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Avocado Root Rot: Steep, Rocky Terrain and Biodiversity Help Protect Small Farmers in Post-Conflict Colombia

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AVOCADO ROOT ROT: STEEP, ROCKY TERRAIN AND BIODIVERSITY HELP PROTECT SMALL FARMERS IN POST-CONFLICT COLOMBIA

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Master of Science

by

Morgan Frankel

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AVOCADO ROOT ROT: STEEP, ROCKY TERRAIN AND BIODIVERSITY HELP PROTECT SMALL FARMERS IN POST-CONFLICT COLOMBIA

by

Morgan Frankel

APPROVED FOR THE DEPARTMENT OF ENVIRONMENTAL STUDIES

SAN JOSÉ STATE UNIVERSITY

May 2019

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Abstract

AVOCADO ROOT ROT: STEEP, ROCKY TERRAIN AND BIODIVERSITY HELP PROTECT SMALL FARMERS IN POST-CONFLICT COLOMBIA

by Morgan Frankel

In recent years, small avocado producers in coastal Colombia returned to their farms after decades of war to discover they must battle the pathogen *Phytophthora cinnamomi* devastating their trees. While this disease is well-described in the U.S., Australia, and Europe, no previous research has examined environmental correlates of *P. cinnamomi* in avocado in the diverse tropical agroecosystem of the Colombian Montes de Maria. I used agroecosystem inventory, in-field diagnostics and disease observations to describe avocado agroecosystem diversity and identify relationships among *P. cinnamomi* presence, root rot disease, slope, pH, soil compaction, elevation, and distance to household in remote smallholder farms in the coastal mountains of Colombia. I also evaluated accuracy of a locally accessible, low-cost bioassay for detecting the pathogen. Although *P. cinnamomi* proved to be ubiquitous in the region, soil compaction and increasing slope were both negatively related to disease incidence at the farm level, and some infected trees appeared healthy. Furthermore, the low-cost bioassay detected *P. cinnamomi* equally well as commercial immunostrips. As conflict reparations are negotiated in this remote region, small farmers should be compensated for retaining their highly diversified genetic stock, diverse cropping palettes, and indigenous techniques, as they may provide a refuge for avocado from the heavy disease burden in the steep and rocky growing terrain.

Acknowledgements

I would like to thank my committee chair, Dr. Rachel O'Malley for her continued guidance, trust, and enthusiastic support throughout this process. I would like to acknowledge and thank my committee members, Dr. Will Russell and Dr. Jenny Broome for their support in designing this research and writing this thesis. This research would not have been possible without the support and guidance of the plant pathology team at Driscoll's and my in-country mentor, Lillian Hall as well as the Sembrando Paz organization, its employees, and all the farmers and their families who graciously allowed me to survey their farms, fed me and housed me. Lastly, I would like to thank my family and especially my fiancé, Dalton, for their continued love and support.

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Introduction

Around the world, social conflict and war disrupts human communities and causes direct social and economic hardship, but the indirect effects of conflict on critical human and ecological systems are less well quantified. Conflict causes displacement, loss of traditional knowledge, and destabilization of agricultural systems, all of which can lead to the spread of disease and other ecological damage. Even after conflicts end, many of these ill effects can persist, and restoring economic livelihoods can be difficult, especially in sectors that depend on healthy ecosystems, such as farming. Yet most agricultural research is done in well-controlled, industrialized settings. Theories developed in these contexts may or may not address the resilience to disturbance needed to understand and assist communities in the transition from conflict. The relationships among displacement, disease spread, and community resilience are paramount in communities recovering from conflict.

Literature Review

Conflict and agriculture. Conflict can forcefully displace farmers from their land and homes, therefore disrupting agricultural production and decreasing regional food security. For example, Kah (2017) examined how the insurgence of Boko Haram in Nigeria and Cameroon has impacted food security since 2009. Along with internal displacement, Kah found that the conflict resulted in the physical destruction of farmland and markets, seizure of crops and livestock by armed militants, and loss of farm labor to fight the war (Kah, 2017). In Latin America, 75 million farmers make up two thirds of the total rural population. These rural farmers produce 40 percent of the food consumed regionally (Altieri, 2009). In Colombia, where illicit crops were grown to fund war efforts, three million hectares of natural forest have been lost to the conflict. International efforts to end the war on drugs used tactics such as aerial spraying of herbicides to decimate coca crops, simultaneously killing food crops and forcing farmers to migrate and clear more forest to start a new crop (Álvarez, 2002; Fjeldsa et al., 2005). Even the ends of conflicts can result in negative consequences for the environment. For example, when rebel groups left their territories in the plains and tropical forests of Colombia, large agricultural companies gained access to clear the land for homogeneous cash crops such as oil palm, soybeans, and rubber (Lavelle et al., 2014).

Agroecological theory. Agroecology applies the concepts of natural ecosystem science to agricultural systems. The basic goal of a sustainable agroecosystem is to mimic a natural ecosystem while maintaining a harvest (Altieri, 1989), but agroecology has evolved to become a scientific discipline, a practice, and a movement (Wezel et al., 2009). The concept of agroecology was first described by Bensin in a 1928 scientific publication about corn varieties, but the term is more recently used in publications about rural livelihoods, food security, and protection of traditional knowledge (Altieri, 2011; Altieri & Nicholls, 2017; Lappé, 2017; Valencia et al, 2018).

In an agroecosystem, many living parts work together to support the food system. For example, an agroecosystem may have a variety of different plant species providing different nutrients to the soil and flowers to attract pollinators. Along with plant species, animals may be integrated into the system for weed control, tillage, fertilizer, or protection against pests. Agroecosystems are intentionally designed to incorporate nonhuman animals and plants in order to reduce human labor, chemical pesticides and herbicides, and synthetic fertilizers. Farmers who practice agroecological methods tend to have close relationships with consumers and tend to be integrated into their local communities. Food sovereignty is more achievable for agroecological farmers because they grow a diversity of foods for their own consumption and have closer relationships with neighboring farmers, who may provide a variety of foods for trade or purchase (Bommarco, 2014; Lappé, 2016).

In contrast, industrial agriculture takes a more reductionist approach, focusing on external inputs to produce high yields of just one market crop (Altieri, 1989). Conventional farmers tend to invest time and money to keep other organisms out of their systems rather than harnessing the ecological functions biodiversity can offer (Lappé, 2017).

The rise of industrial agriculture after World War I introduced chemical pesticides and herbicides, synthetic fertilizers, and reliance on fossil fuels into agricultural production. Monocropping allowed industrial farmers to efficiently apply nutrient amendments, pesticides, and herbicides tailored to the needs of just one plant. Although industrial farming practices can increase yield of the target crops in the short term, this method of production is not without major consequences. Monocrop systems decrease genetic diversity, therefore decreasing the resistance and resilience of the system to pathogens and pests and creating a “pesticide treadmill” of increasing inputs (Helps et al., 2017). As the global population continues to grow along with economies and changing food preferences, food production will face many environmental challenges.

Agroecology is a proposed alternative to common industrial methods which may not be sustainable for the future of food production (Altieri et al., 2012; Kremen & Miles, 2012; Wezel & Soldat, 2009).

Biodiversity is a hallmark of agroecology. Genetic variation, species richness, presence or absence of key species, relative abundance, and species composition all intertwine to describe the biodiversity of an agroecosystem (Hooper et al., 2005). Increased biodiversity in agricultural systems helps increase resistance and resilience to pathogens and insect pests (Bianchi et al, 2006; Bonin & Tracy, 2012; Kieck et al., 2016). In his seminal 1973 study on herbivory in simple collard monocultures versus diverse polyculture systems, Root found that homogeneous stands hosted more insect pests than diverse stands. Root also showed that herbivores find and stay on plants that are grown in simple stands, while diverse stands suffer from fewer insect pests and a more diverse community of natural enemies. Known as the resource concentration hypothesis, Root's work was paramount to the emergence of agroecological thought. In a 2019 review by Dainese et al., the importance of species richness and abundance on pollination, pest resistance, and crop yield in 89 systems was evaluated. The researchers concluded that landscape simplification had a negative impact on pest management and crop yield and recommended the use of biodiverse agricultural systems in the development of sustainable food production.

Disease theory. Along with insects, pathogens can be yield-limiting factors in agroecosystems. Unlike animals (including humans), plants lack the ability to physically relocate to disease-free environments making the relationship between pathogen presence, plant host, and environment even more important to research (Francl, 2001). Even before Louis Pasteur's work on germ theory in animals, scientists interested in plant pathology were aware of the relationships among susceptible hosts, disease pathogens, and the abiotic environment. For example, during the Great Potato Famine in Ireland and most of northern Europe during the mid-19th century, scientists discovered the cause of the potato rot when they made the connection between the pathogen *Phytophthora infestans* and the rain and fog creating a damp, cool environment (Kelman & Peterson, 2002; Turner, 2005). In 1960, Stevens formally described the interactions among susceptible hosts, pathogens, and the abiotic environment in what is now commonly known as the "disease triangle" (Figure 1). The disease triangle recognized that disease incidence requires a susceptible host, the presence of a pathogen, and an optimal environment for the pathogen to successfully attack the host. Like Root's agroecological work with insect pests, research now recognizes that density and diversity of susceptible hosts affects the development of disease , much as crop biodiversity affects insect pest outbreaks (Bell et al., 2006). Modern research is now able to explore the complex interactions among biological and chemical plant processes and pathogens (Thomas & van der Hoorn, 2018), and increasingly researchers are exploring how our changing climate will change the interactions among host, pathogen, and environment (Velásquez et al., 2018).

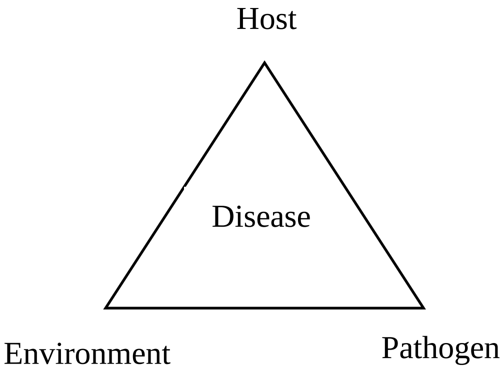

Figure 1. Disease triangle showing the relationships between host, pathogen, and environment

While theories of conflict and agriculture have documented the direct negative relationships between war and food security, and agroecological and disease theories help understand interactions within and among different components of intact farm ecosystems, the relationships between agroecology and disease in small farming communities that have suffered conflict and displacement are not well described in the literature. This research describes indirect agroecological influences of the environment on crop disease in a rural tropical ecosystem that has suffered debilitating conflict in the modern era. Examining Colombia as a model system may help expand our understanding of complex and indirect human/natural/food system interactions between and among a virulent pathogen, a susceptible host, and the abiotic environment in a post-conflict agroecosystem.

