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The effectiveness of cover crops as a control for root knot nematodes (Meloidogyne spp.) in coffee (Coffea arabica)

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The Effectiveness of Cover Crops as a Control for Root Knot Nematodes (*Meloidogyne* spp.) in coffee (*Coffea arabica)*

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ABSTRACT

Root Knot Nematodes are members of the family *Meloidognidae* and can be detrimental to the overall health and productivity of infected crops. Evidence of the presence of these nematodes can be seen by the galls or "swollen nodules" that form where these worms have been feeding. In order to avoid the use of harmful nematicides some farms have chosen to use cover crops as a more sustainable approach to combating these pests. For my study I decided to test if the cover crops used at LIFE Monteverde were effective in reducing the prevalence of root knot nematodes in the roots of their coffee trees. Using two different plots of coffee I was able to analyze a total of 235 roots from 56 trees. After extracting these roots, they were taken back to the lab, examined, and then categorized by the number of galls found on each individual root collected. The first plot that was sampled lacked evidence of root knot nematodes regardless if cover crops were used on the tree or not. The second plot's roots were clearly infested with root knot nematodes from both trees that used cover crops and those who did not. Both plots clearly indicated that cover crops did not affect the presence of nematodes. This data also suggested that perhaps age, proximity to neighboring farms, and topography play a role in the soil's suitability for root knot nematodes. In addition to these results, I also noticed that the coffee trees were the only roots being parasitized. The roots of the cover crops used showed no signs of nematode infestation and were found in the same soil of the roots that were severely affected by these parasites. This evidence could suggest that this species of root knot nematodes are a specialized species that feed only on the roots of *Coffea arabica*.

Efectividad del uso de cobertura vegetal del suelo como control de nematodos de raíz (Meloidogyne spp.) en plantas de café (Coffea arabica)

RESUMEN

Los nematodos de raíz son miembros del género Meloidogyne y pueden ser dañinos para la salud y productividad de los cultivos infectados. La formación de agallas o nudos en raíces son evidencia de la presencia de estos nematodos. Con el fin de evitar el uso de nematicidas nocivos, algunas fincas han optado por utilizar cobertura vegetal de suelo como una alternativa más sostenible para combatir estas plagas. Mi objetivo con este estudio fue probar si la cobertura vegetal de suelo utilizada en LIFE Monteverde es efectiva en reducir la prevalencia de nematodos de raíz en las plantas de café. Usando dos parcelas diferentes de café, analicé un total de 235 raíces de 56 árboles. Las muestras fueron clasificadas según el número de agallas encontradas en cada raíz individual recolectada. La primera parcela que se muestreó carecía de evidencia de nematodos de raíz, independientemente de la presencia de cobertura vegetal de suelo. Las raíces de la segunda parcela estaban claramente infestadas con nematodos de raíz, tanto los árboles rodeados de cobertura vegetal de suelo como de los que no. Ambas parcelas indicaron claramente que la cobertura vegetal de suelo no afectó la presencia de nematodos. Estos datos también sugieren que tal vez la edad y la topografía juegan un papel en la prevalencia de nematodos de raíz en plantas de café. Además de estos resultados, también noté que los cafetos eran las únicas raíces parasitadas. Las raíces de los cultivos de cobertura utilizados no mostraron signos de infestación de nematodos a pesar de encontrarse junto a las plantas de café infestadas. Esto podría sugerir que esta especie de nematodo de raíz es específica de Coffea arabica.

Coffee has become an essential staple of millions of lives worldwide. Its popularity has allowed for it to become the second largest export commodity in the global market (Swift 2013.) The majority of coffee is produced in regions of our planet known as the “coffee belt”, which is found along the equator, in areas with temperatures between 17-18 Celsius and volcanic dark soils. Costa Rica therefore, is an obvious candidate for the coffee industry. However, only one of the two coffee varieties, *Coffea arabica*, is produced in Costa Rica. This variety is part of 70% of the world’s production of coffee and is far more susceptible to disease (Scott 2015.)

