A study of abiotic and biotic factors affecting coffee rust infection rates in a shade-grown organic coffee farm.

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Research Thesis

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ABSTRACT

Coffee rust disease causes significant losses to coffee bean production, and producers can incur heavy costs managing it with fungicides. Given the potential for coffee rust infection rates to increase with climate change, a better understanding of the factors influencing coffee rust infestation could help coffee producers manage coffee rust cost-effectively. In this study, the effect of the abundance of *Lecanicillium lecanii*-infected scale insects, shade, variety type, and plant density were compared, with spatial and temporal effects taken into consideration. *L. lecanii*-infected scales surveyed the year previous to the coffee rust survey decreased coffee rust intensity, while *L. lecanii*-infected scales surveyed the same year to the coffee rust survey showed a positive relationship under some conditions. Shade decreased coffee rust intensity in 2009 but not in 2010, and varieties had differing probabilities of being infected by coffee rust. Coffee plant density had no effect on rust incidence, but it did seem to affect the dispersal of rust through space. The results suggest that ecological management of *L. lecanii*-infected scales and variety type may help to decrease coffee rust intensity and incidence.

Introduction

Coffee rust, caused by the fungus *Hemileia vastatrix* Berkeley and Broome, is one of the most important diseases of Arabica coffee in the world. In the late 1800's, coffee rust epidemics reduced coffee bean yields by as much as 90 per cent in places like Sri Lanka, Java, and Sumatra (Hein and Gatzweiler, 2006). It reached the Americans in the 1960 and until recently it caused losses of 20 – 25% per annum in some regions (McCook, 2006). In late 2012 it emerged in Mesoamerica, Northern South America and the Caribbean as a major threat to coffee production with yield losses predicted to reach 50 percent for the 2013 harvest season (Cressey, 2013).

There are two main methods of managing coffee rust. First is the use of completely or partially resistant species or varieties (Brito et al., 2010; Romero et al., 2010). The second main management technique is the application of the environmentally hazardous fungicide, copper oxychloride (McCook, 2006).

Despite the use of resistant varieties and fungicides, worldwide losses and control efforts are still estimated to cost approximately US $1-2 billion annually (Hein and Gatzweiler, 2006). Some researchers and producers speculate that coffee rust infection rates will increase due to climate-induced changes in precipitation and temperature that extend the geographic range of coffee rust to the higher altitudes where *C. arabica* is often grown (Ghini et al., 2011). Given this concern, and the actual recent increase in the disease in Latin America, it is necessary to improve our understanding of cost-effective management techniques that are economically and ecologically sustainable. This is especially so given that therapeutic interventions, such as the application of fungicides, are effective only in the short term, since these are frequently neutralized by countermoves within the system, such as evolution of resistance.

It has been suggested that long term pest control can best be achieved by managing the system to maximize "build-in" preventive strengths within the agroecosystem (Lewis et al., 1997). This approach is similar to "conservation biological control", where agroecosystems are managed to provide habitat and conserve natural enemies of pests (Letourneau et al., 2011). Many studies have been published on conservation

biological control and the role of plant diversity, natural enemies, and reduction of pest damage (Barbosa, 1998; Fiedler et al., 2008; Letourneau et al., 2011; Tscharntke et al., 2007)**,** however few studies have examined this approach for the management of crop diseases. Within shade-grown coffee agroecosystems it has been suggested that complex ecological interactions may reduce pest damage and infection rates of coffee diseases, including coffee rust (Vandermeer et al., 2010). But to manage the ecosystem service of rust control, it is important to understand the biotic and abiotic factors affecting the dispersal of coffee rust spores, the susceptibility of coffee plants, and the infection cycle. Much of the previous work on the ecology of coffee rust fungus has focused on three factors: the genes that confer resistance in different coffee varieties (Brito et al., 2010; Diniz et al., 2012; Diola et al., 2011; Romero et al., 2010), the abiotic factors affecting the germination and dispersal of coffee rust spores (Becker and Kranz, 1977; Kushalappa and Eskes, 1989; Avelino et al., 2006) and the impact of potential bio-control organisms on coffee rust infection rates (Carrión and Rico-Gray, 2002; Vandermeer et al., 2009).

A common method of managing coffee rust infection rates is by planting cultivars with total or partial resistance to coffee rust (Kushalappa and Eskes, 1989; Romero et al., 2010). At least nine known genes confer complete resistance to coffee rust, and there may be up to five genetic regions affecting partial resistance (Romero et al., 2010). As a result, coffee varieties have varying levels of resistance to coffee rust infections. For example, *Coffea arabica* var *arabica* and *C. arabica* var. *bourbon* are highly susceptible to coffee rust fungus, while varieties cultivated from *Hibrido de Timor,* a hybrid of *Coffea arabica* and *Coffea canephora,* tend to be more resistant due to the larger number of resistant genes (Diniz et al., 2012; Romero et al., 2010). However, varietal resistance is not stable – varieties that were once considered "resistant" to coffee rust have since become susceptible due to the evolution of new *H. vastatrix* races (Silva et al., 2006).

Abiotic factors that affect germination and dispersal of coffee rust spores also have been studied in order to discern how management of these factors can affect coffee rust infection rates. The three most studied factors are wind, precipitation, and temperature (Avelino et al., 2006; Becker and Kranz, 1977; Kushalappa and Eskes, 1989). Spore germination requires a wet leaf surface; consequently, high daily

precipitation tends to cause higher rust infection rates (Kushalappa and Eskes, 1989). Temperatures between 20 and 30 degrees Celsius are ideal for spore germination, although direct solar irradiance can kill spores (Kushalappa and Eskes, 1989). Wind is an important long-distance dispersal factor, however wind can also decrease leaf-wetness via evapotranspiration, thereby decreasing germination rates (Becker and Kranz, 1977; Kushalappa and Eskes, 1989).

Research concerning these abiotic factors has affected coffee management. For example, some producers have eliminated shade trees in an effort to increase solar irradiance and decrease leaf wetness, with the assumption that this will reduce the prevalence of coffee rust infections (Avelino et al., 2004). However, coffee rust infections can still occur in sun-coffee plantations because the plants are often in close proximity and can shade each other, thereby increasing leaf wetness on lower leaves and increasing the possibility of rust spore germination (Avelino et al., 2004). Moreover, shade trees can actually decrease wind dispersal of spores, suggesting that shade has a complicated effect on coffee rust infection rates (Avelino et al., 2004).

Figure 1 *L. lecanii* infecting *H. vastatrix* spores. Photo by John Vandermeer, 2010.

Shade trees are also sources of ecological diversity that can help producers manage a naturally occurring biological control agent, *Lecanicillium lecanii* (Zimmerman). This mycoparasitic and entomopathogenic fungus, which has a global distribution, can parasitize *Hemileia vastatrix* spores (Carrión and Rico-Gray, 2002; Eskes et al., 1991; Silveira and Rodriques, 1972) (Figure 1). Field studies in the past have suggested it is an ineffective biological control when sprayed on rust-infected coffee plants (Kushalappa and Eskes, 1989). However, in Cuba reduction rates of rust with *L. lecanii* were similar to what was achieved with applications of copper oxichloride (Gonzalez, 2006). Furthermore, a large-scale (45-hectares) survey in a shade-grown coffee showed that *L. lecanii* may act as a naturally-found biological control through a complex set of ecological interactions centred around the keystone ant species, *Azteca instabilis* (Perfecto and Vandermeer,

2008).