Model System

A Long History of Conflict

Colonial conflict in Colombia dates to the 16th century, when the Spaniards began their conquest of the many different indigenous Colombian tribes. Civil unrest continued in the 1800s when what was initially one sovereign land under the Spanish, broke into the countries of Colombia, Venezuela, Panama, and Ecuador. More recently, political corruption and violence of the mid-20th century, termed *La Violencia*, resulted in the creation of rebel groups, and war between leftist guerrillas and the right-wing government began (Murillo, 2004). Colombians lived in this conflict for more than eighty years. Millions of people were displaced, and thousands have been kidnapped, injured, or killed (Felter & Renwick, 2017; Murillo, 2004). The two most prominent rebel groups are the Revolutionary Armed Forces of Colombia (FARC) and the Leftist National Liberation Army (ELN). These groups were largely against the privatization of natural resources and claimed to be fighting for peasant and property rights (Murillo, 2004). Both guerrilla groups and paramilitary groups produced and exported cocaine to fund their war efforts, and they forced many peasant farmers off their land for the cultivation of coca and production of cocaine (Álvarez-Salas & Gálvez-Albadía, 2014). During U.S. president Ronald Reagan’s fight to end communism and war on drugs in the 1980s, the U.S. became an ally of the Colombian government, labeling the FARC and ELN as terrorist groups. In the 1990s under the Clinton administration, the U.S. gave US$7.5 billion, known as Plan Colombia, to aid the Colombian government’s effort to eradicate coca production and stop rebel group activities (Felter & Renwick, 2017).

Although all Colombians have been affected by the war, indigenous and afro-Colombians in the coastal mountains and along the coast suffer the greatest discrimination and violence. Many indigenous and black communities were victims of massacres, and even more were forced to flee their rural communities and live in cities where they may have no family, no home, nor the necessary skills to get a job (Álvarez-Salas & Gálvez-Albadía, 2014). In 2005, military and rebel groups began to demobilize their troops in these rural areas, however, and people started returning to their homes and farms (Lillian Hall, personal correspondence).

A Virulent Pathogen

Phytophthora is an Oomycete, commonly referred to as a water mold. The name of this pathogen is derived from Greek, meaning plant destroyer. *Phytophthora* is distinguished from other fungi by its diploid reproduction, cell wall made of cellulose and β-glucans. After the initial infection, *Phytophthora* can spread rapidly due to its quick regeneration time and swimming zoospores (Erwin & Ribeiro, 1996).

There are many different species of *Phytophthora* which infect different hosts. *Phytophthora cinnamomi*, however, infects over 1,000 different species worldwide, with a large host range that includes crops, forests, and ornamentals worldwide, from the tropics to the Mediterranean to semi-arid plains. *P. cinnamomi* has been isolated from eucalyptus in Australia, chestnuts in North America, oaks in Spain, and avocados in Colombia (Davis et al., 2014; Ramírez-Gil et al., 2017; Rhoades et al., 2003; Sena et al., 2018). Due to its ability to affect a diverse array of species and, in turn, impact the livelihoods of many producers and consumers, *P. cinnamomi* has been widely studied, but the pathogen produces different symptoms on its wide range of hosts and is often mischaracterized by growers and conservationists. The exact origin of *P. cinnamomi* is still unknown, but the earliest cases of the pathogen have been tracked to cinnamon trees in Sumatra (Sena et al., 2018a).

A Susceptible Host

Persea americana (avocado) is a fruit-bearing tree originating from southern Mexico (Chen et al., 2009). Archaeological studies provide evidence of avocado consumption in Mesoamerica as early as 8000 BC. Although *Persea americana* is now distributed in many places including Australia, North America, South America, Europe, the Middle East, and Asia (Morton, 1987), the domestication of avocados began around 5000 BC by the Aztec, Maya, Olmec and Toltec civilizations (Boza et al, 2018). The first account of European introduction to avocado was in the early 16th century in Santa Marta, a town on the Caribbean coast of Colombia. Spanish conquistadors studied the plant and brought the seeds back to the Old World (Aceituno and Loaiza, 2018; Zentmyer et al., 1987).

Avocado trees can produce from 100 to 400 fruits per season and, when healthy, can live and produce for hundreds of years. Avocados are a healthy source of unsaturated fats and contain the antioxidant Glutathione and the anti-cholesterolemic, Beta-Sitosterol (Duester, 2000). Avocado is not only beneficial to the consumer, but to the producer as well. As a fruit grown inside an impermeable skin, the avocado requires little processing during production. In addition, some varieties do not ripen until harvested from the tree, reducing production loss from pre-harvested over-ripe fruit (Duester, 2000). *Persea americana* flowers contain both the male and female function, but the stamen and pistil open on different days, preventing homogeneous genetics and encouraging cross-pollination (Bringhurst, 1952). According to the Food and Agricultural Organization (FAO) under the United Nations, in 2017, 5.9 million tons of avocado were grown, produced on 590,000 hectares of land globally.

A Conducive Environment

Direct effects on the host. Studying avocado root growth in clay and sandy soils, Salazar-Garcia and Cortes-Flores (1986) found that trees in sandy soils grew almost four times the number of roots as the trees growing in clay soils. The researchers also found that trees growing in sandy soils were taller and had a larger trunk circumference. Soil compaction can lead to insufficient crop growth, due to poor water uptake, drainage, lack of oxygen, and decreased root growth, and therefore trees growing in clay soils are typically less developed than their sandy soil counterparts (Salazar-Garcia & Cortés-Flores, 1986).

Effects on pathogen presence. Soil compaction and slope are also important factors in creating a suitable environment for *Phytophthora* spp., because compact soils on low slopes discourage water drainage, allowing the oomycete to persist (Fonseca, 2004; Sevillano et al, 2017). Because *Phytophthora* spp. reproduce and move in water, soils with high moisture content create more suitable environments for the pathogen (Duque-Lazo et al., 2018; Rhoades et al., 2003), but the temporal distribution of moisture may be important. Two studies by Corcobado et al. (2013, 2014) found that root rot caused by *Phytophthora cinnamomi* in oak stands in Spain increased in drought conditions following a rainy season. In contrast, researchers in Kentucky found pathogen presence was more common in warm, dry soils (Sena et al, 2018b), while Rhoades et al. (2003) found that root rot in American chestnut trees increased in wetter and more compact soil, although the seedlings grew best in wet, loose soil.

Effects on disease severity. More research is needed to understand how *Phytophthora* interacts with the environment in specific field conditions to cause disease. In 2014 Lavelle et al. found that *Phytophthora* root rot disease outbreaks in alkaline soils tend to be more severe than in acidic soils. Tropical soils typically have a relatively low pH, which may reduce the severity of *Phytophthora* root rot outbreaks in tropical hosts. This study also found that greater soil compaction inhibited communities of beneficial microorganisms thought to help suppress certain soil pathogens, suggesting that complex and indirect interactions may mediate effects of the environment on root rot disease outbreak (Lavelle et al., 2014).

Managing the Disease

The goal of understanding the disease triangle in this post-conflict agroecosystem is important both to help returning farmers manage the disease burden in their farms, and to resurrect successful indigenous practices for managing the agroecosystems. In Colombia, large cash crop farmers amend acidic soils with lime to increase pH, a practice that could create better conditions for *Phytophthora* to invade (Lavelle et al., 2014.). In 2018, Ramírez-Gil et al. (2018) investigated different disease management strategies in commercial avocado farms located in the northwest department of Antióquia, Colombia. The researchers found that “integrated disease management” was most successful in decreasing disease, however the methods used include injecting and irrigating with fungicides, amending the soil with potassium silicate, and the application of high nutrient mulches. While these practices may support industrialized avocado production in Colombia, the on-the-ground experience of small resource-poor farmers producing crops in diversified hillside agroecosystems could be quite different.

Research Questions and Hypotheses

The objective of this research was threefold: 1) to document the on-the-ground biodiversity of post-conflict Colombian avocado-based agroecosystems, 2) to assess the effects of environmental variables on the likelihood of *Phytophthora cinnamomi* infection and on the development of root rot disease in avocado in this agroecosystem, and 3) to evaluate the effectiveness of a low-tech approach for farmers to sample for the pathogen in these remote mountain communities.

RQ1: What is the crop diversity of agroecosystems in post conflict regions of Colombia?

RQ2: Is there a relationship between pathogen presence and a) soil compaction depth, b) slope, c) distance to the household, d) elevation or e) soil pH?

H_A1: Presence of *P. cinnamomi* in soils and root will:

a: be more common as soil compaction increases.

b: be less common as field slope increases.

c: be less common as the distance from household increases.

d: be less common as soil pH decreases.

e: vary with elevation.

RQ3: Is there a relationship between disease incidence and a) soil compaction depth, b) slope, c) distance to the household, d) elevation or e) soil pH?

H_A2: Avocado root rot disease incidence will:

a: increase as soil compaction increases.

b: decrease as field slope increases.

c: decrease as the distance from household increases.

d: decrease as soil pH decreases.

e: vary with elevation.

RQ4: Does pathogen presence predict disease?

H_A3: Pathogen presence will predict disease incidence, but not all infected trees will respond equally.

RQ5: Is a low-cost bioassay comparable to Agdia ImmunoStrip® tests in detecting *Phytophthora cinnamomi*?

Methods

Study System

Farmers in the coastal mountains surrounding the town El Carmen de Bolívar, Colombia, who in 1996 left their healthy and productive land to escape the conflict raging through that region, returned home ten years later to diseased crops suffering from avocado root rot in. El Carmen de Bolívar is known for avocado production, both for local consumption and for export to other regions of Colombia. With a total of 40,000 hectares of sick and dying avocado trees, the disease decimated the region. The virulent pathogen responsible for the disease is *Phytophthora cinnamomi.*

The town of El Carmen de Bolívar is a small town within the department of Bolívar in northern Colombia (Figure 2), 120 km from the city of Cartagena on the Caribbean coast. The town is surrounded by the Serranía de San Jacinto sub-mountainous region of the Montes de Maria, an extension of the Andes. The maximum elevation of these mountains is around 600 m above sea level (Figure 3). The total area of El Carmen de Bolívar is 954 km^2 and it has a total population of around 160,000 people. The rainy season lasts from April until September, averaging approximately 13 millimeters of rain per day. Annually, temperatures range from 22-35° Celsius. The farmers in this region are mostly campesino farmers, of indigenous or Caribbean descent, producing largely for their own subsistence and for local markets.

