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A Comparative Plague Study of Cacao Fungal Disease in Cacao Pods Within Monocultures and Indigenous Agroforests in Ecuador's Napo Province

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SIT Study Abroad

A Comparative Plague Study of Cacao

Fungal Disease in Cacao Pods Within Monocultures and Indigenous Agroforests in Ecuador's Napo Province

Ground view of an indigenous chakra system of Theobroma cacao near Tena, Ecuador

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Abstract
This study analyzes the composition of three major fungal diseases in *Theobroma cacao* fruits compared between monocultures and *chakra* agroforests in the Napo province of Ecuador with the goal of noting similarities and differences in the disease composition between the two systems, as well as investigate possible variation within this poorly understood category of agroforest to better structure future studies. Cacao pods on sampled trees were counted and fungal infections identified visually and by touch. *Chakra* systems were selected in the communities of Cinco de Enero and Seis de Marzo to the Southwest of Tena, Ecuador. Monoculture data was collected from Chonta Punta, Ecuador, East of Tena. Black pod rot (*Phytophthera spp.*) was the most prevalent disease affecting cacao pods in *chakra* systems, followed closely by frosty pod rot (*Moniliopthera roreri).* Monocultures exhibited significantly lower proportions of black pod rot and instead were dominated by infections of frosty pod rot. Witches' broom (*Moniliopthera perniciosa*) was the least present in both. Overall, monocultures had a much lower rate of infection than *chakra* systems, likely explained by differences in management intensity, lower rainfall, and differences in cacao. More than half of young pods in both systems were lost due to cherelle wilt, a poorly understood physiological condition with tenuous ties to fungal disease. Subvarieties of cacao nacional exhibited little difference in disease composition, with similar slight differences being observed along an age gradient of cacao trees. Overall, these findings suggest that *chakra* cacao systems in Tena's climate suffer greater losses to disease than neighboring monocultures to the East under and would benefit from continued research on responsible factors and the increased application of cultural management practices.

Resumen
Este estudio investiga la composición de las tres enfermedades principales de hongos que afectan las frutas de *Theobroma cacao* en monocultivos y sistemas *chakras* en la provincia Napo en Ecuador. La meta fue aprender y anotar las similitudes y diferencias de la composición de estas enfermedades de hongo, y también investigar las variaciones dentro de las *chakras* para obtener un mejor entendimiento de este sistema y fortalecer estudios futuros de *chakras*. Las mazorcas de cacao en árboles seleccionados se contaron y categorizaron por su estado de infección. Las *chakras* se seleccionaron en las comunidades Cinco de Enero y Seis de Marzo al suroeste de Tena, Ecuador. Datos de monocultivos se colectaron de Chonta Punta, al este de Tena. La mazorca negra (*Phytophthora spp.)* fue la enfermedad principal en los sistemas *chakras*, con una presencia más baja de monilia (*Moniliopthera roreri*). Monocultivos tuvieron niveles bajos de mazorca negra y se dominaron por la monilia. Ambos sistemas tuvieron niveles bajos de la escoba de bruja (*Moniliopthera perniciosa)*. Los monocultivos tuvieron una tasa de infección mucho más baja que las *chakras*, explicado por diferencias de la intensidad de manejo, precipitación, y variedades diferentes de cacao. Más de una mitad de las mazorcas jóvenes se perdieron al fenómeno de cherelle wilt, una condición fisiológica mal entendida con relación posible a las enfermedades. Las dos variedades de cacao nacional tuvieron pocas diferencias con respeto a su composición de enfermedades. Lo mismo se encontró con árboles de edades diferentes en las *chakras.* En todo, este estudio encuentra que sistemas *chakras* cerca de Tena tienen más vulnerabilidad a las enfermedades que los monocultivos en las zonas al este, y podría beneficiar de investigaciones continuadas y la aplicación de manejo cultural más intensiva.

Acknowledgements

I would like to thank the family of Lupita and Pedro for welcoming me into their home as I conducted this research, answering all my questions about *chakra* systems and the details included in this study surrounding their management, and for guiding me to and through *chakra* sites. I would like to thank Rogelio Camión and Ivan Merino for their assistance in providing monoculture sites for me to sample, and to Ivan for receiving me for two nights while I conducted my monoculture data collection. I extend this gratitude to Geovani Grefa, Liliana Grefa, and the rest of their family for housing me between visits to monoculture sites, as well as for hosting me as I recovered from a sickness. Additional gratitude goes to Geovani for organizing the logistics of site selection and organizing my homestay for the duration of this study. Much gratitude also to Xavier Silva, Diana Serrano, and Ana Maria Ortega for offering all their services in support the smooth development of this project, and to Natalia Latorre for assisting me in disease identification, the details of site selection, and providing useful and timely feedback on this paper. Many thanks to Don Germán for transporting me from Quito to Tena and vice versa at the start and end of my study period.

Introduction

Background and Context

The tropical understory tree *Theobroma cacao* is native to the Amazon rainforest of Northern Ecuador and Southern Columbia and has a history of cultivation dating back at least 5,300 years (Zarillo et al. 2018). Cultivated today primarily for chocolate production, cacao is one of the most valuable cash crops worldwide; the cacao industry was valued globally at $14.5 billion as of 2022, with Ghana and the Ivory Coast accounting for over half of global production. While Ecuador produces only 7% of the world's cacao, the crop is one of the country's major exports. Ecuador is also the main grower of the cacao variety *nacional*, a form of high-quality, aromatic cacao with similar genetics to wild-type cacao. This form of cacao can fetch a premium price compared to bulk varieties such as CCN-51, the most widely cultivated variety in Ecuador.

Figure 1.1: Close-up on pods of the cacao variety *nacional* in an indigenous *chakra* agroforestry system. Author: Seamus McCarthy

The cacao cultivar CCN-51 was developed in the 1960s in the Naranjal region of Ecuador as a response to an intense outbreak of witches' broom (*Moniliophthora perniciosa*) and has since become widespread in Ecuador and Latin America due to its adaptability to a diversity of climates and high levels of disease resistance (Jaimez et al. 2022). While cacao is traditionally grown with the addition of shade trees, the physiology of

CCN-51 allows it to better tolerate full-sun conditions and as such is most often grown in monocultures, defined as pieces of land on which only a single crop is grown. Monocultures are currently the norm in all forms of large-scale agricultural production. Monoculture yields can be extremely high due to intense inputs of capital and chemicals in the form of fertilizer and pesticides.

The monoculture expansion of cacao, palm oil, banana, and more is a significant driver of deforestation and biodiversity loss both in Ecuador and globally (Walsh et al. 2002). In the case of Ecuadorian cacao, expansion and homogenization of production has also led to cultivar loss and damage to the fine cacao market (Melo et Hollander 2013). Monocultures are also exceptionally vulnerable to disease due to their structure and must almost always be managed with intensive pesticide, herbicide, and fungicide inputs to avoid significant losses. Heavy fertilizer input is also necessary to extract high yields from the same crop over many seasons. These chemical inputs are passed through farms into the surrounding environment, where ecological and environmental consequences are severe and spill over into human health (Carvalho 2017).