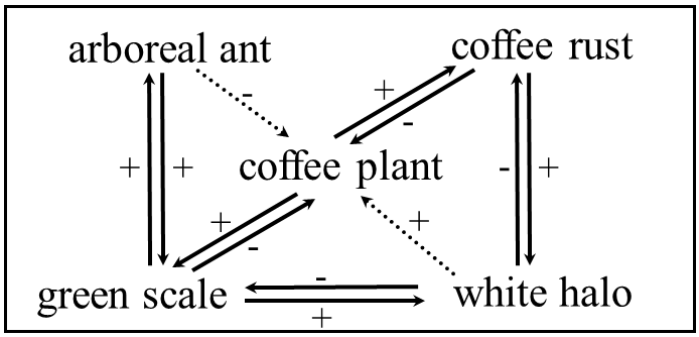

Figure 2 The ecological interactions between the main organisms discussed in this study. The positive interactions are denoted with a plus sign, and the negative interactions are denoted with a negative sign.

Figure 2 shows the direct interactions among the main organisms within the shade-grown coffee agroecosystem in Chiapas, Mexico that affect coffee rust infection rates. *Azteca instabilis* is an arboreal ant that forms a mutualism with the green coffee scale, *Coccus viridis* Green (Hemiptera: Coccidae) by eating excess honeydew and protecting the scales from predators and parasitoids (Jha et al., 2012; Vandermeer and Perfecto, 2006). The ants' protection often allows green coffee scale populations to become quite dense on coffee plants. Dense populations of green coffee scale can become infected by the entomopathogenic white halo fungus, *L. lecanii,* leading to an epizootic that kills all scales in the vicinity (Jackson et al., 2012a; Kouvelis et al., 1999; Vandermeer et al., 2009). Vandermeer and colleagues (2009) reported a decreased prevalence of coffee rust fungus in sites near *A. instabilis* nests, and also a negative relationship between the distance from *L. lecanii* epizootics on *C. viridis* and rust lesions. Their study suggests that high abundances of *L. lecanii* spores caused by local epizootics on green scale insects decreases the intensity of coffee rust infections.

Most of the studies concerning coffee rust ecology have not considered the importance of first order or second order spatial processes that may affect coffee rust dynamics in space (e.g. see reviews Kushalappa and Eskes, 1989; Avelino et al., 2006). First order spatial processes are generally underlying differences in space that affect the intensity of events over space, and second order interactions are inter-event interactions that affect the distribution of events in space (Bailey & Gatrell, 1995). De Carvalho de Alves and colleagues (2009) used geo-statistical analyses to show that the intensity of coffee rust is not constant over space; rather, coffee rust intensity has a clustered spatial distribution in their study site, a sun-grown coffee plantation in Columbia. The authors suggest that an understanding of this clustering pattern will improve our ability to manage the disease, but do not discuss the ecological processes

or factors that may be affecting this spatial heterogeneity. This begs the question: what causes the uneven distribution of coffee rust incidence in space, and can the management of spatial processes limit coffee rust infection rates?

Most studies on coffee rust focus on the broad scale effects over large areas, which necessitate sampling a few branches per coffee plant and sampling a few coffee plants per area. In this study we performed complete spatially-explicit surveys of all coffee plants in two study sites over a two year period. This allowed us to study the impact of a number of factors at a finer scale, including the impact of *L. lecanii*-infected scales, shade, and coffee variety type. In addition, these complete surveys allowed us to study fine-scale spatial dynamics of coffee rust over time.

There are two main goals of this study. The first is to compare the relative effect of *L. lecanii*-infected scales on coffee rust incidence to other important factors such as shade level, precipitation, and variety type. The second goal is to expand current knowledge concerning the spatial dependency in some potential factors that affect coffee rust incidence in a shade-grown organic coffee agro-ecosystem using common spatial analysis tools.

1 Methods and Materials

Study Site

The study was conducted in two sites in *Finca Irlanda*, a 300 hectares certified organic, shade-grown coffee farm in the Soconusco region of Chiapas, Mexico[1]. Site A contains 483 coffee plants in an area of approximately 40 by 50 meters. Site B contains 425 coffee plants in an area of approximately 40 by 40 meters. Both sites are sloped, west facing, and irregularly shaped. Site B is fully situated on an incline, whereas Site A flattens out at the "top" of the site, and approximately 200 of the

[1] Since this study was conducted Finca Irlanda abandoned its organic certification and is no longer certified organic.

coffee plants in Site A are in this flat region. The coffee plants in both sites were mapped in 2009 using an x-y coordinate system and meter tape.

1.1 Data Collection

1.1.1 Coffee rust

Surveys of *H. vastatrix*-infected leaves were conducted in both sites in May, September, and November of 2009 and June, September, and November of 2010. The number of infected leaves per plant was determined by turning over every coffee leaf in every coffee plant and inspecting them for yellow-orange lesions containing pustules of orange spores (Figure 1, page 6). In order to reduce false positives, only orange lesions with obvious rust spores were counted.

An allometric regression equation was used to determine the total number of leaves per plant. This equation was created from a random selection of coffee bushes in Site B in 2009. For each coffee plant in the random selection, researchers measured the total number of leaves and the sum of the heights of all the stems. A regression line was then fit to the relationship between the sum of all stem heights and the number of leaves. The best-fit regression line was a power law, with an r^2 value of 0.8294. The following power regression equation from the correlation between the number of leaves and stem heights was used to estimate the number of leaves per bush in both sites:

$$L = 107.03H^{1.8733}$$

where L denotes the number of leaves per coffee plant and H denotes the sum of the heights of all the stems in the coffee plant. The lengths of all the main stems of each coffee plant were measured in June 2009 and July 2010.

1.1.2 Lecanicillium lecanii-infected scales

Surveys of *L. lecanii*-infected *C. viridis* were conducted in both sites in June, September, and November of 2009. The abundance of adult *C. viridis* (greater than 0.7 mm in width) on all coffee plants in each site was estimated using a rapid-survey protocol adapted from Vandermeer & Perfecto (2006), as described in Jackson *et al.* (2012). The survey method was adapted from a preliminary study by Vandermeer & Perfecto (2006), in which the total number of scales of 21 trees was compared to various methods of estimation. A log-log regression of the estimated and actual counts had an r^2 value of 0.926 (Vandermeer & Perfecto, 2006).

The prevalence of *L. lecanii* was also assessed using the decision tree described in Jackson *et al.* (2012). The total prevalence of *L. lecanii* was calculated as the estimated number of *C. viridis* individuals multiplied by an estimated fraction of infected *C. viridis* individuals. The fractions infected for the four categories were: zero = 0.0; low = 0.05; medium = 0.15; high = 0.35; and very high = 0.75.

1.1.3 Coffee Variety

The manager of the coffee seedling nursery at *Finca Irlanda* provided a key to determine the variety of each coffee plant, shown in Table 1. The five varieties in the two sites are varieties or cultivars of the species *Coffea arabica*. Of these, only *C. arabica var. catimor* is known to carry coffee rust-resistant genes (Diniz *et al.*, 2006).

Table 1 The key used to distinguish coffee varieties in sites A and B.

1. **The young leaves are olive to red-colored**: *typica* or *catimor*
    a. long internodes, thin stems, new shoots growing out of the middle of the stem: *typica*
    b. short internodes, thick stems: *catimor*
2. **The young leaves are green-colored:** *bourbon, caturra* or *catuai*
    a. long internodes, thin stems: *bourbon*
        b. short internodes, larger leaves, more than one stem from the base: *caturra*
        c. short internodes, larger leaves, only one stem from the base: *catuai*
3. **No leaves:** *'unknown'*

1.1.4 Shade

The percent cover of shade was measured with a hand-held densiometer. Three readings of percent shade were taken at every second plant, and then averaged.

