Effect of shade trees on microclimate conditions and coffee leaf rust

by

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Abstract

Coffee leaf rust is a common agricultural disease in the tropics, caused by the fungal pathogen *Hemileia vastatrix*. Recent epidemics throughout Latin America has caused devastating economic loss and re-invigorated research into new disease management. Because *H. vastatrix* uses wind for transmission and humidity for germination, I propose that coffee leaf rust disease dynamics can be influenced by shade trees that modify the abiotic environment. Specifically, I hypothesize that tree stands will disrupt wind transmission of fungal spores but that increasing canopy cover will reduce evaporation, increase local humidity, and increase rust germination. I explored the effect of trees on rust density on a highland coffee farm in Chiapas, Mexico where I measured and modeled the influence of tree density, canopy cover, evaporation rate, and coffee plant density on disease incidence and severity.

Coffee plants were significantly less likely to become infected at higher tree and coffee plant densities, but canopy cover increased the likelihood of infection. The proportion of leaves infected was influenced only by higher coffee densities, and evaporation rates had no correlation with infection or other structural variables. These results suggest that vegetation structures – including both trees and coffee plants themselves – reduce the probability of plants contracting the coffee leaf rust, potentially by blocking spore dispersal. However, once infected, the disease severity is not influenced by humidity, as previously proposed. I suspect that areas with higher coffee densities are more likely to contain different varieties, some of which are more resistant than others, so that disease severity is influenced by the dilution effect. These results suggest that tree stands have complex, multidimensional effects on the coffee leaf rust, and that their use in disease management may not be straightforward.

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Introduction

Coffee leaf rust is an agricultural disease commonly found on coffee farms throughout the tropics, but the recent epidemic (2012-onwards) in Mesoamerica has caused significant economic loss, threatening farmers' livelihood and increasing food insecurity in the region (Cressey, 2013; Avelino *et al.*, 2015). The disease is caused by the fungal agent *Hemileia vastatrix*, whose uredospores penetrate the coffee plant stomata and form orange lesions on the leaves' undersurface (Diniz *et al.*, 2011). New uredospores emerge on the leaf's surface and are dispersed by wind, rain splash, or physical contact with uninfected leaves (Kushalappa, 1989). The lesions eventually become necrotic, leading to defoliation and, in severe cases, death of branches and significant crop loss. Given the importance of this disease, farmers, researchers, and government entities are keen to find effective management strategies.

Current disease management largely falls under three categories: chemical control, developing resistant coffee plant varieties, and agroecological control (McCook & Vandermeer, 2015). Chemical control is a common strategy with substantial drawbacks: fungicides are expensive, they eliminate potentially beneficial mycoparasites, and proper application requires a strict spraying regime at critical points during the growing season (Belan *et al.*, 2014). Given their costs, farmers are more likely to abandon fungicide application or switch to cheaper, less effective alternatives when profits are low, increasing disease incidence (Avelino *et al.*, 2015). Alternatively, a number of new coffee varieties have been developed to be resistant to coffee leaf rust (de Brito *et al.*, 2010; Caicedo *et al.*, 2013; Shigueoka *et al.*, 2014; van der Vossen *et al.*, 2015), many derived from the popular "Hibrido de Timor" cultivar (Diola *et al.*, 2011; Del Grossi *et al.*, 2013; Romero *et al.*, 2014). However, *H. vastatrix* have evolved to infect previously resistant varieties (Gichuru *et al.*, 2012; Diola *et al.*, 2013), indicating that new resistant varieties will be continuously needed.

Agroecological control is the deliberate use of ecological interactions to control pest abundance. Coffee farms contain a variety of ecological interactions (e.g. competition, multitrophic interactions, and trait-mediated indirect interactions) that prevent coffee plant enemies from becoming pests (Perfecto *et al.*, 2014). *H. vastatrix* is parasitized by the white halo fungus, *Lecanicillium lecanii* (Vandermeer *et al.*, 2009; Jackson *et al.*, 2012), and its spores are predated upon by the *Mycodiplosis hemileiae* larvae (Hajian-Forooshani *et al.*, 2016). There is also evidence that shade trees may alter the germination and dispersal dynamics of *H. vastatrix* (Avelino *et al.*, 2012; López-Bravo *et al.*, 2012). Relative to chemical control and the development of resistant coffee varieties, much less is known about the agroecological control of the coffee leaf rust. However, it may be cheaper and more ecologically sound for farmers to leverage pre-existing species interactions as natural pest control (Vandermeer *et al.*, 2010). This creates agricultural systems that are likely to be more autonomous and resilient (Lewis *et al.*, 1997).

