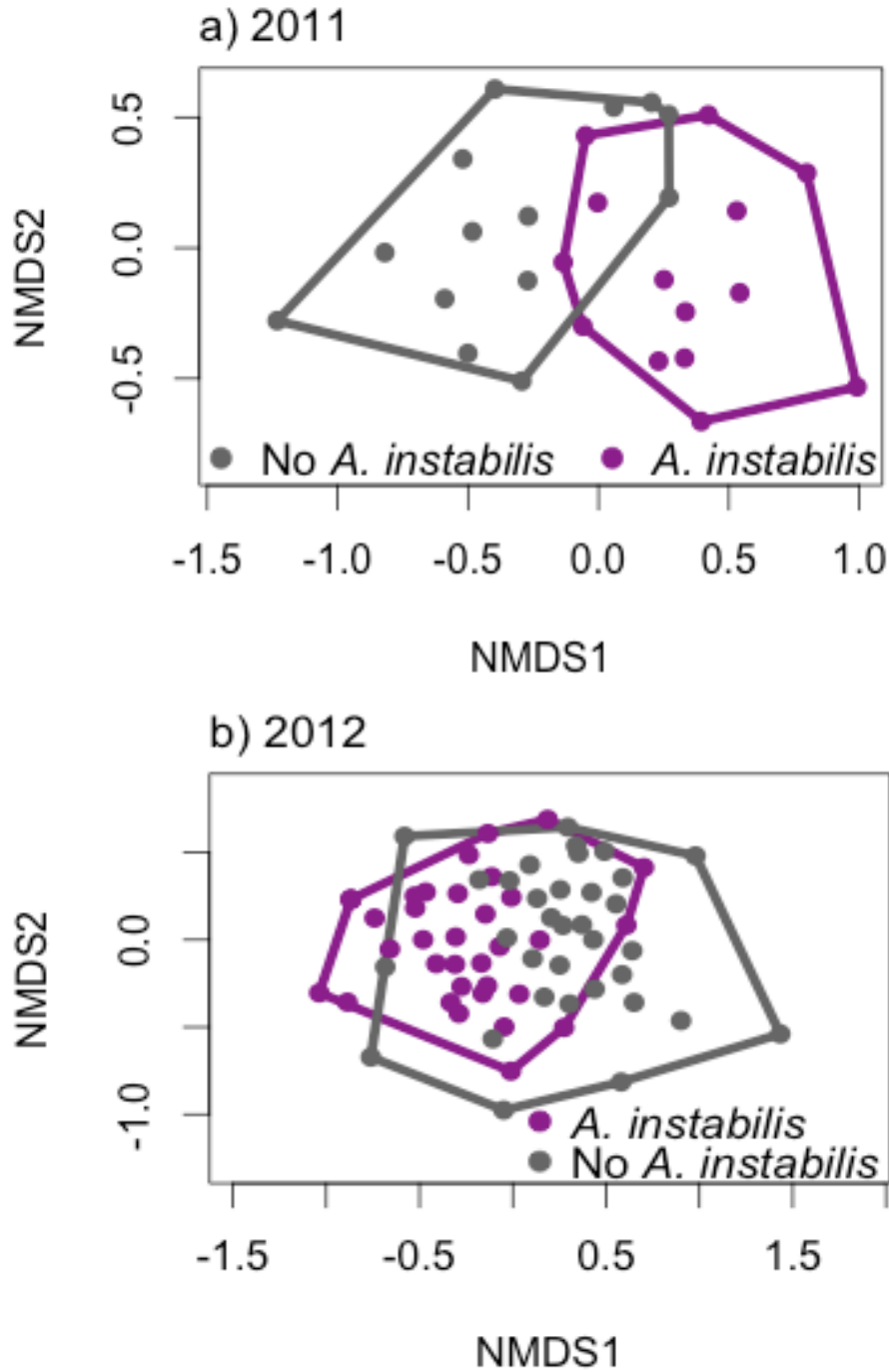


Figure 2.3. Non-metric multidimensional scaling plots showing spider species composition between trees with an *Azteca instabilis* nest and trees without it for 2011 (a) and 2012 (b)

Table 2.1. Associations between spider richness and tree characteristics, percent of canopy cover, distance to forest, elevation and the presence of *Azteca instabilis* ants

Year	Variable	Estimate	Std. Error	t-value	P-value	R ²	F statistic
2011	Intercept	16.03	4.448	3.60	0.001	0.572	9.03
	<i>A. instabilis</i>	1.71	0.865	1.98	0.058 .		
	Number of concavities	0.16	0.072	2.26	0.032 *		
	Diameter at the breast height (cm)	-0.0104	0.021	-0.50	0.621		
	Elevation (m)	-0.0150	0.004	-3.68	0.001 *		
2012	Intercept	4.37	3.02	1.45	0.15	0.23	3.09
	<i>A. instabilis</i>	-1.14	0.65	-1.75	0.08 .		
	Diameter at the breast height (cm)	0.020	0.015	1.35	0.18		
	Number of concavities	0.072	0.033	2.18	0.03 *		
	Elevation (m)	-0.002	0.003	-0.70	0.49		
	Distance to forest (m)	0.002	0.002	1.23	0.22		
	Canopy cover (%)	0.002	0.015	0.15	0.88		

Table 2.2. Associations between spider abundance and tree characteristics, percent of canopy cover, distance to forest, elevation and the presence of *Azteca instabilis* ants

Year	Variable	Estimate	Std. Error	z-value	p-value	% of change
2011						
	Intercept	4.79	1.39	3.4	0.0006	
	<i>A. instabilis</i>	1.36	0.27	5.0	<0.0001 ***	74.3
	Number of concavities	0.076	0.02	3.4	<0.001 **	7.87
	Diameter at the breast height (cm)	0.002	0.006	0.4	0.72	
	Elevation (m)	-0.005	0.001	-3.5	<0.0005 ***	-0.45
2012						
	Intercept	0.77	0.91	0.85	0.4	
	<i>A. instabilis</i>	0.45	0.19	2.35	0.02 *	57.45
	Number of concavities	0.03	0.01	3.13	0.002 **	3.05
	Diameter at the breast height (cm)	0.02	0.0044	3.45	0.0006 **	1.54
	Elevation (m)	0.0002	0.0009	0.24	0.8	
	Distance to forest (m)	-0.0004	0.0005	-0.82	0.41	
	Canopy cover (%)	0.004	0.0046	0.80	0.42	
2011						
Excluding <i>I. digitata</i> and <i>A. guatemalensis</i>						
	Intercept	7.51	1.623	4.63	<0.0001	
	<i>A. instabilis</i>	0.34	0.314	1.09	0.28	
	Number of concavities	0.07	0.026	2.57	0.01 *	7
	Diameter at the breast height (cm)	-0.004	0.007	-0.52	0.6	
	Elevation (m)	-0.007	0.002	-4.65	<0.0001 ***	-0.71
Excluding <i>I. digitata</i>						
2012						
	Intercept	0.93	0.78	1.19	0.23	
	<i>A. instabilis</i>	0.02	0.17	0.14	0.89	
	Number of concavities	0.03	0.01	3.15	0.002 **	2.7
	Diameter at the breast height (cm)	0.008	0.004	2.09	0.037 **	0.8
	Elevation (m)	0.0002	0.0007	0.23	0.82	
	Distance to forest (m)	0.0001	0.0004	0.35	0.73	
	Canopy cover (%)	0.0041	0.0039	1.06	0.29	

Table 2.3. Associations between spider biomass and tree characteristics, percent of canopy cover, distance to forest, elevation and the presence of *Azteca instabilis* ants

Year	Variable	Estimate	Std. Error	t-value	P-value	R ²	F
2011	Intercept	10.65	4.27	2.49	0.019 *	0.57	9.1
	<i>A. instabilis</i>	3.19	0.83	3.84	0.0007 ***		
	Number of concavities	0.177	0.069	2.57	0.016 *		
	Diameter at the breast height (cm)	0.011	0.020	0.57	0.57		
	Elevation (m)	-0.006	0.004	-1.52	0.14		
2012	Intercept	1.06	1.53	0.69	0.49	0.46	8.92
	<i>A. instabilis</i>	1.39	0.33	4.23	< 0.0001 ***		
	Number of concavities	0.056	0.017	3.30	0.002 **		
	Diameter at the breast height (cm)	0.012	0.0077	1.57	0.12		
	Distance to forest (m)	-0.0005	0.0008	-0.64	0.52		
	Elevation (m)	-0.0011	0.0015	-0.74	0.46		
	Canopy cover (%)	-0.0026	0.0078	-0.34	0.73		
Excluding <i>I. digitata</i> and <i>A. guatemalensis</i>							
2011	Intercept	16.30	3.57	4.6	<0.0001	0.43	5.12
	<i>A. instabilis</i>	-0.26	0.69	-0.4	0.7		
	Number of concavities	0.085	0.06	1.5	0.15		
	Diameter at the breast height (cm)	-0.006	0.02	-0.4	0.7		
	Elevation (m)	-0.011	0.003	-3.3	0.003 *		
2012	Intercept	1.03	2.09	0.49	0.62	0.16	2.13
	<i>A. instabilis</i>	0.06	0.45	0.13	0.90		
	Number of concavities	0.055	0.024	2.34	0.023 *		
	Diameter at the breast height (cm)	0.006	0.011	0.57	0.57		
	Distance to forest (m)	-0.0004	0.0010	-0.37	0.71		
	Elevation (m)	-0.001	0.002	-0.60	0.55		
	Canopy cover (%)	0.003	0.011	0.26	0.80		

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CHAPTER 3

SPIDER DIVERSITY IN COFFEE AGROECOSYSTEMS: THE INFLUENCE OF AGRICULTURAL INTENSIFICATION AND AGGRESSIVE ANTS

Abstract

Spiders are a very diverse group of invertebrate predators found in agroecosystems and natural systems. However, spider distribution, abundance and ultimately their ecological function in ecosystems can be influenced by abiotic and biotic factors such as agricultural intensification and dominant ants. Here we explore the influence of both agricultural intensification and the dominant arboreal ant *Azteca instabilis* on the spider community in coffee agroecosystems in Southern Mexico.

In order to evaluate the influence of the arboreal ant *Azteca instabilis* on the spider community inhabiting the coffee layer of coffee agroecosystems, spiders were collected from coffee plants that were and were not patrolled by the ant in sites differing in agricultural intensification. In 2008, generalized linear mixed models showed that spider diversity was positively affected by agricultural intensification but not by the ant. However, results suggested that some spider species were associated with *A. instabilis*. Therefore, in 2009 we concentrated our research on the effect of *A. instabilis* on spider

diversity and composition. In 2009, generalized linear mixed models showed that spider richness and abundance per plant were significantly higher in the presence of *A. instabilis*. In addition, visual counts of insects and sticky trap data show that more prey resources were present on plants patrolled by the ant. The positive effect of *A. instabilis* on spiders seems to be caused by at least two mechanisms: high abundance of insects and protection against predators.

Key words: Araneae, Formicidae, *Azteca instabilis*, predators.

Introduction

Agroecosystems with a low degree of intensification (high crop diversity, little use of synthetic agrochemicals), can act as reservoirs for biodiversity (Perfecto et al. 1996, Matson et al. 1997, Donald et al. 2001, Perfecto and Vandermeer 2002, Perfecto et al. 2003, Klein et al. 2008, Jha and Dick 2010). For example, coffee agroecosystems with low levels of agricultural intensification have been shown to support higher species richness of a variety of vertebrates, invertebrates and plants (Perfecto et al. 1996, Moguel and Toledo 1999, Armbrrecht and Perfecto 2003, Philpott et al. 2006, Moorhead et al. 2010). However, some evidence suggests that spiders seem not to be affected by the intensification of agriculture. For example, Clough et al. (2005) reported that spider diversity did not decrease in intensified cereal plots, whereas Pinkus et al. (2006) showed that spider richness at the coffee layer was higher in more intensified coffee ecosystems (low canopy cover). However, Pinkus et al. (2006) did not show species accumulation curves, making it impossible to assess if this result was due to a sampling effect. On the other hand, Stenchly et al. (2011b) reported that the abundance of webs in cocoa trees increased as the number of shade trees per plot increased. This literature suggests that spider diversity and abundance are not heavily influenced by the degree of intensification of the agroecosystem.

Because of their sociality and recruitment capabilities, ants are thought to play a central role in biological control in agroecosystems (Holldöbblers and Wilson 1990). For example, in coffee production, Vandermeer and colleagues (2010) have elucidated the complex ecological network that revolves around the mutualism between the arboreal ant

Azteca instabilis and the hemipteran, *Coccus viridis* (the green coffee scale) (Jha et al. 2012) and its effects on the biological control of herbivores that feed on coffee (Vandermeer et al. 2008, Liere and Perfecto 2008, Vandermeer et al. 2010, Pardee and Philpott 2011, Philpott et al. 2012). In coffee plantations, *Azteca instabilis* ants build their nests in the shade trees and patrol nearby coffee plants where these ants tend the green coffee scale and chase away other herbivores (Vandermeer et al. 2002, Vandermeer et al. 2010).