Figure 1.2: The cacao variety CCN-51 grown in monocultur. Pods are of a darker red/purple than *nacional* varieties of similar color. Author: Seamus McCarthy

While monocultures are common for the bulk-variety cacao of CCN-51, the tree is traditionally an understory crop grown in agroforests, loosely defined as a cultivated area which includes trees as well as a primary crop or crops. One such form of agroforest is the *chakra*, a traditional polyculture system of Amazonian Kichwa people. *Chakras* consist of subsistence crops, fruit trees, timber species, medicinal and market crops grown together with intention to provide ecological benefits to the farm as well as a diversity of useful yields from a small-scale agricultural system. This diverse agricultural system has scored highly on sustainability metrics (Heredia-R et al. 2021) and often produces high-quality fine aroma cacao if included in the system. Farms can be broken up into multiple chakras of different composition. For example, one very common *chakra* is an interplanting of plantain and yucca for subsistence purposes.

The *chakra* of interest to this study reaches its final form as a cacao agroforest. This cacao agroforest is constructed to support cacao *nacional* and has distinct phases of growth and management, with a high likelihood of variation between farms on the finer details of cultivation. In the case of this study's sample sites, newly planted cacao *nacional* is intercropped with plantain and yucca to offer a subsistence crop and minimize weed growth. Shade trees are not planted by hand but allowed to grow naturally, seeds being carried by birds, wind, or insects. During this successional process shade trees which pose a threat to cacao trees or farmers due to poor location and heavy leaning are removed. The remaining trees grow in conjunction with the cacao and form a taller overstory as subsistence crops make way for mature, productive cacao agroforests. This successional process takes five years, after which its state is maintained for the duration of production time. Weed control is performed with machete, weed whackers, or a combination of the two.

Figure 1.3: Chakras of cacao nacional in developmental and mature stages. A more obvious interplanting of plantain was visible to the left of the developing chakra. Author: Seamus McCarthy

Of particular interest to this study were the *chakras* of cacao *nacional* owned by associates of Kallari, a cooperative of approximately 850 Kichwa families of 21 different communities which seeks to preserve indigenous agricultural systems and culture and give farmers fair compensation for their products. The organization's main market focus is cacao, which is bought from associates, processed into chocolate in Quito, and sold by the organization locally and internationally. For farmers to become associates of Kallari, they must be Kichwa, utilize the *chakra* system in their cacao production, preserve uncultivated forest on their property, and farm without input of synthetic chemicals. The marriage of quality products with ecologically sound agriculture presents a powerful opportunity for the world of conservation, as these same agroforests have been found to contain high levels of biodiversity (Ezra Epstein 2022), combining market value with sound environmental principles. Well-managed agroforests can also provide a diversity of ecosystem services to farms and the surrounding environment through more efficient nutrient cycling, habitat for pest predators, and water retention (Mortimer et al. 2017). However, yields in *chakra* systems and organic cacao farming in general are limited by fungal disease which has the potential to reduce farmer income and the economic stability of the cooperative.

Cacao yields in Ecuador and beyond face their greatest threat from fungal diseases which proliferate in the moisture-laden environment of the Amazon. Specific fungal strains damaging cacao in Ecuador are witch's broom (*Moniliophthora perniciosa*), black pod rot (*Phytophthora* spp.), and frosty pod rot (*Moniliophthora roreri*), the latter being considered the most destructive disease in Latin American cacao production. Witches' broom affects both pods and branches, killing and drying out developing shoots and flower patches. Pods which grow from these infected patches are deeply malformed and harden considerably, with most pods only reaching several centimeters before ceasing growth. Black pod rot affects the wood and pods of cacao trees, resulting in sickly lesions on patches of bark and rapid blackening and breakdown of affected cacao pods. Infected pods are soft with crumbly interiors. Frosty pod rot exclusively affects pods, mummifying the pod and producing patches of white spores. Pods remain full and heavy, with spores surviving on the infected pod for up to a year. Young pods on cacao trees, called cherelles, are also subject to a poorly understood physiological known as Cherelle wilt, in which anywhere from 50% to 100% of newly developing pods shrivel and blacken due to apparent abandonment by the plant. These pods are later infected by fungus and colonized by insects, but the two are not often considered the causal agents of cherelle wilt (Anzules Toala et al. 2022). It's posited that increased average temperatures accelerate the diseases' sporulation cycle, while lower temperatures promote faster spore formation (Leandro-Muñoz et al. 2017). Acceleration of the hydrological cycle from climate change is projected to lead to increased intensity of rainfall events, creating a more favorable environment for spore distribution and subsequent infection. These same changes are expected to expand the range of frosty pod rot across a wider swathe of South America (Ortega Andrade et al., 2017), whereas the disease is currently limited to Central America and the Western Amazon. These projected increases in disease presence coupled with cacao's economic importance to many South American countries makes research on disease mitigation imperative.

Figure 1.4: On-site photographs of witches' broom, black pod rot, frosty pod rot, and cherelle wilt in order of top left to bottom right. Pictured cherelles were infected with frosty pod following fruit abandonment by the tree. Author: Seamus McCarthy

Objective/Purpose

The main objective of this study was to determine whether significant differences exist in the overall fungal disease composition of cacao monocultures and *chakras* of cacao *nacional*. Upon arrival and analysis of potential sample sites, a secondary objective was developed to analyze the details of disease composition within *chakras*. The two cultivation systems have many important differences in areas such as chemical use, crop density, presence of shade trees, labor inputs, and farm size, all of which influence overall disease vulnerability. Moreover, it is unknown the extent of variation of these factors between different sites which meet the definition of *chakra*. Monocultures are well-known to offer ideal sites for disease proliferation due to the high-density plantings of genetically identical crops (Shiva 1993). Incorporation of shade trees into cacao farms has been studied extensively, but mixed findings. Some find increased levels of disease such as black pod rot while others point to no significant difference in levels of frosty pod rot (Armengot et al. 2020). Others furthermore point to management practices such as removal of infected pods from trees as being more important (Armengot et al. 2020). Research is further complicated by differences in cacao variety between monocultures and agroforestry systems, adding factors such as genetic resistance and physiology to the list of differences.