1.2 Statistical Methods

1.3.1 Spatial Analysis

1.3.1.1 Coffee Rust Incidence

The distribution of coffee rust incidence was zero-inflated and highly right-skewed, and the variance of the distribution was greater than the mean, suggesting that the data followed a zero-inflated negative binomial distribution (Figure 3). Therefore, two separate tests were used to analyze the spatial distribution of coffee rust.

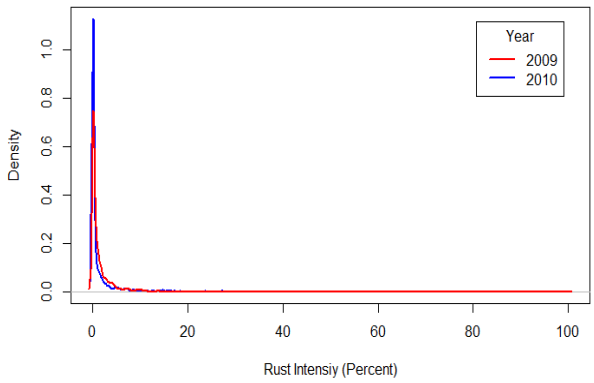

Figure 3 The zero-inflated, right-skewed distribution of rust intensity values for both sites in 2009 and 2010.

The first was a cross-Ripley's K, which tested if rust-infected plants were closer or farther away from uninfected plants than would be found in a completely spatially randomized distribution (Bailey & Gatrell, 1995). This test was implemented using the R statistical package spatstat, with a "translated" edge correction generally used for irregularly-shaped sites (Baddeley & Turner, 2005).

Second, the spatial distribution of coffee rust intensity was analyzed with global and local Moran's I analyses using the freeware program GeoDa (Anselin *et al.*, 2006). For this analysis, the focus was on the autocorrelation between the intensity of infection on infected plants, therefore all uninfected plants were removed from the analysis and the rust incidence per plant was log-transformed to better approximate a normal distribution.

1.3.1.2 *Coffee plant spatial dependence*

The distribution of coffee plants in space was analyzed with a density map, created created using the ArcGIS 10.0 Kernel Density Function. The minimum output cell size was calculated as 2 meters, which roughly correlates to an average of 1.6 plants

per cell. A search radius of five meters was used so that small-scale clusters could be evaluated. Both linear and logistic regressions were used to test for correlations between coffee rust intensity or presence and plant density, as calculated by the kernel density function. These regressions were performed within each month and year in the two sites.

1.3.2 Regression Model

The model objective was to compare the effect of the abundance of *L. lecanii*-infected scales on coffee rust incidence with percent shade coverage over each coffee plant, coffee plant density, and coffee variety type (a categorical variable).

Previous work has shown that coffee rust intensity increases with distance from areas with high *L. lecanii* – infected scales and that this relationship can be captured, on average, at a scale of 10 meters distance from *L. lecanii*-scale epizootic sites (Vandermeer *et al*., 2009). We found multiple plants with differing amounts of *L. lecanii*-infected scales scattered within in each site, and so decided to calculate the effect of *L. lecanii* as the total distance-weighted abundance of *L. lecanii* - infected scales relative to each coffee plant. An R-based model (R Development Core Team, 2011) calculated the abundance of *L. lecanii* – infected scales to a maximum of 10 meters distance of each plant using the following equation:

$$d \leq 1{:}\,10\,; \qquad T_i = \sum\left(d_{ij} + A_j\right)$$

where T_i is the distance-weighted abundance of *L. lecanii* –infected scales at plant *i*, d_{ij} is the distance from plant *i* to plant *j*, and A_j is the abundance of *L. lecanii*-infected scales at plant *j*.

Recent work also suggests that coffee rust infection rates are significantly lower in areas with high *L. lecanii* –infected scale abundances the previous year, such that there is a possible 'time lag' relationship between *L. lecanii* abundance and coffee rust incidence (Jackson *et al.*, 2012). Therefore, models used in this study compared

the effect of *L. lecanii* abundance the same year with the *L. lecanii* abundance the previous year on coffee rust intensities in 2009 and 2010.

Although shade is spatially dependent on the location of trees within the plot, this model used shade as a local effect that might impact micro-climate conditions. The values were calculated from an ordinary kriging model, which was created using the ArcGIS 10.0 Geostatistical Tool to interpolate percent shade values for all coffee plants within each site (Figure 4). Therefore, each shade value corresponded to the average percent shade within a one meter diameter of the centroid of each plant.

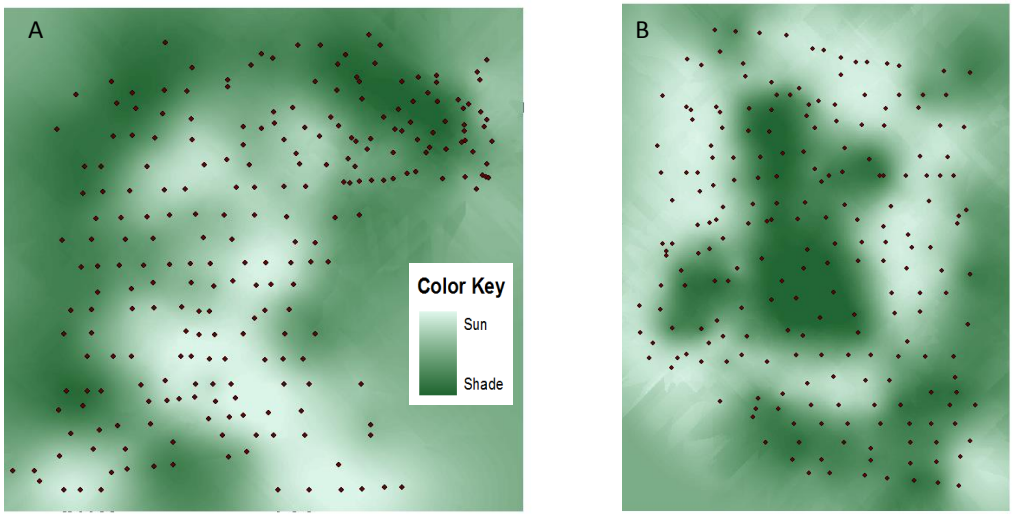

Figure 4 Maps of percent shade cover for Site A (3a) and Site B (3b). Darker green denotes a higher per cent shade. Maps were generated using the Simple Kriging function in ArcGIS (created for a project in NRE540 in November, 2010)

Similarly, plant density was created from the previously discussed kernel density model by attaching the kernel density value of a location in space to the identification number for the coffee plant in that location.

All quantitative explanatory variables were standardized as z-scores $(A_j - A_{mean})/A_{St.Dev.}$ so their effect size on coffee rust infections would be comparable.

Coffee variety is a simple categorical variable. The variety types of 39 coffee plants were indistinguishable due to lack of leaves or other characteristics and so were removed from further analysis.

In addition to the predictor variables discussed above, site, month, and year were also included in the models as categorical predictor variables because they were highly correlated with coffee rust intensity. Two sites were surveyed in three months and two years. The months were May (2009) or June (2010), which was the beginning of the rainy season, September (the end of the rainy season) and December (the height of coffee rust incidence). It is likely that the relationship between these categorical variables and rust intensity were caused by variation in precipitation, temperature, and wind speed – variables that change depending on location and time. Although these variables are known to be very important for rust germination and dispersal, we were unable to measure them at the micro-climate scale, so they were excluded from the analysis.

Coffee rust incidence is not normally distributed within the sites. There are a greater number of uninfected plants (i.e. 'zeros') -- as well as a greater number of plants with a low amount of infection -- than would be expected in a normal distribution. Given that this distribution more closely follows a zero-inflated negative binomial distribution (Figure 3, p 11). A general linear model, which assumes a normal distribution, would be inappropriate because it would under-predict the number of uninfected plants and over-predict the number of highly infected plants (Zuur et al., 2009).