Although *A. instabilis* is an evident biological control agent, it could also negatively impact other predators, specifically spiders. Indeed, preliminary research on spiders in coffee agroecosystems suggested some negative interactions between *A. instabilis* and spiders (Vandermeer et al. 2002). The role of ants as intraguild predators and spiders as intraguild prey has been well documented in other systems (Pętal and Breymeyer 1969, Kajak et al. 1972, Kaplan and Eubanks 2005, Moya-Laraño and Wise 2007, Styrsky and Eubanks 2007, Sanders and Platner 2007, Sanders et al. 2011). Alternatively, some spiders themselves are intraguild predators, preying directly on ants (Gibb 2003).

On the other hand, *A. instabilis* could also facilitate some spider species by protecting them from other predators or by increasing the amount of resources available to spiders (Cushing 2012, Schuch et al. 2008). Indeed, there is evidence that some spiders use ants as a protection from other predators (MacIver and Stonedahl 1993). Furthermore, because *A. instabilis* forms a mutualism with *C. viridis* (Jha et al. 2012), many other insects are attracted to this association (Vandermeer et al. 2010). If those insects are prey for spiders, the mutualism could potentially increase spider abundance.

Indeed, other researchers have shown that some spiders do exploit rich resource areas patrolled by dominant ants (see Meehan et al. 2010).

Here we investigate the effect of coffee intensification and the presence of *A. instabilis* on species richness, abundance and species composition of the spider assemblage in coffee agroecosystems of the Soconusco region in Chiapas, Mexico. We expect to find that spider diversity does not change in the coffee layer across farm type, as has been documented in previous studies. Based on the negative interactions between ants and spiders reported in other studies (Petal and Breymeyer 1969, Kajak et al. 1972, Halaj et al. 1997, Lenoir 2003, Sanders and Platner 2007) and in particular within our study system (Vandermeer et al. 2002), and based on the high aggressiveness of *A. instabilis*, we expect to find lower spider richness in the presence of the ant. However, given the presence of the mutualistic relationship between *A. instabilis* and *C. viridis* we also hypothesize that the mutualism will result in higher insect abundance in the presence of the ant and therefore higher abundance of some spider species.

Materials and Methods

Study Area

The research was conducted in the Soconusco region, located in the Sierra Madre Mountains of Chiapas, Mexico during July 2008 and June-July 2009. The Soconusco is a rugged region with pronounced slopes covered by coffee farms, which are characterized by their large size (~300 ha) and management type. Most of the coffee farms found in this region use trees for providing shade to coffee plants. However, the type of shade (monospecific/diverse), pruning frequency and pesticide application (yes/no) define whether or not farms are undergoing agricultural intensification. The research took place

in a large area of approximately 600 hectares that contain a variety of management types, ranging from very low to very high shade. The area is divided into two large farms, *Irlanda* and *Hamburgo*, located at 15° 11' N, 92° 20' W and 15 ° 10' N, 92° 19' W respectively. *Finca Irlanda* is approximately 300 hectares and tends toward the diversified farm with a complex canopy that provides shade to the coffee plants (Vandermeer et al. 2008), while *Finca Hamburgo* generally contains a less diversified canopy cover ranging more toward the intensified end of the spectrum (Moguel and Toledo, 1999). The more intensive areas correspond to the monospecific shade (MS) system and were characterized by high density of coffee plants and the scarce presence of shade trees mostly in the genus *Inga*. The less intensive areas correspond to diverse shade systems (DS) with lower density of coffee plants and higher density and diversity of shade trees. Moorhead and colleagues (2010) in a study carried out in the same sites and simultaneously to this study, reported that the DS sites had higher mean canopy cover (72.75% versus 30.56%) and mean basal area (30.1 m²/ha versus 12.23 m²/ha) than the MS sites. However, given the rather homogenous but contrasting management imposed by the owners of each farm, all replicates in each type of management were spatially contiguous, thus not allowing for true replication of the agricultural management factor.

Spider Sampling

Spiders were sampled in the coffee layer of these coffee agroecosystems in the summers of 2008 and 2009. In 2008 we sampled spiders on coffee plants patrolled and not patrolled by the arboreal ant *A. instabilis*. In coffee plantations, *A. instabilis* are dominant ants that build their nest in shade trees and patrol nearby coffee plants for tending scale insects (Vandermeer et al. 2008). Vandermeer et al. (2008), studying the

spatial distribution of *A. instabilis* in a 45 ha plot, reported that the spatial distribution of this ant species showed a clustered pattern. Given that the spatial distribution of *A. instabilis* nests was already known and mapped for one area of 45 ha, we used this information for selecting nine *A. instabilis* nests that were of relatively easy access and that had similar ant activity level. We only selected *A. instabilis* nests that were separated from each other by a minimum of 30 m. In the field, we corroborated the presence of *A. instabilis* by its swarming behavior, which was elicited by knocking each tree with a stick. We also selected nine trees that did not have *A. instabilis* nests and that were at least 20 meters apart from their paired *A. instabilis* nest.

Azteca instabilis usually forages and tends the green coffee scale on coffee bushes close to the tree where its nest is located (Vandermeer et al. 2008). Therefore, we decided to sample spiders in the nearest four coffee plants to the selected trees. We sampled the spiders by examining the entire coffee plant branch by branch. In order to improve the visualization of spider webs, at the beginning of each spider search we sprayed coffee plants with water using a hand held sprayer, then collected spiders by hand. Each search lasted approximately 15 minutes per coffee plant.

We collected spiders over the entire month of July alternating between DS and MS farms and between plants with and without *A. instabilis*. By identifying independent *A. instabilis* nests and by setting 30 m minimum distance among them, we ensured that our sampling points were not correlated. All spiders were placed in 70% ethanol and taken to the laboratory for future identification.

In 2009 we conducted the study only in the DS farm and we slightly modified spider sampling. Spiders were sampled on two (instead of 4) coffee plants nearest to a

shade tree with and without *A. instabilis* via visual counts and collections. We selected trees with and without *A. instabilis* nests taking care to avoid overlap with the trees used in 2008. Initially 22 trees (11 with and 11 without *A. instabilis*) and their respective coffee plants (22 with and 22 without *A. instabilis*) were selected, two weeks later 8 more trees were added (4 with and 4 without *A. instabilis*) for a total of 15 trees and 30 coffee plants with and 15 trees and 30 coffee plants without *A. instabilis*. Spiders were recorded weekly for 6 weeks on the same coffee plants over the months of June and July of 2009 using the same method described above for the 2008 sampling, except that we removed spiders from one of the two sampled plants on a weekly basis and left all spiders on the other plant.

This spider removal took place because the two coffee plants sampled per tree were used for another study on the effects of spiders on arthropods. Thus in each weekly survey the identity of each species and its respective abundance was recorded for one of the plants (control plants) whereas all spiders on the other plant were removed and collected (removal plants) for future identification. Therefore, in the analyses carried out we accounted for the potential spider removal effect on spider richness and abundance by including the variable "spider removal" (yes/no). In addition, given that plant structure is important for spider web attachment (Wise 1993, Sanders et al. 2008), in 2009 we accounted for plant effects by counting the number of branches of each coffee plant.

Collected spiders were identified to family using the Spiders of North America (Ubick et al. 2005) and when possible to species level using selected publications (see Platnick 2009). Overall, all spiders were identified to family and morphospecies. Careful

observations were made in the field in order to correctly identify the males and females belonging to the same morphospecies. Collected adult specimens were measured and voucher specimens were deposited at the Arachnological Collection of El Colegio de la Frontera Sur in Tapachula, Mexico.

Insect Abundance

To test the hypothesis that *A. instabilis* benefits some spiders by increasing the amount of prey resources available to them, in 2009 we quantified insect abundance on coffee plants with and without *A. instabilis* with visual counts and sticky traps. Visible insects were counted at the beginning of June of 2009 on the initial 44 coffee plants in which we sampled spiders. We counted all visible insects present in the coffee plants except *C. viridis*. Insects were identified to order and in some cases to family. In order to estimate insect abundance with sticky traps, at the end of June of 2009 we selected twenty-two coffee plants, 11 patrolled and 11 not patrolled by *A. instabilis*, and one small sticky trap (7 x 11 cm) was horizontally placed on each plant at an approximate height of 1 m above the ground. Each sticky trap was attached to its respective plant with two small wires. Sticky traps were collected 48 hours later and insects were counted using a dissecting microscope.

Data Analyses

Effect of A. instabilis and Agricultural Intensification on Spider Diversity

We analyzed the accumulation of spider species found in coffee plants with and without *A. instabilis* and in the two agricultural management systems (MS and DS) with rarefaction curves constructed using the program EstimateS (Colwell 2009). EstimateS uses the function Mao Tau for generating the expected species richness; in addition, the

program also returns a variety of estimators that calculate the total species richness estimated for a specific condition (Colwell 2009). More specifically, we used the 95% confidence intervals generated around the expected species richness (i.e. Mao Tau) for comparing the accumulated species richness between conditions (e.g. agricultural systems and presence/absence of *A. instabilis*) (Colwell 2009). In addition, we compared the estimated total species richness between conditions by using the Chao1 estimator and its 95% confidence intervals calculated by Estimates.

In addition, for 2008 we evaluated the influence of *A. instabilis* presence and agricultural intensification on the average spider richness and spider abundance per coffee plant with generalized linear mixed models (GLMMs). We decided to use GLMM's because these models allow the incorporation of both fixed and random factors as well working with response variables non-normally distributed. Random effects are variables that are included in a model in order to account for the effect of natural variation among subjects on the prediction of response variable (Kleinbaum et al. 2008). Furthermore, the incorporation of random effects is recommended when data are clustered either at one point in time or when data have been taken on the same individuals over time (Kleinbaum et al. 2008, Faraway 2006).

In the models run for 2008 the response variable was either average spider richness or average spider abundance per coffee plant, which was a function of the fixed factors, presence/absence of *A. instabilis* and agricultural system (DS versus MS). In addition, given that the sampled plants were grouped by shade tree we included the variable tree as a random effect.

For 2009, we also used GLMMs for analyzing the effect of *A. instabilis*, spider removal (yes/no), and number of branches on the average richness and spider abundance per coffee plant. Thus, average spider species richness or spider abundance per coffee plant was the response variable, whereas *A. instabilis* presence (yes/no), spider removal (yes/no) and number of branches were the fixed effects, and coffee plant and shade tree were the random effects. We considered coffee plant and shade tree as random effects because over six weeks weekly abundance and richness data were taken on the same coffee plants, which in turn were spatially related to one another as defined by the tree close to which the coffee plants were sampled. For testing fixed effects in either year we used the Wald Z test (the default method in R) and its corresponding Z value (Bolker et al. 2005).

Species Composition

We compared spider species composition between agricultural systems (DS and MS) and between the presence/absence of *A. instabilis* with non-metric multidimensional scaling (NMDS) and one-way analysis of similarities (ANOSIM) using Past software (Hammer et al. 2001). NMDS is an analysis that allows comparing a multivariate data set (in this case spider species) under two or more conditions (e.g. monocultural shade vs. diverse shade). The analysis is based on a similarity distance matrix, which in this case was calculated with the Jaccard similarity index. ANOSIM, a non-parametric version of ANOVA, estimates the dissimilarity among samples belonging to different conditions. ANOSIM uses the R-value as indicator of similarity, a high R value (maximum = 1) indicates high dissimilarity, whereas a low R value indicates low dissimilarity. In

addition, ANOSIM generates a p-value associated to each R value, which is the probability that the similarity is or not significant.

Species associated with Azteca instabilis

In order to determine if specific spider species were related to the presence of *A. instabilis*, we used rank abundance curves as an exploratory tool. Those species that showed an increase or decrease in their abundance in the presence of *A. instabilis* were chosen for further GLMMs analysis. We only included those species that had a total abundance equal or higher than 29 for 2008 and 40 in 2009. For GLMMs carried out on the 2008 data, the response variable used was the abundance of individual species whereas *A. instabilis* presence (yes/no) was the fixed factor and tree was included as random effect. For GLMMs carried out on the 2009 data, the response variable was the abundance of individual species whereas *A. instabilis* presence (yes/no), spider removal (yes/no) and number of branches were the fixed factors, and coffee plant and tree accounted for the random effects.

Given that carrying out multiple individual tests could increase the probability of wrongly rejecting the null hypothesis (spider abundance is the same in the presence/absence of *A. instabilis*) we applied the Bonferroni correction (Kleinbaum et al. 2008), which is to say, divided the standard α value of 0.05 by the number of comparisons carried out. In our case, the standard α value was divided by the number of spider species that were tested for an *A. instabilis* effect. For 2008 the standard α value (0.05) was divided by 6, thus the α corrected value was 0.008 whereas for 2009, the standard α value was divided by 14 species and the α corrected value was 0.004. We decided to divide by those species numbers rather than for the total number of species

reported each year, since in both years many singletons were recorded. Dividing by the total number of species would have artificially lowered the cutoff α value since many species were recorded just once.

