Factors Impacting American Leaf Spot Disease Incidence and Intensity in a Shaded Coffee Farm in Chiapas, Mexico

by

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Abstract

American leaf spot disease, known in Latin America as ojo de gallo, is a coffee fungal disease caused by the fungal agent *Mycena citricolor*. Recent increase in the disease and its ability to cause severe yield losses in coffee raised questions about our understanding of the disease dynamics in coffee farms, and our ability to predict and prepare for possible outbreaks and avoid the economic losses similar to those caused by recent outbreaks of the coffee leaf rust across Latin America. In this thesis, I investigate some of the factors that might impact patterns of American leaf spot disease in an organic shaded coffee farm in Chiapas, Mexico.

In chapter 1, I assess the co-occurrence pattern of American leaf spot (ALS) disease with the coffee leaf rust, another fungal disease caused by *Hemileia vastatrix*, which caused severe outbreaks in Latin America since 2012/2013. Based on seven months of sampling of ALS and coffee leaf rust incidence and intensity in an organic coffee farm in Chiapas, Mexico, results from chi-squared analyses of contingency tables of disease presence suggest that the incidences of the two diseases are not independent. Significantly fewer coffee plants are infected with both diseases than expected at random, while significantly higher number of coffee plants are infected with only one disease than expected at random. The patterns show evidence that there may be trade-offs between the control of the two pathogens. Specifically, the planting of coffee leaf rust resistant varieties may be more susceptible to American leaf spot disease and may lead to an outbreak of this fungal disease.

In chapter 2, I explore the possible effects of shade trees and a dominant ant on ALS. As nitrogen fixing trees can usually add nutrients to the soil and possibly improve plant health, I proposed that coffee plants under nitrogen fixing shade trees will have a lower incidence and intensity of ALS. In addition, due to the complex ecological interactions that the dominant ant, *Azteca sericeasur*, can have with many organisms in the system and maintaining stability through biocontrol, I hypothesized that trees with *A. sericeasur* nests will have a negative impact on ALS disease. Using data of American leaf spot incidence and intensity collected in Finca Irlanda, Chiapas, Mexico, along with shade tree types and *Azteca* ant nest presence, I ran chi-squared analyses, Kruskal-Wallis rank sum test, and t-tests to understand the disease patterns with respect to three shade tree types (*Inga* spp [a n-fixing genus in the family Fabacea], *Alchornea latifolia* [the second most common species after Inga], and all other trees). I found that shade tree types do not significantly impact American leaf spot incidence and intensity in the same way. Coffee plants around *Inga* spp., the nitrogen fixing trees in the coffee farm, have significantly higher disease intensities than other shade trees but are not different from *Alchornea*. The presence of *Azteca* nests in shade trees nearby coffee bushes did not have a significant effect on the incidence of ALS. The results suggest that shade trees have complex impact on American leaf spot disease, and multiple factors, including nutrients, shade level and microclimate, and possible ecological interactions can have a combined effect. Future research focusing on independent factors associated to shade trees can further improve our understanding of American leaf spot disease patterns.

Chapter 1: Early signs of a secondary outbreak of Mycena citricolor in an organic farm in Southern Mexico

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1.1 Abstract

Coffee leaf rust caused by *Hemileia vastatrix* and American leaf spot disease by *Mycena citricolor* are important coffee fungal diseases that can cause severe yield losses. While coffee leaf rust received much attention from the recent regional outbreak in Latin America, here we report on an associated pattern of American leaf spot with the presence of coffee leaf rust in a coffee farm in southern Mexico. Based on seven months of sampling for disease incidence and severity of both coffee leaf rust and American leaf spot, we show that the incidences of the two diseases are not independent. Chi-square analyses of contingency tables of coffee leaf rust and American leaf spot presences show significantly fewer coffee plants infected with both diseases and more plants infected with only one of the diseases than expected at random. The resulting patterns of infection are consistent with the hypothesis that there may be trade-offs in controlling these pathogens, where the resistant varieties to the coffee leaf rust that were planted in response to the recent outbreak may be more susceptible to American leaf spot. These results suggest that management strategies for the control of the coffee rust should not rely on planting homogenous resistant varieties and must take into consideration the community of pathogens that infect coffee.

1.2 Introduction

In 2012/2013 the coffee leaf rust (CLR) (*Hemileia vastatrix*) suddenly burst onto the coffee landscape from southern Mexico to Peru (McCook and Vandermeer 2015), causing significant economic damage throughout the region. This infamous "big rust" (McCook 2020) ushered in a period of intense search for adequate responses, both cultural and chemical (Talhinhas et al. 2017). One important class of response was the planting of newly developed resistant varieties of coffee, mainly based on Catimor genetic material. It has been reported that varieties resistant to CLR are themselves susceptible to American leaf spot disease (*Mycena citricolor*) (Julca-Otiniano et al. 2018), known in Latin America as "ojo de gallo" or "gotera del cafeto". American leaf spot (ALS) is a coffee disease found in Latin America that can infect not only leaves, but also branches and fruits of coffee, leading to defoliation, branch deaths, and fruit fall (Avelino et al. 2018). Complicating matters, *M. citricolor* has a broad range of hosts on coffee farms, including shade trees and weeds, which makes it difficult to contain and can cause severe yield losses (Avelino et al. 2018). Relatively little is currently known about how these two diseases interact in the field or how these new varieties resistant to CLR may impact the dynamics of ALS.

Although it is appreciated that the selection for resistance to a single pathogen can potentially result in higher susceptibility to another, resulting in a trade-off between the two diseases (Sopel et al. 2007), it is unknown if such a trade-off exists between CLR and ALS for these new resistant coffee varieties. If the resistance to CLR comes at the cost of vulnerability to ALS, then the widespread implementation of these varieties as the main strategy for the control of the CLR in Mexico and Central America may contribute to an outbreak of the American leaf spot disease, which may be more difficult to control due to its diversity of hosts. Furthermore, the benefit of controlling CLR via these resistant varieties may be relatively short-lived, as it has been reported that resistance to the CLR is beginning to break down in some areas (Jibat 2020). For example, in 2017, the World Coffee Research Organization reported that the CLR resistant variety Lampira (a cross between Timor hybrid 832/1 and Caturra), which was widely planted in Honduras and other Central American countries after the 2012/2013 CLR outbreak, was no longer resistant to the CLR (Kraft 2017).

In this study, we report on the relationship between CLR incidence and the incidence of ALS in Mexico after the CLR outbreak led to an extensive planting of CLR resistant varieties to better understand how these two diseases are related to each other in the field. Based on systematic sampling of groups of coffee bushes in a contiguous area of 45 hectares (128 50 x 50 m plots), we report on the recent increase in the incidence and intensity of *M. citricolor* for seven months, along with *H. vastatrix*.

1.3 Methods

Surveys of CLR and ALS were conducted at Finca Irlanda, a ~300 ha shaded organic coffee farm in the Soconusco region of Chiapas, Mexico (15° 20′ N, 90° 20′ W). Finca Irlanda is located at elevations ranging from 900 to 1,200 meters above sea level and receives an annual rainfall of 4,500 mm (Philpott and Bichier 2012). The farm has diverse shade trees as canopy cover, with *Inga* being the dominant shade tree (Philpott and Bichier 2012). Many coffee cultivars are planted on the farm, including recently planted rust-resistant varieties.

Sampling was conducted each month from March to November in 2020, with the exception of April and May (due to the COVID-19 pandemic lockdown). Each of the 45 hectares within this plot was subdivided into four 50 × 50 m subplots, for a total of 128 subplots. A central shade tree was identified as a point location for each subplot. The five coffee plants nearest the central shade tree were sampled, and the presence of CLR and ALS were visually inspected and recorded. The intensity of ALS was estimated as a percentage infected for each plant and the intensity of CLR was estimated by counting the number of leaves infected with the fungus and dividing by the estimated total number of leaves per plant. In total, 640 coffee plants were examined each month. We conducted a Pearson's Chi-Square test on a contingency table of presence and absence of the two diseases followed by post hoc analyses to confirm the direction of deviation from expected incidences for each month. We visualized the co-occurring intensity of the diseases over all observations through the seven months by plotting their relative intensities scaled with the z-score method ($z = (x-\mu)/\sigma$).

1.4 Results

Incidence of ALS on coffee bushes was relatively constant over the sampling period. The number of infected bushes decreased from 128 in March to 105 in June and increased slightly from June to November to 127 infected plants, around 19.8% of total coffee bushes sampled (Table 1.1). The number of bushes with CLR decreased from 366 in March to 245 in September, and increased again to 334 in November, around 52.2% of total coffee bushes sampled (Table 1.1). More coffee bushes were infected with CLR than ALS across all months sampled (Table 1.1). Chi-squared results for independence show significant deviation from expected at random for incidences of the two diseases in all months examined ($p < 0.01$ for June, and $p < 0.001$ for all other months, Table 1.1). Each month, the observed numbers of plants with both diseases were significantly lower than expected from a random distribution (post hoc analysis, $p < 0.01$ for June and $p < 0.001$ for all other months). Observations of plants without either disease were significantly lower than expected at random, while observations of plants with only one disease were significantly higher than expected at random, especially for coffee plants with only ALS (Fig. 1.1). In addition, the relative intensities of the two diseases were negatively associated, especially at high disease intensities. Plants with high intensity of ALS had a low intensity of CLR and vice versa (Fig. 1.2).