Meloidogyne species or more commonly known as Root-Knot Nematodes, are amongst the most prevalent parasites found in coffee production and can greatly impact the productivity and health of a coffee field (Taylor 1978.) Nematodes are a very small, white to transparent type of unsegmented worm that can be found in almost every habitat on earth. Depending on the species, these worms can be either beneficial or parasitic to the plant (Insect-Parasitic Nematodes 2015.) Root-Knot Nematodes however, are considered to be very parasitic to coffee trees because of the effects they can have on coffee yields if left untreated (McSorley 1999.) These nematodes invade the roots of coffee trees and obtain their nutrients directly within the roots, creating swollen nodules or galls where they have been (Taylor 1978.) In order to save crop yields and combat the incidence of these nematodes, companies have created several types of nematicides and synthetic fertilizers. However, these nematicides have been shown to drastically reduce the number of galls found on coffee roots, but also have altered the natural state of the soils they are found in by killing beneficial microorganisms in the soil and increasing the development of genetically resistant parasitic nematodes (Meher 2009.) Studies demonstrate that using a cover crop provides multiple benefits and can reduce formation of root nodules (McSorely 1999.) In addition to reducing erosion, helping retain soil moisture, and adding nitrogen to the soil some cover crops, like Marigolds and Sudangrass, can naturally produce chemicals similar to the effects of nematicides (Harsimran 2017.) Using a natural solution would be more advantageous because it could prevent the possibility of parasitic nematodes becoming genetically resistant to nematicides, killing beneficial microorganisms, and refrain from introducing more chemicals to the environment. Therefore, the purpose of this study is to investigate if cover crops are an effective way to combat the incidence of root-knot nematodes in *Coffea arabica*.

MATERIALS AND METHODS

Study Sites:

For my experiment I worked with two different coffee plots at LIFE Monteverde, a sustainable coffee farm in in Cañas, Guanacaste Costa Rica. Both plots I took samples from had areas which used cover crops and other areas that did not. These plots provided the ideal conditions in order to test my hypothesis on the effectiveness of cover crops in preventing the presence of *Meloidogyne spp.* found on the roots of the coffee trees.

The first plot, known as the Guayabo Plot, is about five years old and was once used as land for a cattle pasture before it was a coffee plot. The topography of this plot is fairly even and flat. There are forest corridors surrounding the entire plot and then another one of LIFE's coffee plots next door to those forest corridors. Due to these conditions this plot did not experience any outside influences from any neighboring farms and was purely controlled by LIFE's farming practices. All of the roots extracted from this plot were from trees of the same age and used the same amount and type of fertilizer.

The second plot I obtained root samples from is known as the Paila Plot. This plot greatly differed from the Guayabo plot. The Paila plot is much older in age and has been used as a coffee plot for over fifteen years. Its age may have altered the soil conditions in which these trees are planted in and perhaps the overall prevalence of *Meloidogyne* found in this plots coffee roots. Its close proximity to a neighboring farm may also influence the integrity of the LIFE's sustainable farming practices and be another clue to why the Paila plot had an abundance of galls on its coffee's roots. Water runoff caused by intense rains and debris blowing in from this neighboring farm could contain chemicals from their use of synthetic fertilizers and threaten the plot from being entirely influenced by LIFE's sustainable practices. The topography of this plot was not as flat as the Guayabo plot, and there were trees that were planted on a slant in some areas of the plot. However, all of the roots extracted from this plot were from trees of the same age and used the same amount and type of fertilizer.