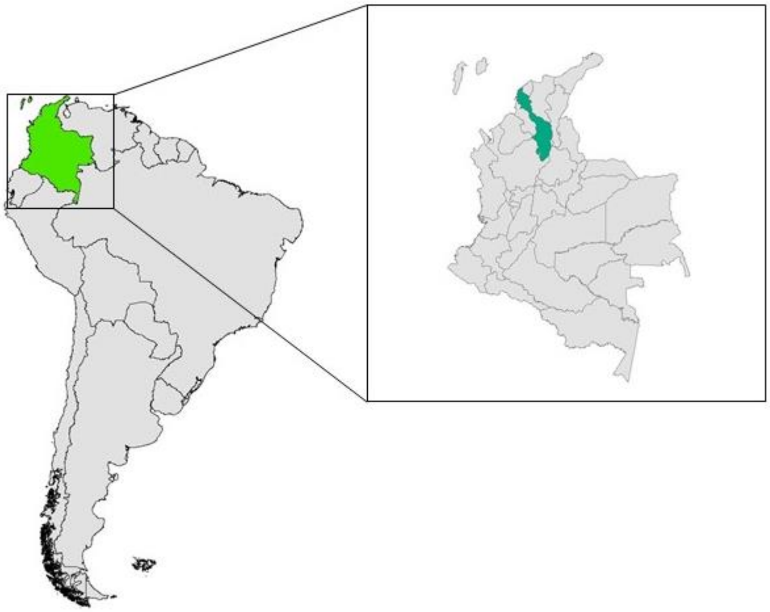

Figure 2. Department of Bolívar located in northern Colombia

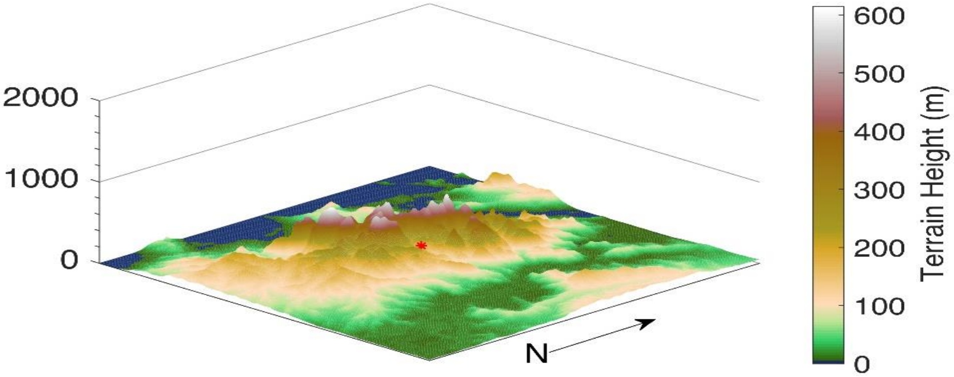

Figure 3. Terrain of study area

In January 2018, I made a preliminary trip to El Carmen de Bolívar to assess the study area and create a relationship with Sembrando Paz (sowing seeds of peace), a non-profit organization that works with coastal peasant communities that have been affected by the conflict. The work of Sembrando Paz primarily focuses on educating community leaders on their political rights to reparations from the government, re-building peace and trust among civilians and ex-combatants, helping ex-combatants re-integrate into the community, and building youth interest in ecological conservation and agriculture. Staff at Sembrando Paz contacted fourteen farmers who agreed to participate in the study (Figure 4).

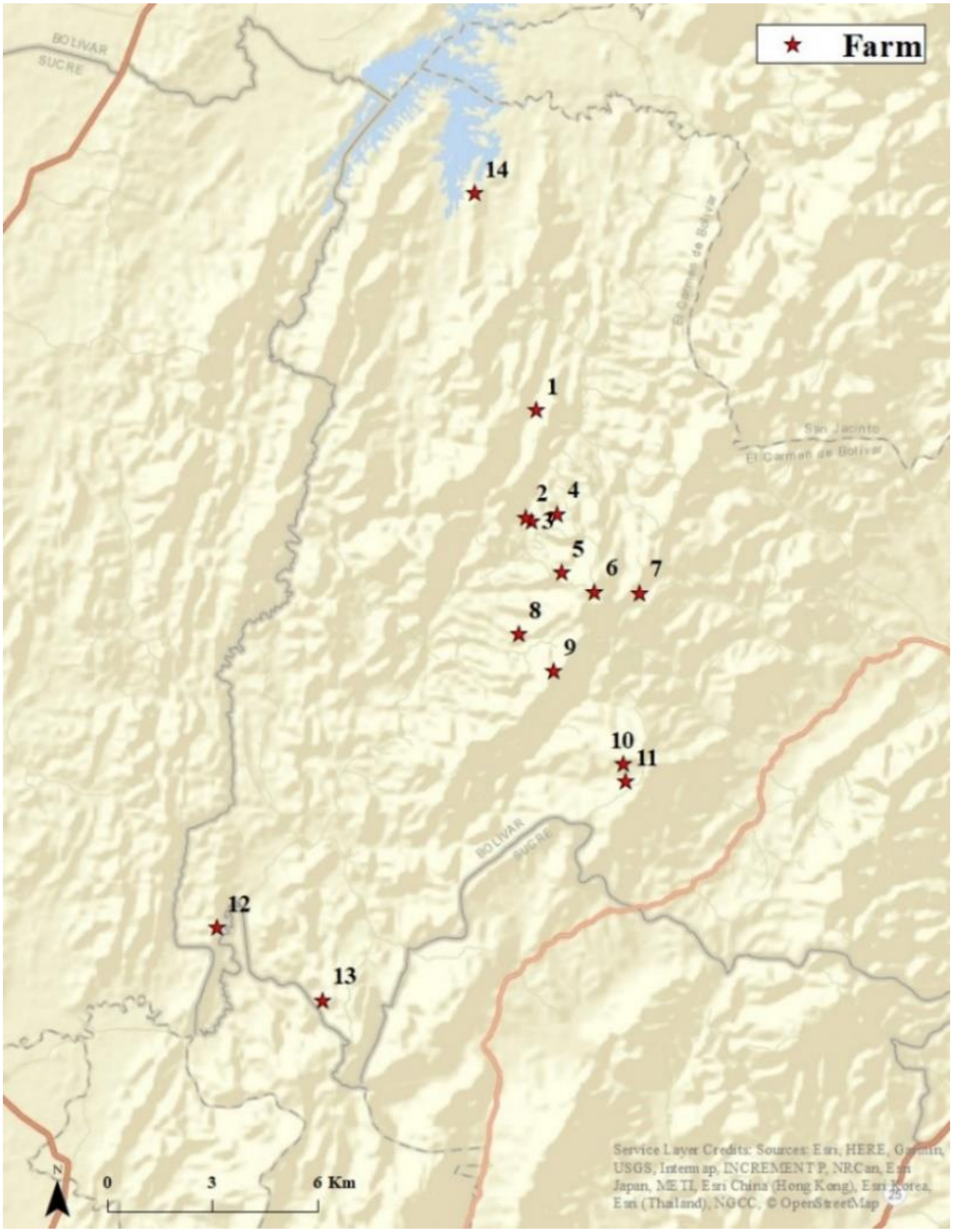

Figure 4. Farm locations surrounding El Carmen de Bolívar. (Base map provided by ESRI)

Research Design

RQ1: To address the first research question regarding crop biodiversity, I visited the 14 farms in the Montes de Maria region in Colombia. At each farm, I inventoried each crop present in the agroecosystem through a combination of observation and casual interactions with farmers.

RQ2 and RQ3: In each of the 14 farms, I selected five to ten trees for a total sample of 72 trees. At each farm site I chose an even mix of 50% healthy and 50% unhealthy trees based on terrain and accessibility, to gather a broad representation of healthy and diseased trees despite the small sample size. Trees that looked dead were not included in the analysis. At each tree, I tested roots for presence/absence of the pathogen and rated disease severity. For research question two, the presence of the pathogen is compared to environmental measurements (Figure 5), while for research question three, disease incidence is compared to environmental measurements (Figure 6).

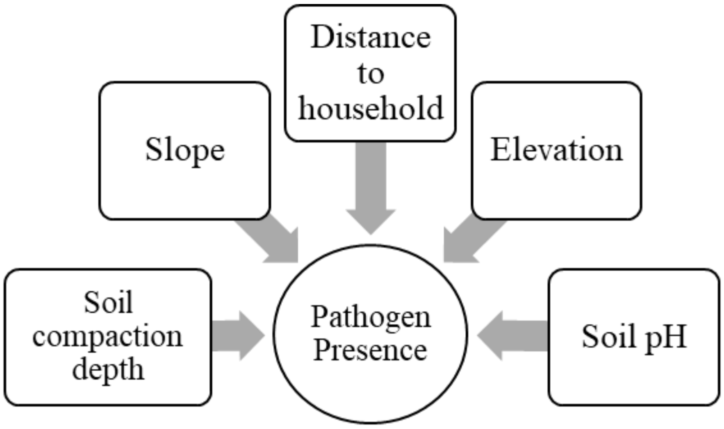

Figure 5. Study design for research question 2. The relationship between environmental variables and pathogen presence

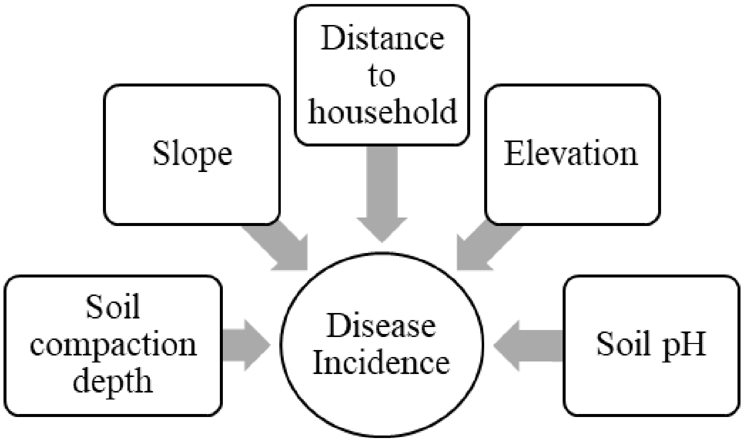

Figure 6. Study design for research question 3. The relationship between environmental variables and disease incidence

RQ4: To determine if pathogen presence predicts disease incidence, I used the data collected for RQ2 and RQ3 for statistical analysis.

RQ5: At the final farm sampled, I conducted a small-scale pathogen baiting experiment to qualitatively compare the accuracy of a low-cost bioassay diagnostic to a more expensive commercially available immunostrip assay technology. I collected soil from underneath the canopy of ten trees and conducted two of each type of assay per soil sample. (Adapted from the University of California Cooperative Extension).

Data Collection

Access to Farm Sites

Different modes of transportation were used to reach the farms. Some farms could only be reached by horseback or an all-wheel drive vehicle. Other sites were accessible by motorcycles and on foot. Local ex-combatants were hired to drive motorized vehicles. To avoid rain and heat, journey times typically began in the early morning around 6 AM.

Research Question 1

In order to collect information about cropping systems, once arriving at a site, introductions were made over café tinto (a small cup of sweet, black coffee) prepared by the woman of the household. A more detailed explanation about the research was given and the farmer explained the issues they have experienced with the disease in their farms.