1.5 Discussions

From our results, it is clear that the incidences of ALS and CLR on coffee bushes were not independent. More bushes were infected with either CLR or ALS than expected, and fewer bushes were infected with both diseases. These patterns support the hypothesis that increased planting of rust-resistant varieties could lead to an increase in ALS, as these varieties may be more susceptible to ALS disease, as observed by Julca-Otiniano and colleagues (2018). Tradeoffs between resistance to one disease and susceptibility to another disease has also been found in other crops. For example, increased use of the powdery mildew resistant gene *mlo* in barley may have caused increased susceptibility to Ramularia leaf spot (*Ramularia collo-cygni*), a serious fungal disease (McGrann 2014). Currently, with increased planting of rust-resistant varieties of coffee, possible outbreaks of ALS could take place in association with a decrease in CLR, causing severe damages to coffee yields. Future monitoring of coffee farms for both CLR and ALS, and their association with coffee bush varieties, would be essential to further inform our understanding of the dynamics of the ALS, and to prevent future outbreaks of the disease.

These results represent an early warning related to control strategies that rely exclusively on planting resistant varieties, and in particular, a single resistant variety instead of a mixture of different resistant varieties and non-resistant varieties. This mixed strategy has yielded good results in reducing rust disease incidence in cereals (Finckh et al. 2000; Zhu et al. 2000). Furthermore, a total system approach (Lewis et al. 1998) that integrates all the factors that contribute to the incidence of the disease and the health of the plant should be taken into consideration. Recent evidence suggests that healthy coffee plants are more resistant to rust attack and that good plant nutrition enhances plant physiological response in fighting both CLR (Toniutti et al. 2017) and ALS (Avelino et al. 2007); however, the relationship between disease incidence and fertilization in coffee is complicated and still under investigation (Vazco et al. 2018; Perez et al. 2019, 2020; Silva et al. 2019). The amount and type of shade within farms and at the landscape level can also influence disease incidence (Staver et al. 2001; Soto-Pinto et al. 2002; Avelino et al. 2012, 2020; López-Bravo et al. 2012) and should be incorporated in a disease management strategy. However, under certain conditions these two diseases can show contrasting responses to shade (Monterroso Salvatierra 1999). Finally, a number of natural enemies of the rust have been identified (Vandermeer et al. 2009; Martins et al. 2015; Hajian-Forooshani et al. 2016, 2020) and could prove important in delaying epidemic development (Flemming 1980). These control and management strategies, in combination with mixtures of CLR-resistant and non-resistant varieties, could prove to be a more sustainable strategy for the control of these two economically important diseases of coffee.

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1.7 References

Avelino, J., Cabut, S., Barboza, B., Barquero, M., Alfaro, R., Esquivel, C., Durand, J. F., & Cilas, C. (2007). Topography and crop management are key factors for the development of American leaf spot epidemics on coffee in Costa Rica. *Phytopathology, 97*(12), 1532–1542. https://doi.org/10.1094/PHYTO-97-12-1532

Avelino, J., Romero-Gurdián, A., Cruz-Cuellar, H.F., & Declerck, F.A. (2012). Landscape context and scale differentially impact coffee leaf rust, coffee berry borer, and coffee root-knot nematodes. *Ecological Applications*, *22*(2): 584-596.

Avelino, J., Allinne, C., Cerda, R., Willocquet, L., & Savary, S. (2018). Multiple-Disease System in Coffee: From Crop Loss Assessment to Sustainable Management. *Annual Review of Phytopathology*, *56*(1), 611–635. https://doi.org/10.1146/annurev-phyto-080417-050117

Avelino, J., Vilchez, S., Segura-Escobar, M.B., Brenes-Loaiza, M.A., Virginio Filho, E.D.M., & Casanoves, F.. (2020). Shade tree Chloroleucon eurycyclum promotes coffee leaf rust by reducing uredospore wash-off by rain. *Crop Protection*, *129*:105038.

Finckh, M., Gacek, E., Goyeau, H., Lannou, C., Merz, U., Mundt, C., Munk, L., Nadziak, J., Newton, A., de Vallavieille-Pope, C., & Wolfe, M. (2000). Cereal variety and species mixtures in practice, with emphasis on disease resistance. *Agronomie, EDP Sciences, 20* (7):813-837.

Fleming, R.A. (1980). The potential for control of cereal rust by natural enemies. *Theoretical Population Biology, 18*(3):374-395.

Hajian-Forooshani, Z., Salinas, I. S. R., 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*(6), 1521–1526. https://doi.org/10.1093/ee/nvw125

Hajian-Forooshani, Z., Vandermeer, J., & Perfecto, I. (2020) Insights from excrement: invasive gastropods shift diet to consume the coffee leaf rust and its mycoparasite. *Ecology, 101*(5):e02966.

Jibat, M. (2020). Review on Resistance Breeding Methods of Coffee Leaf Rust in Ethiopia. *International Journal of Agriculture and Forestry, 7* (6): 32-41.

Julca-Otiniano, A., Alarcón-Águila, G., Alvarado-Huamán, L., Borjas-Ventura, R., & Castro-Cepero, V. (2018). Behavior of three cultivars of coffee (Catimor, Colombia and Costa Rica 95) in the valley of el Perené, Junín, Perú. *Chilean Journal of Agricultural and Animal Sciences*, *34*(3), 205–215. https://doi.org/10.4067/S0719-38902018005000504

Kraft, K. (2017). Coffee leaf rust rear its ugly head again in Honduras. *Daily Coffee News, Coffee Roast Magazine*, May 8, 2017.

López-Bravo, D.F., Virginio-Filho, E.D.M., & Avelino, J. (2012). Shade is conducive to coffee rust as compared to full sun exposure under standardized fruit load conditions. *Crop Protection, 38*:1-29.

Martins, S.J., Soares, A.C., Medeiros, F.H.V., Santos, D.B.C., & Pozza, E.A. (2015). Contribution of host and environmental factors to the hyperparasitism of coffee rust under field conditions. *Australasian Plant Pathology, 44*(6): 605-610.

Mccook, S., & Peterson, P. D. (2020). The Geopolitics of Plant Pathology: Frederick Wellman, Coffee Leaf Rust, and Cold War Networks of Science. *Annual Review of Phytopathology 2020*, *58*, 181–199. https://doi.org/10.1146/annurev-phyto-082718

McCook, S., & Vandermeer, J. (2015). The Big Rust and the Red Queen: Long-Term Perspectives on Coffee Rust Research. *Phytopathology*, *105*(9), 1164–1173. https://doi.org/10.1094/PHYTO-04-15-0085-RVW

McGrann, G.R.D., Stavrinides, A., Russell, J., Corbitt, M.M., Booth, A., Chartrain, L., Thomas, W.T.B., Brown, J.K.M. (2014) A Trade off between Mlo Resistance to Powdery Mildew and Increased Susceptibility of Barley to a Newly Important Disease, Ramularia Leaf Spot. *Journal of Experimental Botany 65*(4): 1025-037. http://www.jstor.org/stable/24043523.

Monterroso Salvatierra, D. (1999) Interacción patosistemas-sombra en el sistema café. In 4. Semana Científica 6-9 Abr 1999, Turrialba (Costa Rica) (No. CATIE ST RT-5). CATIE, Turrialba (Costa Rica). Programa de Investigación.

Pérez, C. D. P., Pozza, E. A., Pozza, A. A. A., de Freitas, A. S., Silva, M. G., & da Silva Gomes Guimarães, D. (2019). Impact of nitrogen and potassium on coffee rust. *European Journal of Plant Pathology*, *155*(1), 219–229. https://doi.org/10.1007/s10658-019-01765-4

Pérez, C. D. P., Pozza, E. A., Pozza, A. A. A., Elmer, W.H., Pereira, A.B., da Silva Gomes Guimarães, D., Monteiro, A.C.A., & de Rezende, M.L.V. (2020). Boron, zinc and manganese suppress rust on coffee plants grown in a nutrient solution. *European Journal of Plant Pathology 156*(3):727-738.

Philpott, S. M., & Bichier, P. (2012). Effects of shade tree removal on birds in coffee agroecosystems in Chiapas, Mexico. *Agriculture, Ecosystems and Environment*, *149*, 171–180. https://doi.org/10.1016/j.agee.2011.02.015

Spoel, S.H., Johnson, J.S., Dong, X. (2007). Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proceedings of the National Academy of Sciences, Nov 20;104*(47):18842-7.