In order to explore potential mechanisms for explaining spider- ant associations we also carried out feeding trials between spiders and ants in the laboratory and conducted extensive field observations. In the laboratory we allowed *A. instabilis* to interact with the spider species that showed a positive association with the ant. Interactions lasted 24 hours and took place in small plastic containers (500 ml) where we put 10 ants and one spider; a small cotton ball dampened with water was added. In the field we observed the behavior of spiders in the presence of *A. instabilis*.

Insect Abundance

To determine if there were differences in insect abundance in the presence/absence of *A. instabilis* we used a generalized linear model. The response variable was insect abundance per coffee plant and the explanatory variable was *A. instabilis* presence/absence.

Statistical Software

We carried out all statistical analyses with the R software. None of the response variables were normally distributed; therefore we used a Poisson distribution for all the analyses run. GLMMs were carried out with the package lmer. The percent change attributable to any predictor variable was obtained by taking the exponent of the corresponding estimate provided by the model. Any value above 1 was taken as a percent

increment with respect to the intercept, any value below 1 was taken as the base for calculating the decrease caused for that variable with respect to intercept. For each hypothesis, at least two models were run; the default model only included a random intercept. The mean values were obtained by using the transformation: mean values = e^x , where x is the estimate provided by the linear model. Models that best explained the data were chosen using the AIC criterion (Faraway 2006).

Results

Overall, 91 spider morphospecies were recorded in the coffee agroecosystems. In 2008 and 2009 61 and 76 morphospecies were collected respectively. These species were distributed in 20 families, 18 families in 2008 and 18 families in 2009.

Effect of Agricultural Intensification on Spider Richness and Abundance

Accumulated spider richness was unaffected by coffee intensification (Fig. 3.1). Fifty-one species were present in the DS sites whereas 48 species were present in the MS sites, but this difference was not statistically significant according to the 95% confidence intervals (Fig. 3.1). Additionally, the estimator Chao1 did not show a significant difference between the MS and DS systems (95% confidence intervals overlapped). However, the GLMM analysis showed that average species richness per plant was affected by coffee management system. Average spider richness decreased by 30 % in the DS system, the least intensified agroecosystem, as compared to MS system ($p=0.04$, Table 3.1).

In addition, the GLMM analysis also showed that average spider abundance per plant decreased by 43 % in the least intensified system (DS) ($p=0.05$, Table 3.1).

Effect of Azteca instabilis on Spider Richness and Abundance

Of the 61 species recorded in 2008, 52 species were recorded from plants with *A. instabilis* and 50 on plants without *A. instabilis*. Accumulation curves showed no significant differences in spider richness in the presence/absence of the ant, according to 95 percent confidence intervals (Fig. 3.2 left). Likewise, Chao1 estimates also show no differences in spider species richness between plants with and without *A. instabilis* (Fig. 3.2 left). Furthermore, results from the GLMM analysis showed that average spider richness per coffee plant was unaffected by *A. instabilis* ($p = 0.86$, Table 3.1).

In 2009, 69 species were recorded in the presence of *A. instabilis*, while only 58 were recorded in its absence. However rarefaction curves as well as the Chao1 estimator showed no significant differences in the number of spider species accumulated in the presence/absence of *A. instabilis* (Fig. 3.2 right). On the other hand, the GLMM analysis for 2009 showed that the average spider richness per coffee plant was significantly affected by the presence of *A. instabilis*. The average spider species richness per coffee plant increased by 27% in the presence of *A. instabilis* ($p = 0.003$, Table 3.2). Spider removal and number of branches did not have an effect on the average spider richness ($p > 0.05$, Table 3.2).

In 2008 GLMM analysis showed that *A. instabilis* did not have an effect on the average spider abundance per coffee plant ($p > 0.82$; Table 3.1). However, in 2009, *A.*

instabilis had a significant effect on spider abundance. On average, plants with *A. instabilis* had 67% more spiders than plants without the ant ($p = 0.004$, Table 3.2). In addition, spider removal but not the number of branches significantly affected spider abundance (Table 3.2). Mean spider abundance decreased by 24% when spiders were removed in comparison to those plants that did not suffer any removal ($p=0.01$, Table 3.2).

Spider species influenced by Azteca instabilis

In 2008, the relative abundance of several spider species, among them *Pocobletus* sp. n. (a new linyphiid species), *Myrmarachne panamensis*, *Anelosimus jucundus* and *Sidusa* sp., changed in the presence of *A. instabilis* (Fig. 3.3). However, a GLMM showed that only the abundance of the salticid *Sidusa* sp. was significantly affected by the presence of the ant. More specifically, the abundance of *Sidusa* sp. decreased by 76 % when *A. instabilis* was present ($p < 0.0001$, Table 3.3).

In 2009, a rank abundance curve suggested that seven species were more abundant in the presence of *A. instabilis* whereas one species was less abundant (Fig. 3.3). GLMMs corroborated that *Pocobletus* sp. n., *Myrmarachne panamensis*, *Lyssomanes spiralis*, *Dolichognatha pentagona*, *Cupennius salei* and *Trachelas* sp. were positively associated with *A. instabilis* whereas *Sidusa* sp. was negatively associated. After the Bonferroni correction the influence of *A. instabilis* on spiders held for *Pocobletus* sp. n., *Myrmarachne panamensis*, *Sidusa* sp., and *Lyssomanes spiralis* (Table 3.3). *Pocobletus* sp. n. abundance increased by 285%, *M. panamensis* by 1144% and *L.*

spiralis by 177% in plants patrolled by *A. instabilis* (Table 3.3). On the other hand, *Sidusa* sp. decreased by 78% in plants patrolled by the ant (Table 3.3). Spider removal did not change the abundance of most spider species except for *Pocobletus* sp. n. (decreased by 81%), *L. spiralis* (increased by 91%) and *Sidusa* sp. (increased by 156%) (Table 3.3). The number of branches in coffee plants did not have an effect on these spiders, except on *Lyssomanes spiralis* therefore this variable was dropped from the models.

Effects of agricultural intensification and Azteca instabilis on spider species composition

Spider species composition between the DS system and the MS system was very similar ($R=0.097$; $p = 0.0001$). The non-metric multidimensional scaling plot showed a high overlap between DS and MS (plot not shown). Likewise, species composition in the presence/absence of *A. instabilis* was similar in both 2008 and 2009, although a more marked difference in species composition was present in 2009. In 2008, the R value was 0.036 ($p = 0.002$) indicating that species composition was very similar, whereas in 2009 the R value was 0.27 ($p = 0.0001$), indicating that the spider community composition showed a greater degree of dissimilarity regarding the presence/absence of *A. instabilis* (Fig. 3.4).

Insect Abundance

Average insect abundance per coffee plant estimated through visual counts was higher in those plants with *A. instabilis* than in plants without *A. instabilis* ($p < 0.001$; Fig. 3.5a). The orders Diptera, Coleoptera, Hemiptera and the family Blattodea, were the

most abundant taxa found on plants patrolled by the *A. instabilis*. Likewise, the results from the sticky traps showed that average insect abundance per sticky trap was significantly higher on plants with *A. instabilis* than on plants without *A. instabilis* ($p = 0.001$, Fig. 3.5b).

Discussion

Contrary to what has been found for other taxa at this locality (Armbrecht and Perfecto 2003, Philpott et al. 2006, Moorhead et al. 2010), agricultural intensification did not result in a reduced richness and abundance of spiders living within the coffee layer. Spider species richness and abundance per coffee plant was lower on plants in the diverse shade system (DS) as compared to the monospecific shade (MS) system (Table 3.1), thus supporting Pinkus et al. (2006) findings. We think that this is partially due to the fact that in the MS sites the canopy cover is significantly reduced (Moorhead et al. 2010) and therefore it is possible that spiders were concentrated in the coffee layer, while in the diverse coffee system spiders have access to a richer canopy thus decreasing the number of spiders found in the coffee layer. Other studies have shown that spiders are vertically structured with well-defined communities in the leaf litter and canopy layers, and an intermediate layer that shows components of both strata (Stenchly et al. 2011a). It is thus possible that the decrease in tree density in the MS could drive the arboreal spiders that otherwise inhabit the shade tree canopy to the coffee layer. Indeed, preliminary results (Marín unpublished) suggest that the spider community found in the canopy of the shade trees in coffee plantations greatly overlap with the spider community found in the coffee bushes.

For species richness we found a discrepancy between the results of the rarefaction curves, which show no significant differences between the systems, and the GLMM results, which do show significant differences. This discrepancy is likely due to high abundance and high species overlap on a per plant basis in the MS. First, the abundance of spiders was on average 43% lower on coffee plants within the DS agroecosystem as compare to the MS agroecosystem (Table 3.1), thus increasing the chances of recording more species in the MS. Second, a higher overlap of species on a per plant basis in the MS system as compare to the DS means that while the average number of species per plant in the more intensive system is higher than in the less intensive system, those species tend to be more similar among plants within the more intensive system, yielding a similar number of total accumulated species in both systems. It is likely that the combination of these two factors resulted in significantly higher species richness per plant in the MS but no significant differences in the total accumulated species richness between the two farms.

Overall, our results support other studies that have reported that spider species richness is not affected by agricultural intensification (Clough et al. 2005, Bruggisser et al. 2010). However, further studies should include the entire spider assemblage, including spiders within the tree canopy and the leaf litter layers to rule out the displacement of spiders from layers that are reduced or non existent in the more intensive systems.

Our study showed a variable effect of *A. instabilis* on the spider community depending on the year of the study. In 2008 we found no significant effect of *A. instabilis* on spider species richness or abundance, while in 2009 we found higher richness and

abundance in coffee plants patrolled by *A. instabilis*. These differences are most likely due to differences in sampling protocols between years. In 2008 we sampled the four closest plants to the tree where *A. instabilis* was nesting. In 2009 we restricted the sampling to only the two closest plants and verified that the ants were actively foraging on those plants. Given these differences, it is likely that the effect of *A. instabilis* was more direct and potentially stronger in the plants sampled in 2009 than those sampled in 2008. Furthermore, in 2009 spider abundance increased by almost four times the abundance in 2008 (Fig. 3.2).

For 2009, GLMM results showed that local spider richness and abundance significantly increased in plants patrolled by *A. instabilis*. However, similar to the results for farm systems, accumulation curves did not reflect the difference in species richness between plants with and without ants. The discrepancy of the results may be due the spider abundance and species overlap in the presence of the ant. Coffee plants patrolled by *A. instabilis* had higher abundance of spiders than those without the ant and even though on average there are more species of spiders on plants patrolled by *A. instabilis*, there was more overlap of species among those plants with ants (notice that in Fig. 3.4, *A. instabilis* points seem to be closer among them than the non *A. instabilis* points, except those extreme points that outline the *A. instabilis* polygon). This qualitative analysis suggests that the presence of *A. instabilis* increases the number of spider species per plant, but that those species tend to be the same in all plants patrolled by ants yielding a similar total number of accumulated species in both systems. Within the patches of *A. instabilis*, the same group of spider species might be taking advantage of the excess of resources present in plants patrolled by *A. instabilis* (Fig. 3.5) or perhaps getting

protection from it. Interestingly, neither in 2008 nor in 2009 did we find a negative effect of *A. instabilis* on the spider community as suggested by Vandermeer et al. (2002). The only negative effect reported in this study was on the salticid *Sidusa* sp. (Table 3.3).

Azteca instabilis also affected specific spider species in a dramatic way. Although, Vandermeer et al. (2002) reported that only linyphiid spiders were positively associated with *A. instabilis* ants, in this study we found that other spiders were also positively associated with these ants. In fact, we found that *Pocobletus* sp. n., is the only linyphiid associated with *A. instabilis*, but we also reported that *L. spiralis* and *M. panamensis*, a generalist and an ant-mimic spider respectively, are positively associated with these ants. We propose that two mechanisms, resource availability and protection from predators, are responsible for these specific associations and also for the higher average spider diversity in the presence of the ants. Indeed, plants patrolled by *A. instabilis* had higher abundance of arthropods (Fig. 3.5), which probably was due to the mutualism between *A. instabilis* and the hemipteran *C. viridis*. Although a growing literature shows that ant-hemipteran and ant-plant mutualisms play a key role in increasing plant fitness and altering the structure of arthropod communities in both natural ecosystems (Izzo and Vasconcelos 2005, Styrsky and Eubanks 2007, Delabie and Fernández 2003) and agroecosystems (Styrsky and Eubanks 2007, Vandermeer et al. 2010) the effects of ant-hemipteran mutualisms on other predators such as spiders have been largely ignored (but see Philpott et al. 2004 and Piñol et al. 2010). We propose that in our study system the *A. instabilis* - *C. viridis* mutualism creates an excess of honeydew and scales that, in turn, attract flying insects. Thus coffee plants that harbor this

mutualism maybe a valuable and tracked food source for spiders. Several studies have reported that spiders are food limited and that they actively track food resources and hunt or built their webs where prey is more abundant (Wise 1993, Harwood et al. 2003).