Research Questions 2 and 3

Environmental measurements. Upon approaching each tree, I used a Dicky-john© soil compaction tester, to record the depth at which the meter read 300 psi. If the soil type did not allow compaction analysis (i.e. rocky), a depth of 0 was recorded. I determined slope using a Suunto© clinometer, recorded coordinates and elevation using a handheld GPS tracker (Garmin©, GPSMAP 64st). I extracted soil samples from under the tree canopy using a JMC© soil probe and took a subsample from the midsection of the core to test soil pH using a simple capsule test kit (Leaf Luster©).

Pathogen presence. I took small feeder roots from four sides at the base of each tree. If necrotic roots were observed, I selected an even ratio of healthy and necrotic roots for analysis. Next, I rinsed the roots with filtered water and placed them in an Agdia

ImmunoStrip® buffer-liquid filled pouch. Using the blunt end of a knife, I macerated the roots before placing a test strip in the buffer-root solution. After no less than five minutes, I read and interpreted the results (negative result yielding one-line, positive result yielding two lines). In the case of a faulty test, I performed another analysis.

Disease incidence. I recorded disease incidence of each tree using a one to nine symptom rating scale (adapted from Julien Mercier, 2018) (Figure 7). On a scale from one to nine, one being healthy and nine being very unhealthy or dead, I observed each tree for symptoms of *Phytophthora cinnamomi*. Common symptoms include yellowing and drooping of foliage, a thinning canopy, visibly dead branches, little or no new growth, and poor fruit production (Figure 8). Because the research was not active during the fruiting season, I asked farmers to describe the quality of the last year's crop from each selected tree.

Observation	Rating
No observed symptoms/healthy	1
Slightly drooping/yellowing canopy	3
Moderate/severe drooping canopy/yellowing & thin canopy no new growth	5
Very thin canopy/some dead branches/no new growth	7
Completely/almost dead/ no foliage	9

Figure 7. Disease incidence rating guide

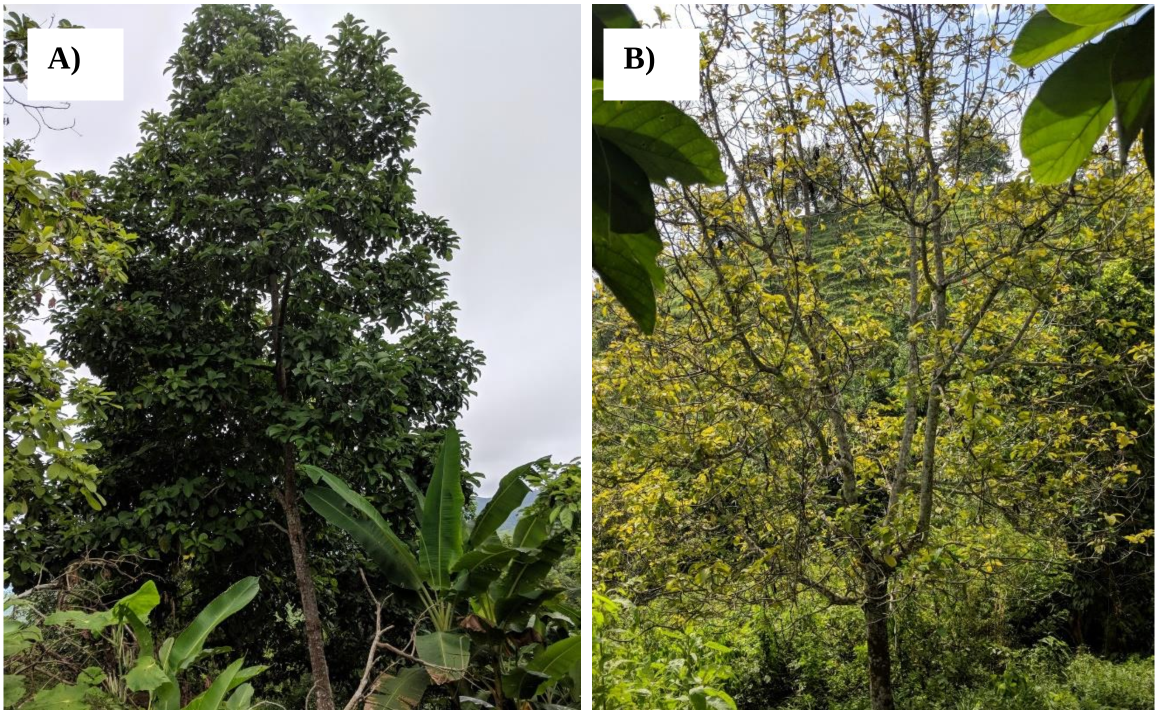

Figure 8. Disease incidence rating example. A) healthy tree rated as a one B) unhealthy tree rated as a seven

Research Question 5

Baiting trial. I collected soil from underneath the canopy of ten trees, placed them in a labeled zip-lock bags and transported them back to the Sembrando Paz office in the city of Sincelejo. I purchased 20 green-skinned avocados from a local market, surface sterilized them in a 10% bleach solution, and labeled them with a permanent marker. I filled 20 wide-mouthed plastic cups with a quarter cup of soil and a half cup of filtered water (two cups per soil sample). I then placed the avocados bottom down in the cup, partially submerged in the water-soil solution (Figures 9A and 9B).

I left the avocados in a dark room for 48 hours before removing them from the cups. After removal I washed them off and placed them on a surface sterilized counter for 48 more hours. After 48 hours out of the soil-water solution, I observed the avocados for purple/brown spots at the water line. The appearance of these spots is indicative of *Phytophthora cinnamomi* in the soil. I took photos of the avocados before and after the assay was complete for my records and observations.

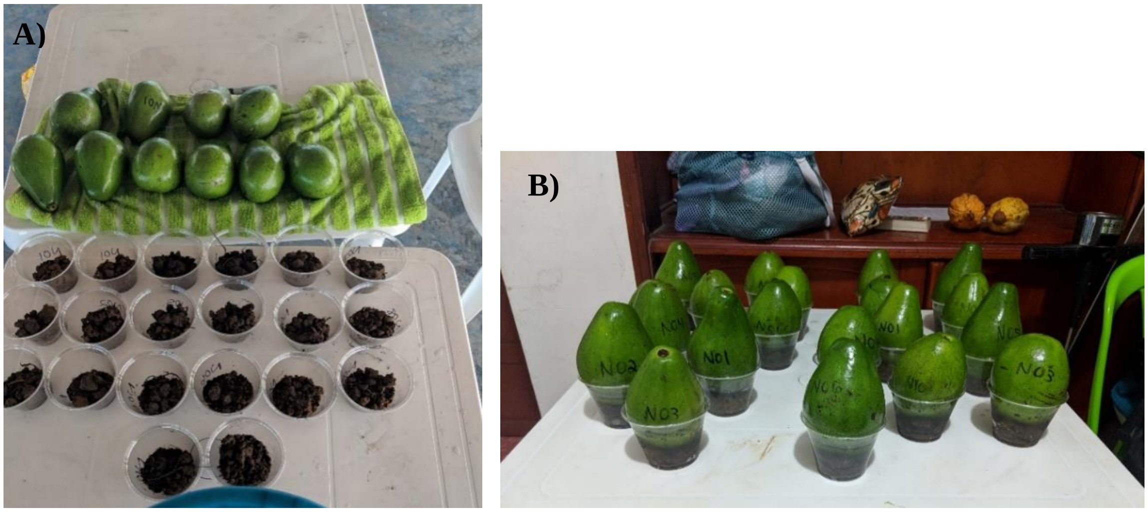

Figure 9. Pathogen baiting bioassay A) Pathogen baiting supplies: green skinned avocados, soil samples from seven trees (with one replication), bleach-water solution for surface sterilization. B) The complete set-up of the pathogen bioassay

Analysis

For research questions one and five, I qualitatively summarized and described information collected, but did not conduct statistical analyses.

For research questions two and three, I used ESRI ArcMap 10.5.1, to map the GPS coordinates of the trees and household locations and calculated the distance to the household for each tree. I ran a Pearson's correlation among soil compaction depth, slope, distance to household, elevation, and soil pH to confirm their independence. I performed a binary logistic regression test for relationships (alpha = 0.05) among the positive/negative immunostrip results and these environmental variables. Due to small sample sizes, I recategorized disease incidence (1-9) into healthy (1) and unhealthy (3-9) and used a binary logistic regression to identify relationships among elevation, compaction depth, slope, soil pH, and distance to the household. I used Mann-Whitney U tests to re-confirm relationships found using the binary logistic regression.

To evaluate research question four, I used a chi-square test of independence to evaluate any relationship between pathogen presence and disease incidence.

Limitations

The main limitation of this research was farm and tree accessibility. Ease of accessibility was mostly dependent on terrain and precipitation. Roads were mostly heavy clay and became extremely perilous with even a small amount of rain. Tree selection was non-random, but the selective methodology should produce conservative results. I sought out healthy trees at each farm to include in the study, in order to gather a broad sample of pathogen incidence. Because the region is heavily infected, random sampling would have produced a much higher sample of unhealthy trees. Nonetheless, the small sample size for several of the questions limited the possible breadth of statistical analysis.

Results

Research Question 1

At least 18 actively cultivated crops were observed in the agroecosystems and described by farmers (Table 1). All farms consisted of many different crop varieties, non-crop trees, and ornamentals. Most farmers were unsure about which varieties of avocado they were growing and referred to all local varieties under the category “criollo,” implying that they represented a locally developed or adapted variety. Avocado seeds are frequently passed down from previous generations, and different varieties have cross-pollinated to create unique varieties. To control the pathogen, farmers mainly used swidden and extreme pruning. Several of the farmers had been given a free chemical fungicide (Propamocarb HCL) from a government agency, which some farmers used, but many would not.

Table 1

Crops Observed and Listed by Farmers

Crop	Common name
Persea spp.	Avocado
Musa spp.	Banana
Musa pardisiaca	Plantain
Mangifera indica	Mango
Carica papaya	Papaya
Cocos nucifera	Coconut
Theobroma cacao	Cacao
Moringa oleifera	Moringa
Coffea spp.	Coffee
Ananas comosus	Pineapple
Dioscorea spp.	Ñame
Manihot esculenta	Cassava
Annona muricata	Guanábana
Hylocereus undatus	Dragon fruit
Zea mays	Corn
Oryza sativa	Rice
Syzygium malaccense	Malay Apple
Spinacia oleracea	Spinach

Research Question 2

A Pearson's correlation showed no significant autocorrelation between soil compaction depth, slope, distance to the household, elevation or soil pH (Table 2). Out of the 72 trees, 29 tested as negative for *P. cinnamomi* and 43 tested as positive (Figure 10). None of the predictors, soil compaction depth, slope, distance to the household, elevation, or soil pH, significantly explain the variation in pathogen presence (Table 3).