Soto-Pinto, L., Perfecto, I., & Caballero-Nieto, J. (2002). Shade over coffee: Its effects on berry borer, leaf rust and spontaneous herbs in Chiapas, Mexico. *Agroforestry Systems*, *55*(1), 37–45. https://doi.org/10.1023/A:1020266709570

Silva, M. G., Pozza, E. A., Vasco, G. B., Freitas, A. S., Chaves, E., Paula, P. V. A. A., Dornelas, G. A., Alves, M. C., Silva, M. L. O., & Pozza, A. A. A. (2019). Geostatistical analysis of coffee leaf rust in irrigated crops and its relation to plant nutrition and soil fertility. *Phytoparasitica*, *47*(1), 117–134. https://doi.org/10.1007/s12600-019-00714-5

Staver, C., Guharay, F., Monterroso, D., & Muschler, R. G. (2001). Designing pest-suppressive multistrata perennial crop systems: Shade-grown coffee in central america. *Agroforestry Systems*, *53*(2), 151–170. https://doi.org/10.1023/A:1013372403359

Talhinhas, P., Batista, D., Diniz, I., Vieira, A., Silva, D. N., Loureiro, A., Tavares, S., Pereira, A. P., Azinheira, H. G., Guerra-Guimarães, L., Várzea, V., & Silva, M. do C. (2017). The coffee leaf rust pathogen *Hemileia vastatrix* : one and a half centuries around the tropics. *Molecular Plant Pathology*, *18*(8), 1039–1051. https://doi.org/10.1111/mpp.12512

Toniutti, L., Breitler, J.C., Etienne, H., Campa, C., Doulbeau, S., Urban, L., Lambot, C., Pinilla, J.C.H. & Bertrand, B. (2017). Influence of environmental conditions and genetic background of Arabica coffee (*C. arabica* L) on leaf rust (*Hemileia vastatrix*) pathogenesis. *Frontiers in Plant Science 8*:2025.

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*(4), 636–641. https://doi.org/10.1111/j.1365-3059.2009.02067.x

Vasco, G. B., Pozza, E. A., da Silva, M. G., Pozza, A. A. A., & Chaves, E. (2018). Interaction of K and B in the intensity of coffee rust in nutrient solution. *Coffee Science*, *13*(2), 238–244. https://doi.org/10.25186/cs.v13i2.1428

Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J., Fan, J., Yang, S., Hu, L., Leung, H., Mew, T.W. (2000). Genetic diversity and disease control in rice. *Nature*, *406*(6797):718-722.

1.8 Tables and figures

Table 0.1 Contingency tables for American leaf spot and coffee leaf rust presence for all months with chi-square test results

		American leaf spot			Pearson's Chi-squared test
		Absent	Present	Total	
Coffee leaf rust (March)	Absent	197	77	274	X^2 = 18.782, p = 1.465e-05
	Present	315	51	366	
	Total	512	128	640	
Coffee leaf rust (June)	Absent	258	68	326	X^2 = 8.9552 p = 0.002767
	Present	277	37	314	
	Total	535	105	640	
Coffee leaf rust (July)	Absent	253	73	326	X^2 = 17.514, p = 2.853e-05
	Present	283	31	314	
	Total	536	104	640	
Coffee leaf rust (August)	Absent	266	83	349	X^2 = 27.814, p = 1.336e-07
	Present	268	23	291	
	Total	534	106	640	
Coffee leaf rust (September)	Absent	305	90	395	X^2 = 24.602, p = 7.047e-07
	Present	227	18	245	
	Total	532	108	640	
Coffee leaf rust (October)	Absent	270	91	361	X^2 = 39.639, p = 3.055e-10
	Present	262	17	279	
	Total	532	108	640	
Coffee leaf rust (November)	Absent	220	86	306	X^2 = 24.171, p = 8.817e-07
	Present	293	41	334	
	Total	513	127	640	

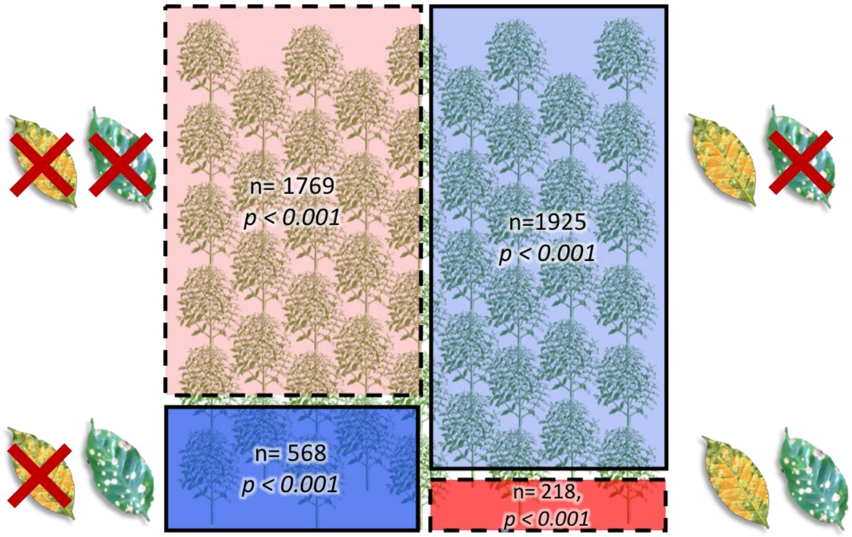

Figure 0.1 Pooled data on the incidence of CLR and ALS. Red rectangles with dashed borders indicate lower number of plants than expected, and blue rectangles with solid borders indicate higher number of plants than expected. Darker shades indicate more deviation from expected.

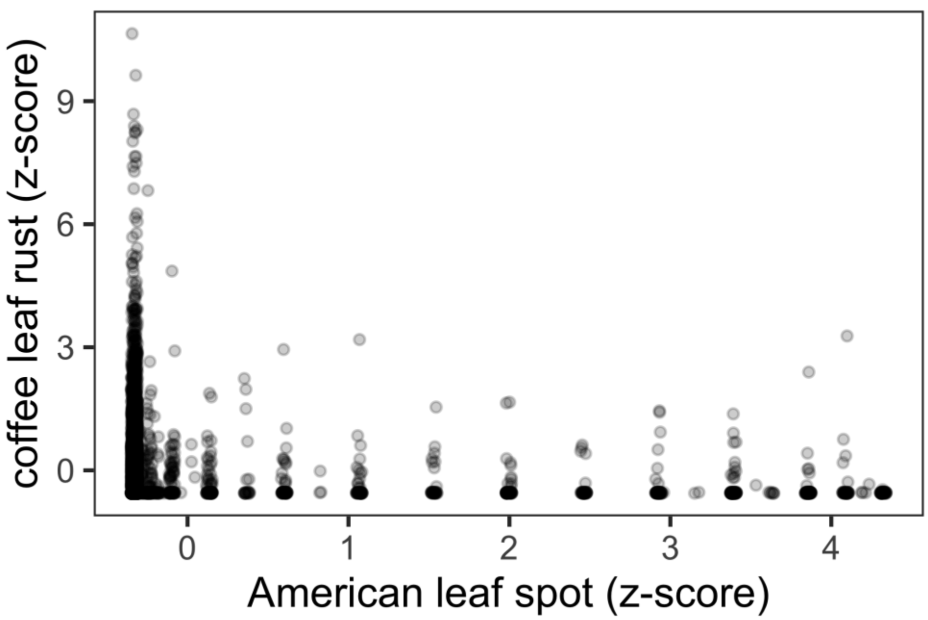

Figure 0.2 Relative intensities of the two diseases on each coffee plant for all samples across the seven months. Points are transparent and jittered to show overlapping samples. Note that when one disease is not present or at low intensities, the other disease is at a higher intensity, suggesting that coinfection of both diseases is not common on our field site.

Chapter 2: American leaf spot disease incidence and intensity among shade trees in coffee agroforestry systems

2.1 Abstract

American leaf spot disease caused by *Mycena citricolor* is a rising coffee fungal disease in South America. Native in the area, American leaf spot can infect not only coffee, but also weeds and shade trees, making it hard to control and can cause severe yield losses in coffee. Relatively little is known about factors that impact the disease incidence and intensity in coffee farms, including the effect of various shade trees. In this study, I report on patterns of American leaf spot disease incidence and intensity with respect to three shade tree types and the presence of a dominant ant in an organic coffee farm in Mexico. Specifically, disease incidence and intensity were compared among *Inga spp.*, nitrogen fixing shade trees commonly found in coffee farms, *Alchornea latifolia*, a second common shade tree in the study site, and other shade trees including fruit trees. Additionally, I examine the potential impact of the presence of *Azteca instabilis*, a dominant ant species that nest in shade trees and forage and tent hemipterans in coffee bushes. *Chi*-squared test and Kruskal-Wallis rank sum test show no significant difference in disease incidence by month for different tree types. T-test on American leaf spot intensity show significant differences by tree types when compared in pairs, with significantly higher intensities under *Inga* spp. trees than other shade trees, possibly due to the favorable microclimate conditions under *Inga* trees in the farm. No statistically significant results for *Alchornea* tree and presence of *Azteca* ants were found in the analyses. The results suggest a possible trade-off between nutrient addition by *Inga spp.* trees and shade levels for nitrogen fixing shade trees, and further studies examining independent factors associated with shade trees including nutrient addition and complex ecological webs can inform management in coffee farms for fungal disease control.