Furthermore, it is likely that some spiders are associated with *A. instabilis* because the aggressive behavior of the ant provides enemy free space to spiders, either through ecological or evolutionary mechanisms (Huang et al. 2011, Sanders et al. 2011). Here, we suggest that the higher abundance of the spider *Myrmarachne panamensis* on plants patrolled by *A. instabilis* is due to Batesian mimicry since these spiders mimic the ant (the model) in both behavioral and physical appearance (L.M personal observations). According to the Batesian evolutionary model, palatable mimics gain protection from aggressive models, because the model's aggressive behavior either chases away the mimics' predators or kills them (Durkee et al. 2011, Huang et al. 2011, Cushing 2012). Although, aggressive mimicry (when a spider mimics an ant to prey on it) also takes place in nature (Castanho and Oliveira 1997), it seems not to be the case for *M. panamensis* given that in six of seven feeding trials carried out in the laboratory *M. panamensis* did not consume the ant. In addition, other studies have reported that spiders in the *Myrmarachne* genus follow the Batesian model (Jackson et al. 2008).

The negative effect of *A. instabilis* on the abundance of the salticid *Sidusa* sp. is in agreement with other studies that have shown that ants have a negative effect on salticids (Halaj et al. 1997, Nelson et al. 2004). Field observations and results suggest that in the case of the *L. spiralis* and *Sidusa* sp., their body size and hunting mode were

influential in defining their interactions with *A. instabilis*. Studies have shown the critical role that body size plays in intraguild predation and competition among predators; small organisms have higher probabilities of being preyed upon or excluded by larger ones (Wise 2006, Eichenberger et al. 2009, Huang et al. 2011). Given that the average body length of *Sidusa* sp., is 2.8 mm and *A. instabilis*' body length is 3.5 mm, it is possible that *Sidusa* individuals may be more prone to attack and injury by the ants than the individuals of *L. spiralis*, which are larger (6 mm). However, other spider species in this study also have body sizes similar to *Sidusa* sp. and their abundances did not decreased in the presence of the ant. Thus, other factors such as foraging mode may be involved in defining whether or not ants negatively affect spiders. Indeed, active hunters are more prone to intraguild predation than sit and wait predators (Denno and Fagan 2003, Rosenheim et al. 2004). Thus, we suggest that the negative association between *Sidusa* sp. and *A. instabilis* is due in part to the active hunting mode of *Sidusa* as well its small body size.

In conclusion, our results provide evidence of the complexity of interactions between spiders and ants in the coffee system, as well the effect of coffee intensification on spider diversity. We document that coffee intensification does not affect the spider community that hunts or builds webs in the coffee layer in a negative way. Furthermore we show that *A. instabilis* ants increase the richness and abundance of spiders in coffee plants that are very close to the main ant nest and that are actively patrolled by the ants. Our results suggest that the positive association between ants and spiders may be caused by at least two mechanisms, protection from predators and indirect effects on resource availability.

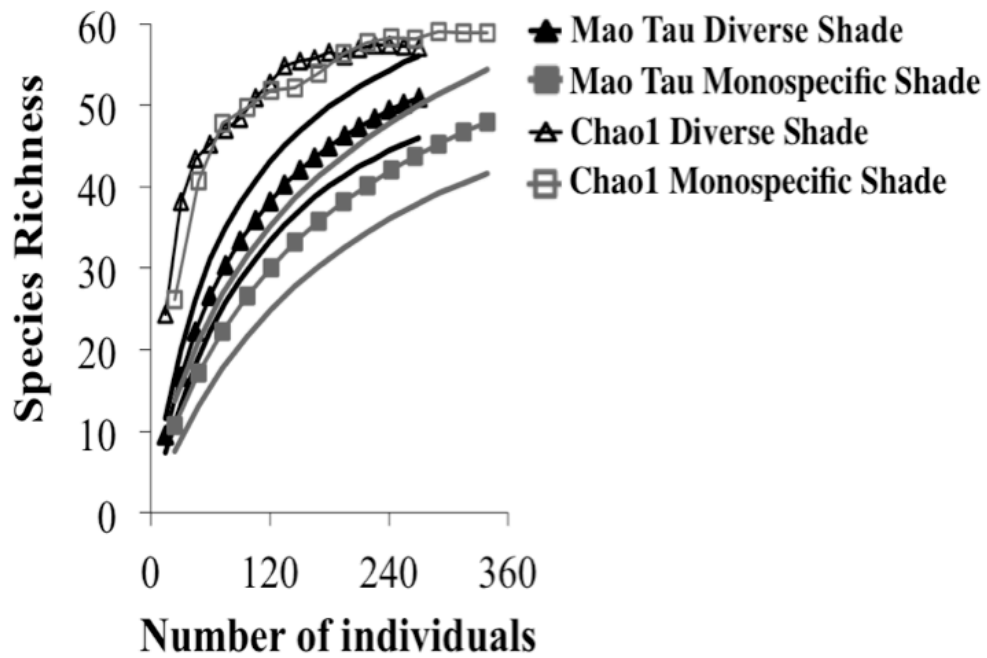


Figure 3.1. Rarefaction curves (Mao Tau) and Chao1 estimates for spider richness in a diverse shade coffee agroecosystem (DS) and a monospecific shade monoculture (MS) in the Soconusco region Chiapas, Mexico. The lines show 95% confidence intervals around the expected richness (Mao Tau) for coffee agroecosystems with the same colored symbol

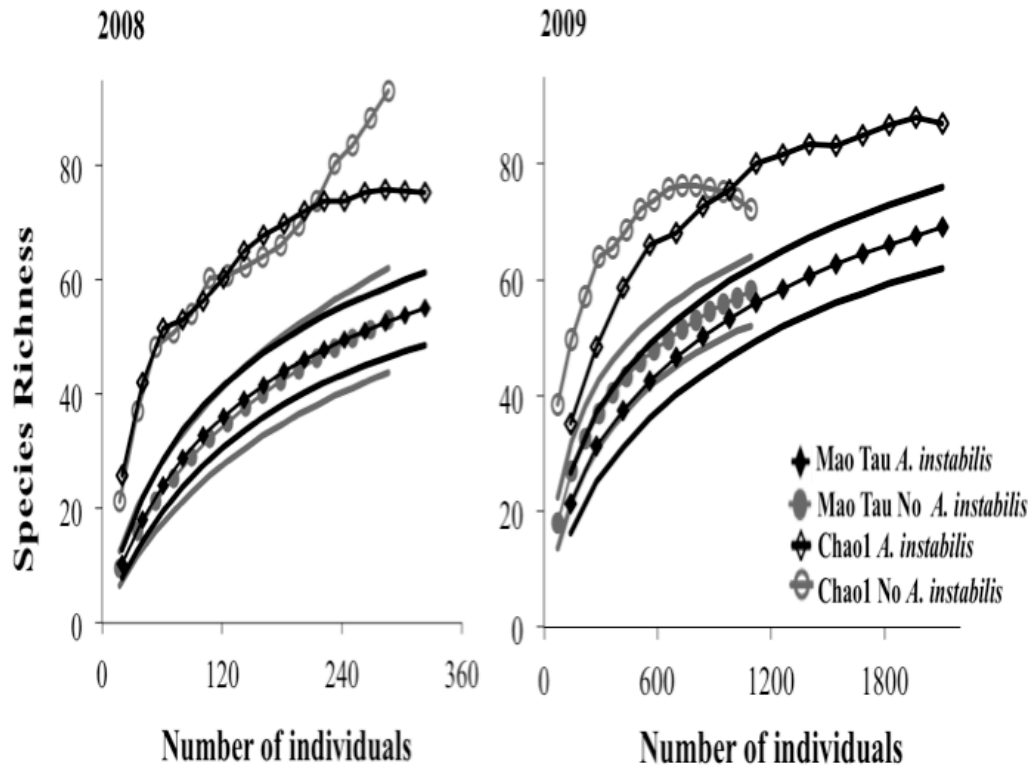


Figure 3.2. Individual Rarefaction curves (Mao Tau) and Chao1 estimates for spider species richness in the presence/absence of *Azteca instabilis* ants in 2008 and 2009. The lines show 95% confidence intervals around the expected richness (Mao Tau) for *A. instabilis* presence with the same colored symbol

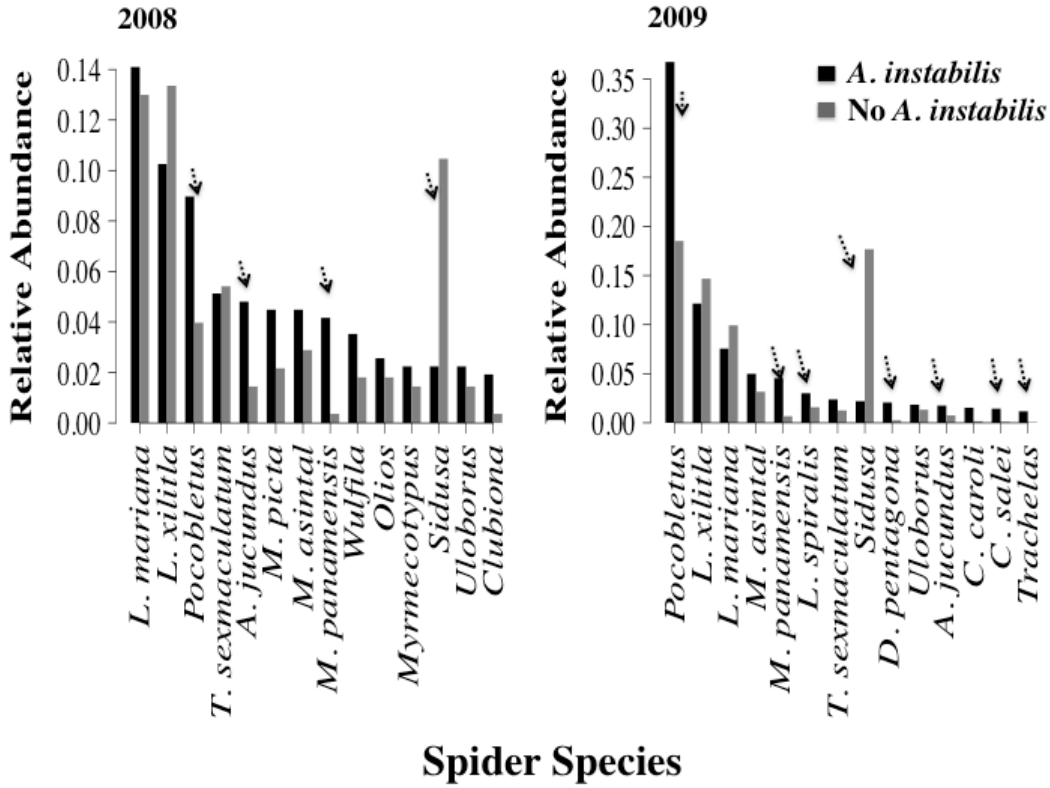


Figure 3.3. Rank abundance of spider species in the presence/absence of *Azteca instabilis* in 2008 (right) and 2009 (left). Dotted arrows indicate the affected species

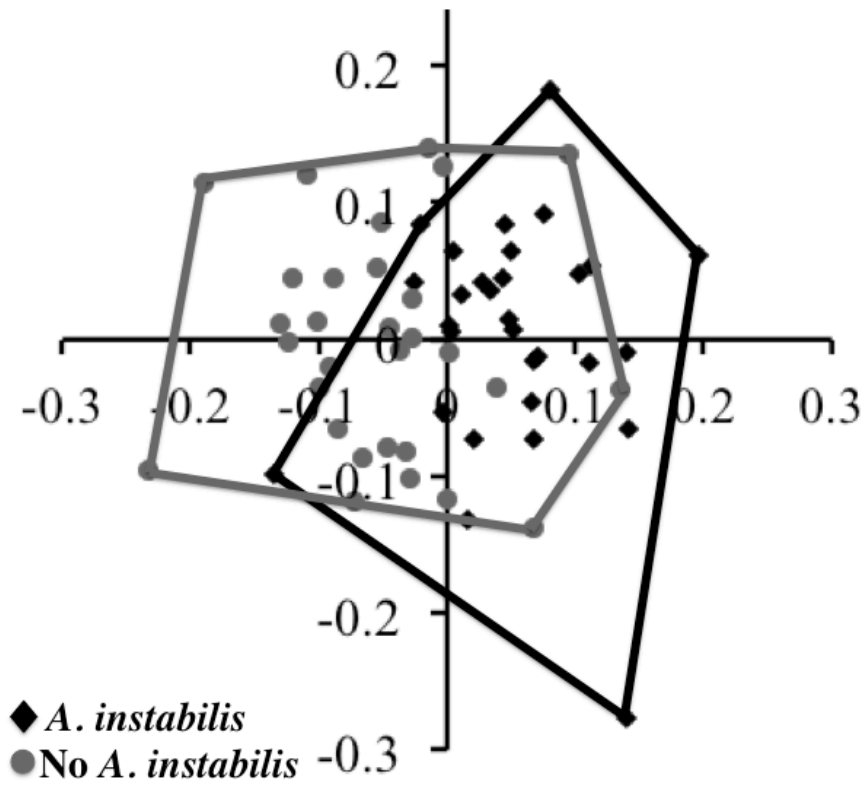


Figure 3.4. Non-metric multidimensional scaling plot showing the similarity in species composition in the presence/absence of *Azteca instabilis* in 2009. The overlapping polygons indicate the degree of similarity