Table 2

Pearson's Correlation Among Predictor Variables

		Compaction depth	Slope	Distance to household	Elevation	Soil pH
Compaction depth	Pearson's correlation	1	0.0A38	0.086	-0.100	-0.181
	p-value		0.754	0.470	0.422	0.128
Slope	Pearson's correlation	0.038	1	-0.058	0.056	-0.103
	p-value	0.754		0.628	0.643	0.387
Distance to household	Pearson's correlation	0.086	-0.058	1	-0.148	0.024
	p-value	0.470	0.628		0.215	0.843
Elevation	Pearson's correlation	-0.100	0.056	-0.148	1	0.116
	p-value	0.402	0.643	0.215		0.331
Soil pH	Pearson's correlation	-0.181	-0.103	0.024	0.116	1
	p-value	0.128	0.387	0.843	0.331	

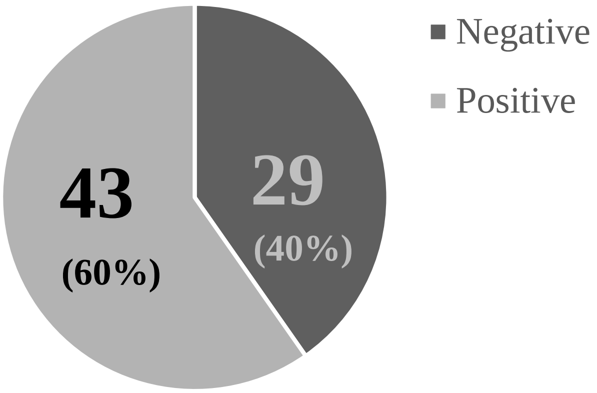

Figure 10. Pathogen presence results. Most the trees (60%) tested positive for the pathogen while 40% of tree showed negative results

Table 3

Relationship Between Pathogen Presence or Absence and Predictor Variables (Binary Logistic Regression)

Independent Variable	B	S.E.	Wald	P-value	Exp(B)
Distance to Household	-0.002	0.006	0.151	0.697	0.998
Soil pH	0.684	0.512	1.782	0.182	1.981
Elevation	0.007	0.014	0.262	0.609	1.007
Slope	0.000	0.001	0.035	0.851	1.000
Soil Compaction Depth	0.000	0.000	0.289	0.591	1.000
Constant	-4.240	3.673	1.333	0.248	0.014

Research Question 3

After observational symptom rating, 37 trees were rated as a one, 20 as a three, eight as a five, and seven as a seven (Figure 11): 37 were thus considered healthy and 35 unhealthy.

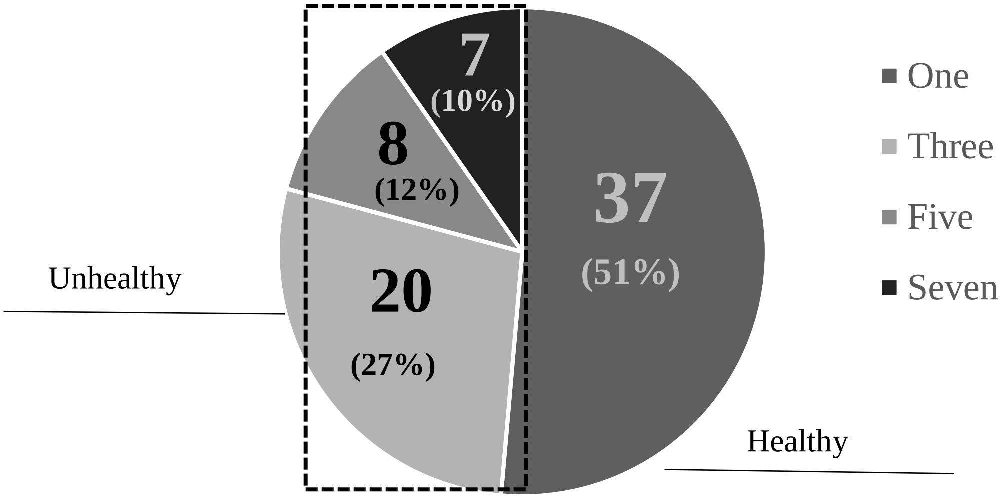

Figure 11. Disease incidence rating results. Half the trees (51%) were rated as a one (healthy) while 49% of trees were rated as a three, five or seven. The area within the dashed lines represent the unhealthy binary category

All five environmental predictors explain about 23.2 percent of the variability in observational tree health. The model correctly predicted 73 percent of cases where healthy trees were observed and 65.7 percent of cases where sick trees were observed. The overall percentage of correct prediction is 69.4 percent (Table 4).

Table 4

Binary Logistic Regression Classification Table

	Predicted		
Observed	Healthy	Unhealthy	% Correct
Healthy	27	10	73
Unhealthy	12	23	65.7
Overall %			69.4

Trees with healthier ratings were more likely to be growing in soils with a shallower compaction level ([chi-square=5.658, df=1, p=0.027 (<0.05)]) (Table 5; Figure 12). This result was verified with a Mann-Whitney U test (p=0.04). Trees growing on steeper slopes were more likely to be rated as healthy ([chi-square=4.256, df=1, p=0.045 (<0.05)]) (Table 5) (Figure 13). Neither elevation (p=0.592), soil pH (p=0.977), nor distance to the household (p=0.365) significantly explained disease incidence (Table 5).

Table 5

Disease Incidence and Predictor Variables (Binary Logistic Regression)

Independent Variable	B	S.E.	Wald	P-value	Exp(B)
Distance to Household	-0.001	0.001	0.820	0.365	0.999
Soil pH	0.016	0.529	0.001	0.977	1.016
Elevation	0.001	0.002	0.287	0.592	1.001
Slope	-0.016	0.007	5.436	0.020*	0.984
Soil Compaction Depth	0.042	0.017	6.161	0.013*	1.043
Constant	-0.254	3.802	0.004	0.947	0.776

* $p < 0.05$

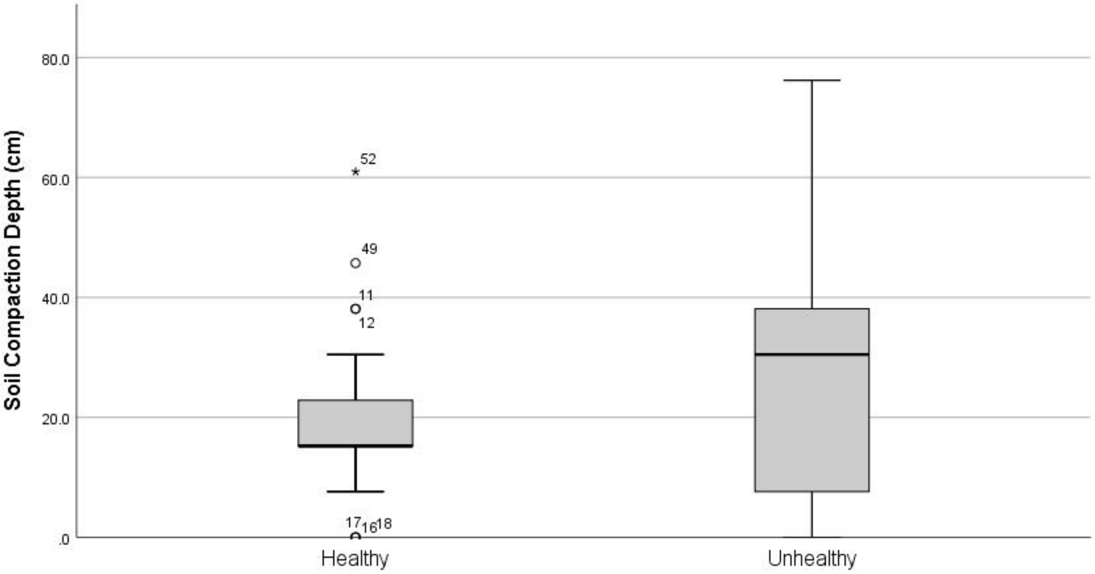

Figure 12. Soil compaction depth in root zone of apparently healthy versus visibly diseased trees

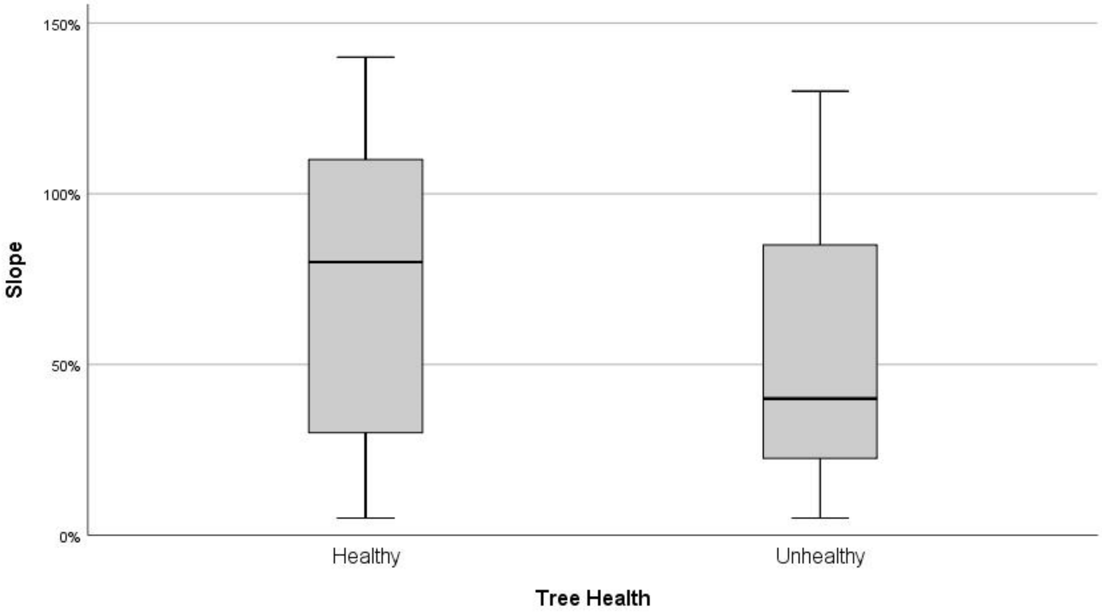

Figure 13. Slope gradient in root zone of apparently healthy versus visibly diseased trees

Research Question 4

Pathogen presence did not predict disease incidence ($\chi^2(1, N=72) = 1.02$, $p=.31$). Twenty out of the 43 trees that tested positive for the pathogen were also rated as healthy (see Appendix A).

Research Question 5

Out of the soil collected from the ten trees, all but two avocados produced signs of *P. cinnamomi* at the end of the trial. The two samples that did not produce a positive sign of the pathogen came from the only tree for which the roots tested negative with an Agdia ImmunoStrip® test (Figures 14A and 14B).