A binomial regression model and a linear regression model were performed to test two hypotheses. The binomial regression model tested the effects of the explanatory variables on the probability of a plant being infected by coffee rust, while the linear regression model tested the effects of the explanatory variables on the intensity of rust infection. A detailed explanation of these models can be found in the supplementary information.

2 Results

2.1 Fine-Scale Spatial Analysis

The spatial pattern of coffee rust intensity was studied in November of both years, when the rust infection rates were high. A Moran's I analysis of spatial autocorrelation found that coffee rust intensity clustered more strongly in Site A compared to Site B (Table 2). As well, the location in which coffee rust clusters in space changed between years within the two sites (Figure 5). Specifically, in Site A, coffee rust intensity moved from the lower section in 2009 to the upper section in 2010. In Site B, coffee rust intensity seemed to move from the center to the edges of the site between 2009 and 2010.

Table 2 Global Moran's I Values for Coffee Rust Intensity in Site A and Site B. All Moran's I values are significant at $P < 0.001$ (M.L., 999 permutations).

	Site A		Site B	
	Moran's I	**Cluster Size (m)**	**Moran's I**	**Cluster Size (m)**
November 2009	0.5274	5	0.0186	7
November 2010	0.3572	5	0.0581	7

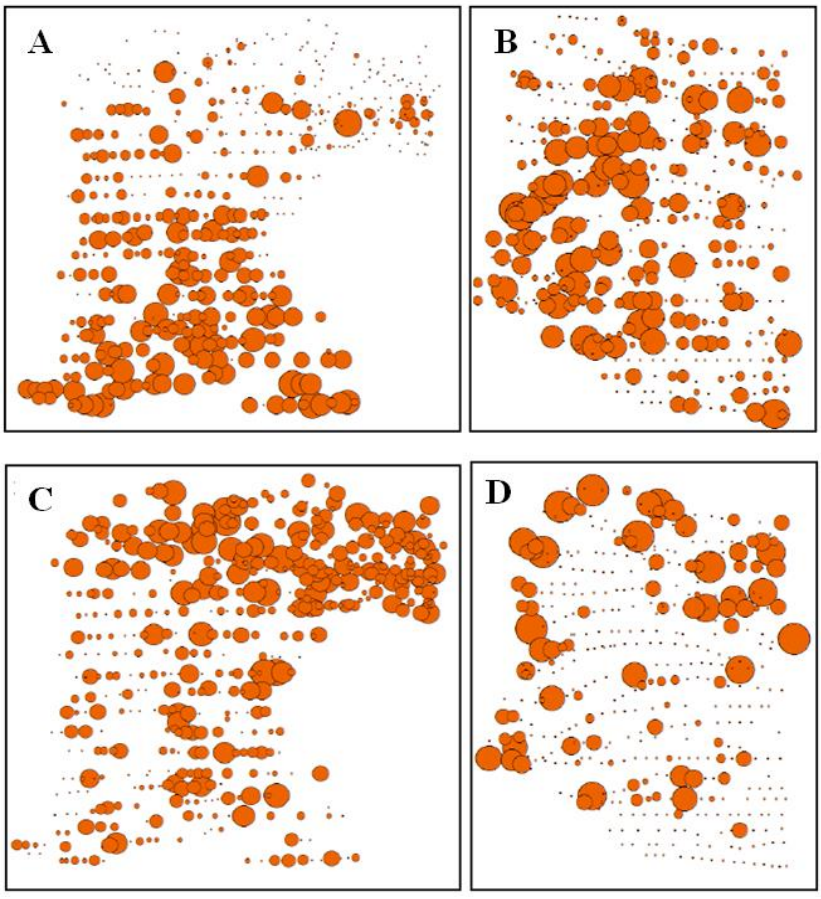

Figure 5 Rust intensity per plant in the month of November for: A) Site A 2009, B) Site B 2009, C) Site A 2010, D) Site B 2010.

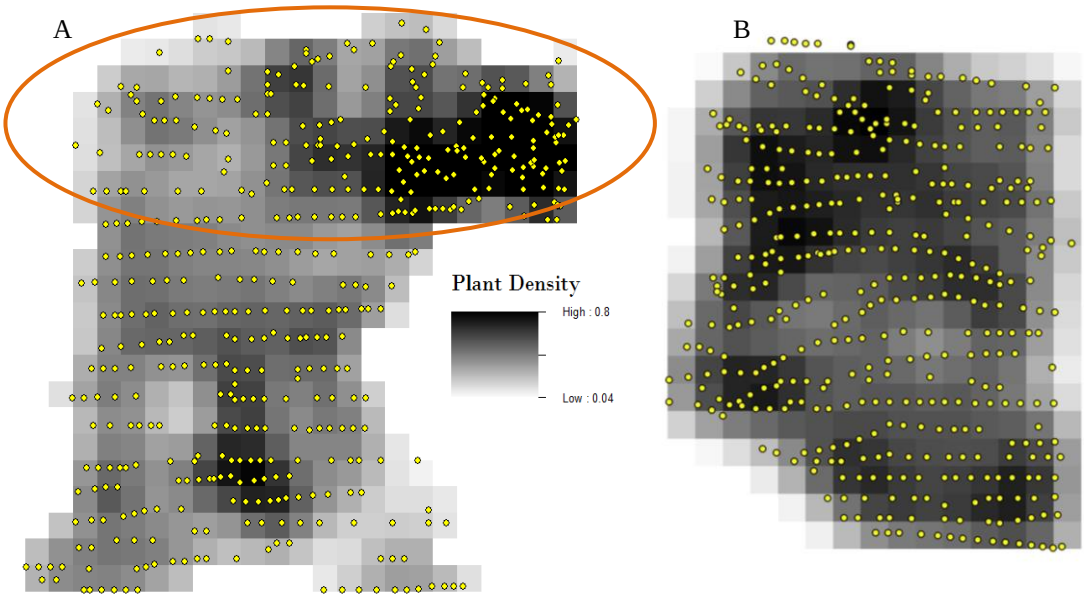

Figure 6 The kernel density distribution of coffee plants in sites A and B, with a cell size of 2 meters and a search radius of 5 meters. Dark denotes higher plant densities per cell while light denotes lower plant densities per cell. Yellow dots show the locations of coffee plants within the site. The orange oval roughly corresponds to the area within Site B that is relatively flat. The rest of sites A and B are on an incline.

To test if coffee rust distribution was affected by the distribution of coffee plants, kernel density functions were calculated to evaluate clustering of coffee plants in the two sites. The relationship between the spatial distribution of coffee rust and plant density, as calculated by a kernel density function was then evaluated using regression models. A kernel density map of Site A shows two clusters of coffee plants with shorter plant-to-plant distances than would be found by chance as well as a number of local gaps that created larger plant-to-plant distances (Figure 6A). The kernel density map for Site B shows more homogeneity in plant-to-plant distances within the site (Figure 6B). The correlation between plant density and coffee rust was negative in 2009 and positive in 2010 (Table 3). The month and site affected the significance of the correlation between coffee rust and plant density, with more significant correlations occurring in Site A compared to Site B. There were stronger correlations in November compared to May-June.

Table 3 The coefficients for the linear relationships between coffee rust intensity and plant density. One asterisk denotes a significance of $p < 0.01$, three asterisks denotes a significance of $p < 0.001$.

Intensity of infection		**Site A**	**Site B**
Year	**Month**	**Coefficient**	**Coefficient**
2009	May-June	-1.2525	-0.5989
	November	-3.2787 ***	0.6938
2010	May-June	1.7906 ***	0.8358
	November	1.7924 ***	-3.8 *

2.2 Regression Models

The results of the linear regression model are shown in Table 4, while the results of the logistic regression model are shown in Table 5. In each model, some variables had a consistent relationship with rust across years, whilst other variables did not. Likewise, some variables had a consistent relationship in both the rust intensity and the probability of infection models, whilst others did not.