Methods and Materials

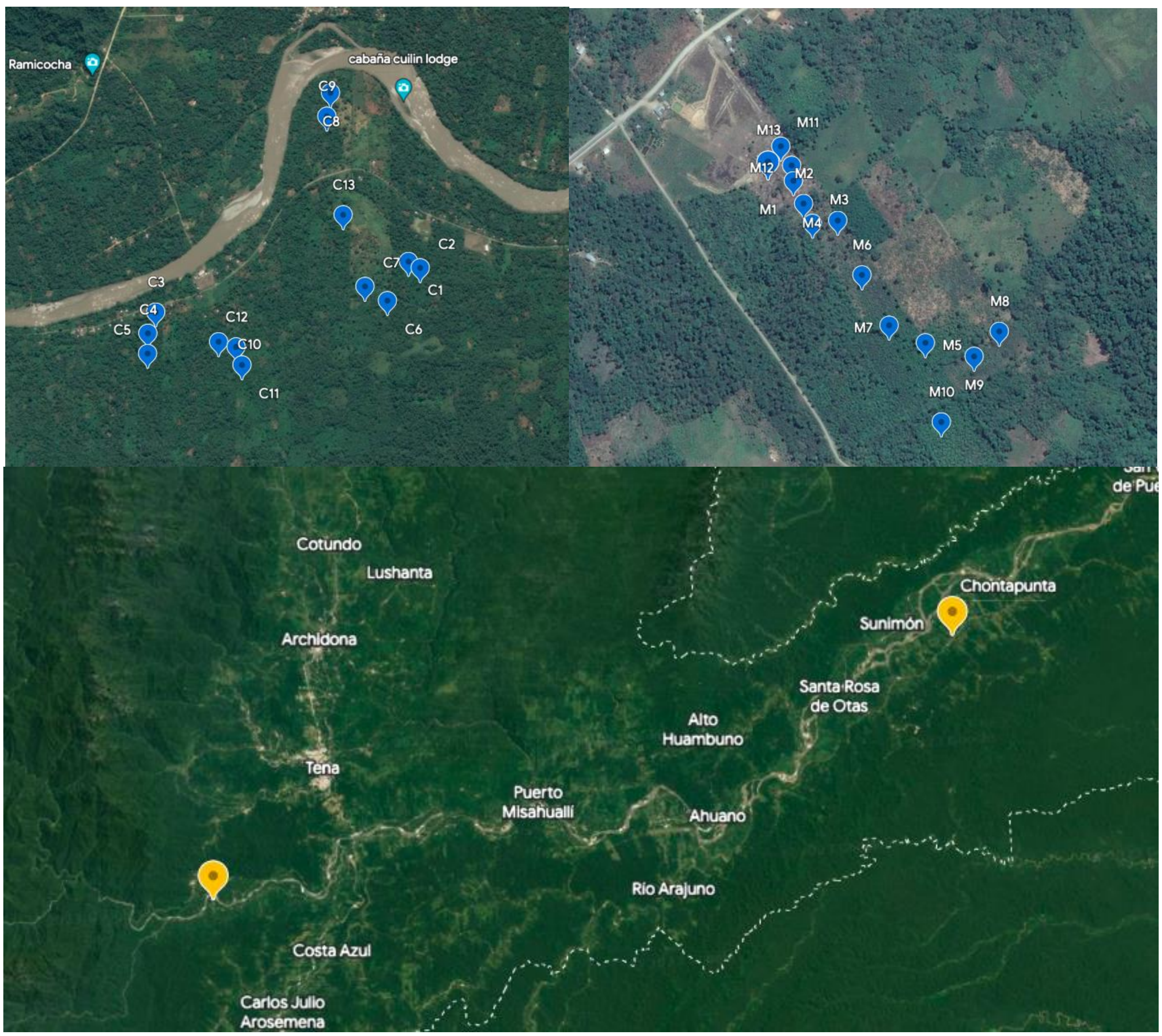


Figure 1.5 Satellite map of all utilized sample sites. Left is chakra systems near Tena, right is monocultures in Chonta Punta, and below covers all sampling sites over a larger view. Sites are numbered chronologically. Chakras are denoted with C and monocultures with M.

Site Descriptions

A total of 15 sites were sampled for chakras and 18 for monocultures, with data collection taking place from late April to early May near the height of the rainy season. 13 sites from each were selected for similar harvest times and varietal consistency to be used in data analysis and comparison. Chakras were located near Tena, Ecuador, South of the Napo River within the rural communities of Cinco de Enero and Seis de Marzo. Average annual rainfall in this area is 4,235mm, with relatively stable low and high temperatures of 18° and 28° C. Altitudes ranged between 500 and 540 meters. Monoculture samples were taken in two neighboring plantations in Chonta Punta, Ecuador with an altitude between 340 and 360 meters, annual rainfall of 2500-3300mm and temperatures of 16° and 28°.

Chakras were selected to be currently maintained agroforests of cacao *nacional* varieties. Tree ages ranged from 15 to 35 years, though it was common to cut trunks and large branches to encourage new growth on older trees. The overstory of *chakras* was dominated by species of the family *lauraceae*, but overstory tree density and diversity varied between farmers and different parts of the *chakra*. Sites were located within 1km of the Napo River. Sites further from the river were always close in proximity to a series of streams 2-3m across. Understory composition ranged from only dead leaf cover to a dense and diverse herbaceous layer. Timing of understory clearing differed between owners, with all sites displaying different levels of growth likely corresponding to the time of the last clearing. All utilized *chakra* sites were harvested 1 to 5 days before data collection, with obvious sick pods also being removed alongside the harvest.

Figure 1.6: Photographs of overstory tree cover on sites …, respectively. These *chakra* agroforests are dominated by laurel species and have variable tree diversity. Understory density is determined by the timing of understory clearing more than method of management.

All monoculture sites cultivated the same variety (CCN-51), had similar tree spacing, and were 2km from the Napo River. All sites utilized Glifopac, paraquat, and Engeo as means of chemical pest control. Tree age ranged from 6 to 12 years. All sites were harvested 1 to 5 days before data collection as part of a mostly consistent 20-day harvest cycle in which highly apparent diseased pods were also removed.

Figure 1.7: Photographs of monoculture sites in Chonta Punta, Ecuador. Differences in the herbaceous layer are due to the timing of herbicide application.

Methodology

A 30x30m quadrat was staked out in each site using a distance tracker app. 15 trees were randomly selected from each quadrat and marked with plant fiber. A representative branch or set of branches was chosen from each tree and the number of diseased and clean pods recorded, with diseased pods being grouped further into black pod, frosty pod, witches' broom, or unidentified/other. The representative branch was then multiplied on a case-by-case basis to represent the total pod count of the tree. Pods on the ground were excluded due to difficulty of identifying and assigning them to specific trees. Diseased pods were identified visually and texturally with the assistance of farmers. When necessary, disease identification was conducted through squeezing or breaking infected pods before disinfecting hands with rubbing alcohol to avoid spreading spores. Pods less than 2cm in length were not recorded due to their identification difficulty. Cacao pods in monocultures were categorized as either cherelle or non-cherelle.