Figure 14. Pathogen baiting bioassay results A) Avocado in contact with soil collected from a tree that tested negative for *P. cinnamomi*. B) Avocado in contact with soil collected from a tree that tested positive for *P. cinnamomi*

Discussion

In the coastal mountains of Colombia, all three sides of the disease triangle are currently present and smallholder farmers are often forced to produce on marginal land. Field tests show that the pathogen in this study, *Phytophthora cinnamomi* is found in the majority (60%) of the trees that were tested, even with an overrepresentation of apparently healthy trees in this study. Because 60% of the trees tested positive for the pathogen, soil compaction depth, slope, distance to household, elevation, and soil pH did not significantly predict the Agdia ImmunoStrip® result. Prior studies have shown that the spatial distribution of *P. cinnamomi* is somewhat predictable, with a larger presence of the pathogen in areas with clay soils and high precipitation (Hernández-Lambraño et al, 2018). The tropical climate of this area creates the optimal environment for many different pathogens to thrive as it is warm and humid. The soils are heavy clay, decreasing movement of water during the rainy season and allowing *Phytophthora cinnamomi* to rapidly spread. Some trees survive and produce, nonetheless, even though disease has affected 40,000 hectares of land in El Carmen de Bolívar and surrounding areas. Clay soils also tend to be nutrient poor and less able to support beneficial soil microbes that have been found to defend again soil borne pathogens. The use of agroecological practices could be an important aspect in the success of the farms and the resilience of their crop to disease.

Studies in controlled settings have found that presence of *Phytophthora* increases in more compact soils along with increased moisture, independent of soil compaction (Fonseca, 2004; Rhoades et al., 2003). For this study, it was predicted that tree health and soil compaction would have a negative relationship. This hypothesis was rejected, and trees tended to look healthier in more compact soils. Trees also looked healthier on steeper slopes. These results suggest that even though the pathogen is ubiquitous, and pathogenicity is likely, trees on steep slopes, where water is unable to be stagnant, are better off than trees growing in less compacted soils on flat ground. The compact soils also include rocky hillsides where filtration of water may be higher and therefore create a less susceptible environment. Due to the large amount of diversity in this study system, it is possible that soil compaction has less of an impact on pathogen presence.

The farms in El Carmen de Bolívar were all structured as agroforests, some being agrosilvopastoral, or the integration of crops, trees, and livestock. An agroforestry system is defined as having 10-30% tree coverage, which describes the homes and farms of around 1.6 billion people worldwide (Motagnini & Metzel, 2017). Allowing trees to grow within the agricultural system has been a long-used method, especially in tropical and sub-tropical regions. Agroforestry provides many services while having a low environmental impact and needing little external agricultural inputs. Growing trees increases diversity and habitat and provides the farmer with wood for building and burning. Trees also provide more fallen leaf litter which is broken down by beneficial microorganisms and supply important nutrients for crop species growing in the low-nutrient clay soils often found in tropical regions. Thick mulch layers can also inhibit the growth of weeds, retain soil moisture, and prevent soil erosion (Gliessman, 2015). The root systems of trees influence soil structure, strengthening hillsides, reducing erosion, and aerating dense clay soil (Motagnini & Metzel, 2017).

Along with pathogen, host, and environment, some pathologists include a fourth disease factor; humans (Francl, 2001). The movement of humans in the coastal mountains increased during the conflict as military and guerrilla groups took over land and moved deeper into the forests. Increased human presence also increased movement of vehicles and animals in the area, further increasing the chances of the pathogen spreading from one location to another. A 2017 study by Ramírez-Gil on avocado disease in Bolívar's neighboring department, Antióquia, suggests absence of management can increase the chances of trees being infected. During the time of conflict, the farmers were not present on their lands to observe disease and act early on. It is possible that the absence of observant farmers who would have been controlling the disease in early stages could explain in part why the disease was extreme upon their return to the land.

Twenty out of the 43 trees that tested positive for the pathogen were rated as healthy, suggesting that even though the pathogen is ubiquitous, trees in this region may have some genetic resistance. In 2018, Colombian president Juan Manuel Santos signed an agreement with the United States to allow Colombian avocado exports to the United States (USDA, 2017). As pressure to grow a more commercial variety (cv. Hass) may increase, indigenous varieties may be threatened. Traditional agricultural practices allow for and encourage gene exchange between crops and wild relatives, creating landraces that are more resistant to pathogens and pests (Galluzzi & Noriega, 2014; Rao et al., 2003). As industrial agriculture expands in developing countries, loss of crop genetic diversity increases. The use of monocropping and GE seeds discourages gene exchange and the use of foreign cultivars increases yield limiting factors such as herbivory and disease. Protection of indigenous genetic resources must be coupled with rural development and the preservation of traditional agricultural practices. Without traditional knowledge and farming techniques, important landraces may not function well or at all (Altieri & Merrick, 1987).

Despite the rich diversity of this system, 40,000 hectares of avocado trees have been lost to disease, therefore diverse agroecosystems provide a different type of resilience for farmers: economic stability. Much like diversifying an investment portfolio, by growing multiple crops farmers create a safety net for when agricultural disturbance occurs. For small farmers to maintain economic stability and autonomy, they must have access to appropriate technology. With little guidance from government and local agencies, it is important that these resource-poor farmers have an affordable and easy method to detect pathogens within their farms. This study suggests that a low-cost pathogen baiting bioassay is comparable to more expensive immunostrip technology. If farmers can identify the presence of the pathogen before visible symptoms occur, they may be able to prevent crop loss. As rural agricultural communities develop, it is important to provide appropriate technology and establish local leadership for continued conservation of natural resources.

Conclusions

Small farmers are impacted by social conflict worldwide. When farmers are displaced from their land, disturbance can occur, and restoration can be difficult once farmers return. This study suggests that in Colombian post-conflict farms, avocado trees growing on steep hillsides in more compact/rocky soils have lower incidence of disease but that although the pathogen was ubiquitous, the indigenous varieties grown may be less susceptible to *Phytophthora cinnamomi.* Therefore, when using agroecological methods of farming and indigenous knowledge, vulnerable systems may be more resistant to disease. Smallholder farms in this study and similar systems globally are important for the future of food production, the success of small farmers, land stewardship, and the health of our global ecosystem. When communities who are protecting traditional knowledge and indigenous varieties are threatened, important genetic information and the future of resistant cultivars could be threatened as well. Further research is needed to study genetics of indigenous avocado varieties and their resistance to disease along with how the protection of vulnerable post-conflict farmers is important for regional food security and ecological preservation.

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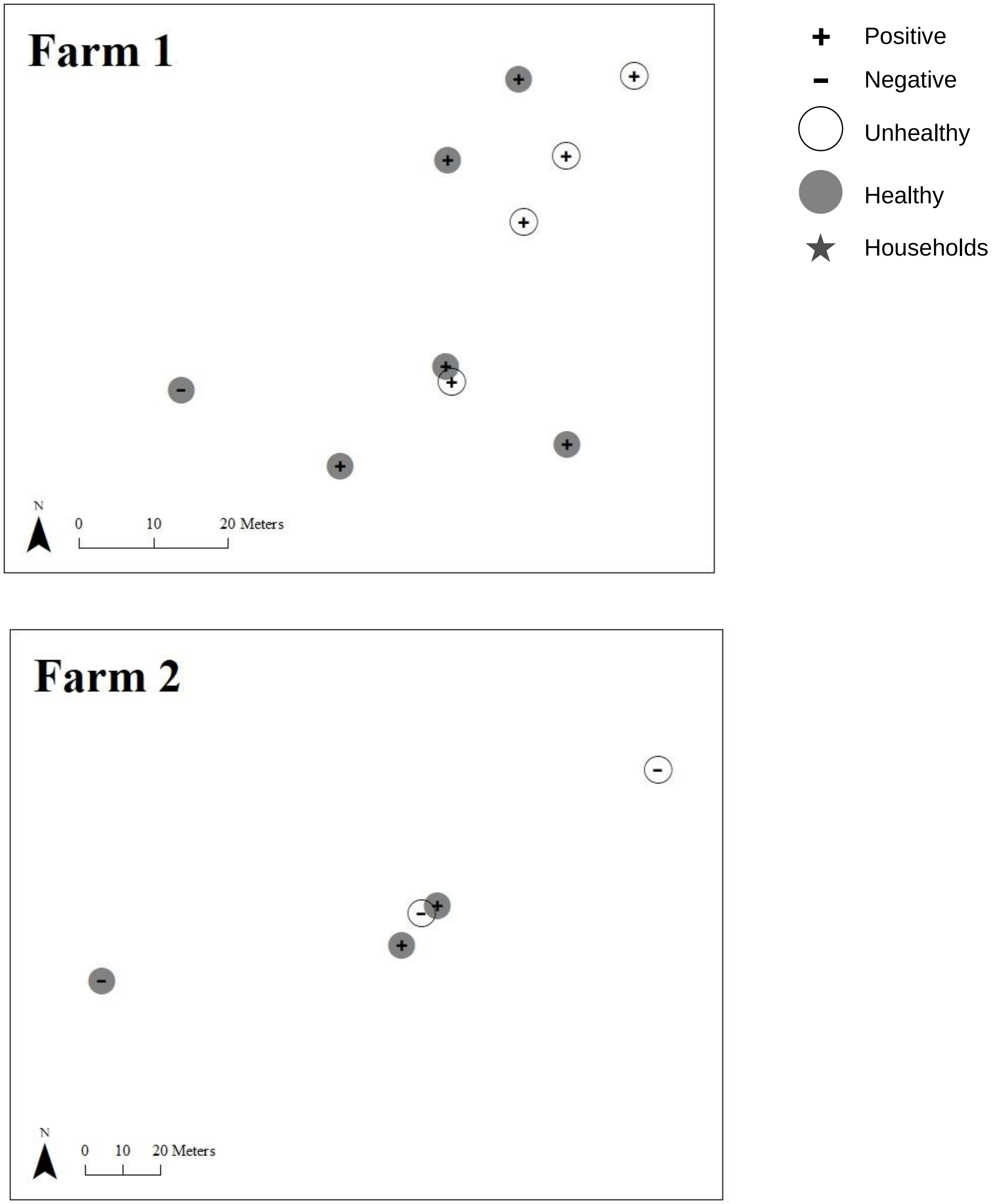
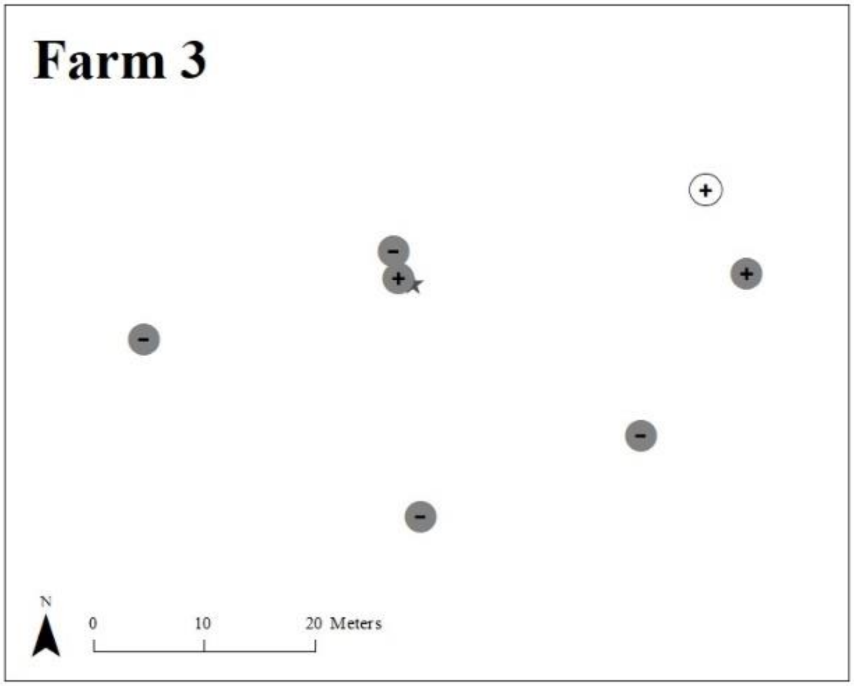
Appendix

Geographic location of trees at each farm by infection status and health.