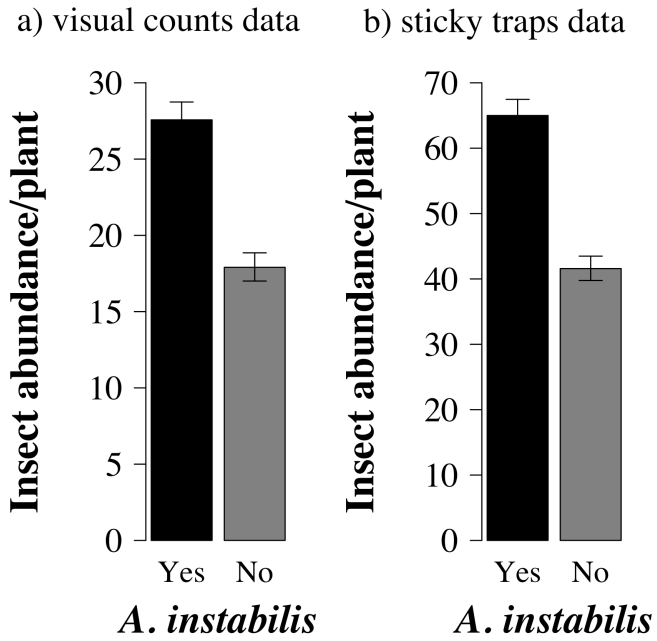


Figure 3.5. Mean insect abundance per plant (\pm SE) in the absence/presence of *Azteca instabilis* ants in 2009. a = visual counts data, b= sticky traps data

Table 3.1. Results of generalized linear mixed models testing the effect of *Azteca instabilis* and agricultural intensification on spider richness and abundance in 2008

Variable	Fixed effects	Estimate	Standard error	Z	p (Z)	% of change	Random effects	Variance	Standard error
Richness	intercept	1.30	0.15	8.42	<0.0001		tree	0.18	0.42
	<i>A. instabilis</i>	0.03	0.17	0.17	0.86	3			
	farm DS	-0.37	0.17	-2.10	0.04	-30			
Abundance	intercept	1.66	0.18	9.34	<0.0001		tree	0.25	0.5
	<i>A. instabilis</i>	0.04	0.20	0.22	0.829	4			
	farm DS	-0.56	0.20	-2.79	0.005	-43			

Table 3.2. Results of best-fitted generalized linear mixed model for testing the effects of *Azteca instabilis*, spider removal and number of branches on spider richness and abundance per coffee plants in 2009

Variable	Fixed effects	Estimate	Standard error	Z	p (Z)	% of change	Random effects	Variance	Standard error
Richness	intercept	1.440461	0.122541	11.755	<0.0001		plant	0.005	0.072
	<i>A. instabilis</i>	0.238061	0.081319	2.928	0.00342	27	tree	0.029	0.17
	spider removal	0.042043	0.047846	0.879	0.37955	4			
	branches	0.002071	0.001609	1.287	0.19822	0.2			
Abundance	intercept	1.389164	0.277381	5.008	<0.0001		plant	0.16	0.4
	<i>A. instabilis</i>	0.512203	0.177724	2.882	0.00395	67	tree	0.128	0.358
	spider removal	-0.263633	0.111796	-2.358	0.01837	-24			
	branches	0.005739	0.003675	1.562	0.11832	0.5			

Table 3.3. Results of best fitted generalized linear mixed models for testing the effects of *Azteca instabilis* in 2008 and 2009 and spider removal in 2009 on specific spiders found in coffee plants

Year	Spider species	Fixed effects	Estimate	Standard error	Z	p (Z)	% of change	Random effects	Variance	Standard error
2008	<i>Sidusa</i> sp.	intercept	-0.79	0.19	-4.26	<0.0001				
		<i>A. instabilis</i>	-1.42	0.42	-3.38	<0.0001	-76	tree	<0.0001	<0.0001
2009	<i>Pocobletus</i> sp. n.	intercept	-0.14	0.34	-0.41	0.682		plant	1.40	1.18
		<i>A. instabilis</i>	1.35	0.42	3.21	0.001	285	tree	0.43	0.66
		removal	-1.67	0.35	-4.81	<0.0001	-81			
	<i>M. panamensis</i>	intercept	-3.38	0.45	-7.47	<0.0001		plant	0.41	0.64
		<i>A. instabilis</i>	2.52	0.48	5.24	<0.0001	1144	tree	0.24	0.49
		removal	-0.10	0.31	-0.34	0.736	-10			
	<i>L. spiralis</i>	intercept	-4.05	0.57	-7.07	<0.0001		plant	0.21	0.46
		<i>A. instabilis</i>	1.02	0.35	2.94	0.003	177	tree	0.14	0.38
		removal	0.65	0.28	2.32	0.020	91			
		branches	0.02	0.01	2.70	0.007	1			
	<i>Sidusa</i> sp.	intercept	-0.81	0.26	-3.16	0.002		plant	0.21	0.46
		<i>A. instabilis</i>	-1.51	0.35	-4.32	<0.0001	-78	tree	0.52	0.72
		removal	0.94	0.21	4.51	<0.0001	156			

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CHAPTER 4

A POSITIVE ASSOCIATION BETWEEN ANTS AND SPIDERS AND POTENTIAL MECHANISMS DRIVING THE PATTERN

Abstract

Biotic interactions play a central role in determining species distributions. Some ants act as keystone species affecting the distribution of other species, including spiders. In coffee plantations small linyphiids are significantly more abundant in coffee plants patrolled by aggressive arboreal ants. Here we examine the abundance and spatial distribution of small linyphiid, *Pocobletus* sp., in relation to the presence and spatial distribution of aggressive ants and coffee plant foliage in coffee agroecosystems from Southern Mexico. We also explore prey availability and enemy free space as potential mechanisms underlying the association reported here. Results show that the spatial distribution of *Pocobletus* in plots of 20 by 20 meters was associated positively with the distribution of the dominant ants. Furthermore, *Pocobletus* sp. abundance was best explained by the presence of the aggressive ants and coffee foliage. Insect abundance was significantly higher in the presence of aggressive ants, both per individual coffee plant and per spider web. The abundance of the predators of *Pocobletus* decreased in the

presence of ants. Thus, higher food resource availability and lower predation risk are two possible mechanisms that may explain the positive association between dominant ants and *Pocobletus* sp.

Key words: agroforests, enemy-free space, linyphiids, plant structure

Introduction

Both abiotic and biotic factors affect species abundance and distribution and therefore the overall impact of organisms on ecosystems and agroecosystems. Indeed, some organisms can have strong effects on the distribution of other species in the ecosystem because they act as keystone species whose effects cascade to other trophic levels (Paine 1966, Davic 2003, Vandermeer et al. 2010).

Ants play a central role in many terrestrial communities because they constitute a large part of the animal biomass and are likely to interact with many other taxa (Risch and Carroll 1982, Hölldobler and Wilson 1990, Stadler and Dixon 2005, Vandermeer et al. 2010). In addition, the effects of ants in terrestrial communities can be amplified by the mutualistic relationships that they establish either with hemipterans or plants (Izzo and Vasconcelos 2005, Styrsky and Eubanks 2007, Zhang et al. 2012). These mutualistic associations can directly and indirectly affect the abundance and presence of other trophic levels, such as herbivores and predators (Styrsky and Eubanks 2007, Zhang et al. 2012) and potentially transform the structure of communities. Furthermore, as predators, ants have been shown to have strong effects on many other arthropod groups (Perfecto 1990, 1991, Halaj et al. 1997, Pinol et al. 2010). These top-down effects can also lead to cascading effects that result in alternate increases and decreases in the abundances of organisms in sequential trophic levels (Risch and Carroll 1982, Dyer and Letourneau 1999).

Intraguild predation, a negative interaction among generalist predators that are also potential competitors, is very common in terrestrial communities (Polis et al. 1989,

Arim and Marquet 2004). Two ubiquitous generalist predators in terrestrial communities are ants and spiders. These two groups comprise the most abundant terrestrial arthropods and they feed on a wide array of other arthropods, including each other. Ants are eusocial insects that for the most part forage in groups, and thus are able to attack and kill prey items that are much larger than any individual worker (Hölldobler and Wilson 1990). For this reason, ant-spider interactions are expected to be asymmetrical with ants having a stronger negative effect on spiders than *vice-versa*. Although many studies that have examined ecological interactions between ants and spiders have documented a negative effect of ants on spiders (Buckley 1990, Halaj et al. 1997, Heikkinen 1999, James et al. 1999, Laakso and Setälä 2000, Lenoir et al. 2003, Pinol et al. 2010, Sanders et al. 2011) a few have also documented neutral (Philpott et al. 2004) and positive effects of ants on spiders at population, and community levels (Cushing 1998, Vandermeer et al. 2002, Moya-Larano and Wise 2007, Schuch et al. 2008, Sanders and van Veen 2011, Stenchly et al. 2011). The mechanisms responsible for these positive effects are ecosystem engineering (Schuch et al. 2008, Sanders and van Veen 2011), predation (Moya-Larano and Wise 2007), and protection against predators, this last as a result of evolutionary processes (Durkee et al. 2011, Huang et al. 2011, Pekar and Jiros 2011) or ecological interactions.

In coffee agroecosystems in Southern Mexico, Vandermeer et al (2010) reported that the aggressive arboreal ants *Azteca instabilis* function as keystone species in coffee production by regulating herbivores. *A. instabilis* also affects the ant community in coffee plants by excluding ants from their nesting sites (Philpott 2010). However, *A. instabilis* has a positive effect on the spider community that inhabits the coffee plants. Indeed,

Marín and Perfecto (2013b) reported that coffee plants patrolled by *A. instabilis* had significantly higher spider richness and abundance. In addition, these authors reported that three spider species, *Pocobletus* sp, *Myrmarachne panamensis* and *Lyssomanes spiralis*, were significantly more abundant in the presence of *A. instabilis*. However, Marín and Perfecto (2013b) did not investigate the contribution of plant attributes to these specific ant-spider associations that took place in coffee plants. Nonetheless, in order to get a better understanding about the ecology of spider-ant associations, plant attributes should be considered since plants can affect spider abundance through altering microhabitat conditions and by providing web attachment, hiding and nesting sites (Wise 1993, Langellotto and Denno 2004, de Souza and Martins 2005). In this paper we ask the following questions: 1) Does the spatial distribution of *Pocobletus* sp. respond to *A. instabilis*?, 2) What is the relative contribution of *A. instabilis* presence and coffee plant structure on *Pocobletus* sp. abundance? and 3) What are the mechanisms underlying the association between *Pocobletus* sp. and *A. instabilis*?

Given that a positive association between *A. instabilis* and *Pocobletus* sp. was reported previously, we predicted that the spatial distribution of *Pocobletus* sp. would be clustered around *A. instabilis* nests. Specifically, we expected that the distance of *Pocobletus* sp. to *A. instabilis* patrolled plants/trees would be less than the distance that would be expected if *Pocobletus* sp. were distributed at random. In relation to the contribution of plant structure and *A. instabilis*, we expected that both variables together would better explain *Pocobletus* sp. abundance than either alone. Finally, we explore resource availability and enemy free space as two potential mechanisms that could be underlying the *Pocobletus* sp. - *A. instabilis* association. First, we hypothesize that the *A.*

instabilis-*C. viridis* mutualism attracts more flying insects that are consumed by spiders; therefore, we expect more insects in the presence of the ant-hemipteran mutualism.

Second, we investigate whether *A. instabilis* ants provide some sort of protection to *Pocobletus* sp. against predation. Specifically, we expect to find lower abundance of the predators of *Pocobletus* sp. in the presence of *A. instabilis*.