2.2 Introduction

Diseases and pests are important factors that can impact agricultural production, reducing yield and leading to ecological and social impacts. Coffee, as one of the most commercially important crops and a major traded commodity in the developing world, have suffered from various disease and pest outbreaks (Avelino et al. 2018). Originated from Africa, coffee has been introduced to the rest of the world including Southeast Asia and Latin America. Approximately 125 million people worldwide depend on coffee to support and maintain their livelihoods, from farmers to retailers (Krishnan 2017). According to the International coffee organization, production in 2018/2019 reached 170.22 million bags, with Latin America currently being the largest producing region of coffee in the world, representing about 48.3% of the world total (International Coffee Organization 2020). However, Latin America has seen severe damages from pests and pathogens. For example, the "Big Rust" – coffee leaf rust outbreak moving from Mexico to Peru since 2012, caused significant economic loss and hardship for coffee growers, and raised much interest in understanding fungal disease ecology and possible responses in coffee systems (McCook and Vandermeer 2015).

As coffee growing zones coincide with hotspots of biodiversity, and producers live in culturally and biologically diverse regions, coffee growers adopt a large range of management practices (Bravo-Monroy et al. 2016, Bacon 2005). On one end of the spectrum, intensified coffee farms grow monocultural coffee with full sun exposure, using synthetic fertilizers and pesticides (Bravo-Monroy et al. 2016). On the other end of the spectrum, coffee is traditionally grown under diverse and dense shade canopy, composing environments similar to natural forests (Toledo and Moguel 2012). In the middle of the spectrum, management practices such as monocultural shade coffee and commercial polycultures are also common. Across various intensities of coffee agriculture, shade trees regularly play an essential role, as shade coffee is desirable as a commodity and shade trees can offer many additional ecosystem services (Muschler 2001, Hernandez-Aguilera et al. 2019). Due to the high incorporation of shade trees in coffee systems, impact of shade cover on coffee have been extensively examined. Shade trees provide many benefits to coffee farms. Studies show that shade trees can improve the quality of coffee, but such effect can vary based on altitude (Muschler 2001, Bosselmann et al. 2009). Shade trees can increase the survival of coffee plants in varying environmental conditions (Beer 1987). They can also suppress weed growth through reduced sunlight reaching the forest floor and competition (Soto-Pinto et al. 2002). Physiologically, some shade trees, such as nitrogen fixing trees, can add nutrients to the soil and increase soil fertility and subsequently support coffee growth (Cerdá et al. 2012). Shade trees can also have negative impacts on coffee plants, such as damaging the plants from branch fall and heavy shading (Beer 1987).

Interactions between shade cover and coffee diseases and pests are complicated. Shade trees that add nutrients to the soil, especially nitrogen fixing trees that are commonly found in coffee systems, have been shown to promote activity of beneficial soil microorganisms and increase soil fertility (Sauvadet et al. 2018). These beneficial soil characteristics then contribute to the health of coffee plants by enhancing growth and increasing resistance to diseases (Ratnadass 2012, Durand-Bessart et al. 2020). Meanwhile, shade trees could also increase disease incidence through the creation of favorable microclimate of high moisture and low light, promoting fungal disease germination and growth (Avelino et al. 2018).

Interactions of shade trees with the agroforestry communities can also impact coffee diseases through potential indirect effect of biological control. Studied on a dominant ant species *Azteca instabilis*, commonly found in coffee systems in Mexico and Puerto Rico, show variations in ant nest frequencies and ant activities by tree species, which further impact the complex ecological systems involving insects, birds and fungi (Philpott et al. 2005; Livingston et al. 2008). *Azteca* ants as a key stone species in a complex ecological web can serve as a potential biological control impacting fungal disease incidence, where such relationships between *Azteca* sp. and coffee leaf rust were found in Mexico and Puerto Rico (Vandermeer et al. 2009; Jackson et al. 2012; Hajian-Forooshani et al. 2016). On the other hand, *Azteca* could also be spreading the spores of the ALS disease.

Recently, a fungal disease, American leaf spot (ALS), has become more common in Latin America, and signs of increasing ALS were found in Mexico (Avelino et al. 2018). American leaf spot disease, caused by the fungal agent *Mycena citricolor*, is a native fungal agent in Latin America. It can infect coffee leaves, branches, and fruits, causing significant damage to coffee plant through defoliation, branch deaths and fruit fall. *M. citricolor* has a broad range of hosts in coffee farms including weeds and shade trees, making it difficult to control (Avelino et al. 2018). A study by Avelino et al. in 2007 showed that forest and fruit trees favor the development of ALS providing propitious conditions for growth, while fertilization was unfavorable for the disease. In this study, I aim to further understand the impact of shade tree types on ALS disease incidence and severity from the mixed impact of various shade trees. I hypothesize that different shade tree types will have different effects on ALS disease, with nitrogen fixing trees having a negative relationship with ALS incidence and severity, due to the increased nitrogen input in the soil and improved coffee health and disease resistance. In addition, shade trees with *Azeteca* ant nest will have a lower incidence and severity of ALS through indirect biological control, resulting in lower ALS in *Alchornea* trees because of the possible high occurrence of *Azteca* ants.

2.3 Method

Study site

To test the hypothesis, surveys of ALS were conducted at Finca Irlanda, Chiapas, Mexico (15°10'6N", 92°20' 29"W). Finca Irlanda is a 280-ha certified organic farm with a high density and diversity of shade trees (for more details on the farm vegetation see Philpott and Bichier 2012). It is located at elevations ranging from 900 meters to 1200 meters above sea level and receives an annual rainfall of 4500 mm. The farm adopts a coffee agroforestry system with diverse shade trees as canopy cover, including dominant shade trees in the genus *Inga*, which are nitrogen fixing plants in the family Fabaceae, allowing for exploration of the impacts of shade tree types on ALS diseases.

Sampling and data collection

Sampling was conducted each month from March to November in 2020, with the exception of April and May on a contiguous 45 hectares study plot. All shade trees bigger than 10 cm in circumference in the plot have been identified and georeferenced for another study (Vandermeer et al. 2008; Jackson et al. 2009; Li et al. 2016). Each of the 45 hectares within this plot was subdivided into four 50 × 50 m subplots, for a total of 128 subplots (all border hectares were excluded from the study). A central shade tree was identified as a point location for each subplot and the presence or absence of *Azteca* ant nest was recorded. The five coffee plants nearest the central shade tree were sampled, and the presence and severity of ALS were visually inspected and recorded. The intensity of ALS was estimated by percentage of infection for each coffee plant. In total, 640 coffee plants around 128 shade trees were examined every month.

Central shade trees in the 128 subplots were identified and categorized into three categories, *Inga* spp., *Alchornea latifolia,* and others, to compare more generally the effects of the shade trees on ALS. With the categorization of the shade trees, ALS presence and intensities were compared for each month. Contingency tables for ALS presence and tree types were constructed and chi-square analysis were run to understand the deviation of disease presence near each type of central shade tree from expected at random. Due to the non-normal distribution of the data for disease intensity, a Kruskal-Wallis rank sum test was used to compare the severity of ALS with respect to tree types for each month. Additional t-tests comparing means for each pair of tree types were conducted to confirm the direction and level of significance.

In addition, similar analyses were run on pooled data of all coffee plants through the entire sampling period, averaged data per subplot each month and with a subset of infected coffee plants. Graphs of ALS incidence and severity were drawn to visually show the disease distribution across tree types.

To understand ALS incidence with respect to *Azteca* ants and the relationship of *Azteca* ant nest presence with tree species, I run chi-squared analysis on nest presence and tree types to confirm any pattern in ant nest distribution, and Kruskal-Wallis rank sum test to compare the severity of ALS with respect to ant nest presence.

2.4 Results

From the 128 subplots in the study site, twenty-two different species of shade trees were found, and eighteen were identified to genus (Table 2.1). Among the eighteen species of shade trees identified, four belong to the genus *Inga*. *Inga micheliana*, a common nitrogen fixing shade tree in the family Fabaceae has the highest number of occurrences, 68 times, in the 128 subplots. Four *Inga* trees were grouped accounting for 85 subplots with *Inga* as a central shade tree. The second most common tree in the site is *Alchornea latifolia*, with a total occurrence of 14 times. The rest 29 subplots with various tree species were grouped together in a category of "Other" for subsequent analysis (Table 2.1).