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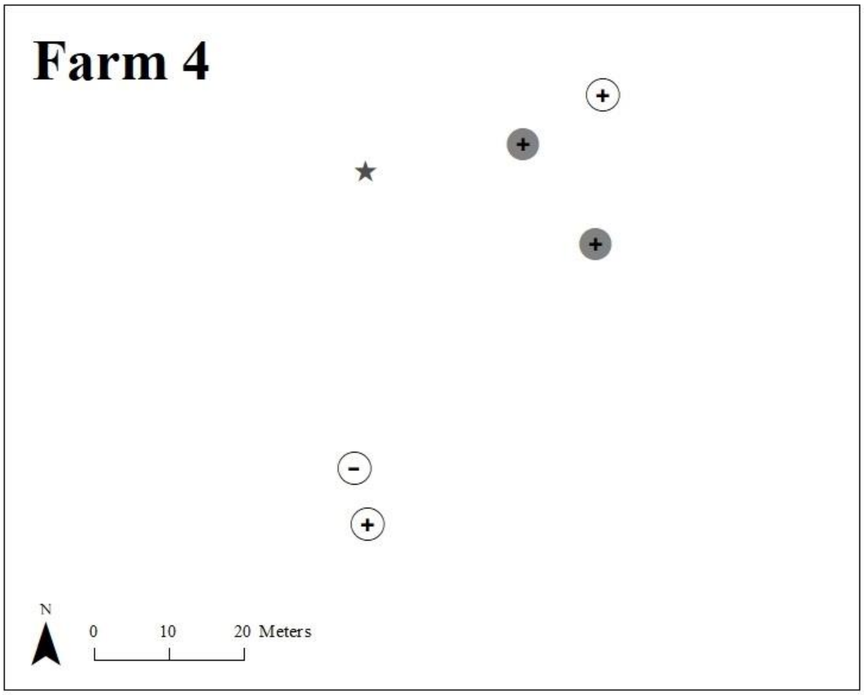
20 Meters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+
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+
N
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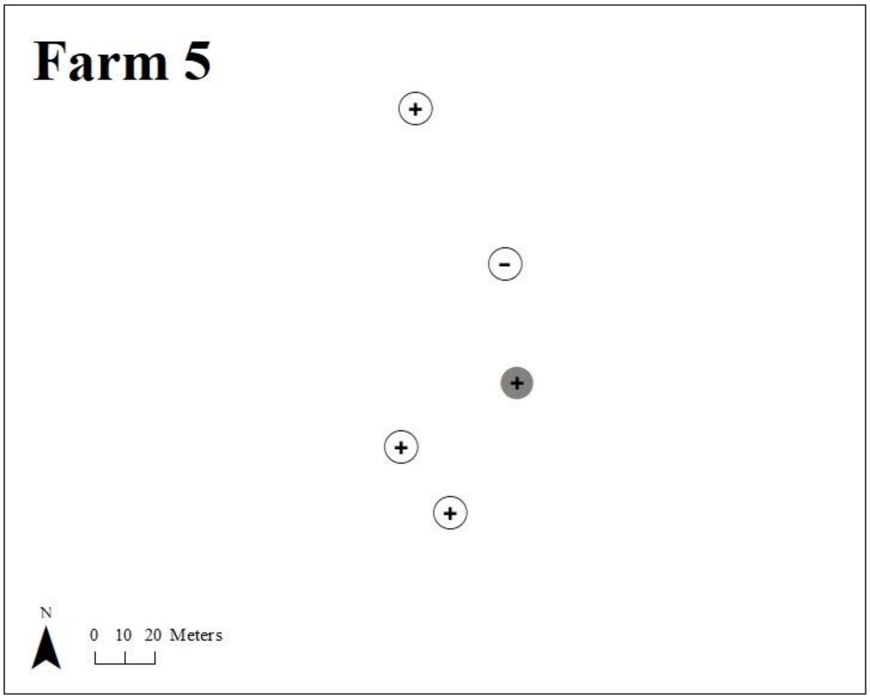
10
20 Meters


Farm 5
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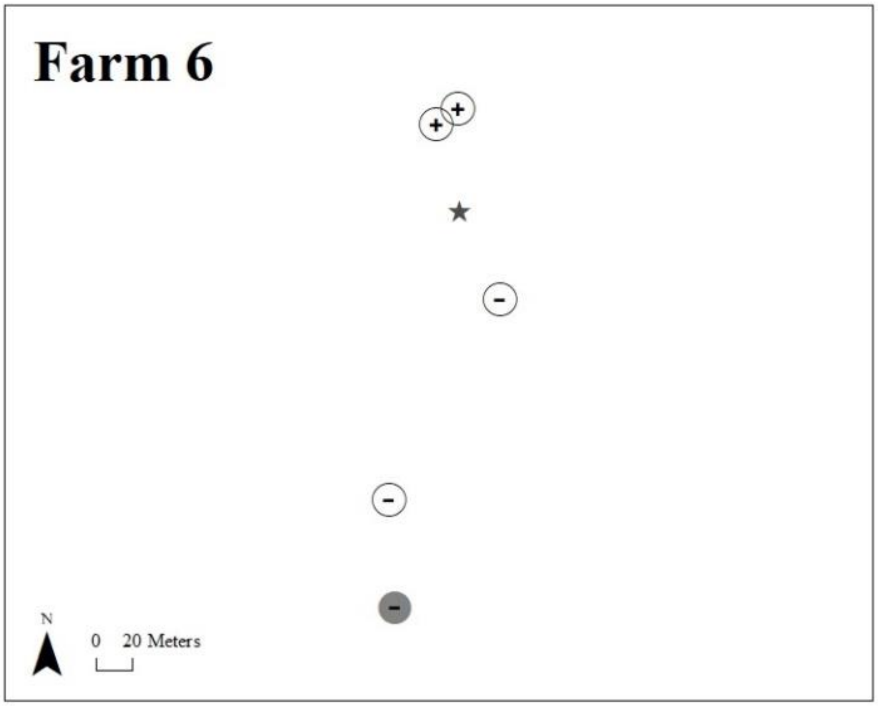
+
+
+
N
0 10 20 Meters


Farm 6
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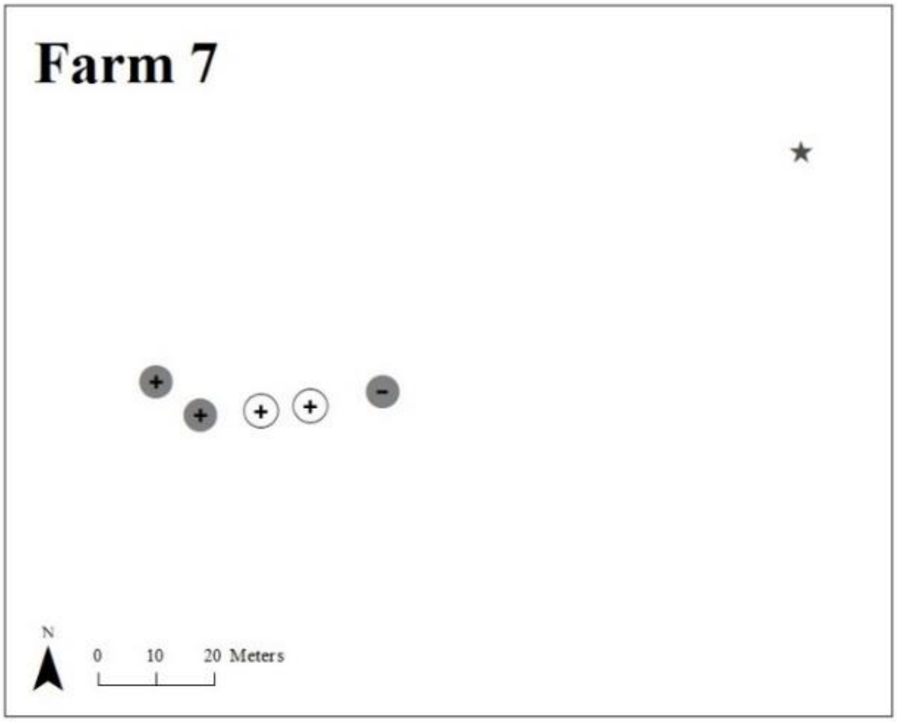
N
0 20 Meters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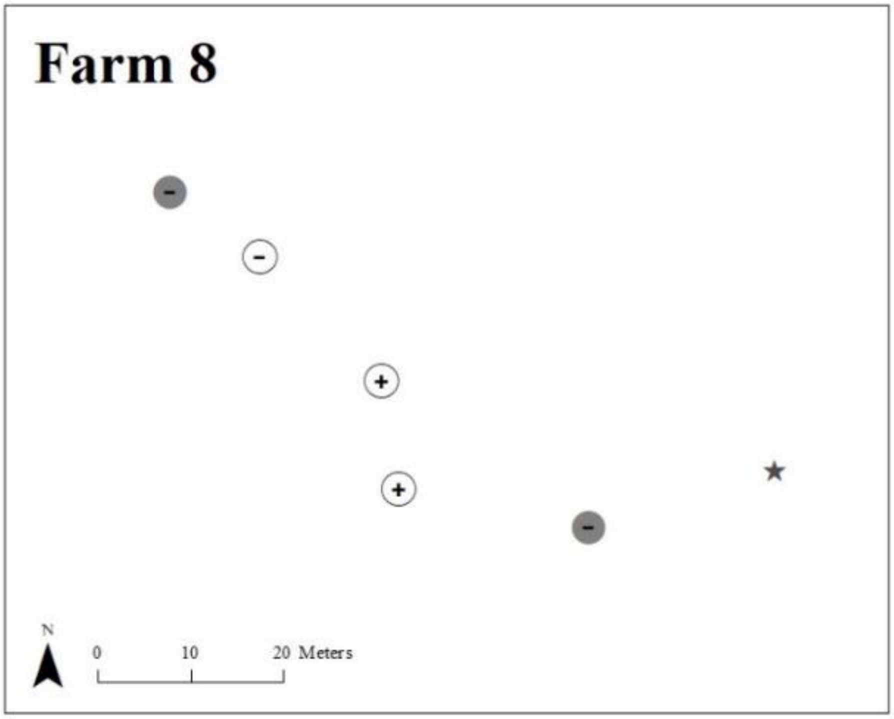
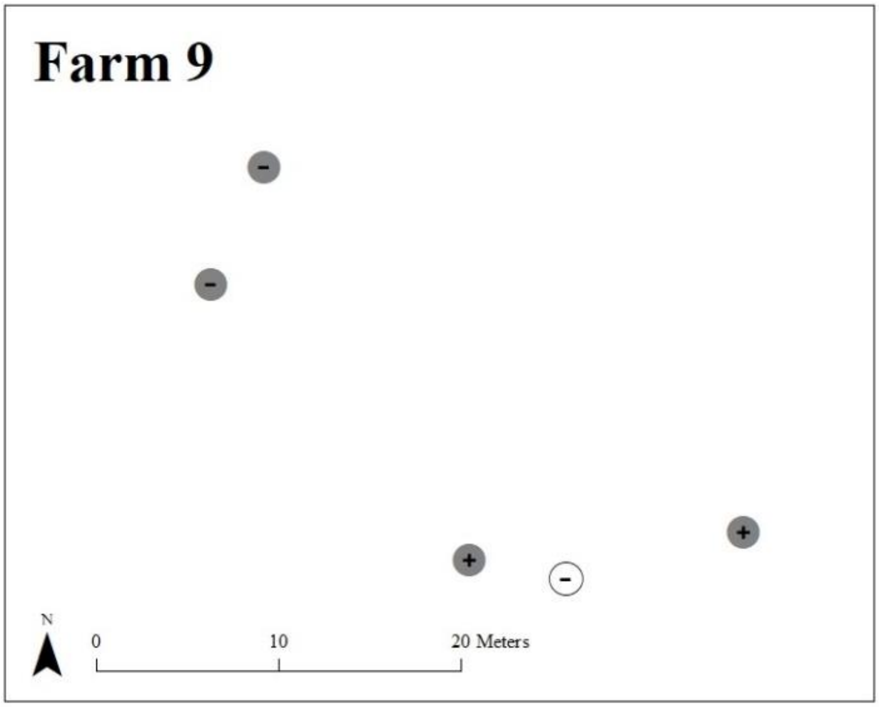
+
+
+
-
N
0
10
20 Meters