Two variables that maintained some consistency across years and across models were the abundance of *L. lecanii*-infected scales surveyed the year previous to the rust survey, as well as the abundance of *L. lecanii*-infected scales survey the same year as

Table 4 The coefficients, standard errors, and p-values for each predictor variable in the linear regression model. "rem" variables were removed from the bootstrap analysis due to lack of significance in non-bootstrapped regression and lack of degrees of freedom.

	2009 and 2010			2009			2010		
Predictor Variables	**Coef.**	**S.E.**	***p***	**Coef.**	**S.E.**	***p***	**Coef.**	**S.E.**	***p***
Intercept	-6.709	0.097	<0.0001	-7.457	0.121	<0.0001	-4.365	0.081	<0.0001
L.lecanii-abundance (previous year)	-0.598	0.039	<0.0001	-0.153	0.089	0.083	-0.534	0.037	<0.0001
L.lecanii-abundance (same year)	0.638	0.041	<0.0001	0.620	0.048	<0.0001	0.022	0.066	0.738
Average Shade Coverage	-0.068	0.029	0.019	-0.107	0.041	0.008	0.021	0.036	0.569
Variety Bourbon : Variety Arabica	0.132	0.067	0.049	rem.	rem.	N.S.	0.065	0.076	0.397
Variety Caturra : Variety Arabica	0.466	0.191	0.015	rem.	rem.	N.S.	0.182	0.206	0.378
Variety Catuai : Variety Arabica	0.396	0.084	<0.0001	rem.	rem.	N.S.	0.457	0.095	<0.0001
Variety Catimor : Variety Arabica	-0.153	0.328	N.S.	rem.	rem.	N.S.	0.014	0.476	0.976
Site 2: Site 1	0.670	0.074	<0.0001	1.874	0.093	<0.0001	-0.580	0.085	<0.0001
September : May	0.292	0.069	<0.0001	1.030	0.107	<0.0001	-0.333	0.073	<0.0001
November : May	1.326	0.069	<0.0001	2.061	0.102	<0.0001	0.591	0.077	<0.0001
Year 2: Year 1	1.816	0.096	<0.0001						

Table 5 The coefficients, standard errors, and p-values for each predictor variable in the logistic regression model.

	2009 and 2010			2009			2010		
Predictor Variables	**Coef.**	**S.E.**	***p***	**Coef.**	**S.E.**	***p***	**Coef.**	**S.E.**	***p***
Intercept	0.352	0.173	0.042	-0.632	0.295	0.032	4.380	0.365	<0.0001
L.lecanii-abundance (previous year)	-0.373	0.066	<0.0001	0.025	0.155	**N.S.**	-0.454	0.095	<0.0001
L.lecanii-abundance (same year)	0.810	0.054	<0.0001	0.816	0.080	<0.0001	0.508	0.143	<0.0001
Plant Density	-0.731	0.332	0.028	-1.438	0.554	0.009	0.437	0.639	N.S.
Variety Bourbon : Variety Arabica	-0.120	0.107	N.S.	0.101	0.174	N.S.	-0.410	0.197	0.038
Variety Caturra : Variety Arabica	-1.012	0.214	<0.0001	-1.036	0.372	0.005	-1.542	0.349	<0.0001
Variety Catuai : Variety Arabica	-0.348	0.125	0.005	0.052	0.209	N.S.	-0.987	0.218	<0.0001
Variety Catimor : Variety Arabica	-2.614	0.376	<0.0001	-3.240	0.687	<0.0001	-3.319	0.572	<0.0001
Site 2: Site 1	-1.285	0.104	<0.0001	-0.780	0.184	<0.0001	-2.346	0.172	<0.0001
September : May	0.190	0.078	0.015	1.526	0.119	<0.0001	-1.426	0.138	<0.0001
November : May	0.768	0.081	<0.0001	2.943	0.136	<0.0001	-1.502	0.138	<0.0001
Year 2: Year 1	2.259	0.149	<0.0001						

the rust survey. In the linear regression model, the abundance of *L. lecanii*-infected scales surveyed the year previous to the rust survey had a negative effect on coffee rust intensity in all three iterations of the model, although in 2009, this predictor variable was only moderately significant (P = 0.083). In the logistic regression model, this variable was also negatively correlated with the probability of infection in 2010, but the relationship was insignificant in 2009.

In contrast, the abundance of *L. lecanii*-infected scales surveyed the same year as rust intensity was positively correlated with rust intensity in 2009, but not a significant predictor in 2010. However in the logistic model, this variable was positively correlated with the probability of rust infections in both 2009 and 2010.

To look at how abundance a whole affected these results, I compared the overall *L. lecanii* abundance and rust intensity each year, and found that the abundance of *L. lecanii*-infected scales was highest in 2009 compared to 2008 or 2010 (Figure 7). According to the regression models, *L. lecanii* – infected scales in 2009, when abundances were the highest, have the strongest relationship with rust infections in 2009 and rust infections in 2010. Coffee rust intensity was also higher in 2009 than in 2010 (Figure 8).

Other variables had a more mixed relationship with rust intensity and the probability of rust infection. Shade was removed from the logistic regression because it was an

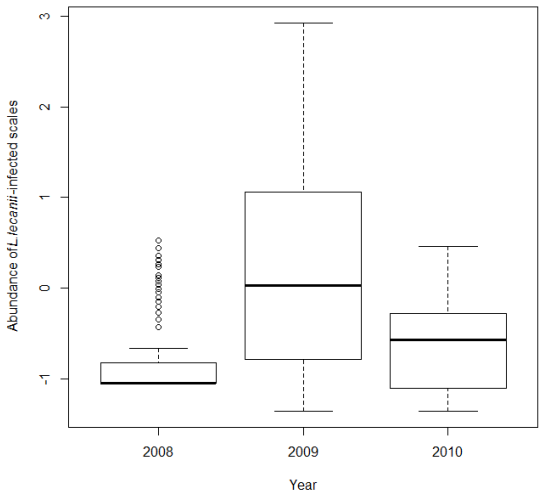

Figure 7 The log-transformed distance-weighted abundance of *L.lecanii* –infected scales in 2008, 2009, and 2010.

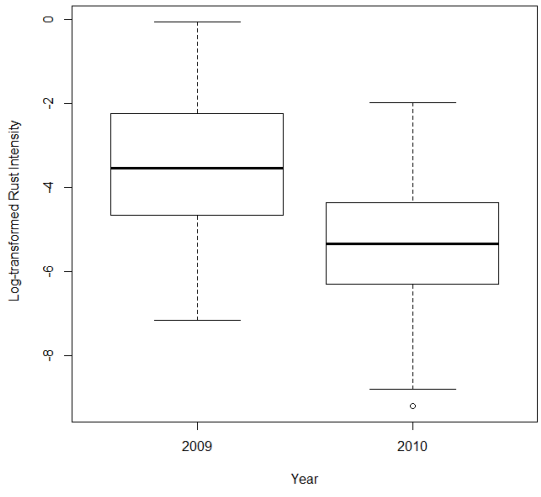

Figure 8 The log-transformed rust intensity in 2009 and 2010.

insignificant variable. Shade was kept in the linear regression, as it was negatively correlated with rust intensity in 2009, although it was an insignificant factor in 2010.

Plant density was removed from the linear regression model because it was an insignificant variable, but was kept in the logistic regression model because it was negatively correlated with the probability of rust infections in 2009.