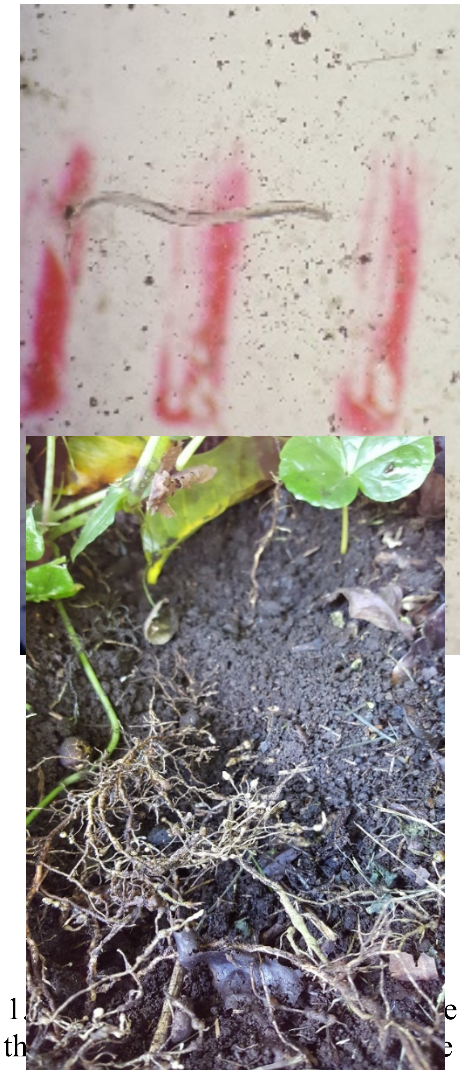
Data Collection and Analysis:

I randomly chose every tree in both plots and began by examining the base of the tree trunk and measuring the width of the trunk and height of the tree to ensure that the trees within each plot I sampled were of similar age. If the tree had a cover crop I labeled each species of cover crop found at that particular tree and also took note on the amount of cover crop coverage present within the twenty-centimeter radius from the base of the tree.

In order to extract the roots from the coffee tree I dug up approximately fifteen centimeters deep within the twenty-centimeter radius of the tree's base. I took a minimum of four roots from every tree and did my best to collect roots that ranged between twelve to thirteen centimeters long. In order to randomize the data, I would collect my root samples from different branches of the larger roots of the coffee tree. I then gathered soil samples from the soil that was dug up from each tree in order to take back to the lab and test for soil moisture content and pH using the corresponding electronic meters.

In the lab the roots were then counted and categorized into the three nematode infection categories. The first category consists of uninfected roots, noting for an absence of any evidence of galls indicating the presence of *Meloidogyne* species. The second category was for roots that found themselves in a transition phase. This category was for roots that showed potential for the development of gall formation, and had at least one to five galls or "swollen nodules" forming on their roots already. The third category was for infected roots and used for roots that had five galls or more and clearly demonstrated signs of obvious root knot nematode infestation.

In the lab, the soil was sieved and measured for soil moisture and pH using a soil moisture meter and pH meter. Besides looking for evidence of root knot nematodes I also looked for the nematodes themselves. Due to their size, root knot nematodes can often be difficult to see without the use of a dissecting scope. The nematodes that are seen tend to be female and can range from 400-1000 micrometers or 0.4-1.0 millimeters long (Mitkowski 2003.) In order to see the actual nematode within the roots and in the soil I used several methods. For the first collection of roots from the Guayabo coffee plot there was little to no indications of the presence of root knot nematodes. In order to be sure of these observations I conducted a Baermann Funnel Technique to ensure that there were no nematodes present. Using this method you must first homogenize your soil samples collected, and then construct a funnel with both a mesh strainer and paper filter, and have a vial connected to the funnel's spout. By homogenizing the soil you are able to obtain a combined soil sample of the entire area you sampled. After thoroughly mixing my soil samples and preparing my funnel, I placed about 50 ml of soil on the filter paper and then poured about 50 ml of water to filter through any microscopic nematodes into the vial over a period of 24-48 hours (Tylka 2017.) This method allows for me to carefully observe any root knot nematodes in their motile J2 stage that I may have missed while sifting through soil and analyzing roots (Mitkowski 2003.) Since root knot nematodes spend the majority of their life living within roots I decided to also split open roots in the soil and placed them in a petri dish under a microscope to try to obtain an actual visual of the nematode itself. I did this for each plot using the soil and the roots collected from both the areas with and without cover crop. By using this method I was able to see the actual body of the root knot nematode in every soil sample taken from the Paila plot, and few that emerged from within the actual root. None were seen in any of the samples taken from the Guayabo plot. Both these outcomes confirmed what we observed about the overall physical state of the roots collected from these plots.