Shade trees provide a variety of ecosystem services, including pest control (Mouen Bedimo *et al.*, 2008; Jonsson *et al.*, 2014); improving soil quality (Meylan *et al.*, 2017); habitat for native tropical species (Moguel & Toledo, 1999); and additional income from fruits and timber resources for farmers (Rice, 2011; Cerda *et al.*, 2014; Somarriba *et al.*, 2014). On coffee farms, shade trees may have additional influences on coffee leaf rust. *H. vastatrix* germinates better in higher relative humidity (Capucho *et al.*, 2012) and leaf wetness (Salustiano *et al.*, 2009), so much so that relative air humidity has been used to predict coffee leaf rust epidemics (Meira *et al.*, 2008). Additionally, wind is a major dispersal mechanism for *H. vastatrix* spores, with the occasional long-distance wind-dispersal hypothesized to have introduced the pathogen to Latin America across the Atlantic Ocean (Schieber & Zentmyer, 1984). Trees alter the local understory humidity and wind conditions experienced by *H. vastatrix*, which in turn may alter the germination and dispersal of the fungus. This suggests a potential for shade trees to provide pest control services for the coffee leaf rust.

Two recent studies have found significant influence of shade trees on microclimate conditions and coffee leaf rust. (Avelino *et al.*, 2012) found higher disease severity in areas with less forest cover and in areas with more open pastures, proposing that tree stands serve as windbreaks that disrupt fungal spore dispersal. However, (López-Bravo *et al.*, 2012) found that increased canopy cover also reduces intra-day temperature variations and increases leaf wetness in the understory, promoting *H. vastatrix* germination. In terms of pest control, there appears to be two conflicting effects of shade trees: they may increase disease severity by increasing understory humidity and fungal germination, while simultaneously reducing disease incidence by blocking wind transmission of fungal spores.

This study builds on previous work by closely examining the mechanisms by which shade trees alter *H. vastatrix* dispersal and germination at the local scale. I conducted a survey in a shaded, highland coffee plantation to measure key structural variables (tree density, canopy cover, and coffee density), their effects on key microclimate factors (evaporation rates and wind velocity), and their correlations with coffee leaf rust disease incidence and severity. I hypothesized that both mechanisms are in play, such that trees, while potentially reducing disease transmission, may promote spore germination.

Methods

Study site

This study was conducted on Finca Irlanda, a 300-hectare certified shaded organic coffee farm in the Soconusco region of Chiapas, Mexico (Figure 1). The farm grows a variety of *Coffea arabica* – including Bourbon, Catimor, Catuai, and Caturra. Trees are maintained throughout the farm with annual pruning. In 2003-2004, a 45-hectare plot was established where all trees greater than 10cm in circumference were tagged and identified. There are roughly a hundred different woody species in the plot, with the five most common species making up 71% of the individuals: *Inga micheliana* (37%), *Alchornea latifolia* (9%), *Inga rodrigueziana* (7%), *Conostegia xalapensis* (7%), *Veronia deppeana* (6%), and *Inga vera* (5%). Every two years, the entire plot is re-surveyed to add new trees as they appear and record those that die.

Survey measurements

A grid of 128 50x50m^2 sites was established within the 45-hectare plot, surrounded by a half-hectare boundary to avoid edge effects (Figure 2). At the center of each site, 5 coffee plants were selected (for a total of 640 plants), and the total number of infected and uninfected leaves were counted. If possible, the coffee variety was identified. All plants were surveyed from July 5 through August 25, 2016.

I measured five predictor variables at the center of each 128 sites, in-between the five monitored coffee plants: tree distance, coffee density, canopy cover, evaporation rate, and wind velocity. Local tree distance is a proxy for tree density, and was measured as the average distance (in meters) of the three trees nearest to the center of the site. Coffee density was measured as the number of coffee plants within a 3-meter radius of the center point. Percent canopy cover was estimated by averaging four readings of a concave densiometer facing north, south, east, and west (Lemmon, 1957) while standing at the center of the site. All canopy cover readings were collected by the same researcher, and care was taken to exclude coffee plant leaves from canopy readings.