Likewise, the effect of variety type on coffee rust intensity and probability of infection varied. When compared with *Arabica,* the varieties *Bourbon, Catuai*, and *Caturra* were more positively correlated with coffee rust intensity when both 2009 and 2010 data were included in the model. Strangely, these varieties showed no significant effect on rust intensity when the model was separated into the years 2009 and 2010, save for *Catuai* in 2010, which was positively correlated with rust intensity. *Catimor* was not a significant factor in any year. The logistic regression model found that *Caturra* and *Catimor* had consistent negative relationships with the probability of infection compared to *Arabica* in all years, whereas *Catuai* had a more negative relationship with the probability of infection in 2010.

3 Discussion

The results of both the linear regression model and the logistic regression model suggest that *L. lecanii* could be acting as a time-lagged conservation biological control, because an abundance of *L. lecanii* consistently had a negative effect on the intensity of rust infections the following year. The exact mechanism causing the time lag between *L. lecanii* abundance and coffee rust is unclear, however some hypotheses can be made based on an understanding of the *L. lecanii* lifecycle.

First, *L. lecanii* can survive in the soil and on multiple hosts, so it is plausible that an abundance of *L. lecanii* from the previous year can survive long enough to have an effect on rust the following year (Brodeur, 2012; Jackson et al., 2012b). Rain splash has also been found to disperse *L. lecanii* spores from *L. lecanii*–infected soil (Jackson et al., 2012b). This provides a mechanism that would allow *L. lecanii* build-up in the soil from previous-year epizootics to infect both *C. viridis* populations and coffee rust spores. Rain splash also aids in rust dispersal (Bock, 1962). When the

rainy season starts, newly dispersed rust spores in areas with a high local abundance of *L. lecanii* spores would have a higher probability of being attacked by a *L. lecanii* spore compared to areas with a low build-up of *L. lecanii* spores, thereby causing a negative relationship between *L. lecanii*-infected scales the previous year and rust intensity the following year. This would also provide a reason as to why *L. lecanii*-infected scales in 2008 had a weaker relationship with rust in 2009 compared to *L. lecanii*-infected scales in 2009 and rust in 2010: there were few *L. lecanii*-infected scales in 2008, and thus fewer *L. lecanii* spores in the environment in 2009 compared to that in 2010.

One complicating factor is that the abundance of *L. lecanii*-infected scales was positively correlated with both coffee rust intensity and incidence when all were surveyed in the same year. This result contradicts previous studies, which have found either a negative correlation between *L. lecanii* and coffee rust disease (Alarcón and Carrión, 1994) or no correlation between the two (Eskes et al., 1991a). However, *L. lecanii* epizootics on scales tend to occur after the rainy season has started and after rust lesions have started to grow (pers. obs.), so the within-year build-up of *L. lecanii* may be too late to have a preventative effect on rust infection rates, hence why there's not a negative relationship between within-year rust intensity and *L. lecanii* abundance. This provides a reason for a lack of relationship between same-year *L. lecanii* abundance and rust intensity, but does not address the positive correlation between the two.

It is possible that this positive correlation is not caused by a direct interaction between rust and *L. lecanii*. Instead, the fitness cost to having high scale populations on a plant may impact its ability to resist coffee rust disease. Although it is known that *C. viridis* infestations result in the growth of a fungal mildew, to my knowledge, the impact of *C. viridis* infestations on the ability of coffee to resist rust infections has not yet been studied. However, coffee plants with large scale populations commonly show signs of stress, with reduced photosynthesis, overall plant weakening, and yield reductions (Fernandes et al., 2011). It is plausible that coffee plants with *C. viridis* infestations have more rust when both are surveyed the same year because the stress of *C. viridis* infestations reduces coffee's ability to resist coffee rust.

These results provide a bit of a conundrum: how do we know that *L. lecanii* –infected scales are a net positive in terms of controlling coffee rust infections? This study does not provide a definitive answer to that question, however, large scale surveys done in both 2009 and 2013 have both found a small, yet significantly negative relationship between the location of *A. instabilis* nests and rust infection rates in surrounding coffee plants (Vandermeer et al., 2009, unpublished). The evidence suggests that *A. instabilis* is the main driver affecting the location of *L. lecanii* epizootics, and so these surveys provide good evidence that in a shade-grown coffee agroecosystem, *L. lecanii* is having a small negative effect on rust infection rates.

Given the recent outbreaks of coffee rust in Central America, a small, yet significant effect may not be enough for producers to focus on this as a preventative resource (Cressey, 2013). However, *L. lecanii* is known to infect multiple insects and fungal hosts besides *H. vastatrix* and *C. viridis,* including a number of aphid species and the fungal genera *Pythium*, suggesting that there may be other options for natural sources of *L. lecanii* spores (Brodeur, 2012). The phenology of *L. lecanii* may also have to be taken into consideration in order for *L. lecanii* to have a stronger negative effect on rust infection rates. The literature suggests that *L. lecanii* only attacks *H. vastatrix* spores (Alarcón and Carrión, 1994; Carrión and Rico-Gray, 2002; Eskes et al., 1991; Gonzalez, 2006). A comprehensive literature review found no evidence that *L. lecanii* can parasitize *H. vastatrix* when *H. vastatrix* is inside the haustorium of a leaf. This may be the reason why *L. lecanii* surveyed the previous year has a negative impact on rust infection rates the following year – *L. lecanii* spores may need to build up their numbers in a region before they have an effect, and this does not happen until later in the season (toward the end of summer), at which time the rust spores have already dispersed. One possibility that has yet to be explored is to enhance the preventative effects of *L. lecanii* by spraying sites with *L. lecanii* spores before the rainy season, and thus before *H. vastatrix* spores begin to disperse and germinate.

Coffee variety type was included in the regression models because resistant varieties are one of the most important management tools for coffee rust (Kushalappa and Eskes, 1989; Romero et al., 2010). Over time, different varieties have been planted within the coffee farm as new varieties have become available, such that during the study, there was a mixture of coffee plant varieties within both sites; on average, each

plant was surrounded by two of the same and two different cultivars. This mixture allowed us to study the interaction of each variety type with coffee rust within the shaded coffee agro-ecosystem. The results of the regression models showed that variety type had a stronger effect on the probability of a plant being infected compared to its effect on rust intensity. Specifically, the varieties *Caturra* and *Catimor* were less likely to be infected by coffee rust than the varieties *Bourbon* and *Arabica.* This is interesting, because *Caturra* is considered a "susceptible" variety (Rozo et al., 2012), while *Catimor* is considered to be partially resistant to coffee rust (Samper, 2010).

The reason for this discrepancy may lie in the gene-to-gene interaction between the coffee plants and *H. vastatrix* races. Romero and colleagues (2010) found evidence of multiple genes conferring partial resistance to rust in the different *C. arabica* varieties and cultivars. There are also over 40 known races of *H. vastatrix,* which can be more or less aggressive depending on the resistant genes present in the coffee varieties (Rozo et al., 2012). Even though the majority of the coffee plants in these sites are susceptible to *H. vastatrix,* the *H. vastatrix* race (or races) in this site may have been less aggressive towards *Caturra* and *Catimor* varieties.

Shade was included as a variable in the regression models because previous studies have found that shade can either increase or decrease rust incidence (Avelino et al., 2004, 2006). In this study, shade either had a negative effect or no effect on rust infection rates, but in no instance did shade result in more rust. Specifically, in the linear regression model, shade was negatively correlated with rust in 2009, but was an insignificant variable in 2010. Shade was removed from the logistic regression because it was an insignificant variable in both years.