Material and methods

Study Area

We conducted our research at the Finca Irlanda coffee farm (15° 11' N, 92° 20' W) in the Soconusco region of the Sierra Madre Mountains of Chiapas, Mexico, in July 2009 and June-July 2010. Finca *Irlanda* is approximately 300 hectares and represents a diversified coffee plantation with trees that provide shade to the coffee plants (Perfecto and Vandermeer 2008).

Pocobletus sp. and coffee plant survey

In the summer of 2010 we carried out two surveys on coffee plants to elucidate a) the spatial distribution of *Pocobletus* sp. in relation to *A. instabilis* and b) the relationships among number of coffee branches, *A. instabilis* presence and *Pocobletus* sp. abundance. The first survey took place in mid May-early June (for simplicity we will call it the June survey) whereas the second survey took place in late July (hereafter the July survey). We conducted surveys in three 20 x 20 m and one 20 x 25 m plots within a 45-hectare plot previously established (Vandermeer et al. 2008) for the study of the aggressive arboreal ants *A. instabilis*, whose nests are distributed in clusters (Vandermeer et al. 2008). We established each plot around the central nest of a given cluster of *A. instabilis* nests. We selected clusters of nests taking into account accessibility and a

minimum distance of 100 m from the next nearest cluster. Each plot was set up using an x, y coordinate system, thus creating a grid wherein the location of each coffee plant and shade tree was recorded.

For each coffee plant within a plot we recorded all spiders present as well the number of webs of *Pocobletus* sp., the number of *Pocobletus* adults (females and males) and ovisacs found on each web. In order to increase the detection of spiders and in particular of the *Pocobletus* sp. webs we sprayed coffee plants with a hand-held water sprayer. In addition, for each coffee plant we recorded its height and number of branches and whether or not the coffee plant was patrolled by *A. instabilis*. After beginning the first survey, we noticed that *Pheidole synanthropica*, a small ant (average body size ~ 2 mm) that nests in the ground was very abundant on some coffee plants and seemed to be associated with *Pocobletus* sp., thus we decided to include *P. synanthropica* as an explanatory variable.

Vacant webs and intruders

We recorded the number of vacant webs, defined as those *Pocobletus* sp. webs that did not have any *Pocobletus* (adults, juveniles or spiderlings) but were not damaged. In addition, we recorded whether or not vacant webs were actually vacant or were occupied by a different spider species. In the case that the web was occupied by another species we recorded the identity and abundance of those intruders per web and their activity (e.g. resting, consuming, etc) and also recorded the identity and abundance of those intruder spiders per coffee plant.

Insect abundance

To explore whether or not *A. instabilis* may benefit *Pocobletus* sp. by increasing the amount of prey resources available to spiders, we quantified insect abundance on coffee plants with and without *A. instabilis* in the summers of 2009 and 2010 with two methods.

First, we used sticky traps placed on coffee plants. On each coffee plant two (7 x 11 cm) sticky traps were placed horizontally approximately 1.5 m above the ground. The horizontal position mimics the placement of *Pocobletus* sp. webs. Sticky traps were attached to coffee plants using wires. After 48 hrs, sticky traps were collected and insects were counted and identified using a dissecting microscope. In 2009, sticky traps were placed on 14 plants with and 14 plants without *A. instabilis*. In 2010, sticky traps were placed on 15 plants, five with and ten without *A. instabilis*. Second, we quantified prey items collected from *Pocobletus* sp. webs. We used index cards as a collecting device for the webs to keep the *Pocobletus* sp. webs in their original horizontal position without major damage or losses. Each web-card was kept in a plastic bag and labeled. Samples were taken to the lab for examination and identification of arthropods under a dissecting microscope. In 2009, 12 plants with and 12 plants without *A. instabilis* were randomly selected and two *Pocobletus* sp. webs were collected from each plant.

Data analyses

Spatial distribution

In order to elucidate the spatial distribution of *Pocobletus* in relation to the presence of *A. instabilis* and *P. synanthropica* we compared the observed weighed mean distance of *Pocobletus* sp. to *A. instabilis* and *P. synanthropica* with a random weighed mean distance that assumed a random distribution for *Pocobletus* sp. First, in each plot

we calculated the minimum distance between each coffee plant and the nearest coffee plant or tree patrolled by *A. instabilis*, with the Pythagoras theorem. Then we calculated the weighted mean distance in each plot according to the formula:

$$W = \frac{\sum_i N_i d_i}{\sum_i N_i}$$

Where W = weighted mean distance, N_i = number of *Pocobletus* (adults or ovisacs) on plant i , and d_i = the distance of plant i to the nearest plant/tree with *A. instabilis*

Thus the weighted mean distance reflected the observed weighted mean distance of *Pocobletus* sp. to *A. instabilis* in each plot. Then, we compared the observed weighted mean distance with a random mean distance. In order to calculate the random mean distances for *Pocobletus* sp. (adults or ovisacs) we randomly sampled *Pocobletus* sp. data (either adults or ovisacs) by using a random sample function and then assigned the random values to coffee plants. These random values were taken and used to calculate the random weighted means following the same equation used for calculating the observed weighed means. We ran this procedure 10, 000 times. We expected that the distance between coffee plants that harbor *Pocobletus* sp. and a *A. instabilis* site (nearest coffee plant or shade tree) would be smaller than the random distance. We therefore calculated the probability of the random distance being less or equal to the observed weighed distance as the sum of the times that random weighted mean distance was less than the observed weighted mean distance and then divided this sum by 10,000. This procedure was repeated for each plot in both months. The same procedure was conducted for

elucidating the spatial distribution of *Pocobletus* sp. in relation to *P. synanthropica* but using the corresponding data (e.g. the minimum distance between each coffee plant and the nearest coffee plant or tree patrolled by *P. synanthropica*).

A. instabilis, plant structure and *Pocobletus* sp. abundance

As a surrogate of plant structure, we estimated the amount of foliage on each coffee bush by dividing the total number of branches of each coffee plant by its own height. Then in order to eliminate influential points coffee foliage was square rooted. For each month, we pooled together the variables recorded in each plot. The dependent variable was *Pocobletus* sp. abundance (adults or ovisacs) and the independent variables were *A. instabilis*, *P. synanthropica* presence and coffee foliage. Given that across plots an excess of zeros characterized *Pocobletus* abundance, we analyzed data with zero inflated models with a Poisson distribution. These models consist of two analytical tests; the first test, the count model, analyzes the dependent variable as a function of the explanatory variables under the assumption that the response variable follows a Poisson distribution. The second test, the zero inflation model, analyzes the probability of having an excess of zeros in the dependent variable as a function of the explanatory variables with the logit function. We compared the zero inflated models to null models using the likelihood test ratio; we also compared the zero inflated models to simple generalized linear models (GLM) with Poisson distribution using the Vuong test (Faraway 2006).

Vacant webs and intruders

In order to test if the number of vacant webs was associated with *A. instabilis* presence, *P. synanthropica* presence, and coffee foliage we used a GLM model with a binomial distribution. In this case vacant webs were considered as a failure of the total

number of counted webs per plant. In this model the number of vacant webs with respect to the total number of webs per coffee plant was a function of *A. instabilis* and *P. synanthropica* presence, and coffee foliage.

In order to analyze the relationship among spider intruders, *Pocobletus* sp. abundance, *A. instabilis* presence and *P. synanthropica* presence we used a zero inflated model. In this case the total number of intruders per plant was dependent upon the abundance of *Pocobletus* sp. adults and *A. instabilis* presence. We did not use the number of intruders per web given the low numbers recorded. However, the field observations allowed us to identify which of the spiders present in coffee plants were potential intruders of *Pocobletus* sp. webs. Indeed, all species (except one) recorded as intruders of *Pocobletus* sp. webs were recorded preying upon *Pocobletus* sp. at least once during spider surveys. Both a tree fall and ongoing management practices slightly decreased the number of coffee plants from June to July in plots I, III and IV. Plot I lost three plants whereas plots III and IV each lost two plants.

All statistical analyses were carried out with the R software (R Development Core Team 2005).

Results

Spatial distribution of *Pocobletus* sp. in relation to *A. instabilis* and *P. synanthropica*

Pocobletus sp. was clustered near to *A. instabilis* such that the average distance of any *Pocobletus* sp. adult or ovisac to a plant patrolled by *A. instabilis* was less than the distance expected if spiders were distributed at random (Table 4.1). The mean weighed distance between *Pocobletus* sp. (adult/ovisac) and a plant with *A. instabilis* varied across the four plots, however, these distances were always significantly smaller than the

expected distance under a random distribution (Table 4.1). This response was strong for both adults and ovisacs of *Pocobletus* sp. for all plots in the June sample (Fig. 4.1). Nonetheless, in plots I and IV, the distribution of *Pocobletus* was more spread in space, suggesting that other factors are involved in shaping the distribution of the spider.

The spatial distribution of *Pocobletus* sp. changed slightly in July. In late July the clustered pattern around *A. instabilis* disappeared from plot I and was marginally significant in plot III, but was still strongly significant in plots II and IV (Table 4.1). In July, the number of coffee plants patrolled by *A. instabilis* decreased in plots I and III.

The response of the spatial distribution of *Pocobletus* sp. to the presence of *P. synanthropica* was very strong and significant but only in Plot IV.

Relationships among *A. instabilis*, coffee foliage and *Pocobletus* sp.

The abundance of *Pocobletus* sp. (adults and ovisacs) was positively associated with the presence of *A. instabilis*, coffee foliage and the presence of *P. synanthropica* (Table 4.2). In all cases the zero inflated models were more accurate in explaining the variance of *Pocobletus* sp. abundance in relation to the presence of *A. instabilis*, coffee foliage and *P. synanthropica* than the GLMs (all Vuong tests $p < 0.0001$).

Over the two surveys, the abundance of *Pocobletus* sp. was explained by the presence of *A. instabilis* and *P. synanthropica* and by coffee foliage ($p < 0.0001$). The abundance of *Pocobletus* increased by at least 100% in the presence of *A. instabilis*, 40% in the presence of *P. synanthropica*, and 20% for each unit of foliage (Table 4.2). The increase in *Pocobletus* sp. abundance (adults and ovisacs) in the presence of *A. instabilis* and *P. synanthropica* was more pronounced during June than in late July, however, the

correlation with coffee foliage showed little variation over surveys (Table 4.2). Over the two surveys the probability of having zero adults or ovisacs decreased when *A. instabilis*, and coffee foliage were considered; however, the effect of *P. synanthropica* was less consistent (Table 4.2, zero inflated model).

Vacant webs and intruders

The probability of finding vacant webs of *Pocobletus* sp. varied across surveys. In June, the probability of finding vacant webs of *Pocobletus* sp. decreased in the presence of *A. instabilis* by a factor of 0.70; however, it did not show any significant variation in regards to *P. synanthropica* and coffee foliage. In July, none of the variables influenced the probability of finding vacant webs (Table 4.3).

In total we found 7 morphospecies of intruder spiders in the vacant webs of *Pocobletus* sp. Two morphospecies were active hunters belonging to the Salticidae family (~3.5 mm) whereas the other 5 species were web-builders belonging to the families Linyphiidae (~ 3 mm), Pholcidae (~ 2 mm) and Theridiidae (~ 2 mm). The three most abundant web builders found in the *Pocobletus* sp webs were *Faiditus dracus*, *Neospintharus* sp., and *Rhomphaea* sp., all of them belonging to the subfamily Argyrodoxinae, well known for its kleptoparasitic members. The most abundant hunter spider found in the webs of *Pocobletus* sp. belonged to the genus *Sidusa*.

The abundance of intruders per plant increased with the abundance of *Pocobletus* adults and coffee foliage, and decreased in the presence of *A. instabilis* and *P. synanthropica* (Table 4.4). The abundance of intruders decreased by 50% in the presence of *A. instabilis* in June ($p = 0.003$) but not in July ($p = 0.3$). In addition, the abundance of intruders was positively correlated to the increase in coffee foliage in both months by at

least 17 %. The zero inflated models showed that the probability of having zero intruders per plant was related to the abundance of *Pocobletus* sp. adults, *A. instabilis* presence and the coffee foliage (Table 4.4). In June, the probability of having zero intruders decreased with the increase in coffee foliage. In July the probability of having zero intruders per plant decreased with the abundance of *Pocobletus* adults and increased with the presence of *A. instabilis*, whereas the presence of *P. synanthropica* was not related to the probability of having zero intruders in any season.

Insect abundance in sticky traps and in spider webs

Insect abundance in sticky traps was higher with *A. instabilis* during both 2009 ($p = 0.0001$) and 2010 ($p = 0.005$). Mean insect abundance and its respective standard error in the presence of *A. instabilis* was 65 ± 2.47 per trap in 2009 and 36.07 ± 1.15 per trap in 2010 whereas mean insect abundance on plants without *A. instabilis* was 41.58 ± 1.90 per trap in 2009 and 31.23 ± 0.87 per trap in 2010 (Fig. 4.2a and b). In addition, the analysis of the content of *Pocobletus* sp. webs showed that overall more insects or residues of insects were present in the webs of *Pocobletus* sp. when the ant was present (13.03 ± 1.05) than when the ant was absent (3.8 ± 1.10 , $p < 0.0001$, Fig. 4.2c). Although a variety of orders were present in *Pocobletus* sp. webs, the higher prey abundance was driven by the abundance of *C. viridis*, the coffee green scale.