Chi-square analysis and Kruskal-Wallis rank sum test for each month found no significant differences on ALS presence and intensity based on central shade tree types except for June, where statistically significant differences of ALS intensities by tree type were found (Kruskal-Wallis chi-squared = 6.7106, df = 2, p-value = 0.0349) (Table 2.2). T-test for ALS intensity comparing *Inga*, *Alchornea* and other trees in pairs show different results (Table 2.3). T-test comparing ALS intensities for coffee plants under *Inga* trees and other trees show statistically significant difference ($p<0.05$) across all months except for March (p = 0.09063). When comparing ALS intensities for coffee plants under *Alchornea* and other, or *Inga* and other trees, no statistical significance was found except for in June, when coffee plants under *Inga* plants have significantly higher intensity of ALS than coffee plants surrounding *Alchornea* trees do. To visualize the data, ALS intensities were plotted by month (Fig. 2.1).

Chi-squared analysis for pooled data across all months show significant deviation of ALS occurrence from expected at random (X-squared = 30.329, df = 2, p-value = 2.595e-07) (Fig. 2.2), and posthoc analysis using Bonferroni method for chi-squared analysis suggests that higher than expected number of coffee plants around *Inga* trees are infected with ALS, while lower than expected coffee plants around other shade trees are infected with ALS. Kruskal-Wallis rank sum test for all samples across the months suggests that ALS intensities are different by tree types (Kruskal-Wallis chi-squared = 32.729, df = 2, p-value = 7.817e-08) and t-test for each pair of shade tree groups show that *Inga* is associated with higher level of ALS intensities compared to other trees (t = 6.398, df = 2472.5, p-value = 1.877e-10). Similar to data by month, no statistical significance was found between ALS intensities in *Inga* and *Alchornea* (t = 0.70968, df = 669.62, p-value = 0.4782), but lower ALS intensities under other shade trees than *Alchornea* were found (t = -3.0488, df = 746.21, p-value = 0.002379).

No statistically significant differences were found in the three tree types in the aggregated data by quadrat where ALS intensities among five coffee plants were averaged (Fig. 2.3), though coffee plants with higher ALS intensities in general are found under *Inga* plants. A subset of coffee plants that are infected with ALS were compared and plotted (Fig.

2.4), and no significant difference in ALS intensities were found in comparisons by tree groups.

Chi-squared analysis of *Azteca* ant nest presence and tree types show no statistically significant results throughout all months, though a pattern of higher *Azteca* ant nest on *Alchornea* shade trees were found in all months, similar to the pattern presented for March, due to the low variability of ant nests within a year (Fig. 2.5). No statistically significant results for Kruskal-Wallis rank sum test were found for *Azteca* ant nest presence and ALS intensities across all months examined (Fig. 2.6).

2.5 Discussions

This study focuses on disease patterns in an organic agroforestry coffee system, and shows that ALS disease presence and intensity are not significantly impacted by all coffee shade trees in the same way. Results show that coffee plants around shade tree *Inga*, although not significantly different from under *Alchornea*, have higher intensities of ALS than under other shade trees, including many fruit trees, rejecting the hypothesis that coffee plants around *Inga* trees would have lower disease incidence and intensity due to their nitrogen fixations (Ratnadass et al. 2012). Presence and intensity of ALS with pooled data amplified the effect due to the multi-month sampling and showed significant differences between *Inga* and other shade trees as well. *Azteca* ant nest occurrences were not significantly higher on *Alchornea* trees than *Inga* or other shade trees in the coffee farm, and ALS intensities were not significantly lower under shade trees with ant nest.

Although the effects are complicated, the results suggest that *Inga* shade trees can have a negative effect on coffee plants by increasing the infection of ALS on coffee plants. The results disagree with previous hypothesis that *Inga* trees will benefit coffee fitness and reduce disease incidence, indicating a more complexed effect. One possible explanation is that nitrogen fixation might not be the same in all *Inga* trees, as a study by Grossman et al. (2006) suggests that nodulation in some *Inga* trees could be relatively slow, and that the *Inga* shade trees might not fix much nitrogen, especially in young trees. Direct quantification on nitrogen fixation in *Inga* and disease incidence should be futher explored. Besides, contrary to the common idea where nitrogen fixation can reduce disease incidence by benefitting plant health (Ratnadass et al. 2012), recent studies focusing on soil nutrients and fungal diseases in coffee show complicated responses to disease from various soil nutrients, where higher nutrient levels of common nutrients might not necessarily have a direct positive impact on disease resistance and reduction (Vazco et al. 2018; Perez et al. 2019; Silva et al. 2019).

The results suggest that additional factors associated with coffee shade trees aside from nutrient addition can interact to have a combined effect on disease incidence and intensity (Durand-Bessart et al. 2020). An important characteristic of shade trees is the capacity to alter microclimate for coffee plants underneath. Reduced airflow, increased shade and moisture can contribute positively to fungal disease development due to the suitable environment for spore germination. In fact, Avelino et al. (2018) found that high shade levels increase ALS intensities, similar to the results from Durand-Bessart (2020) where an antagonistic effect was shown of shade cover on coffee foliar diseases. In the coffee farm where the study was conducted, *Inga* and *Alchornea* trees are regularly pruned, resulting in lower heights of tree canopies compared to other shade trees in the farm. Pruning of shade trees could have led to higher shade level and lower air flow, maintaining higher moisture for coffee plants, but also providing better opportunities for the germination and growth of *Mycena citricolor*. This hypothesis could also explain the insignificant difference on intensity and incidence of the disease for coffee plants under *Inga* and *Alchornea*, due to the possible similarities for microclimate conditions under the similarly pruned shade trees. Furthermore, both of these types of trees produce a fairly dense shade due to their broad leaves. The trade-off between the positive effect from nitrogen fixation and negative effect from microclimate alteration under *Inga* trees could explain the results of the study, and the independent and opposite effect of nitrogen fixing shade trees should be further examined.

Although we did not see any significant effect of *Alchornea* trees compared to the other shade trees, average ALS disease intensities between *Inga* and other trees is present across months. With similar microclimates created to pruned *Inga* trees, *Alchornea* can promote growth of ALS as well. Although no significant effect from *Azteca* ant nest presence were found, possible biological interactions within the system should be further explored by research focusing on possible natural predators of ALS in coffee systems and their distributions with respect to shade trees. Other shade trees in our study only consist of a small proportion of all shade trees in the plots, and the possibly similar tree architecture and microclimate conditions under less dense shade due to higher tree canopies can be less favorable for ALS development.

Spatial and temporal differences of ALS disease were not explicitly removed from the study and could have an impact on the comparison of disease incidence and severity reflected in coffee plants under the same shade trees for each month. As five coffee plants were sampled around each central shade tree, the spatial proximity of the coffee plants could have impacted ALS severity and persistence. When any number of coffee plants were infected under a central shade tree, the other plants surrounding the same shade tree could have a higher probability of getting infected, and further develop the disease. Coffee plants that are already infected in a month might be more likely to stay infected, resulting in persistent infection under the same shade trees. These effects should be further examined to understand the independent impact of different shade trees species.

ALS as a coffee fungal disease rising in Latin America can raise problems on coffee production, especially under the current management of coffee leaf rust by planting resistant varieties, which are more susceptible to ALS disease (Staver et al. 2001, Allinie et al. 2016; Su et al., in review). Management of coffee farm landscapes with focus on shade tree species, architecture and ecological interactions between the fungus *Mycena citricolor* and other biological links in the ecosystem is essential, and better understanding of the mixed effect of shade trees on ALS incidence and intensity will be beneficial to guide management.

2.6 References

Allinne, C., Savary, S., & Avelino, J. (2016). Delicate balance between pest and disease injuries, yield performance, and other ecosystem services in the complex coffee-based systems of Costa Rica. *Agriculture, Ecosystems and Environment*, *222*, 1–12. https://doi.org/10.1016/j.agee.2016.02.001

Avelino, J., Cabut, S., Barboza, B., Barquero, M., Alfaro, R., Esquivel, C., Durand, J. F., & Cilas, C. (2007). Topography and crop management are key factors for the development of American leaf spot epidemics on coffee in Costa Rica. *Phytopathology*, *97*(12), 1532–1542. https://doi.org/10.1094/PHYTO-97-12-1532

Avelino, J., Allinne, C., Cerda, R., Willocquet, L., & Savary, S. (2018). Multiple-Disease System in Coffee: From Crop Loss Assessment to Sustainable Management. *Annual*

Review of Phytopathology, *56*(1), 611–635. https://doi.org/10.1146/annurev-phyto-080417-050117

Bacon, C. (2005). Confronting the coffee crisis: Can Fair Trade, organic, and specialty coffees reduce small-scale farmer vulnerability in Northern Nicaragua? *World Development*, *33*(3), 497–511. https://doi.org/10.1016/j.worlddev.2004.10.002

Beer J. (1987). Advantages, disadvantages and desirable characteristics of shade trees for coffee, cacao and tea. In *Agroforestry Systems* (Vol. 5).