For *chakras*, individual tree variety and age were recorded categorically and numerically, respectively. A rapid assessment of 13 sites was conducted later in the collection process to visually ascertain the percentage of pods in the cherelle stage, the percentage of diseased cherelles to clean cherelles, and the percentage of older diseased pods to older clean pods.

Farmers on each site were asked about management methods including the time of the last harvest, how weed control is conducted, and whether and when they remove infected pods from the trees. Weed control was split into the categories of machete, weed whacker, or both. Harvest timing and pod removal were noted more generally and not as categorizable data points.

In total, data was collected from 15 sites in chakra systems belonging to 7 different owners, and 19 sites from 4 different owners in monocultures.

Analysis

Data was compiled in Microsoft Excel and reviewed to select sites suitable for a comparative study. 13 of the sampled chakra sites were included in comparative analysis due to uniformity of harvest time. 13 monoculture sites were included based on uniformity in variety, management methods, and harvest time. Tree totals per site were 15 each for a total of 195 trees per system for use in data analysis. Totals were compiled using the SUM function, averages with the AVERAGE function, and various divisions of individual categories and blanket totals were conducted to get percentage values for disease composition of each system. Comparative tables displayed total pod count among categories as well as average pod count per tree along these same categories. Comparative charts included total pod count broken into diseased and clean pods, overall clean pod/infected pod status between the two systems, disease composition within diseased pods, cherelle percentage as a proportion of total pods, percentages of clean and diseased cherelles, and scatterplots of pod count values for individual trees. Analysis of *chakra* systems consisted of two parts: a variety comparison and an age comparison. The two main varieties of cacao *nacional* were charted by number of trees, with the same chart being made for 6 tree subvarieties to better represent the variety composition of sampled *chakras.* Another bar chart was made to compare disease composition between the two main varieties. To compare trees of different ages, a table of average values between age groups with sufficient data was constructed, as well as bar charts displaying infection rate and disease composition between ages.

Ethics

This research did not involve the use of human subjects. During this study, no damage was done to sampled cacao trees and surrounding plants were cut only when clearing lines for sample quadrats. Guides, farmers, and hosts were treated respectfully, and permission was obtained from landowners before conducting on-site analysis. Hands were routinely disinfected with an alcohol solution after touching infected pods to minimize the spread of fungal disease by hand.

Results

System Comparisons

According to **table 1**, the *chakra* system had a pod count of 6,597 when accounting for all categories, a significantly higher value than the monoculture total of 5,778, but this number diminishes significantly when accounting only for clean pods. Clean pod count was nearly double the *chakra* value for monocultures, with 2,558 clean pods in 195 trees and 1,411 clean pods for the same number in *chakras*. *Chakras* had a higher count of diseased pods for every category save for other/unknown, since all but 2 pods were fully identified with the help of an experienced farmer. **Figure 2** covered the total diseased pod count between systems compared to their clean pod count. The diseased pod total in *chakras* was significantly higher than monocultures, contributing to the lower clean pod count despite having a higher total pod count.

Table 1: Pod count totals and their classification status

	Black Pod	Frosty Pod	Witches' Broom	Other/Unknown	Clean	Total Count
Chakras	2177	1760	1247	2	1411	6597
Monocultures	539	1625	351	705	2558	5778

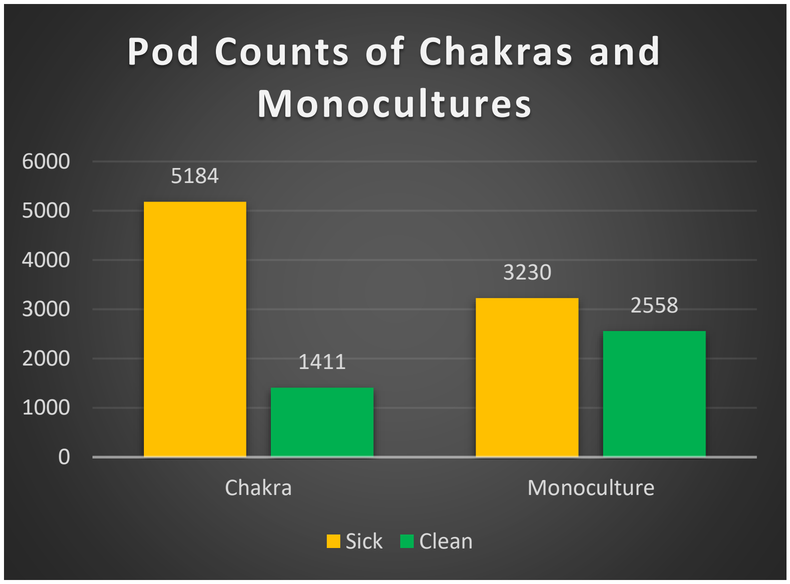


Figure 2: Pod counts of both cultivation forms categorized as either sick or clean.

Nearly every cacao tree had both cherelles and non-cherelle pods. According to **figure 3,** a greater percentage of monocultures' overall pod count was cherelles at 64% and was lower for *chakras* at 55%, though both contained more cherelles than older pods, an indicator that the harvest season will continue for several months more. The greater cherelle percentage of monocultures communicates the potential for a larger future harvest than *chakras* in the coming months.