Previous studies have found that the effect of shade on rust intensity is dependent on a number of factors, including fruit load, precipitation, and temperature. High fruit loads one year are known to increase rust incidence and severity the following year by affecting the physiology of the coffee plant (Avelino et al., 2004, 2006; Costa et al., 2006). Shade mitigates this interaction by decreasing overall fruit load, thus allowing for more evenly sized crops from year to year, and indirectly decreasing coffee rust intensity (López-Bravo et al., 2012). However, when comparing between coffee

plants in shade and sun conditions with similar fruit loads, coffee plants in shade are found to have higher coffee rust incidence and severity (López-Bravo et al., 2012). This is likely caused by the shade providing a wetter environment than the sun.

Fruit load was not surveyed for 2009 and 2010 in these sites, but it is possible that this was a confounding factor that affected coffee rust intensity each year, as well as the interaction between coffee rust intensity and shade.

Previous studies have found that shade may increase the amount of *L. lecanii* within coffee agroecosystems, indirectly decreasing coffee rust incidence in shaded agroecosystems (Staver et al., 2001). *L. lecanii*-infected scales are more likely to be found under shade because the ants that farm and protect the scales live in the shade trees. In these two sites, coffee plants infested with *L. lecanii*-infected scales did have a higher probability of being located in high shade compared areas as compare to coffee plants without *L. lecanii*-infected scales (Binomial Regression Model; $P < 0.0001$). Shade trees undoubtedly increase the number of *L. lecanii* epizootics, by increasing the amount of habitat for *A. instabilis*, the ant that tends the scales, thus allowing them to reach high population densities. However, this interaction was only picked up in the correlation between shade and rust intensity in 2009.

Besides the regression models, this study also focused on fine-scale spatial trends that may be affecting the presence of infected plants or coffee rust intensity. The spatial relationship between plant density and shade was studied because closely spaced coffee plants can increase plant-to-plant dispersal of coffee rust, thereby increasing coffee rust incidence (Paiva et al., 2011; Schieber, 1975). Spatial clustering of plants within Site A may have affected spore dispersal. This is inferred from the results showing that plant density significantly correlates with rust intensity in November in Site A in both 2009 and 2010.

One potential explanation for the significant relationship between plant density and coffee rust intensity is that coffee rust dispersed more readily in the two local clusters within Site A because plants were closer together. This is plausible, as rust intensity does center around one of these two sites each year (Figures 5 and 6).

An alternative explanation for why plant density affects Site B more so than Site A may have to do with the differences in slope in the two sites, and how changes in slope may be affecting the pattern of local spore dispersal via wind and rain. The large cluster of coffee plants in Site A roughly corresponds to a region that's relatively flat in the top third of the plot (Figure 6). This flat region had considerably less rust in 2009 compared to the bottom half, and considerably more rust than the bottom half in 2010. The bottom half is on an incline. It is plausible that the changes in slope are affecting dispersal dynamics more so than the actual differences in plant density, although this is purely speculative given that we were unable to measure wind velocity or direction in these two sites.

The fine-scale spatial analysis focused on only one factor that could affect coffee rust infection rates, while the regression models allowed for a comparison of factors, one of which was plant density. Plant density did not have a significant effect on rust intensity, and in 2009, plant density was correlated with a higher probability of infection, although this correlation was not significant in 2010. Although plant density may have had a small impact on rust infection rates, it seems to have had more impact on the location of dispersal, rather than intensity or probability of infection.

4 Conclusion

This study compared the effects of multiple factors on coffee rust infection rates, including temporal and small-scale spatial effects. The results of this study provides support for the hypothesis that long-term ecological management of *L. lecanii* – infected scales may be able to decrease both coffee rust intensity and the probability of rust infection in shaded agroecosystems. It also provides evidence for the effect of coffee plant density on dispersal (but not coffee rust intensity), and the effect of variety type on the probability of a coffee rust infection. More work is needed to better understand how *L. lecanii* both decreases and increases the probability of rust infecting a particular plant, and how producers can effectively increase the positive effects of *L. lecanii* in their coffee farm systems.

5 Supplementary Methods

Statistical Methods for Linear and Logistic Regressions

The dataset of rust intensity (the number of infected leaves divided by the total number of leaves per plant) had a zero-inflated negative binomial distribution (Figure 4). In order to fully evaluate this dataset, it was split into two sets and two different regression models were used to evaluate the model. First, the glmer function in the R package "lme4" was used to create a binomial regression model that tested the effect of the predictors on the probability that each plant is infected with coffee rust (Bates *et al.*, 2012). For this analysis, rust was converted into a binary "infected" vs. "uninfected" dataset. For the second model, the gls function in the R package "nlme" was used to create a general linear regression model that tested the effect of the explanatory variables on the intensity of coffee rust infection within infected plants (Pinheiro *et al.*, 2012). All uninfected plants were removed from this analysis, and the data were log-transformed to approximate a normal distribution. The Gls function in the "rms" R package generated cluster bootstrap re-samples in order to test the models predictive capability (Harrell, 2012).

Each site was surveyed six times, so covariance structures were added to the linear model to account for temporal correlations between repeated measurements on the same plants. AIC information criterion determined that a compound symmetrical correlation structure, whereby repeated measures have equal variance, and the correlations between any two measurements are identical, gave the best fit. For the logistic regression model, repeated measurements were accounted for by adding plant number as a random group effect.

For the linear model, both coffee rust intensity and the abundance of *L. lecanii*-infected scales were log-transformed to improve the fit of the linear regression model. All quantitative predictor variables in both models were z-transformed to modulate their effect on rust intensity. "Plant Density" was removed from the linear regression model because it was not significantly correlated with rust intensity over multiple years. The residuals of the final linear regression model were normally distributed, with no significant heteroskedasticity, and no significant multicollinearity.

The linear regression model focused on the relationship between rust intensity and the predictor variables, whereas the logistic regression model focused on the relationship between the predictor variables and the probability of a coffee plant being infected by coffee rust. In order to test the predictive capability of the linear regression model, the model was cluster bootstrapped (n = 1000), and run three times: once with both years, once using only the 2009 data, and once using the 2010 data. Cluster bootstrapping is used for mixture models, whereby sampling with replacement occurs for each cluster (Field & Welsh, 2006). In this model, all values for one plant at every time point are considered one cluster. It was not possible to cluster bootstrap the logistic regression model because a suitable R statistical program was not available, however the logistic regression model was run three times as in the linear regression model.

6 References

Alarcón, M., and Carrión, G. (1994). Uso de *Verticillium lecanii* en cafetales como control biológico de la roya del cafeto. Fitopatología (Perú) *29*, 82–85.

Avelino, J., Willocquet, L., and Savary, S. (2004). Effects of crop management patterns on coffee rust epidemics. Plant Pathology *53*, 541–547.

Avelino, J., Zelaya, H., Merlo, A., Pineda, A., Ordoñez, M., and Savary, S. (2006). The intensity of a coffee rust epidemic is dependent on production situations. Ecological Modelling *197*, 431–447.

Barbosa, P.A. (1998). Conservation Biological Control (Academic Press).

Becker, S., and Kranz, J. (1977). Comparative studies on the dispersal of *Hemileia vastatrix* in Kenya [coffee]. Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz *84*.

Bock, K.R. (1962). Dispersal of uredospores of *Hemileia vastatrix* under field conditions. Transactions of the British Mycological Society *45*, 63–74.

Brito, G.G. de, Caixeta, E.T., Gallina, A.P., Zambolim, E.M., Zambolim, L., Diola, V., and Loureiro, M.E. (2010). Inheritance of coffee leaf rust resistance and identification of AFLP markers linked to the resistance gene. Euphytica *173*, 255–264.

Brodeur, J. (2012). Host specificity in biological control: insights from opportunistic pathogens. Evolutionary Applications *5*, 470–480.