Farm 8
-
-
+
+
-
N
0
10
20 Meters


Farm 9
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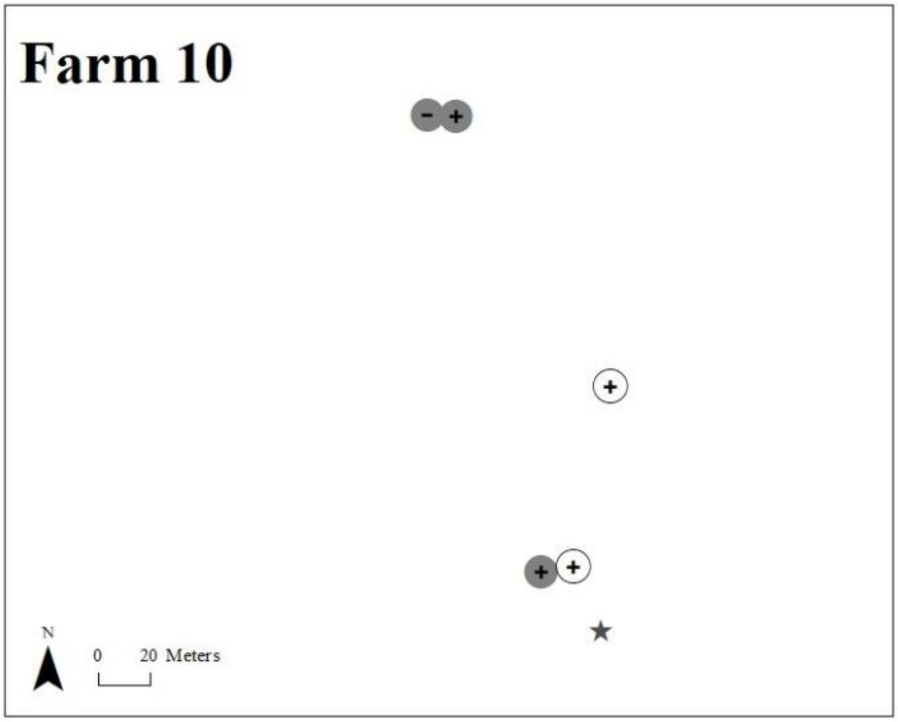
10
20 Meters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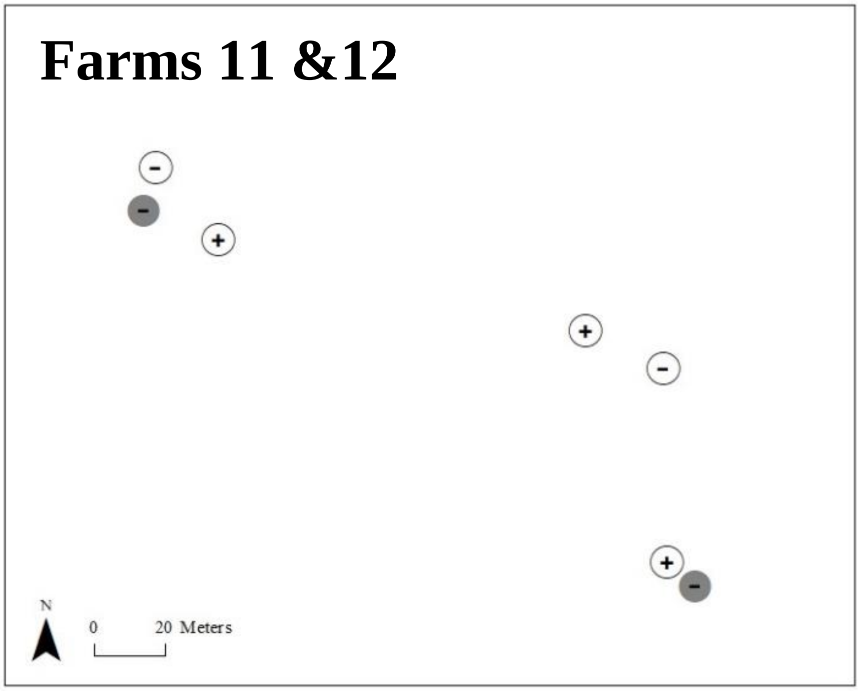
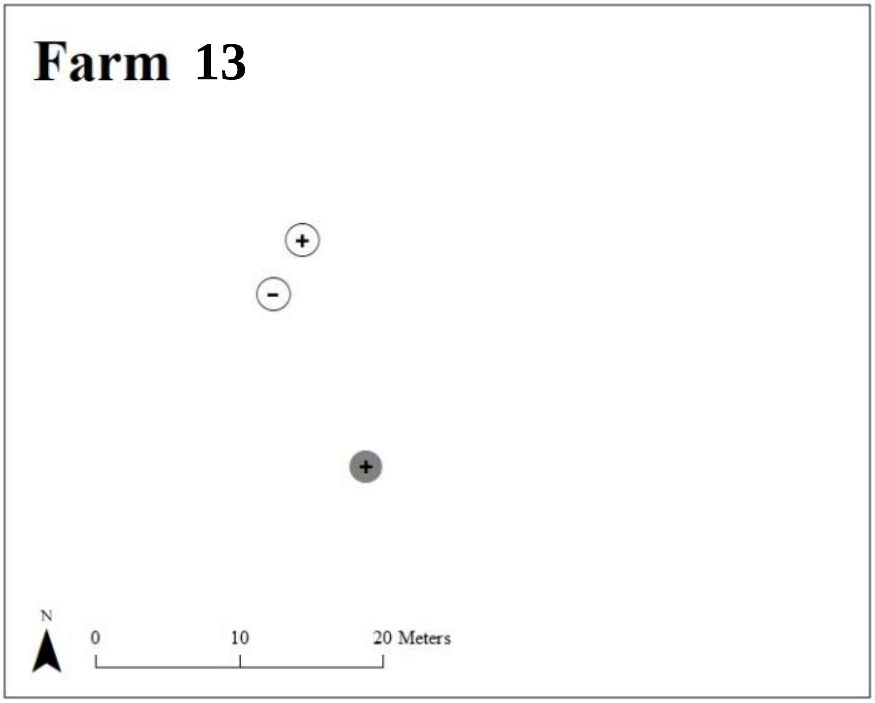
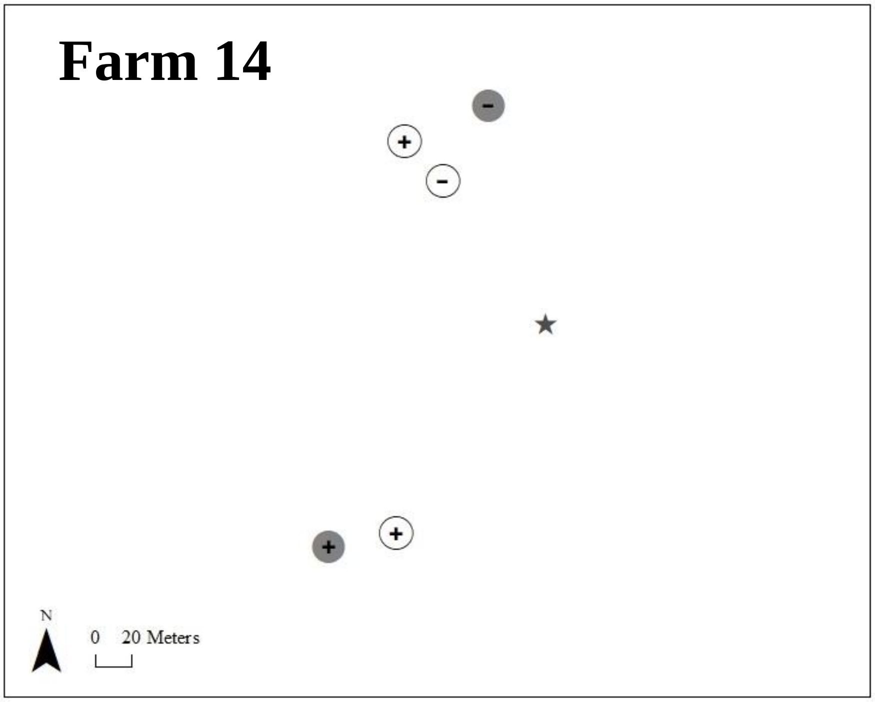
N
0
20 Meters


Farms 11 &12
N
0
20 Meters


Farm 13
N
0
10
20 Meters


Farm 14
N
0 20 Meters