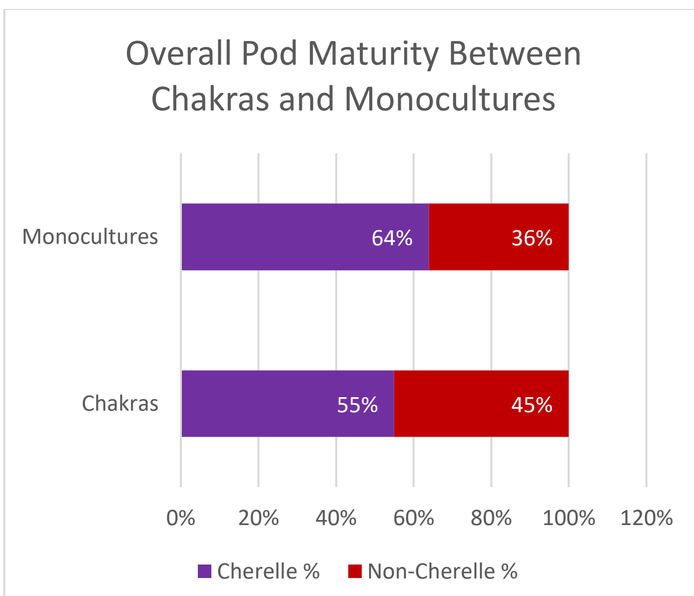
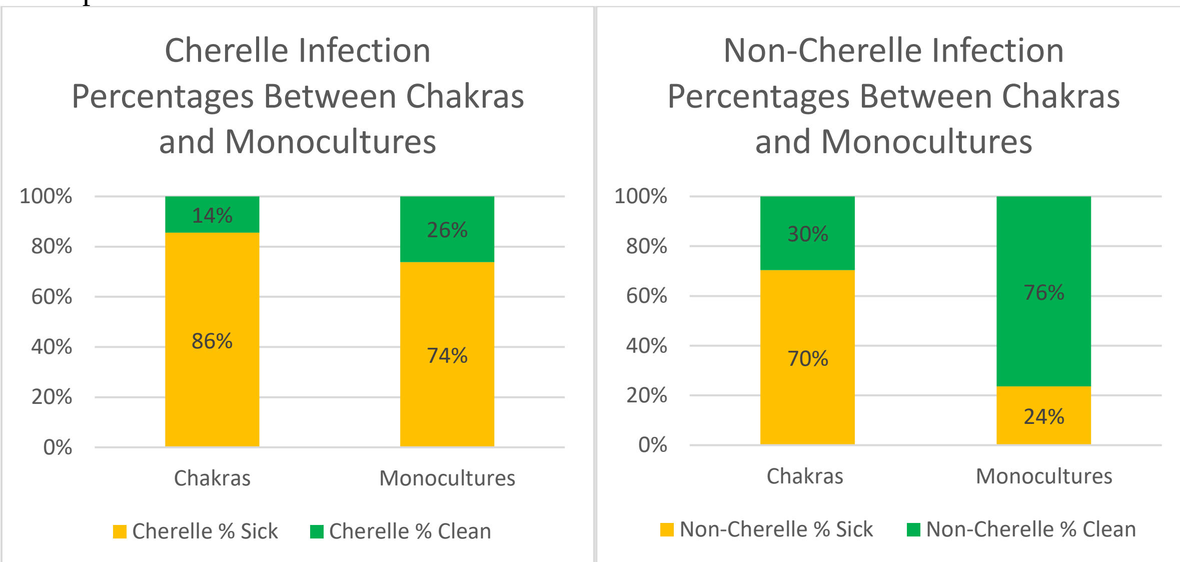


Figure 3: Overall pod maturity levels as seen between *chakras* and monocultures. Cherelles were classified as pods between 2 and 8 centimeters in length, and non-cherelles as greater than 8cm.

As newly developing pods, cherelles are highly susceptible to fungal infection and a host of other pests and diseases, not to mention a high probability of being abandoned by the tree itself. Both *chakra* and monoculture data reflect this increased vulnerability in the infection percentage counts between cherelles and non-cherelle pods, with 86% of *chakra* cherelles being diseased and 70% of monoculture cherelles being found the same. This high loss rate drops slightly in *chakras* and significantly in monocultures when looking at the infection status of non-cherelle pods.



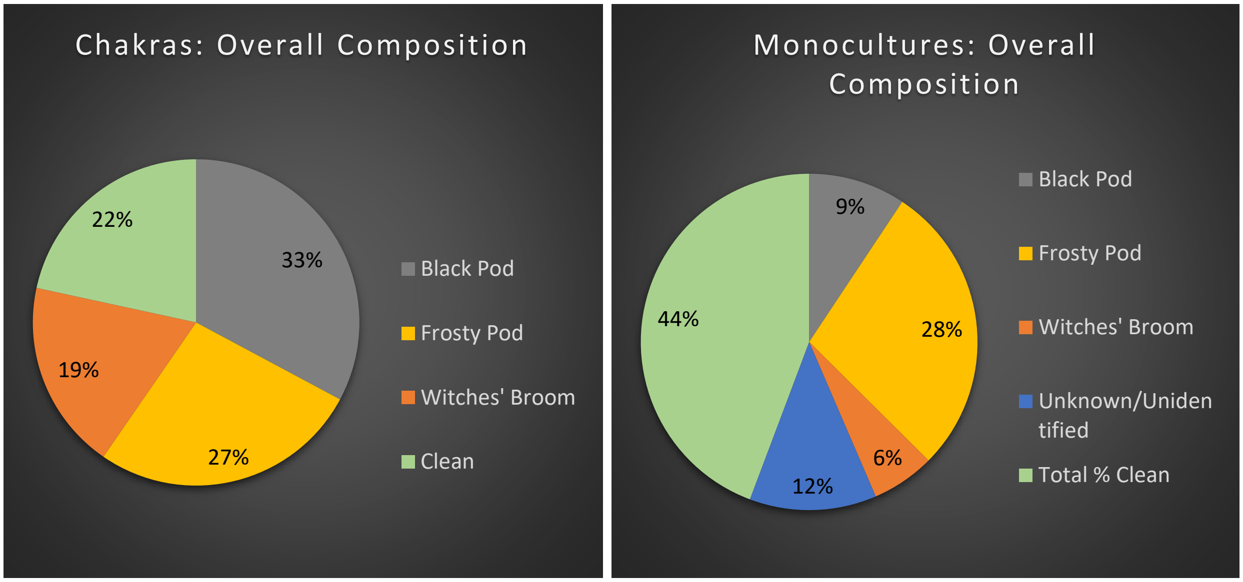
Figures 4.1, 4.2: Overall infection status of cherelles and non-cherelles as seen between *chakras* and monocultures.

Average pod count reflected total pod count in *chakra* and monoculture systems. **Table 2** indicates the average cacao *nacional* tree in a *chakra* system had approximately 4 more pods than the average CCN-51 tree in cacao monocultures and had higher counts of every identified fungal disease.

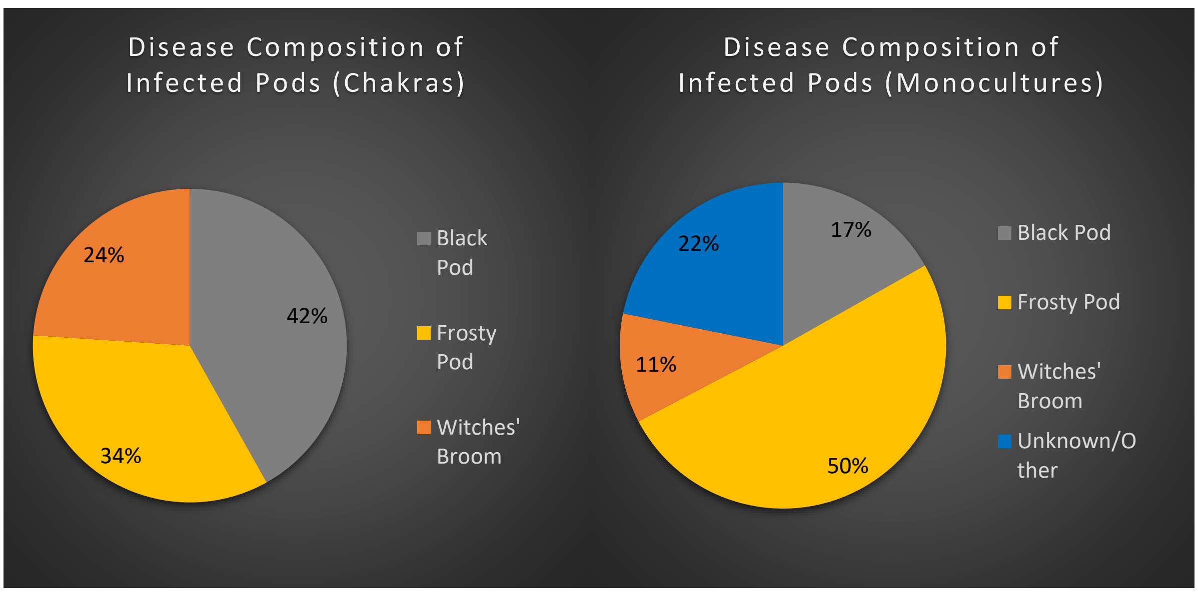
Table 2: Average pods per tree classified by disease status, compared between monocultures and *chakras.*