A

B

Figure 1. … e of the *Meloidogyne* spp under a disecting scope from one of th… Paila Plot. Photo B is of roots that have a clear presence of root galls created by root knot nematodes.

RESULTS

From the first plot, the Guayabo plot, I obtained a total of 122 root samples from 18 trees that used cover crops and 18 trees that did not. All the trees found here were no older than five years old and an average height of 200cm and an average trunk width of approximately 5.2cm. After analyzing these roots in a lab I found that only four roots from one of the trees sampled showed evidence of five or more galls formed. Of the remaining roots only 19 showed the presence of one to five galls, and the remaining 99 roots were healthy and showed no evidence of being affected by root knot nematodes. Meaning only 3.4% of the roots collected at this plot had significant galling and overwhelming evidence of being infected by root knot nematodes. The roots collected from both trees with and without the cover crops had a very similar distribution of roots that had no galling, some galling, and overwhelming number of galls (Figure 2.) This information suggests that cover crops do not play an effective role when it comes to controlling root knot nematode abundance.

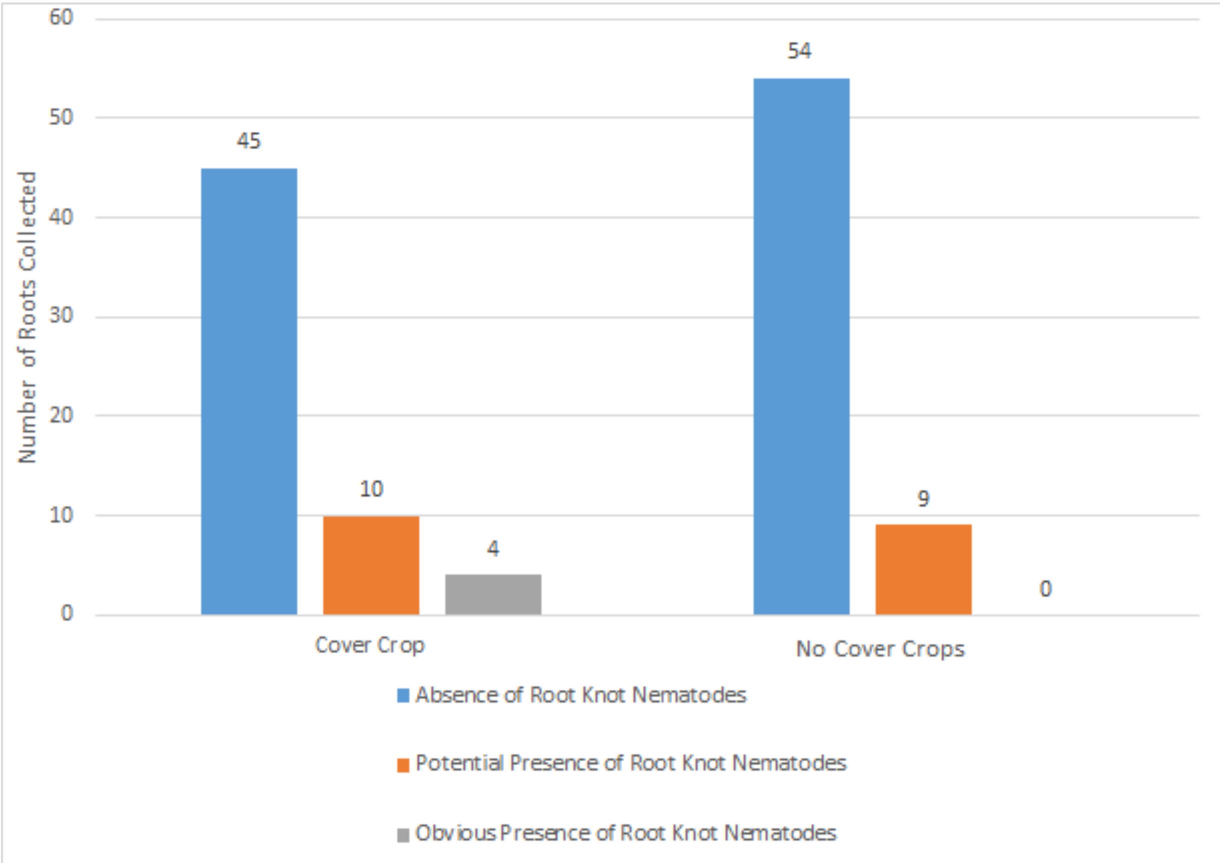


Figure 2: This graph is of the number of roots collected from the Guayabo plot and those found with an absence of evidence of root knot nematodes, a potential presence of root knot nematodes, and finally an obvious presence of root knot nematodes indicated by an abundance of galls on the roots. Guayabo Plot had N=36 trees, n=122 roots (X^2= 1.779 df = 1 $p > 0.05$)

In order to test the validity of these results from the first plot, I took samples from a field that was significantly impacted by the presence of root knot nematodes and see if cover crops played any significant role in the presence of root knot nematodes found here. The second plot, the Paila plot, had a total of 113 roots collected from 20 randomly selected trees. These trees were much older in age and had an average height of 250 cm and an average trunk width of about 7 cm. Of this plot's roots 19 had no signs of nematodes, 16 had at least one to five galls forming, and the remaining 78 were heavily infected with galls. Meaning about 70% of the roots collected at this plot had significant galling and overwhelming evidence of being infected by root knot nematodes. The roots collected from both trees with and without the cover crops had a very similar distribution of roots that had no galling, some galling, and overwhelming number of galls (Figure 3.) This information suggests that cover crops do not play an effective role when it comes to controlling root knot nematode abundance.