I measured evaporation rate as the water weight lost from qualitative filter paper, controlled for surface area, over five minutes ($g/mm^2/s$) at each site. Sites were measured at different times and on different days, so I expected evaporation rates to vary within the day (e.g., evaporation rates were highest around noon) and between days (e.g., some days were warmer and drier). I controlled for hourly variation by generating a polynomial regression from all evaporation measurements as a function of time, calculating the residual of a datum at the time the measurement was taken, then using that residual to estimate the evaporation rate of that site at 10:00 AM UTC-6:00. To account for daily variation, I re-sampled a site every day. I controlled for daily variations by dividing the evaporation rate of each site by the evaporation rate of the site that was re-measured that day. Adjustments for daily variation were made after adjustments for hourly variations were accounted for.

I measured wind velocity using a Kestrel 200 anemometer held at 1.5m height, and took the velocity as an average over 5 minutes (m/s). Wind velocities were also corrected for hourly and daily variations using the same method as was used for evaporation rates. All five measurements were taken between 07:00 AM and 02:00 PM UTC-6:00, from June 1 through July 20, 2016.

Analysis

To understand the influence of tree distance and canopy cover on understory wind velocity and evaporation rates, respectively, I calculated Pearson's correlation coefficient for all pairs of predictor variables. If there was a significant relationship between predictors, I examined their relationship using a linear regression. I modeled the disease incidence and severity on the five predictors using a general linear model for a binomial distribution with zero inflation, controlled for the total number of leaves on the plant (Zeileis *et al.*, 2008). This analysis examines the relationship between the landscape variables and the probability of disease incidence (as determined by the presence of infected leaves) and disease severity (as measured by the proportion of leaves infected). Disease incidence is indicative of wind dispersal, since increasing wind velocity brings more spores to the area and increases the likelihood that at least one spore will infect a coffee plant. Disease severity is indicative of ideal germination conditions, where sites with abiotic conditions more ideal for germination will result in a greater number of leaves infected.

Results

All landscape structure variables were not evidently deviant from a normal distribution except for evaporation rate and wind velocity; evaporation rate was log-transformed for all subsequent analyses (Table 1). During field surveys, I determined that the Kestrel 200 handheld anemometer did not provide an accurate characterization of the wind velocity in the region. The instrument was not sensitive enough for measurements below 0.3m/s, resulting in an over-abundance of zero values, and wind velocities can change dramatically between 5-minute intervals. Therefore, I excluded wind measurements from subsequent analyses.

Pearson correlation coefficient and linear regression showed significant negative relationship between tree distance and canopy cover. There was no significant relationship between evaporation rate and tree distance or canopy cover (Figure 3, Table 2).

Of the 640 coffee plants measured between July 5 through August 25, 121 plants were either dead or had no leaves, leaving 519 plants for subsequent analyses. A general linear model showed that disease incidence (i.e. presence of infected leaves) was positively correlated with tree distance ($p=0.0015$) and canopy cover ($p=0.0016$), and negatively correlated with coffee density ($p<0.001$) (Table 3). There was no correlation between disease incidence and evaporation rate. There was a negative relationship between the disease severity (i.e. the proportion of leaves infected) and coffee density that is almost significant ($p=0.0521$). There were no correlations between disease severity and tree distance, canopy cover, or evaporation rate.

Discussion

This is one of the first studies to examine the effects of continuously increasing tree density and canopy cover on coffee leaf rust disease at a local scale, and the influence of vegetation density on disease agrees with previous work comparing shaded vs unshaded sites. Increasing tree distance (i.e. decreasing tree density) significantly increased coffee leaf rust incidence. These results agree in part with (Avelino *et al.*, 2012), who found a significant positive relationship between the proportion of open pasture and coffee leaf rust incidence, likely due to open pasture facilitating wind dispersal of *H. vastatrix* spores. However, they did not find a correlation between tree density and coffee leaf rust incidence. This may be due to the larger scales at which their study was conducted (50m, 100m, 150m, and higher), and suggests that the effect of trees on disease transmission may change at different spatial scales. I was unable to connect these trends with wind velocity and establish more support for the influence of trees as a windbreak. Future studies may combine structural measurements with characterization of wind velocities, as well as measurements of aerial spore load, to establish stronger connections between vegetation density and wind transmission of fungal pathogens.