	Black Pod	Frosty Pod	Witches' Broom	Unknown/Unidentified	Clean	Total
Average Pods Per Tree (Monocultures)	2.78	8.36	1.800	3.615	13.12	29.68
Average Pods Per Tree (Chakras)	11.16	9.026	6.395	0.01026	7.236	33.83

Overall, monocultures had more than twice the percentage of clean pods (44%) than *chakras* (22%) relative to their total pod counts. This higher infection rate in *chakras*, shown clearly in **figure 5.2,** allowed pods contaminated by black pod rot and witches' broom to constitute a far greater percentage of total pods at 33% and 19%, respectively, when compared to monocultures. Monoculture proportions of the same diseases were significantly lower at 9% and 6%, shown in **figure 5.3**. Percentage of frosty pod rot differed by only 1% between systems but was by far the most impactful disease in monocultures.



Figures 5.2, 5.2: The above pie chart represents the total volume of pods occupied by each category. Other/unidentified was excluded from the chakra chart due to negligible presence.



Figures 6.1, 6.2: The above pie charts show the overall composition of diseased pods between chakra and monoculture systems.

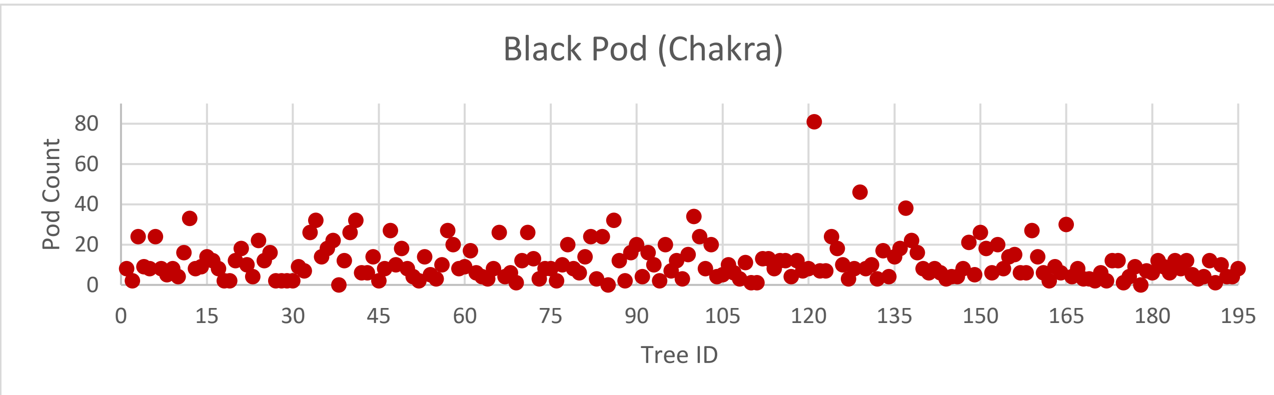
Scatterplots

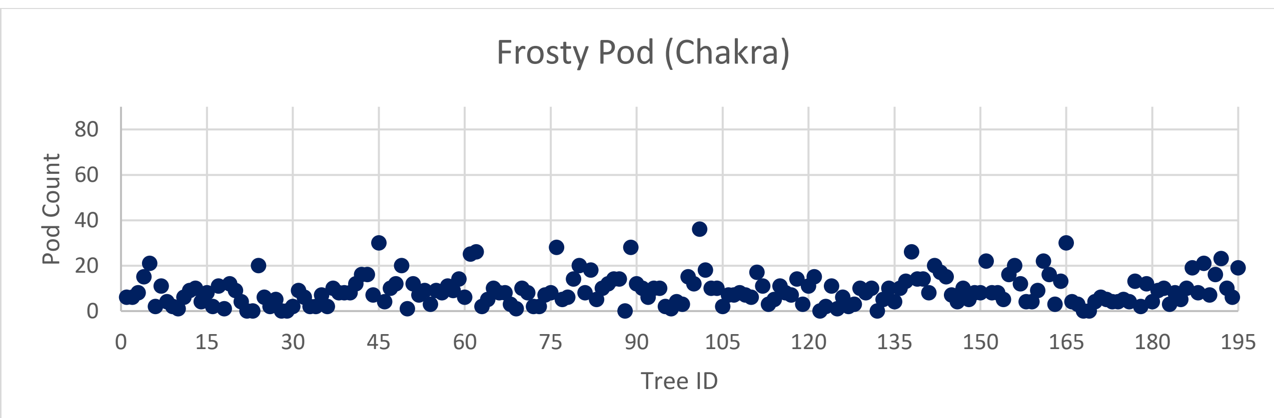
The four scatterplots below correspond to *chakra* system data, in which trees had an average of 33.83 pods per tree. According to **figure 7.1,** most black pod rot infections ranged from values of 2 to 20, with some trees hosting an exceptionally high number of infected pods. The highest count for black pod in a single tree was 81, the vast majority coming from diseased cherelles. This value was an extreme outlier and accompanied another less significant outlier in the same site. 3 sites had counts exclusively under 20, while all others were consistent in their composition: mostly values under 20 alongside a handful of higher-count individuals.