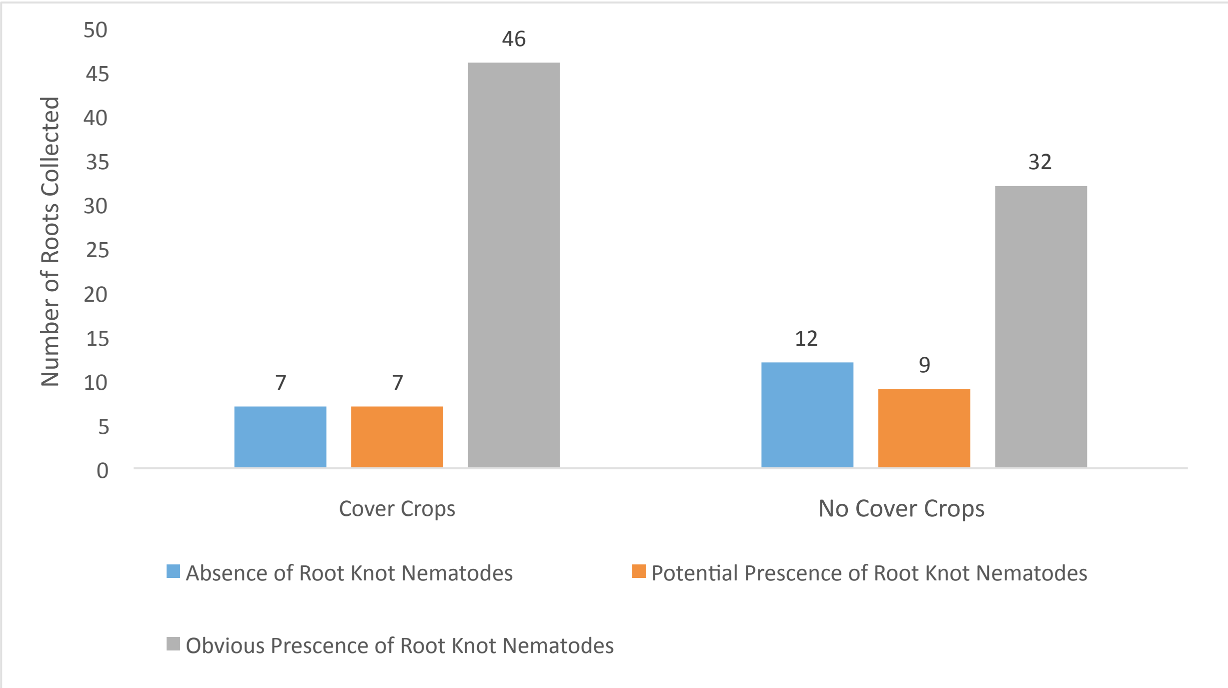


Figure 3. This graph is of the number of roots collected from the Paila plot and those found with an absence of evidence of root knot nematodes, a potential presence of root knot nematodes, and finally an obvious presence of root knot nematodes indicated by an abundance of galls on the roots. N=20 trees, n=113 roots ($X^2 = 2.43$, df = 1, $p > 0.05$)

The first plot that was sampled lacked evidence of root knot nematodes regardless if cover crops were used on the tree or not. The second plot's roots were clearly infested with root knot nematodes from both trees that used cover crops and those who did not. Both plots clearly indicated that cover crops did not affect the presence or absence of nematodes.

I also measured the pH and soil moisture content from each plot by using the soil that was homogenized and observed any differences in their values. The pH and soil moisture content values of both these plots turned out to be very similar. The pH of both these plots soils ranged from 5.52 - 6.20, and had a soil moisture content range of 8.07%-8.54% and were all taken at about 19.9 Celsius. pH is an important variable to consider for nutrient availability, the more acidic it is the tighter the nutrients are bound in the soil (Pais 2015). It is also important to note the soil moisture content because nematodes need a film of water on the soil particles in order to move through the soil to infiltrate root tips (Insect-Parasitic Nematodes 2015.) Testing these variables for both the plots allowed for my study to confirm that they both are treated with the similar amounts of fertilizers and compost.

The crop species diversity also ranged from as little as one species of crop to a maximum of six morpho species of grasses and clovers as cover crop per tree. The diversity of species of cover crop did not show any correlation to the density of roots infested with nematode galls. This observation suggests that diversity of these cover crops does not notably impact root knot nematode abundance. In addition to this, none of the cover crop's roots showed any signs of nematode infestation despite being in the same soil that trees with heavily infected roots were found in. This data could infer the possibility of a species specialization between the *Meloidogyne spp.* found at LIFE Monteverde and *Coffea arabica.*

DISCUSSION

There was no correlation in the use of cover crops as a natural and effective combatant against nematodes. Both plots either had either an absence or an overwhelming abundance of root knot nematodes present within their coffee plots roots regardless of whether the cover crop were used or not. The cover crop's species diversity on each tree also did not show any correlation to the number of roots found with galls. These results seem to suggest that perhaps there is another important factor to consider when studying the presence of root knot nematodes in coffee plots. The two plots were the same species of coffee, treated with the same farming methods, fertilizers, and climate. The biggest difference between the two plots was perhaps the age, the outside influences they may experience, and topography.