Discussion

In this study we found a positive relationship among *Pocobletus* sp., *A. instabilis*, *P. synanthropica* and coffee foliage. Indeed, the spatial distribution of *Pocobletus* was associated with *A. instabilis* presence, whereas *Pocobletus* abundance was strongly and positively associated with *A. instabilis*, *P. synanthropica* and coffee foliage. In none of

the studies reporting significant effect of ants on spiders (either positive or negative) has the relationship been made spatially explicit. Analysis of the spatial distribution of the associates can help us to better understand the strength of the interaction and the ecological forces that shape the spatial distribution of the associates. This is particularly important when the interactions are not between models and their mimics or specialist predators and prey. The spatial distribution of *Pocobletus* in the four sites illustrates that these tiny spiders are linked to *A. instabilis* ants; however, it also illustrates that other factors beside *A. instabilis* influence *Pocobletus* abundance.

Indeed, coffee foliage and the presence of *P. synanthropica* also contributed to explaining variation in the abundance of *Pocobletus*. The correlation between coffee foliage and *Pocobletus* sp. abundance is not surprising considering that these spiders build long lasting hammock webs. Coffee plants with more foliage may offer more sites for attaching webs. *Pocobletus* also increased in the presence of *P. synanthropica*; however, although significant, this increase was four times lower than the increase associated with the presence of *A. instabilis*.

A growing literature has documented that ants may positively influence spider populations and distributions through direct and indirect effects. Indirect effects include the cascading effects of their mutualistic interactions with hemipterans (Styrsky and Eubanks 2007, Zhang et al. 2012), plants (Meehan et al. 2009), and those resulting from ecosystem engineering (Schuch et al. 2008, Sanders and van Veen 2011). Positive direct effects of ants on spiders are due to their predation by spiders (Gibb 2003).

We propose that the association between *A. instabilis* and *Pocobletus* sp. can be explained by 1) the mutualism between *A. instabilis* and *C. viridis* and its indirect effects

and 2) *A. instabilis* aggressiveness and the enemy free space that may create for *Pocobletus* sp. We suggest that the mutualism between *A. instabilis* and *C. viridis* attracts insects that can exploit the honeydew resource secreted by the homopterans or even the homopterans (Volk 1992, Liere and Perfecto 2008). In turn, this increase in insects attracts spiders that prey upon them. Linyphiids are known for efficiently tracking resources and setting up their webs in prey-rich sites (Harwood et al. 2001, 2003, Harwood and Obrycki 2007) and our results from both sticky traps and web surveys indicate that more prey resources were recorded in the presence of *A. instabilis*.

Ant aggressiveness plays a central role in some food webs (Sanders and Platner 2007, Vandermeer et al. 2010, Pardee and Philpott 2011, Sanders et al. 2011, Sanders and van Veen 2011) because in addition to its potential negative effects on predators involved in biological control (Sanders et al. 2011) ant aggressiveness may create enemy free space for other taxa besides their mutualists (Volk 1992, Dejean et al. 2001, Liere and Perfecto 2008).

Indeed, the analyses of vacant webs and number of intruders per coffee plant suggest that *A. instabilis* protects *Pocobletus* sp. from predators. Marín and Perfecto (2013b) reported that *Sidusa* sp., one of intruders recorded in the coffee plants, was less abundant on plants with *A. instabilis*. Interestingly, the effects of *A. instabilis* on the probability of finding vacant webs and on the abundance of intruders per plant held only for June, indicating that the influence of *A. instabilis* may vary over time. Although, we found a positive correlation between *P. synanthropica* and *Pocobletus* sp. abundance, we did not find any relation between these ants and the number of vacant webs or the abundance of intruders per plant, thus suggesting that *Pocobletus* would not get any

enemy free space from *P. synanthropica*. Indeed, Ibarra-Nunez et al. (2001) reported that the webs of *Pocobletus* sp, then referred as Linyphiidae sp.1, had a great abundance of hemipterans and ants as prey. We think that these ants might have been *P. synanthropica* or any other small ant present at that time in the coffee plants, but not *A. instabilis* since these ants are twice as large (3.5 mm) as *Pocobletus* (1.4mm), and can get out the webs relatively easy (L. M. personal observations).

In summary, this study documents the positive relationships among the abundance of *Pocobletus*, coffee foliage and the presence of *A. instabilis* and *P. synanthropica*. Specifically, this study shows that the spider *Pocobletus* sp. and *A. instabilis* have a strong spatial association. Variation in the abundance of *Pocobletus* sp. is likely due to the combination of both coffee foliage, a factor that might directly affect web attachment, and to *A. instabilis* ants that may play a dual role by increasing resource availability and reducing the number of potential predators of *Pocobletus* sp.

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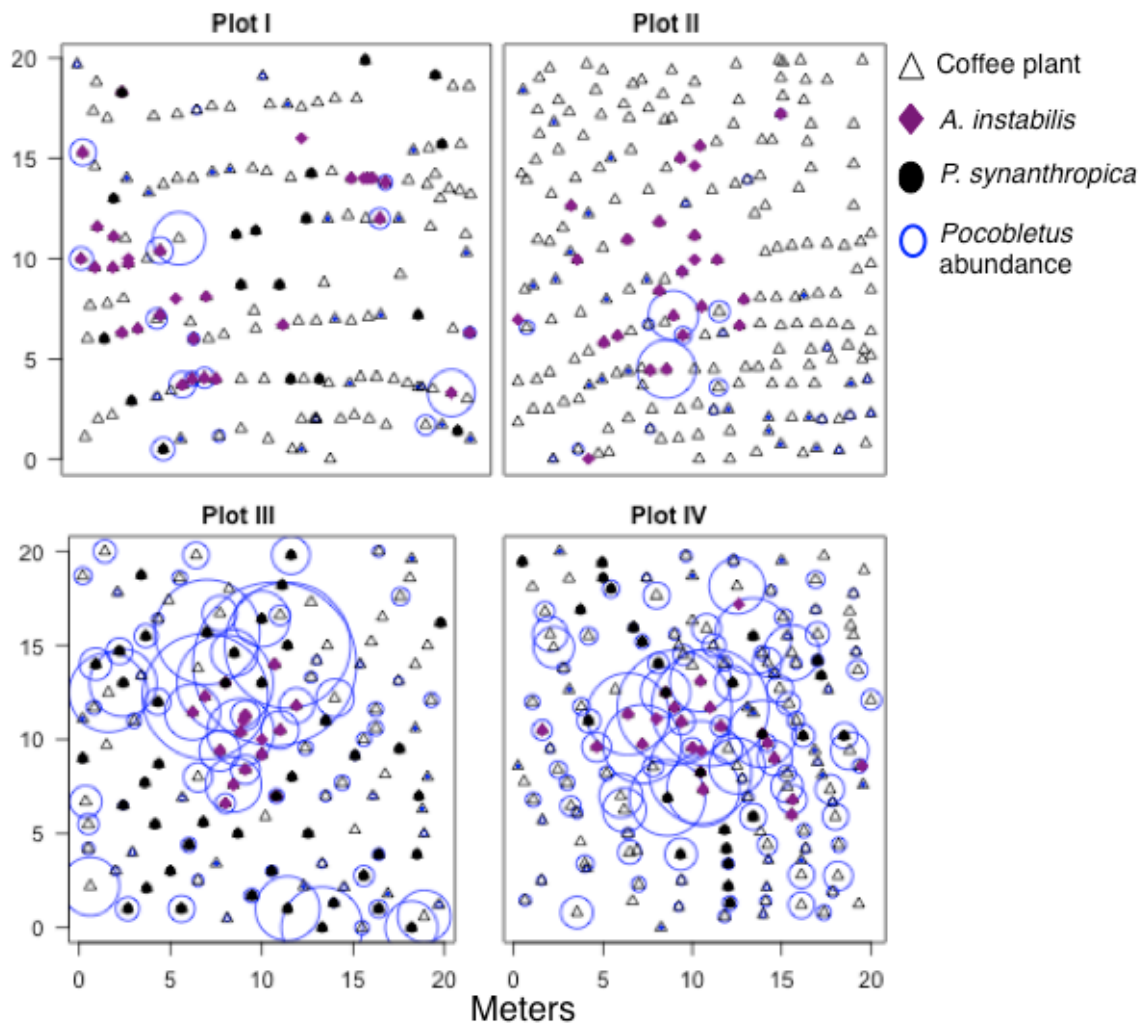


Figure 4.1. Spatial distribution of the abundance of *Pocobletus* sp. adults in the presence of *Azteca instabilis* and *Pheidole synanthropica* in three 20 x 20 m plots and one 20x 25 m plot in a coffee farm in Southern Mexico. a) June, b) July

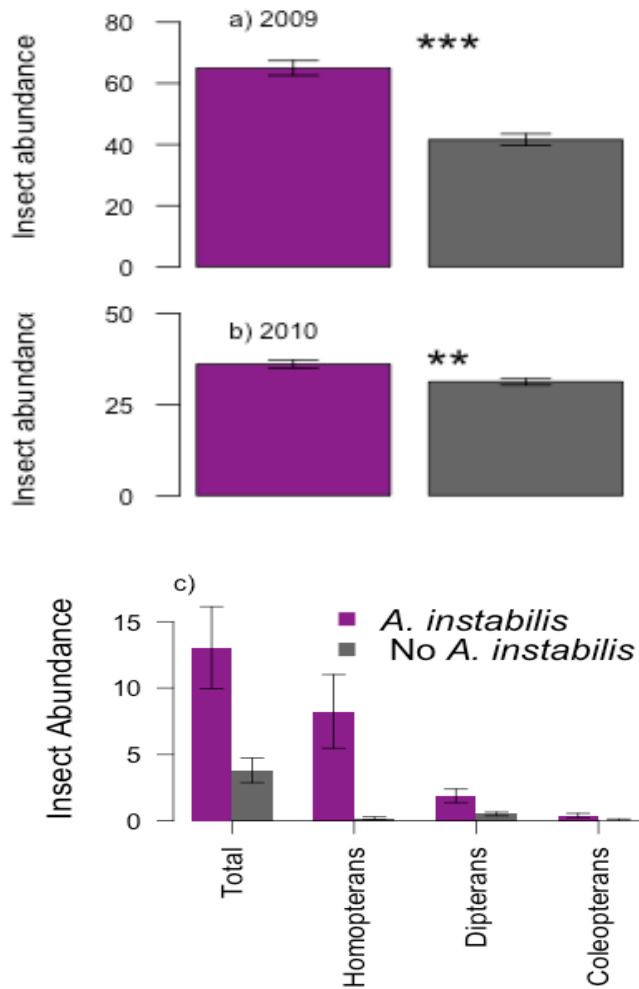


Figure 4.2. Insect abundance per coffee plant (mean \pm SE) in the presence and absence of *Azteca instabilis* in 2009 (a) and 2010 (b) and mean insect abundance per web of *Pocobletus* sp. collected in plants patrolled and not patrolled by the ant (c)

Table 4.1. Observed and random weighted mean distances (m) of *Pocobletus* sp. to the nearest coffee plant/tree patrolled by *Azteca instabilis* and *P. synanthropica* in the summer of 2010

Ants	Plot	<i>Pocobletus</i>	Observed	Random	Observed	Random	P-value	
			distance	distance	distance	distance	June	July
			June		July		June	July
<i>A. instabilis</i>	I	Adults	1.13	2.65	3.85	3.98	<0.0001	0.31
	II	Adults	1.92	3.19	1.41	3.21	0.001	<0.0001
	III	Adults	3.84	4.71	5.03	5.64	<0.0001	0.05
	IV	Adults	2.25	3.38	2.47	3.37	<0.0001	<0.0001
	I	Ovisacs	0.8	2.67	4.2	4	<0.0001	0.65
	II	Ovisacs	2.04	3.2	1.27	3.21	0.008	<0.0001
	III	Ovisacs	3.26	4.7	4.75	5.64	<0.0001	0.023
	IV	Ovisacs	2.21	3.38	2.37	3.37	<0.0001	<0.0001
<i>P. synanthropica</i>	I	Adults	3.03	2.5	2.79	2.56	<0.977	0.9
	II	Adults	NA	NA	NA	NA	NA	NA
	III	Adults	1.43	1.72	1.66	1.79	0.11	0.52
	IV	Adults	1.92	2.48	1.9	2.6	0.0014	<0.0001
	I	Ovisacs	3.06	2.41	2.73	2.14	0.95	0.83
	II	Ovisacs	NA	NA	NA	NA	NA	NA
	III	Ovisacs	1.45	1.5	1.64	1.51	0.23	0.48
	IV	Ovisacs	1.85	2.22	1.84	2.2	<0.0001	0.0012