Bosselmann, A. S., Dons, K., Oberthur, T., Olsen, C. S., Ræbild, A., & Usma, H. (2009). The influence of shade trees on coffee quality in small holder coffee agroforestry systems in Southern Colombia. *Agriculture, Ecosystems and Environment*, *129*(1–3), 253–260. https://doi.org/10.1016/j.agee.2008.09.004

Bravo-Monroy, L., Potts, S. G., & Tzanopoulos, J. (2016). Drivers influencing farmer decisions for adopting organic or conventional coffee management practices. *Food Policy*, *58*, 49–61. https://doi.org/10.1016/j.foodpol.2015.11.003

Cerda, R., Allinne, C., Krolczyk, L., Mathiot, C., Clement, E., Harvey, C. A., Aubertot, J.-N., Tixier, P., Gary, C., & Avelino, J. (2015). Ecosystem services provided by coffee agroecosystems across a range of topo-climatic conditions and management strategies. *FSD5 Proceedings: Multi-Functional Farming Systems in a Changing World*.

Durand-Bessart, C., Tixier, P., Quinteros, A., Andreotti, F., Rapidel, B., Tauvel, C., & Allinne, C. (2020). Analysis of interactions amongst shade trees, coffee foliar diseases and coffee yield in multistrata agroforestry systems. *Crop Protection*, *133*, 105137. https://doi.org/10.1016/j.cropro.2020.105137

Grossman, J.M., Sheaffer, C., Wyse, D., Bucciarelli, B., Vance, C. and Graham, P.H.. (2006). An assessment of nodulation and nitrogen fixation in inoculated Inga oerstediana, a nitrogen-fixing tree shading organically grown coffee in Chiapas, Mexico. *Soil Biology and Biochemistry*, *38*(4), pp.769-784.

Hajian-Forooshani, Z., Salinas, I. S. R., 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*(6), 1521–1526. https://doi.org/10.1093/ee/nvw125

Hernandez-Aguilera, J. N., Conrad, J. M., Gómez, M. I., & Rodewald, A. D. (2019). The Economics and Ecology of Shade-grown Coffee: A Model to Incentivize Shade and Bird Conservation. *Ecological Economics*, *159*, 110–121. https://doi.org/10.1016/j.ecolecon.2019.01.015

International coffee organization. (2020). Coffee Development Report 2020 (CDR2020). https://www.internationalcoffeecouncil.com/cdr2020

Jackson, D., Vandermeer, J., & Perfecto, I. (2009). Spatial and Temporal Dynamics of a Fungal Pathogen Promote Pattern Formation in a Tropical Agroecosystem. *The Open Ecology Journal*, *2*(1), 62–73. https://doi.org/10.2174/1874213000902010062

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*(1), 89–97. https://doi.org/10.1016/j.biocontrol.2012.01.004

Krishnan, S. (2017). Sustainable Coffee Production. In *Oxford Research Encyclopedia of Environmental Science*. Oxford University Press. https://doi.org/10.1093/acrefore/9780199389414.013.224

Li, K., Vandermeer, J. H., & Perfecto, I. (2016). Disentangling endogenous versus exogenous pattern formation in spatial ecology: A case study of the ant Azteca sericeasur in southern Mexico. *Royal Society Open Science*, *3*(5). https://doi.org/10.1098/rsos.160073

Livingston, G. F., White, A. M., & Kratz, C. J. (2008). Indirect Interactions Between Ant-Tended Hemipterans, a Dominant Ant Azteca instabilis (Hymenoptera: Formicidae), and Shade Trees in a Tropical Agroecosystem. *Environmental Entomology*, *37*(3), 734–740. https://doi.org/10.1093/ee/37.3.734

McCook, S., & Vandermeer, J. (2015). The Big Rust and the Red Queen: Long-Term Perspectives on Coffee Rust Research. *Phytopathology®*, *105*(9), 1164–1173. https://doi.org/10.1094/PHYTO-04-15-0085-RVW

Muschler, R. G. (2001). Shade improves coffee quality in a sub-optimal coffee-zone of Costa Rica. *Agroforestry Systems*, *51*(2), 131–139. https://doi.org/10.1023/A:1010603320653

Pérez, C. D. P., Pozza, E. A., Pozza, A. A. A., de Freitas, A. S., Silva, M. G., & da Silva Gomes Guimarães, D. (2019). Impact of nitrogen and potassium on coffee rust. *European Journal of Plant Pathology*, *155*(1), 219–229. https://doi.org/10.1007/s10658-019-01765-4

Philpott, S. M., Greenberg, R., & Bichier, P. (2005). The Influence of Ants on the Foraging Behavior of Birds in an Agroforest1. *Biotropica*, *37*(3), 468–471. https://doi.org/10.1111/j.1744-7429.2005.00063.x

Philpott, S. M., Perfecto, I., & Vandermeer, J. (2006). Effects of management intensity and season on arboreal ant diversity and abundance in coffee agroecosystems. *Biodiversity and Conservation*, *15*(1), 139–155. https://doi.org/10.1007/s10531-004-4247-2

Philpott, S. M., & Bichier, P. (2012). Effects of shade tree removal on birds in coffee agroecosystems in Chiapas, Mexico. *Agriculture, Ecosystems and Environment*, *149*, 171–180. https://doi.org/10.1016/j.agee.2011.02.015

Ratnadass, A., Fernandes, P., Avelino, J., & Habib, R. (2012). Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: A review. In *Agronomy for Sustainable Development* (Vol. 32, Issue 1, pp. 273–303). Springer. https://doi.org/10.1007/s13593-011-0022-4

Sauvadet, M., den Meersche, K. Van, Allinne, C., Gay, F., de Melo Virginio Filho, E., Chauvat, M., Becquer, T., Tixier, P., & Harmand, J. M. (2019). Shade trees have higher impact on soil nutrient availability and food web in organic than conventional coffee agroforestry. *Science of the Total Environment*, *649*, 1065–1074. https://doi.org/10.1016/j.scitotenv.2018.08.291

Silva, M. G., Pozza, E. A., Vasco, G. B., Freitas, A. S., Chaves, E., Paula, P. V. A. A., Dornelas, G. A., Alves, M. C., Silva, M. L. O., & Pozza, A. A. A. (2019). Geostatistical analysis of coffee leaf rust in irrigated crops and its relation to plant nutrition and soil fertility. *Phytoparasitica*, *47*(1), 117–134. https://doi.org/10.1007/s12600-019-00714-5

Soto-Pinto, L., Perfecto, I., & Caballero-Nieto, J. (2002). Shade over coffee: Its effects on berry borer, leaf rust and spontaneous herbs in Chiapas, Mexico. *Agroforestry Systems*, *55*(1), 37–45. https://doi.org/10.1023/A:1020266709570

Staver, C., Guharay, F., Monterroso, D., & Muschler, R. G. (2001). Designing pest-suppressive multistrata perennial crop systems: Shade-grown coffee in central america. *Agroforestry Systems*, *53*(2), 151–170. https://doi.org/10.1023/A:1013372403359

Su. C., Hajian-Forooshani, Z., Li, K., Vandermeer, J.H., & Perfecto, I.. Early signs of a secondary outbreak of *Mycena citricolor* in an organic farm in southern Mexico. *Journal of plant pathology.* In review.

Toledo V.M., Moguel P.. (2012). “Coffee and sustainability: the multiple values of traditional shaded coffee.” *Journal of Sustainable Agriculture*. 36:353–77

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*(4), 636–641. https://doi.org/10.1111/j.1365-3059.2009.02067.x

Vasco, G. B., Pozza, E. A., da Silva, M. G., Pozza, A. A. A., & Chaves, E. (2018). Interaction of K and B in the intensity of coffee rust in nutrient solution. *Coffee Science, 13*(2), 238–244. https://doi.org/10.25186/cs.v13i2.1428

2.7 Tables and figures

Table 0.1 Central shade tree species and count in the 128 subplots.

Shade tree species	Count	Shade tree species	Count	Shade tree species	Count
Inga		**Others**		*Critonia morifolium*	1
Inga micheliana	68	*Yucca elephantoides*	7	*Ficus* sp.	1
Inga vera	8	*Conostegia xalapensis*	3	*Ficus* sp.-2	1
Inga sapindoides	8	*Cordia* sp.	3	*Hibiscus* sp.	1
Inga punctata	1	*Aspidosperma cruentum*	2	*Micropholis (crotoniodes)*	1
		Citrus sinensis	2	miritz sin pelos	1
Alchornea		*Albizia* sp.	1	*Ocotea* sp.	1
Alchornea latifolia	14	*Codiaeum variegatum*	1	*Persea americana*	1
		Eriobotrya japonica	1	*Trema micrantha*	1

Table 0.2 Chi-squared test and Kruskal-Wallis rank sum test results by month of ALS presence and intensity by tree types. Significant results are in bold and marked by * (p<0.05).