Increasing canopy cover significantly increased disease incidence, but did not influence disease severity. On the other hand, (López-Bravo *et al.*, 2012) found that shaded sites (with 29% and 57% canopy cover on average) had significantly less disease severity, as measured by proportion of leaves infected accumulated over time. In both cases, it seems that canopy cover is having some positive effect on *H. vastatrix* abundance. In this study, differences in methodology might be responsible for the lack of significant influence of canopy cover on disease severity. For example, this study measured canopy cover as a continuous variable ranging from 21.74% to 94.8% and did not include an unshaded state (Table 1), which has been found to have significant less disease severity compared to shaded conditions (Mouen Bedimo *et al.*, 2008; López-Bravo *et al.*, 2012). Future studies should take care to include the full range of canopy cover when possible.

Canopy cover did not correlate with evaporation rates in this study, and evaporation rates did not correlate with disease incidence or severity. This suggests that the effect of canopy cover on coffee leaf rust disease may not be through modification of understory humidity. Baseline humidity levels in the tropics may be high enough that increasing canopy cover does not sufficiently alter germination conditions for *H. vastatrix*. Experts also suggest that daily morning dew may be sufficient to promote rust germination (Graciela Huerta, personal communications, 2016). Canopy cover may be promoting coffee leaf rust by altering other environmental variables that were not measured in this study, such as temperature, radiation, and light exposure, all of which have optimal ranges for *H. vastatrix* germination (Salustiano *et al.*, 2008; Capucho *et al.*, 2012). Additionally, we know very little about the influence of canopy cover on *H. vastatrix* spore dispersal via the splash effect, where rainfall scatters spores to infect neighboring plants. Increasing canopy cover may pool rainwater into larger droplets, which would fall with greater force and scatter fungal spores more effectively. While canopy cover may not influence fungal spores via altering understory humidity levels, it may still alter other physical variables relevant to germination and dispersal of coffee leaf rust.

Coffee plant density was negatively correlated with both disease incidence and severity. This effect is counterintuitive, as higher concentrations of coffee plants might be expected to amplify infection rates by increasing the availability of hosts. However, managers at Finca Irlanda often remove older coffee plants when their productivity declines, then replant at higher densities while adding new resistant varieties over time. Thus, areas with higher coffee densities may contain more resistant varieties (personal observations), which would lower the rates of disease incidence and severity observed in the field. Coffee plant density explained much of the variation in our data: removing it eliminated any significant correlations observed with tree density and canopy cover for both disease incidence and severity. Future surveys should take care to include the effect of resistant varieties as a factor influencing disease, whenever they are present. These findings also suggest that breeding resistant coffee plant varieties will continue to play a key role in coffee leaf rust management strategies (Talhinhas *et al.*, 2017).

Results from this study support the hypothesis that the physical structure of local vegetation has a significant influence on the coffee leaf rust disease. Higher tree density may disrupt wind-transmission of fungal spores, though these effects may be counter-balanced by the positive relationship between canopy cover and disease incidence. Unlike other ecological interactions (Jackson *et al.*, 2012; Hajian-Forooshani *et al.*, 2016), shade trees have a multidimensional interaction with *H. vastatrix* where different components (tree density, canopy cover) have independent and opposite effects on the pathogen. Given these opposing effects on the pathogen, much care should be taken when using these results to inform management recommendations. More studies should be done to understand the mechanism by which canopy cover influences disease incidence. Altering abundance and structure of shade trees may also affect its influence on 1) other coffee pests, 2) other ecosystem services (Staver *et al.*, 2001), and 3) the effect of shading on primary coffee production. Shade trees exist in a complex web of ecological interactions. The use of agroecological management strategies requires a holistic evaluation of this interaction web that, while more difficult, may create more resilient agricultural systems in the long term (Lewis *et al.*, 1997).