These results lead to the idea that perhaps age is a significant factor in the abundance of root knot nematodes found. This argument is strengthened by the manner that root knot nematodes infiltrate tree roots. During their short-lived motile life stage these nematodes invade the nearest neighboring roots and therefore if they are left unchecked or untreated can over time spread like a cancer throughout a coffee plot (Insect-Parasitic Nematodes 2015.) The Paila plot demonstrated a trend similar to this idea. Throughout the entire plot almost every tree's roots were infected with an overwhelming number of galls on every root. The few trees that did not show any signs of contamination on their roots seemed to be found in only one specific area of the plot. All the trees were randomly selected throughout the plot, and all but the one corner containing those three uncontaminated trees lacked the presence of nematodes in their roots. The age of this plot suggests that these nematodes have over time slowly spread throughout this plot and because of its age has had the time to infiltrate the roots of almost every tree one by one and has therefore simply not had the time to spread to this specific area of the plot. This observation is strengthened by the fact that nematodes travel very slowly and have a short stage of their life as a juvenile when they are free living and can move through soil. This short period of time only allows for nematodes to infect the nearest available roots, which is most likely very close to the roots they were born in (Insect-Parasitic Nematodes 2015.) Therefore, the three trees without cover crops that also lacked root knot nematode galls in their roots had little to do with the lack of cover crop, and more to do with the area in which they were found.

The area in which these trees was neighboring a larger patch of banana leaves and several coffee trees that did not show any signs of health defects. Some agricultural studies have shown that the use of banana leaves as mulch has helped restore nutrients in the soil and prevent the prevalence of coffee pests (Waller 2007.) I would like to further study the effects that banana trees planted in between coffee crops can have on the prevention of root knot nematodes or if these trees simply have not had the time to be infiltrated by root knot nematodes.

I also observed the overall health of the tree was not telling of the amount of roots infected with root knot nematodes. Some of the trees that had full leaves and an abundance of coffee berries were also some of the trees that had the most galls on their roots. In addition to these observations, I also noticed there were trees that were very physically damaged above ground and displayed very yellow leaves, but did not show significant damage by root knot nematodes to their roots, suggesting that it perhaps was being parasitized by another coffee pest. However, these observations could be considered arbitrary because they are limited to my observations of only two coffee plots, but do inspire a more in depth analysis of abundance of healthy leaves and berries versus the presence of galled roots in *Coffea arabica*.

If I were given the opportunity and an extended period of time to do a further study on the effectiveness of cover crops as combatant against root knot nematodes I would like to test several more variables. I would first like to control the type of cover crop planted on the coffee trees and see if some worked better than others or were effective at all. Despite my results, it has been shown that there have been cover crops that have effectively reduced the number of *Meloidogyne* species found on crops and improved crop yields (Viaene 1996.) Marigolds for example are known to produce an allelochemical called alpha-terthienyl that effectively combat the presence of various plant pests including root knot nematodes (Harsimran 2017.) I then would like to compare the above the ground health of the coffee crop versus the below the ground health.

Due to my observations at the Paila plot, I would also like to study the effectiveness of banana trees as a potential control for root knot nematodes. Upon other studies, it would also be useful to identify which species of *Meloidogyne* was found at LIFE Monteverde and expose it to different plant's roots in order to determine if there was a species specialization. Many of the *Meloidogyne* species previously studied like *M. Incognita and M. hapla* have been shown to have specific preferences on the crops they decide to infiltrate, and could be useful to determine if the species at LIFE has a similar relationship (Moens 2009.) For these future studies I find it necessary to sample a larger number of coffee trees from a variety of coffee plots at the farm. This is important to consider in order to compare a greater variety of plots of different ages, proximity to neighboring farms, and topography.

According to this study the cover crops used at LIFE Monteverde do not effectively reduce or increase the number of root knot nematodes found on their coffee trees. However, other studies have shown certain species of cover crop to be effective in combatting root knot nematodes. This suggests that LIFE Monteverde would benefit in investing in a different cover crop like Marigolds or something similar in order to effectively combat the growth and spread of root knot nematodes in their coffee plots. The difference in the presence of root knot nematodes found in the roots of the Guayabo plot and the Paila plot suggest that other factors like the age of the plot, proximity to neighboring coffee farms, or topography may be important to understanding the prevalence of root knot nematodes in some plots and not in others.

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