Months	χ^2 test (Tree Type and ALS Presence)	Kruskal-Wallis rank sum test (Tree type by ALS Intensity)
March	X-squared = 2.7308, df = 2, p-value = 0.2553	Kruskal-Wallis chi-squared = 3.5141, df = 2, p-value = 0.1726
June	**X-squared = 6.3218, df = 2, p-value = 0.04239***	**Kruskal-Wallis chi-squared = 6.7106, df = 2, p-value = 0.0349***
July	X-squared = 5.1111, df = 2, p-value = 0.07765	Kruskal-Wallis chi-squared = 5.785, df = 2, p-value = 0.05544
August	X-squared = 4.5049, df = 2, p-value = 0.1051	Kruskal-Wallis chi-squared = 5.0972, df = 2, p-value = 0.07819
September	X-squared = 4.68, df = 2, p-value = 0.09633	Kruskal-Wallis chi-squared = 4.6364, df = 2, p-value = 0.09845
October	X-squared = 4.6752, df = 2, p-value = 0.09656	Kruskal-Wallis chi-squared = 4.9493, df = 2, p-value = 0.08419
November	X-squared = 3.8017, df = 2, p-value = 0.1494	Kruskal-Wallis chi-squared = 4.317, df = 2, p-value = 0.1155

Table 0.3 T-test results by month of ALS intensity on coffee plants with comparisons between each pair of shade trees - Inga, Alchornea and other. Significant results are in bold and marked by *(p<0.05) and **(p<0.01).

Months	*Inga vs.* Other	*Inga vs. Alchornea*	Other *vs. Alchornea*
March	t = 1.6976, df = 300.09, p-value = 0.09063	t = -0.46673, df = 87.974, p-value = 0.6418	t = -1.3868, df = 105.96, p-value = 0.1684
June	**t = 2.4071, df = 377.04, p-value = 0.01656***	**t = 2.3896, df = 256.74, p-value = 0.01759***	t = -0.23729, df = 205.99, p-value = 0.8127
July	**t = 3.1058, df = 428.71, p-value = 0.002024****	t = 0.021468, df = 94.257, p-value = 0.9829	t = -1.6612, df = 93.539, p-value = 0.1
August	**t = 3.2205, df = 419.21, p-value = 0.001379****	t = 0.40001, df = 95.695, p-value = 0.69	t = -1.3996, df = 96.079, p-value = 0.1649
September	**t = 2.0093, df = 325.53, p-value = 0.04533***	t = -0.091085, df = 89.436, p-value = 0.9276	t = -1.198, df = 102.94, p-value = 0.2337
October	**t = 2.7749, df = 365.1, p-value = 0.005806****	t = 0.13592, df = 92.416, p-value = 0.8922	t = -1.4135, df = 100.17, p-value = 0.1606
November	**t = 2.4102, df = 325.05, p-value = 0.0165***	t = 0.80466, df = 96.465, p-value = 0.423	t = -0.72399, df = 114, p-value = 0.4706

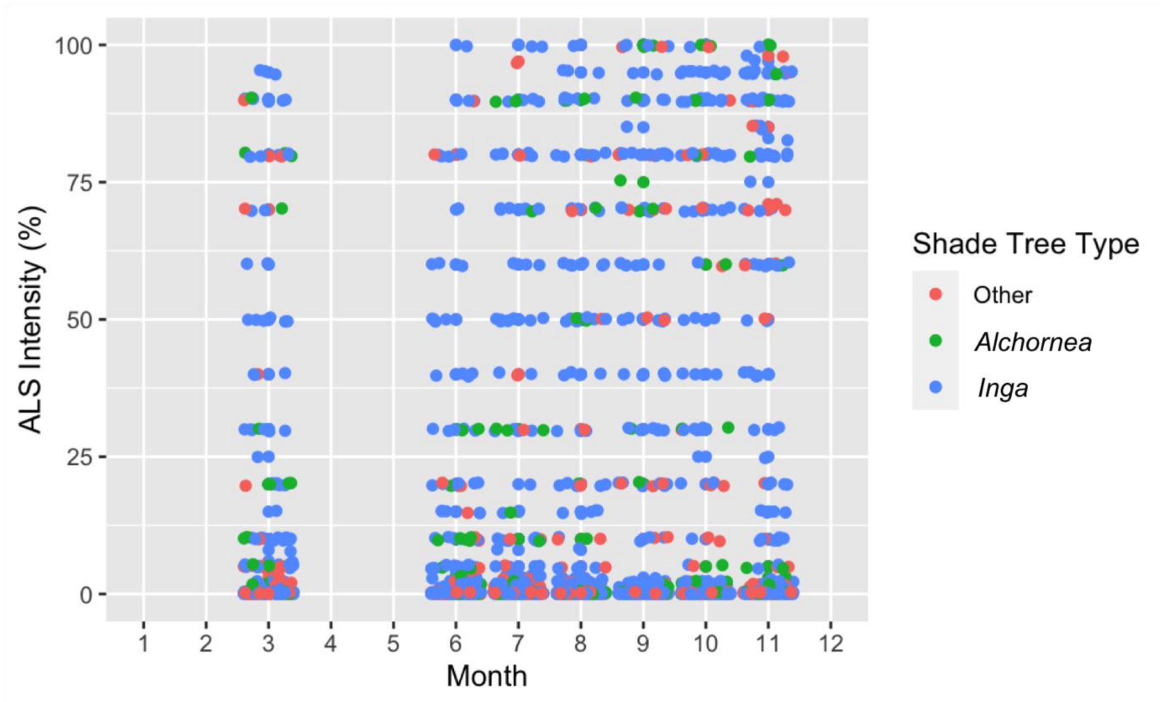

Figure 0.1 ALS intensity distribution of coffee plants by month. Color represents central shade tree types for each coffee plant.

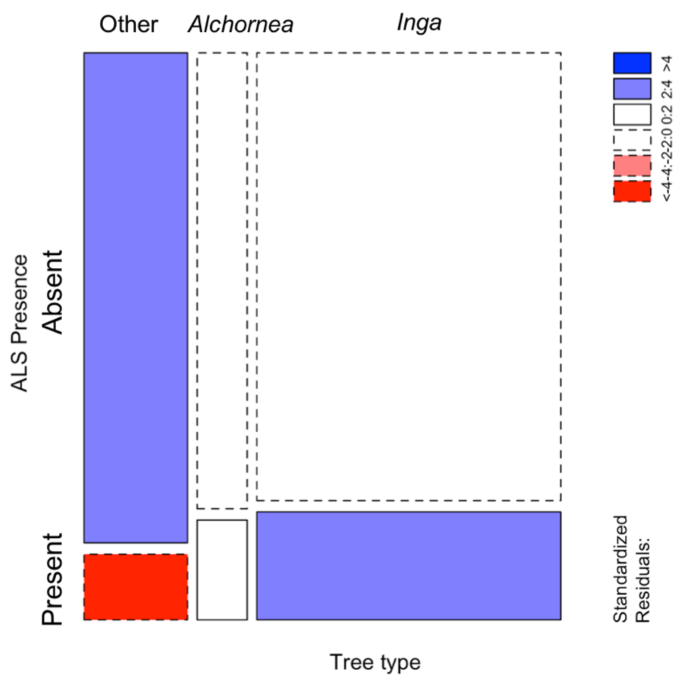

Figure 0.2 Pooled data on the incidence of ALS by tree types. Red rectangles with dashed borders indicate lower number of plants than expected, and blue rectangles with solid borders indicate higher number of plants than expected.

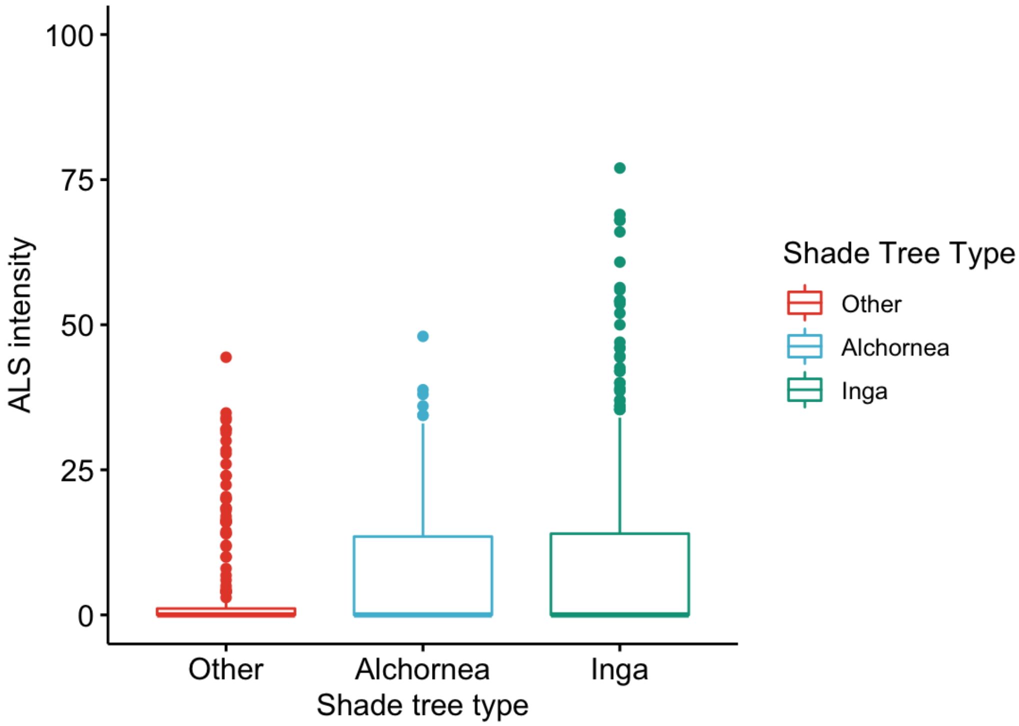

Figure 0.3 Box plot of pooled data aggregated by quadrat for average ALS intensity distributions with respect to tree types.