Tables and Figures

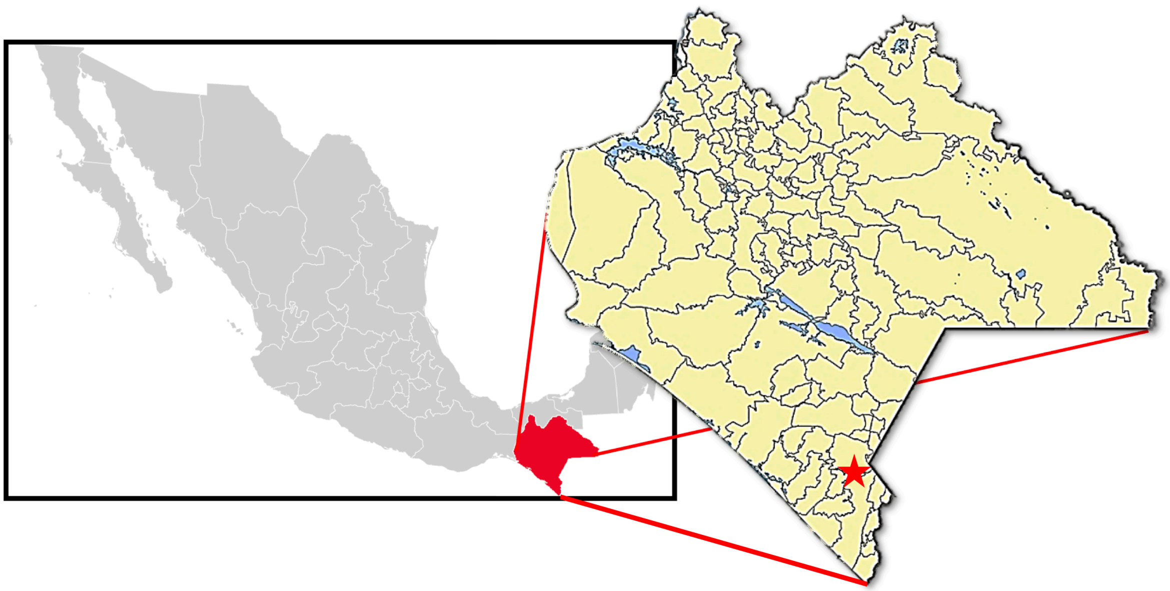

Figure 1. Locator map of study site (red star) in Chiapas, Mexico.

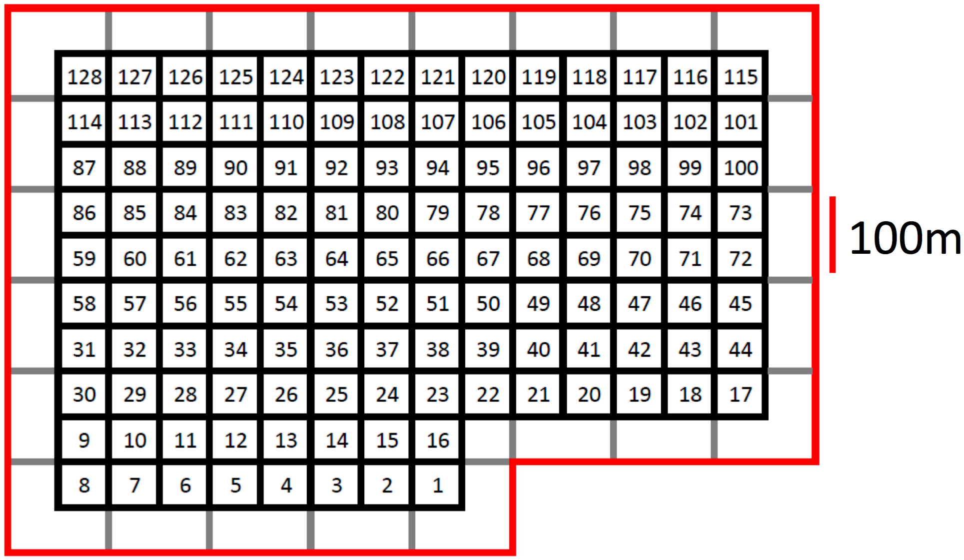

Figure 2. Diagram of the 45-hectare study plot, with 128 sampling sites, on the coffee farm.

Table 1. Data value distribution for independent and dependent variables measured.