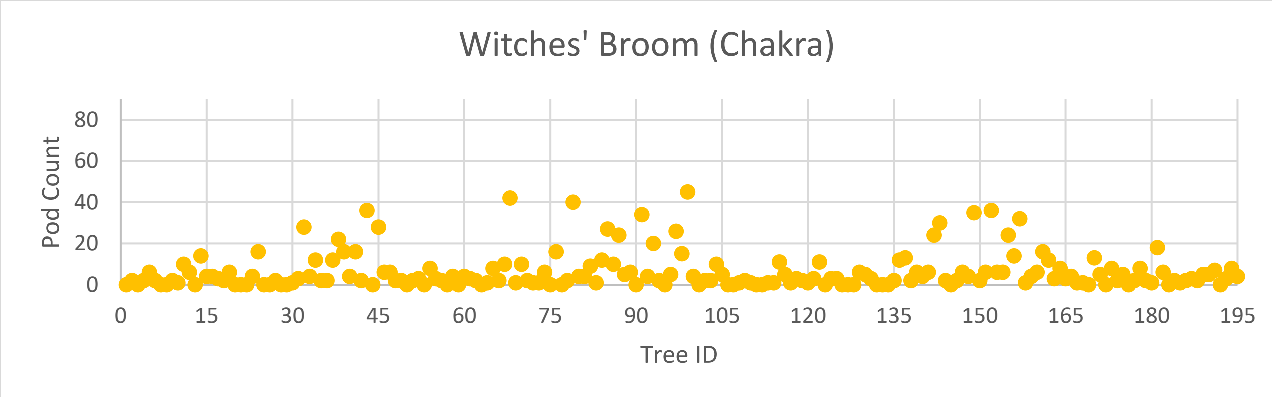
Frosty pod rot in **figure 7.2** displayed similar consistency to black pod rot with a smaller presence of outliers and slightly lower range of common values. Nearly all counts were between 0 and 20 with a diversity of counts within this range being found at each site.

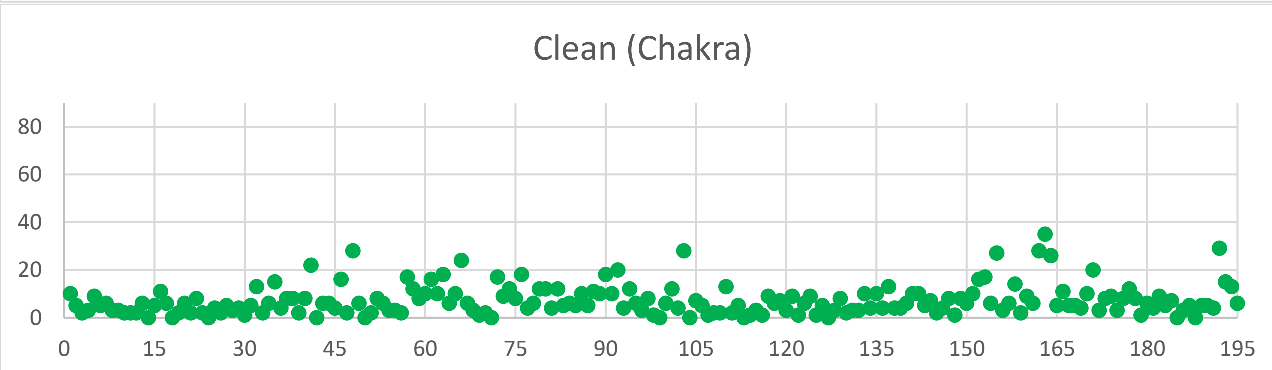
Witches' broom in **figure 7.3** had significantly lower common values, with most counts staying below 10, but also had higher numbers of high values. Most trees had only a few witches' broom pods, but a handful had intense outbreaks in which anywhere from 20 to 45 pods could be lost to the disease. These pods were almost always malformed cherelles found in clumps. The low common values and the high outlier values mean that trees with outbreaks significantly influenced the total count of witches' broom pods and have the possibility to overrepresent the disease.

Most clean pod counts stayed at 10 or less. Counts over this threshold had a diversity of values and were concentrated at sites 3, 4, 5, 7, and 11, according to **figure 7.4**. High counts of clean pods overlapped slightly with instances of intense witches' broom in the same sites, but were often found on different trees.









Figures 7.1, 7.2, 7.3, 7.4: Scatterplots of infections and clean pods on individual trees. X-axis values correspond to the individual tree number, and vertical gridlines create 13 sections representing the 13 sample sites used in data analysis. The Y-axis corresponds to the pod count a given tree for the title category.

The below scatterplots represent data collected from monoculture sites where the average pod count per tree was 29.68. Counts of black pod on individual trees in **figure 8.1** almost never passed 10 and exhibited even lower common values for sites 9 and 10. Multiple values over 10 were found only in sites 5 and 7.

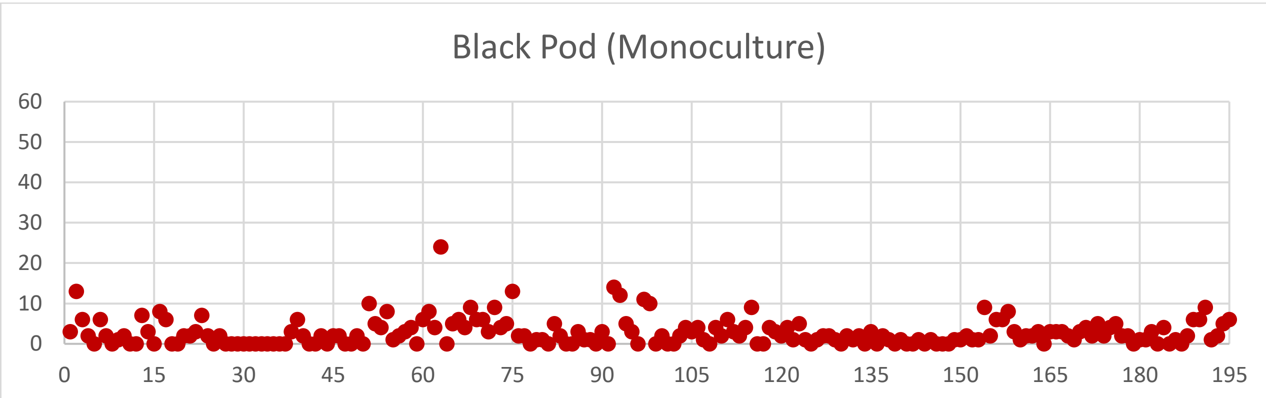
Frosty pod rot had consistently higher values than black pod according to **figure 8.2**, with the average pod having a range of 0 to 20, similar numerically to *chakra* systems but more significant in impact due to the overall lower pod count of monoculture sites. An abnormally low range of values was found in sites 5 and 7, consistent with the pattern of black pod rot in the same site.