Table 4.2. Abundance of *Pocobletus* sp. adults and ovisacs in relation to coffee foliage and the presence of *Azteca instabilis* and *Pheidole synanthropica* in June and July 2010

<i>Pocobletus</i> adults~ <i>A. instabilis</i> + <i>P. synanthropica</i> + Foliage				
June				
Count model				
	Estimate	Standard error	P-value	% of change
Intercept	-0.46	0.10	<0.0001 ***	
<i>A. instabilis</i>	0.96	0.05	<0.0001 ***	161.8
<i>P. synanthropica</i>	0.43	0.05	<0.0001 ***	54.4
Foliage	0.29	0.01	<0.0001 ***	33.6
Zero-inflation model				
	Estimate	Standard error	P-value	
Intercept	5.45	0.54	<0.0001 ***	
<i>A. instabilis</i>	-1.43	0.35	<0.0001 ***	
<i>P. synanthropica</i>	-1.03	0.32	0.001 **	
Foliage	-0.97	0.09	<0.0001 ***	
July				
Count model				
	Estimate	Standard error	P-value	% of change
Intercept	0.11	0.10	0.27	
<i>A. instabilis</i>	0.75	0.05	< 0.0001 ***	111.7
<i>P. synanthropica</i>	0.38	0.05	< 0.0001 ***	46.4
Foliage	0.23	0.01	< 0.0001 ***	26.0
Zero-inflation model				
	Estimate	Standard error	P-value	
Intercept	4.70	0.47	< 0.0001 ***	
<i>A. instabilis</i>	-1.60	0.36	< 0.0001 ***	
<i>P. synanthropica</i>	-0.63	0.29	0.03 *	
Foliage	-0.82	0.08	< 0.0001 ***	
<i>Pocobletus</i> ovisacs~ <i>A. instabilis</i> + <i>P. synanthropica</i> + Foliage				
June				
Count model				
	Estimate	Standard error	P-value	% of change
Intercept	0.43	0.12	0.0002 ***	
<i>A. instabilis</i>	0.85	0.05	< 0.0001 ***	134.3
<i>P. synanthropica</i>	0.20	0.06	0.0003 ***	22.4
Foliage	0.18	0.02	< 0.0001 ***	0.3
Zero-inflation model				
	Estimate	Standard error	P-value	
Intercept	5.79	0.52	< 0.0001 ***	
<i>A. instabilis</i>	-1.35	0.30	< 0.0001 ***	
<i>P. synanthropica</i>	-0.75	0.28	0.006 **	
Foliage	-0.90	0.09	< 0.0001 ***	
July				
Count model				
	Estimate	Standard error	P-value	% of change
Intercept	0.41	0.11	<0.0001 ***	
<i>A. instabilis</i>	0.87	0.05	<0.0001 ***	139.7
<i>P. synanthropica</i>	0.17	0.06	0.001 *	19.2
Foliage	0.20	0.01	<0.0001 ***	22.5
Zero-inflation model				
	Estimate	Standard error	P-value	
Intercept	4.99	0.48	<0.0001 ***	
<i>A. instabilis</i>	-1.42	0.32	<0.0001 ***	
<i>P. synanthropica</i>	-0.74	0.28	0.028 *	
Foliage	-0.77	0.08	<0.0001 ***	

Table 4.3. Vacant webs of *Pocobletus* in relation to coffee foliage, and the presence of *Azteca instabilis* and *Pheidole synanthropica* in June and July 2010.

Vacant webs~ <i>A. instabilis</i> + <i>P. synanthropica</i> + Foliage			
	Estimate	Standard error	P-value
June Intercept	-2.05	0.40	<0.0001 ***
<i>A. instabilis</i>	-0.72	0.20	0.0004 ***
<i>P. synanthropica</i>	-0.03	0.18	0.85
Foliage	-0.095	0.06	0.07 *
July Intercept	-3.40	0.44	<0.0001 ***
<i>A. instabilis</i>	-0.08	0.21	0.68
<i>P. synanthropica</i>	-0.09	0.21	0.65
Foliage	0.05	0.06	0.37

Table 4.4. Abundance of spider intruders per coffee plant in relationship to coffee foliage and the presence of *Azteca instabilis* and *Pheidole synanthropica* in June and July 2010

Intruders~ <i>Pocobletus</i> adults + <i>A. instabilis</i> + <i>P. synanthropica</i> + Foliage				
June				
Count model				
	Estimate	Standard error	P-value	% of change
Intercept	-0.47	0.19	0.02	
<i>Pocobletus</i> adults	0.01	0.01	0.06	1.20
<i>A. instabilis</i>	-0.66	0.23	0.005 **	-48.1
<i>P. synanthropica</i>	0.02	0.12	0.83	
Foliage	0.16	0.03	<0.0001***	17.8
Zero-inflation model				
	Estimate	Standard error	P-value	
Intercept	0.50	0.62	0.42	
<i>Pocobletus</i> adults	-0.01	0.03	0.80	
<i>A. instabilis</i>	0.42	0.62	0.50	
<i>P. synanthropica</i>	0.40	0.37	0.27	
Foliage	-0.27	0.11	0.01*	
July				
Count model				
	Estimate	Standard error	P-value	% of change
Intercept	-1.37	0.34	<0.0001 ***	
<i>Pocobletus</i> adults	-0.008	0.0100	0.4	
<i>A. instabilis</i>	0.28	0.26	0.3	
<i>P. synanthropica</i>	0.13	0.19	0.5	
Foliage	0.20	0.05	<0.0001 ***	22.4
Zero-inflation model				
	Estimate	Standard error	P-value	
Intercept	1.12	0.81	0.16	
<i>Pocobletus</i> adults	-0.13	0.05	0.015 *	
<i>A. instabilis</i>	1.23	0.59	0.038 *	
<i>P. synanthropica</i>	-0.21	0.60	0.73	
Foliage	-0.21	0.14	0.12	

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CHAPTER 5

CONCLUSION

In my dissertation, I studied spider diversity in a coffee landscape in Southern Mexico with the goal of gaining a better understanding of the ecology of predators in tropical agroforestry systems. I hoped to help unravel the richness of ecological interactions that take place in agricultural settings. Specifically, the analysis of spiders distributed on the ground, on coffee plants, and on tree trunks, and their correlations with coffee management, aggressive ants and distance to forest, shows that even though the spiders found in these three strata exhibit different behaviors, a general pattern in diversity is present. Overall, the local diversity of ground dwelling spiders (Chapter 1) and coffee inhabiting spiders (Chapter 3), but not the local diversity of tree-trunk dwelling spiders (Chapter 2) tends to reach higher values in the most intensified coffee agroforestry systems (e.g. in those coffee systems with less canopy shade). Second, *Azteca instabilis* ants have a strong correlation with the local spider richness, abundance and biomass found in the coffee and tree layer (Chapter 2, Chapter 3 and Chapter 4). Third, plant characteristics seem to strongly influence spider abundance in the coffee and tree trunk layers (Chapter 2 and Chapter 4). Fourth, distance to forest is not a crucial

variable for explaining the richness of spiders in this agroforested landscape (Chapter 1 and Chapter 2), though forests sites might contribute to spider assemblages.

In addition, an important technical consideration regarding species diversity also emerged. This study has pointed out the need to consider both the local species richness and the accumulated species richness and used both of them to better understand the interplay between diversity, management type and aggressive ants. Indeed, in most cases of my research across spider communities the observed patterns in local spider richness differed from the total accumulated spider richness patterns. For example, in both the coffee layer and the tree trunk layer the patterns of accumulated species richness did not differ in the presence and absence of *A. instabilis*; however, local spider richness was higher in the presence of *A. instabilis*. Thus, although in terms of total accumulated species richness the presence of *A. instabilis* did not affect spider diversity, at the local level (either coffee plant or shade tree) the average spider richness increased. The same is true in the case of the coffee dwelling spider community and its contrasting results in species accumulation patterns and local richness in relation to coffee management type.

In Chapter 1, I showed that local spider richness and abundance were higher in low shade coffee systems across the dry and rainy seasons. However, the pattern of the accumulated species richness across systems was more complex. Indeed, accumulated species richness was higher in the high shade coffee systems over the dry season, whereas in the rainy season the pattern reversed. Accumulated species richness was higher in the low shade coffee systems than in any other systems. Thus in the ground

dwelling spider community there is a clear trend to increase diversity in the most intensified systems at least in the rainy season. The positive association between low-shade coffee systems and spiders seem to be due to a variety of causes including dispersal capabilities and prey availability, but only further studies will elucidate the underlying mechanisms. In Chapter 1, I also reported that distance to the forest was a poor predictor of spider richness and abundance. However, in the dry season, probably the harshest season in the tropics, the forest sites were the most dissimilar in terms of species composition when compared with low and high-shade coffee sites. Other studies have found stronger correlations and effects on spider diversity with distance to forest. However, those studies have been conducted in landscapes with a high contrast between forest and agricultural systems. I suggest that in agroforestry landscapes, the effect of forest fragments on spider diversity is less pronounced (see also Stenchly et al. 2011, 2012).

In chapter 2, I showed that local spider richness, abundance and biomass were positively correlated with the number of concavities found in the trunks of shade trees and that spider abundance and biomass increased in the presence of *A. instabilis*. However, I did not find any correlation with canopy cover as I did with the ground dwelling spiders. It seems that for the tree trunk spiders the identity of the tree on which they build their webs is more important than canopy cover. Indeed, I found that local spider richness; abundance and biomass were higher in non-*Inga* trees than in *Inga* trees, probably because non-*Inga* trees were structurally more complex than *Inga* trees, which in the region are characterized by their straight trunk and small tree diameter. Given that intensification of coffee agroecosystems implies an increase in the proportion of *Inga*

trees and consequently a decrease in the proportion of other tree genera my study suggests that in order to keep a diverse spider community, a diverse template provided by the shade trees is required. Regarding *A. instabilis*, I found that although spider richness was not correlated with *A. instabilis*, spider abundance had a strong positive correlation with the ants. Indeed, spider abundance increased by at least 50% when *A. instabilis* were present. This correlation was also reflected in spider biomass, which also increased in the presence of these ants. These correlations were driven by *Ischnothele digitata* and *Azilia guatemalensis*, the two most abundant spider species found in 2011 and 2012, that seem to prefer *A. instabilis* individuals as prey.

In Chapter 3, I showed that although the accumulated species richness in coffee plants did not differ between coffee management nor in the presence/absence of *A. instabilis*, the local spider richness and abundance were highest in the most intensified coffee system and in the presence of *A. instabilis*. Indeed, I found that at least three spider species, *Pocobletus* sp., *Myrmarachne panamensis*, and *Lyssomanes spiralis*, were positively correlated with *A. instabilis*. I also reported that the positive effect of *A. instabilis* on spiders seems to be caused by at least two mechanisms: higher abundance of insects and protection against predators.

In Chapter 4, I showed that the spatial distribution of *Pocobletus* sp., a small linyphiid spider was positively associated to the presence of *A. instabilis*. In addition, I found that the abundance of *Pocobletus* ovisacs and adults was positively related to coffee foliage, and to the presence of the ants *A. instabilis* and *Pheidole synanthropica*. Although, *Pocobletus* abundance increased in the presence of both ants, the magnitude of the correlations shows that *A. instabilis* seems to be a key factor for *Pocobletus*. Indeed, I

showed that in the presence of *A. instabilis*, the number of predators of *Pocobletus* decreased, thus suggesting that these linyphiid spiders might gain enemy-free space in the presence of *A. instabilis*. In addition, I reported that in the presence of *A. instabilis*, more potential prey insects were found in the webs of *Pocobletus*, thus suggesting that this tiny spider might take advantage of an increase in insect abundance.

Thus, my research on local and landscape factors and their relationship with spider communities and diversity in tropical agroforestry systems at least opened three new areas that require further research. First, the positive correlation between low-shade coffee systems and local spider diversity found in the ground and coffee layers may be linked with dispersal capabilities and prey availability, whereas the positive correlation with plant characteristics (either coffee foliage or tree trunk characteristics) shows that spiders may prefer to build their webs in more structurally complex plants and trees. Second, the positive associations between *A. instabilis* and local spider richness and abundance seem to be due to a combination of indirect effects, such as the higher abundance of resources recorded in the presence of the ants and potential enemy-free space, and direct effects, such as predation of the ants. Third, the low correlation value between distance to forest and local spider richness and abundance suggest that distance to forest is not an important variable in this agroforested landscape. I suggest that in agroforestry landscapes, the effect of forest fragments on spider diversity is less pronounced.

Overall, my research adds to the growing literature in agroforestry systems that explores the influence of agricultural management, aggressive ants and landscape effects on natural agents of biological control.