Measurement	Min.	Median	Max.	Mean
Coffee density (3m radius)	0	6	21	7.40
Average tree distance (m)	1.30	4.33	20.08	4.64
Canopy cover (%)	21.74	74.91	94.80	70.46
Evaporation rate ($g/mm^2/s$)	5.36×10^{-9}	2.42×10^{-8}	5.13×10^{-8}	5.13×10^{-8}
Wind velocity (m/s)	0.00	0.15	0.90	0.22
# leaves infected (per plant)	0	1	695	19.89
Proportion of leaves infected (%, per plant)	0	0.47	63.18	3.68

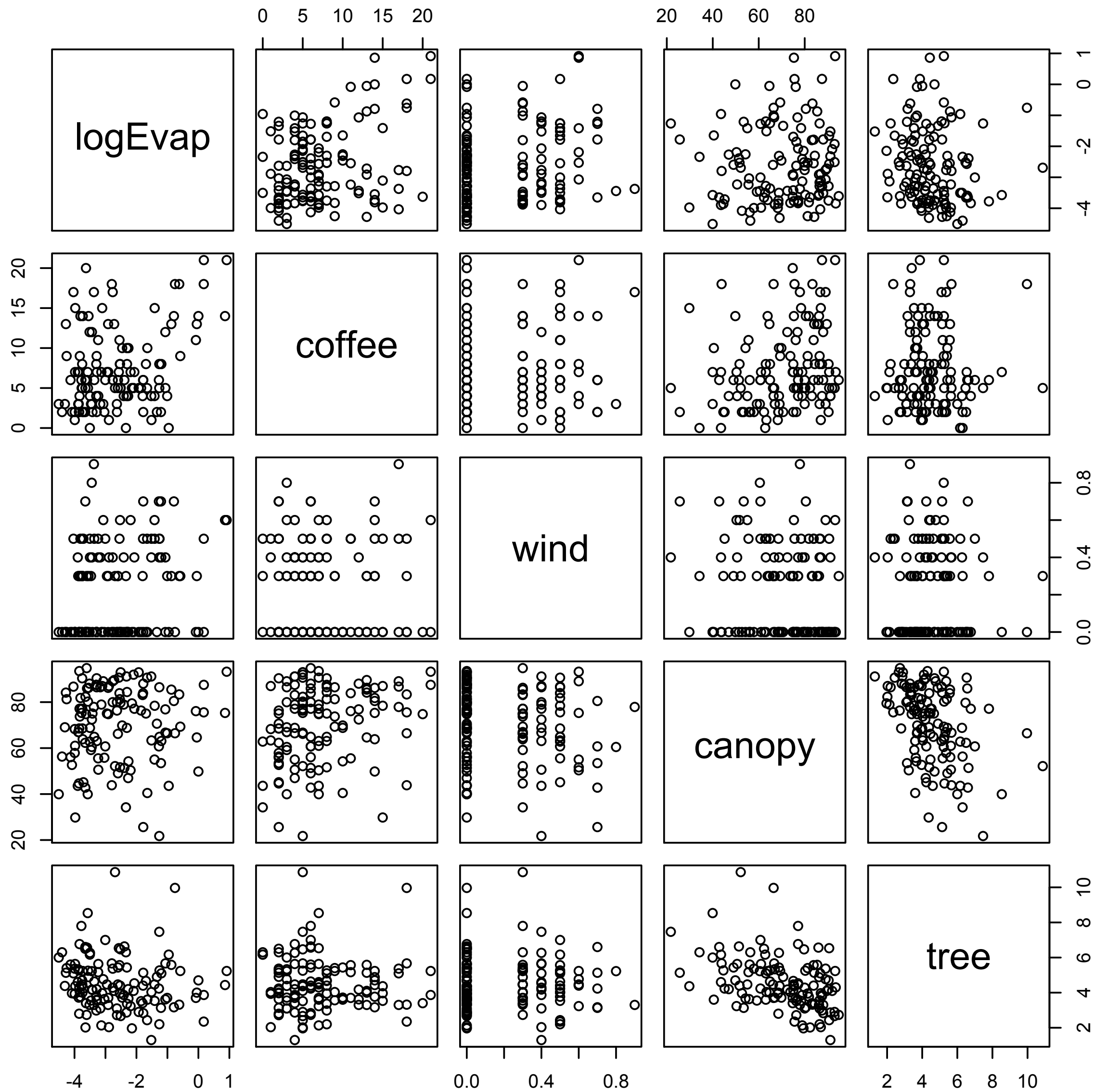

Figure 3. Correlation between independent variables, with standardized evaporation rate log-transformed. One outlier datum was removed for average tree distance. Refer to Table 1 for summary statistics.