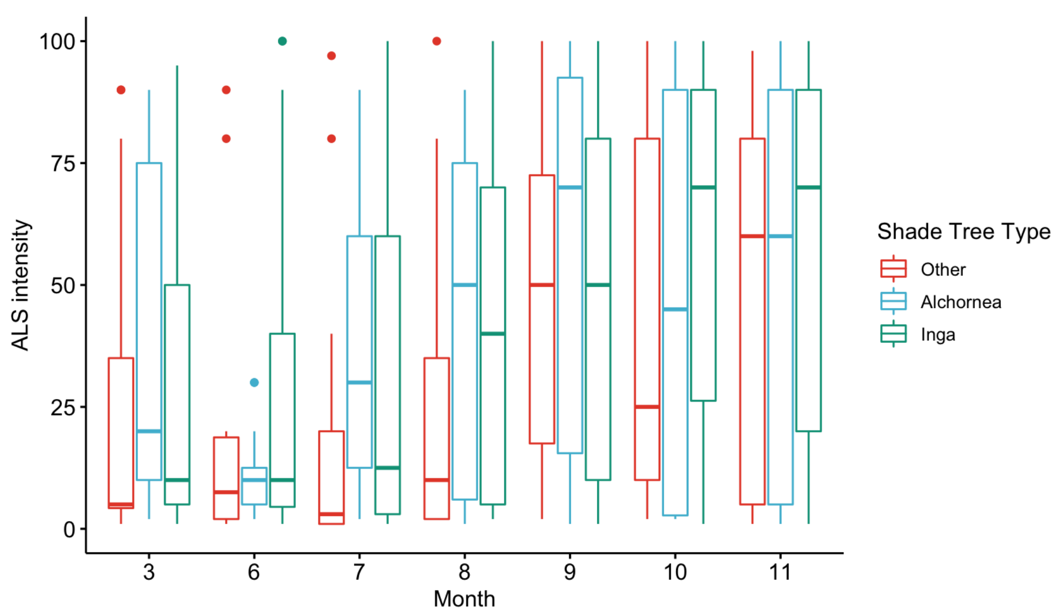

Figure 0.4 Box plot of ALS intensities for only infected plants by month and central shade tree types.

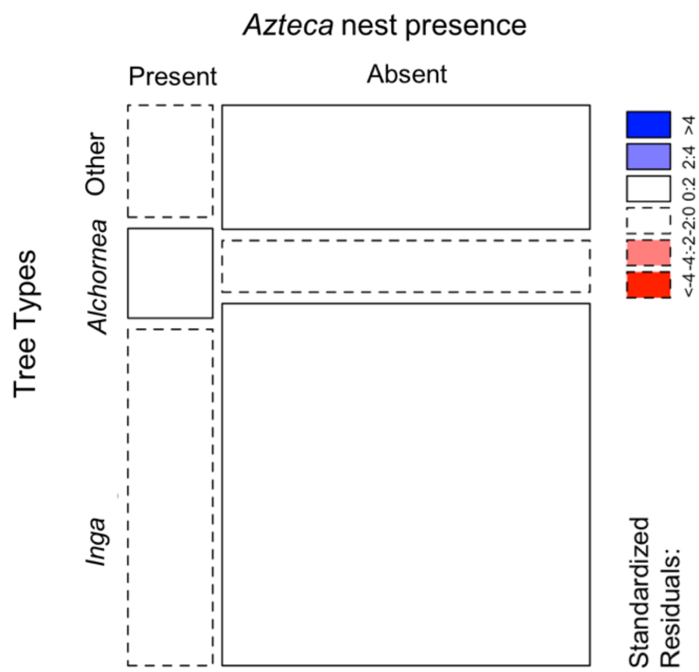

Figure 0.5 March Azteca ant nest presence by tree type.

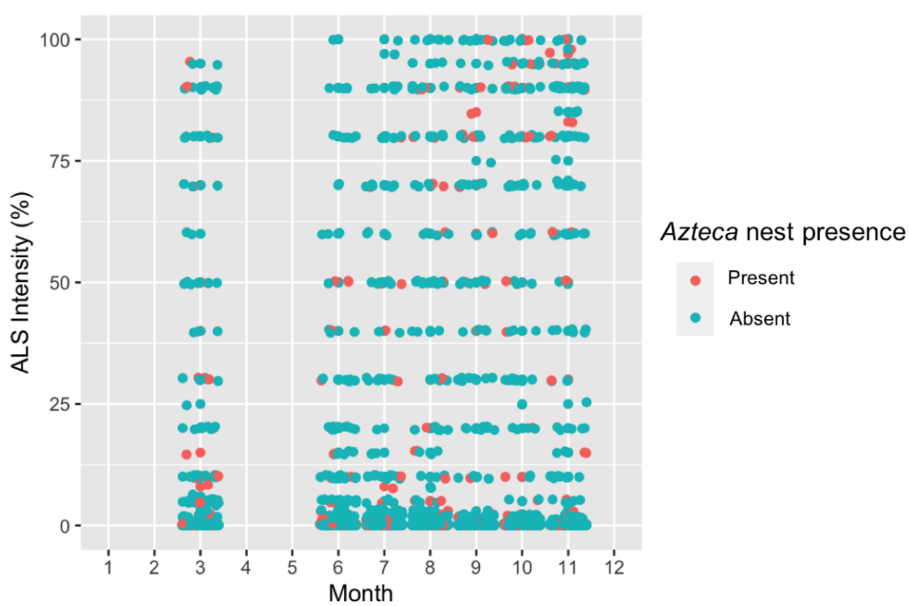

Figure 0.6 Scatter plot of ALS intensities by month and Azteca ant presence on central shade trees.

Conclusions

Understanding fungal disease patterns in agricultural settings is essential for better prediction and planning for future outbreaks. This thesis focuses on the American leaf spot diseases in coffee agroforestry systems and builds on our knowledge on fungal diseases in coffee. Using a variety of methods, I investigated the patterns of American leaf spot disease with respect to coffee leaf rust presence and types of shade trees. I found clear indication that American leaf spot disease presence is not independent from coffee leaf rust. Plants without coffee leaf rust have a higher probability of being infected with the American leaf spot disease than expected at random. Shade trees on disease incidence and intensity of American lead spot is complex. *Inga* spp. as nitrogen fixing plants in the coffee farm are associated with higher American leaf spot disease incidence and intensity than other tree types aside from *Alchornea* including many fruit trees, possibly due to the variability in the nodulation and actual nitrogen fixed for *Inga* plants, and the favorable microclimate conditions to fungal development due to the pruning practices in the farm. *Alchornea latifolia* trees in the farm are associated with higher incidence of *Azteca* ant nest, though the relationship is not significant. However, coffee plants around *Alchornea* trees have American leaf spot disease incidence and intensity between *Inga* trees and other shade trees, with mean American leaf spot intensity closer to *Inga*, and cannot support the hypothesis of lower disease due to more active ecological interactions around *Azteca* ants. In addition, the evidence points to the possibility that microclimate conditions may be a more important factor in shade trees impacting fungal disease than the factors explored including nutrients and natural predators, as *Inga* and *Alchornea* are similarly pruned with lower canopy heights than other shade trees, which could result in higher moisture and lower light, beneficial for American leaf spot germination and growth.

The negative relationship between the coffee leaf rust and the American leaf spot diseases suggest that rust resistant varieties are more susceptible to American leaf spot than plants that are not resistant. This has important implications for disease management given the massive plantings of rust resistant varieties after the 2012 outbreak of the coffee leaf rust in Northern Latin America. The mixed effect of shade trees on coffee suggests that management for fungal diseases can be very complicated, and considerations for interactions of factors that may have opposing effects are essential. Future research exploring possible competition of the American leaf spot disease with coffee leaf rust aside from the indirect effect from resistant varieties can add to our understanding of disease management. In addition, research on independent factors impacting fungal diseases associated with coffee shade trees can inform management strategies in coffee farms.