Carrión, G., and Rico-Gray, V. (2002). Mycoparasites on the coffee rust in Mexico. Fungal Diversity *11*, 49–60.

Costa, M.J.N., Zambolim, L., and Rodrigues, F.A. (2006). Effect of levels of coffee berry removals on the incidence of rust and on the level of nutrients, carbohydrates and reductor sugar. Fitopatologia Brasileira *31*, 564–571.

Cressey, D. (2013). Coffee rust regains foothold. Nature *493*, 587–587.

Diniz, I., Talhinhas, P., Azinheira, H.G., Várzea, V., Medeira, C., Maia, I., Petitot, A.-S., Nicole, M., Fernandez, D., and Silva, M. do C. (2012). Cellular and molecular analyses of coffee resistance to *Hemileia vastatrix* and nonhost resistance to *Uromyces vignae* in the resistance-donor genotype HDT832/2. Eur J Plant Pathol *133*, 141–157.

Diola, V., Brito, G.G. de, Caixeta, E.T., Maciel-Zambolim, E., Sakiyama, N.S., and Loureiro, M.E. (2011). High-density genetic mapping for coffee leaf rust resistance. Tree Genetics & Genomes *7*, 1199–1208.

Eskes, A., Mendes, M., and Robbs, C. (1991). Laboratory and field studies on parasitism of *Hemileia vastatrix* with *Verticillium lecanii* and *V. leptobactrum*. Café Cacao Thé *35*, 275–282.

Fernandes, F.L., Picanço, M.C., Gontijo, P.C., de Sena Fernandes, M.E., Pereira, E.J.G., and Semeão, A.A. (2011). Induced responses of *Coffea arabica* to attack of *Coccus viridis* stimulate locomotion of the herbivore. Entomologia Experimentalis Et Applicata *139*, 120–127.

Fiedler, A.K., Landis, D.A., and Wratten, S.D. (2008). Maximizing ecosystem services from conservation biological control: The role of habitat management. Biological Control *45*, 254–271.

Ghini, R., Bettiol, W., and Hamada, E. (2011). Diseases in tropical and plantation crops as affected by climate changes: current knowledge and perspectives. Plant Pathology *60*, 122–132.

Gonzalez, E. (2006). *Lecanicillium* (= *Verticillium*) *lecanii* as an antagonistic agent of *Hemileia vastatrix*., *Lecanicillium* (= *Verticillium*) *lecanii* como antagonista de *Hemileia vastatrix* causante de la roya anaranjada del cafeto. In Fitosanidad,.

Hein, L., and Gatzweiler, F. (2006). The economic value of coffee (*Coffea arabica*) genetic resources. Ecological Economics *60*, 176–185.

Jackson, D., Skillman, J., and Vandermeer, J. (2012a). Indirect biological control of the coffee leaf rust, *Hemileia vastatrix*, by the entomogenous fungus *Lecanicillium lecanii* in a complex coffee agroecosystem. Biological Control *61*, 89–97.

Jackson, D.W., Zemenick, K., and Huerta, G. (2012b). Occurrence in the soil and dispersal of *Lecanicillium lecanii*, a fungal pathogen of the green coffee scale (Coccus viridis) and coffee rust (*Hemileia vastatrix*). Tropical and Subtropical Agroecosystems *15*.

Jha, S., Allen, D., Liere, H., Perfecto, I., and Vandermeer, J. (2012). Mutualisms and population regulation: mechanism matters. PLoS ONE *7*, e43510.

Kouvelis, V.N., Zare, R., Bridge, P.D., and Typas, M.A. (1999). Differentiation of mitochondrial subgroups in the *Verticillium lecanii* species complex. Letters in Applied Microbiology *28*, 263–268.

Kushalappa, A.C., and Eskes, A.B. (1989). Advances in coffee rust research. Annual Review of Phytopathology *27*, 503–531.

Letourneau, D.K., Armbrecht, I., Rivera, B.S., Lerma, J.M., Carmona, E.J., Daza, M.C., Escobar, S., Galindo, V., Gutiérrez, C., López, S.D., et al. (2011). Does plant diversity benefit agroecosystems? A synthetic review. Ecological Applications *21*, 9–21.

Lewis, W.J., Lenteren, J.C. van, Phatak, S.C., and Tumlinson, J.H. (1997). A total system approach to sustainable pest management. PNAS *94*, 12243–12248.

López-Bravo, D.F., Virginio-Filho, E. de M., and Avelino, J. (2012). Shade is conducive to coffee rust as compared to full sun exposure under standardized fruit load conditions. Crop Protection *38*, 21–29.

McCook, S. (2006). Global Rust Belt: *Hemileia Vastatrix* and the ecological integration of world coffee production since 1850. Journal of Global History *1*, 177–195.

Paiva, B.R.T.L., Souza, P.E. de, Scalco, M.S., and Santos, L.A. (2011). Progress of rust in coffee plants in various densities of cultivation in irrigated planting after pruning. Ciência e Agrotecnologia *35*, 137–143.

Perfecto, I., and Vandermeer, J. (2008). Biodiversity conservation in tropical agroecosystems. Annals of the New York Academy of Sciences *1134*, 173–200.

Romero, G., Alvarado, G., Cortina, H., Ligarreto, G., Galeano, N., and Herrera, J.C. (2010). Partial resistance to leaf rust (*Hemileia vastatrix*) in coffee (*Coffea arabica L.*): genetic analysis and molecular characterization of putative candidate genes. Molecular Breeding *25*, 685–697.

Rozo, Y., Escobar, C., Gaitán, Á., and Cristancho, M. (2012). Aggressiveness and genetic diversity of *Hemileia vastatrix* during an epidemic in Colombia. Journal of Phytopathology *160*, 732–740.

Samper, M. (2010). Costa Rica's response to the coffee crisis. Latin American Perspectives *37*, 72–92.

Schieber, E. (1975). Present status of coffee rust in South America. Annual Review of Phytopathology *13*, 375–382.

Silva, M. do C., Várzea, V., Guerra-Guimarães, L., Azinheira, H.G., Fernandez, D., Petitot, A.-S., Bertrand, B., Lashermes, P., and Nicole, M. (2006). Coffee resistance to the main diseases: leaf rust and coffee berry disease. Brazilian Journal of Plant Physiology *18*, 119–147.

Silveira, H., and Rodriques, C. (1972). Bursting of rust uredospores caused by *Verticillium Hemileia*e Bour. culture filtrates. Agronomía Lusitana *34*, 391–396.

Staver, C., Guharay, F., Monterroso, D., and Muschler, R.G. (2001). Designing pest-suppressive multistrata perennial crop systems: shade-grown coffee in Central America. Agroforestry Systems *53*, 151–170.

Tscharntke, T., Bommarco, R., Clough, Y., Crist, T.O., Kleijn, D., Rand, T.A., Tylianakis, J.M., Nouhuys, S. van, and Vidal, S. (2007). Conservation biological control and enemy diversity on a landscape scale. Biological Control *43*, 294–309.

Vandermeer, J., and Perfecto, I. (2006). A keystone mutualism drives pattern in a power function. Science *311*, 1000–1002.

Vandermeer, J., Perfecto, I., and Liere, H. (2009). Evidence for hyperparasitism of coffee rust (*Hemileia vastatrix*) by the entomogenous fungus, *Lecanicillium lecanii*, through a complex ecological web. Plant Pathology *58*, 636–641.

Vandermeer, J., Perfecto, I., and Philpott, S. (2010). Ecological complexity and pest control in organic coffee production: uncovering an autonomous ecosystem service. BioScience *60*, 527–537.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., and Smith, G.M. (2009). Mixed effects models and extensions in ecology With R (Springer).