Table 2. Pearson correlation coefficients for between predictor variables.

	Avg. tree distance	Canopy cover	Coffee density	Evaporation rate
Avg. tree distance	1.0000	-0.3089	0.0889	0.0142
Canopy cover		1.0000	0.2309	0.0573
Coffee density			1.0000	0.3373
Evaporation rate				1.0000

Table 3. GLM output for disease incidence (the presence of infected leaves) and disease severity (the proportion of leaves infected).

	Disease incidence		Disease severity	
	Estimate	**p-value**	**Estimate**	**p-value**
Avg. tree distance	0.9891	**0.0015**	-0.0533	0.5235
Canopy cover	0.1026	**0.0016**	-0.0026	0.7580
Evaporation rate	0.5365	0.1206	0.0532	0.5158
Coffee density	-0.5380	**0.0009**	-0.0400	0.0521

Bibliography

Avelino J, Cristancho M, Georgiou S *et al.*, 2015. The coffee rust crises in Colombia and Central America (2008–2013): impacts, plausible causes and proposed solutions. *Food Security* **7**, 303–321.

Avelino J, Romero-Gurdián A, Cruz-Cuellar HF, Declerck FAJ, 2012. Landscape context and scale differentially impact coffee leaf rust, coffee berry borer, and coffee root-knot nematodes. *Ecological Applications* **22**, 584–596.

Belan LL, de Jesus Junior WC, de Souza AF *et al.*, 2014. Monitoring of leaf rust in Conilon coffee clones to improve fungicide use. *Australasian Plant Pathology* **44**, 5–12.

de Brito GG, Caixeta ET, Gallina AP *et al.*, 2010. Inheritance of coffee leaf rust resistance and identification of AFLP markers linked to the resistance gene. *Euphytica* **173**, 255–264.

Caicedo B, Guerrero C, Roux J, Wingfield MJ, 2013. New coffee (*Coffea arabica*) genotypes derived from *Coffea canephora* exhibiting high levels of resistance to leaf rust and Ceratocystis canker. *Tropical Plant Pathology* **38**, 485–494.

Capucho AS, Zambolim L, Cabral PGC, Maciel-Zambolim E, Caixeta ET, 2012. Climate favourability to leaf rust in Conilon coffee. *Australasian Plant Pathology* **42**, 511–514.

Cerda R, Deheuvels O, Calvache D *et al.*, 2014. Contribution of cocoa agroforestry systems to family income and domestic consumption: looking toward intensification. *Agroforestry Systems* **88**, 957–981.

Cressey D, 2013. Coffee rust regains foothold. *Nature News* **493**, 587.

Del Grossi L, Sera T, Sera GH *et al.*, 2013. Rust resistance in Arabic Coffee cultivars in northern Paraná. *Brazilian Archives of Biology and Technology* **56**, 27–33.

Diniz I, Talhinhas P, Azinheira HG *et al.*, 2011. Cellular and molecular analyses of coffee resistance to *Hemileia vastatrix* and nonhost resistance to *Uromyces vignae* in the resistance-donor genotype HDT832/2. *European Journal of Plant Pathology* **133**, 141–157.

Diola V, de Brito GG, Caixeta ET, Maciel-Zambolim E, Sakiyama NS, Loureiro ME, 2011. High-density genetic mapping for coffee leaf rust resistance. *Tree Genetics & Genomes* **7**, 1199–1208.

Diola V, de Brito GG, Caixeta ET, Pereira LFP, Loureiro ME, 2013. A new set of differentially expressed signaling genes is early expressed in coffee leaf rust race II incompatible interaction. *Functional & Integrative Genomics* **13**, 379–389.

Gichuru EK, Ithiru JM, Silva MC, Pereira AP, Varzea VMP, 2012. Additional physiological races of coffee leaf rust (*Hemileia vastatrix*) identified in Kenya. *Tropical Plant Pathology* **37**, 424–427.

Hajian-Forooshani Z, Salinas ISR, Jiménez-Soto E, Perfecto I, Vandermeer J, 2016. Impact of regionally distinct agroecosystem communities on the potential for autonomous control of the coffee leaf rust. *Environmental Entomology* **45**, 1521–1526.

Jackson D, Skillman J, Vandermeer J, 2012. 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.

Jonsson M, Raphael IA, Ekbom B, Kyamanywa S, Karungi J, 2014. Contrasting effects of shade level and altitude on two important coffee pests. *Journal of Pest Science* **88**, 281–287.

Kushalappa AC, 1989. Biology and epidemiology. In: Kushalappa AC,, Eskes AB, eds. *Coffee Rust: Epidemiology, Resistance, and Management*. Boca Raton, FL, USA: CRC Press, 13–80.

Lemmon PE, 1957. A new instrument for measuring forest overstory density. *Journal of Forestry* **55**, 667–668.

Lewis WJ, van Lenteren JC, Phatak SC, Tumlinson III JH, 1997. A total system approach to sustainable pest management. *Proceedings of the National Academy of Sciences* **94**, 12243–12248.

López-Bravo DF, de M. Virginio-Filho E, 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, Vandermeer J, 2015. The big rust and the red queen: long-term perspectives on coffee rust research. *Phytopathology* **105**, 1164–1173.

Meira CAA, Rodrigues LHA, Moraes SA, 2008. Analysis of coffee leaf rust epidemics with decision tree. *Tropical Plant Pathology* **33**, 114–124.

Meylan L, Gary C, Allinne C, Ortiz J, Jackson L, Rapidel B, 2017. Evaluating the effect of shade trees on provision of ecosystem services in intensively managed coffee plantations. *Agriculture, Ecosystems & Environment* **245**, 32–42.

Moguel P, Toledo VM, 1999. Biodiversity conservation in traditional coffee systems of Mexico. *Conservation Biology* **13**, 11–21.

Mouen Bedimo JA, Njiayouom I, Bieysse D, Ndoumbè Nkeng M, Cilas C, Nottéghem JL, 2008. Effect of shade on Arabica coffee berry disease development: toward an agroforestry system to reduce disease impact. *Phytopathology* **98**, 1320–1325.

Perfecto I, Vandermeer J, Philpott SM, 2014. Complex ecological interactions in the coffee agroecosystem. *Annual Review of Ecology, Evolution, and Systematics* **45**, 137–158.

Rice RA, 2011. Fruits from shade trees in coffee: how important are they? *Agroforestry Systems* **83**, 41–49.

Romero G, Vásquez LM, Lashermes P, Herrera JC, 2014. Identification of a major QTL for adult plant resistance to coffee leaf rust (*Hemileia vastatrix*) in the natural Timor hybrid (*Coffea arabica* x *C. canephora*). *Plant Breeding* **133**, 121–129.

Salustiano ME, Pozza EA, Filho F, Carlos A, 2009. Differences in the intensity and pustule expansion of coffee rust for two *Hemileia vastatrix* accesses. *Ciência e Agrotecnologia* **33**, 1837–1841.

Salustiano ME, Pozza EA, Filho F, Carlos A, Botelho AO, Alves E, 2008. Variability in ten populations of *Hemileia vastatrix* for germination and germinative tube length under four temperatures. *Ciência e Agrotecnologia* **32**, 1651–1656.

Schieber E, Zentmyer GA, 1984. Coffee rust in the Western Hemisphere. *Plant Disease* **68**, 89–93.

Shigueoka LH, Sera GH, Sera T *et al.*, 2014. Selection of Arabic coffee progenies with rust resistance. *Crop Breeding and Applied Biotechnology* **14**, 88–93.

Somarriba E, Suárez-Islas A, Calero-Borge W *et al.*, 2014. Cocoa–timber agroforestry systems: *Theobroma cacao–Cordia alliodora* in Central America. *Agroforestry Systems* **88**, 1001–1019.

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

Talhinhas P, Batista D, Diniz I *et al.*, 2017. The coffee leaf rust pathogen *Hemileia vastatrix*: one and a half centuries around the tropics. *Molecular Plant Pathology*.

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

van der Vossen HAM, Bertrand B, Charrier A, 2015. Next generation variety development for sustainable production of arabica coffee (*Coffea arabica* L.): a review. *Euphytica* **204**, 243–256.

Zeileis A, Kleiber C, Jackman S, 2008. Regression models for count data in R. *Journal of Statistical Software* **27**, 1–25.