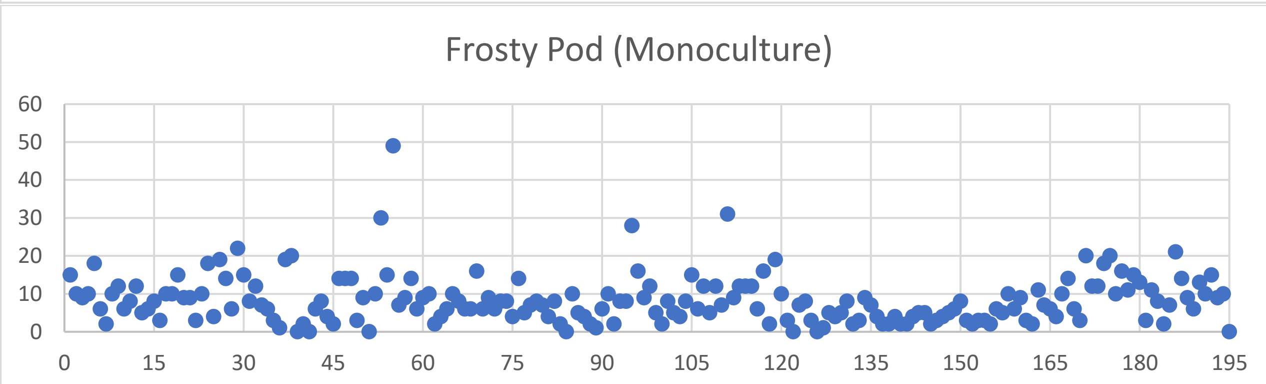
Witches' Broom had the lowest count and most even distribution between trees, seen in **figure 8.3**. Most values ranged from 0 to 5, making the few trees with high counts more influential in affecting the overall disease composition of monocultures. This trend of few pod infections for most trees and intense outbreaks in a few mirrors the results seen in *chakras*, but monocultures contained fewer outbreaks within an identical tree count.

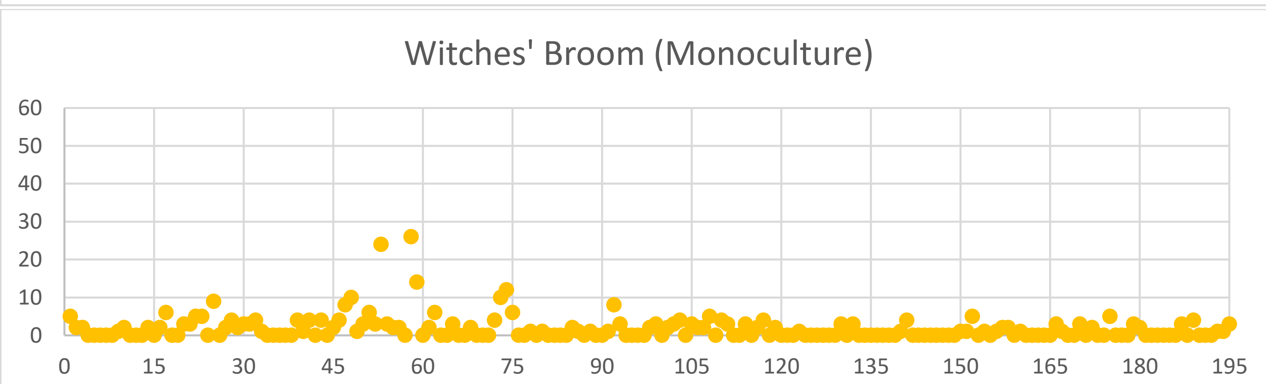
Counts of unknown/unidentified pods in **figure 8.4** had significantly higher fluctuations than the counts of other disease pods. These unidentified pods, all cherelles, had interiors which had been too damaged by time or by insects to accurately identify with limited equipment. The interiors of these pods were most often broken down by a mold different from the three main diseases or by worms or ants. Cherelles eaten on the inside by worms often corresponded to minor leaf damage by the same worms in the crowns of affected cacao trees. The other common cause of unidentifiable pods was colonization by ants who raised their larvae in the hollowed-out pods. Most counts ranged from 0 to 10, and fluctuations within this range of values was consistent with fluctuations of average values for black pod and frosty pod, most obviously seen once again in sites 9 and 10. Outliers and spikes in count did not correspond consistently with spikes in other diseases.

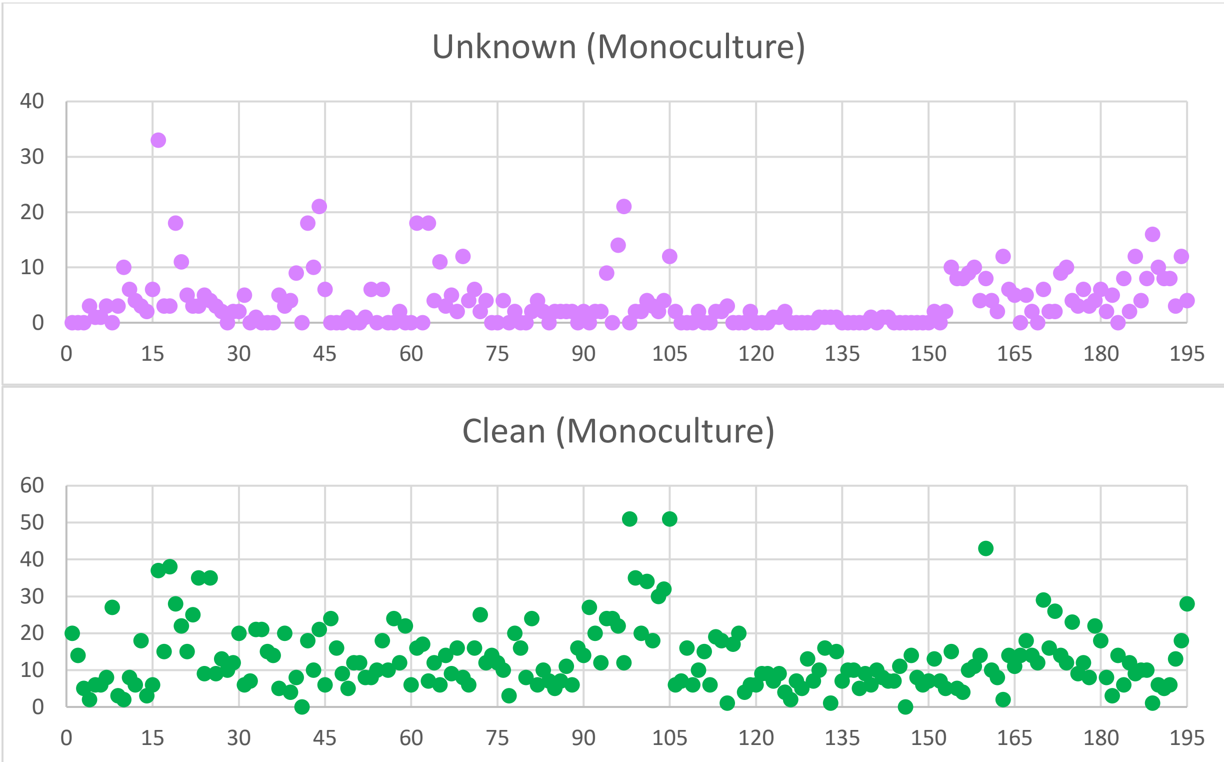
Clean pod counts had a wide range of consistent values, with nearly all counts being between 5 and 30. This diversity of values in **figure 8.5** stays consistent when looking at individual sites, and counts only drop in sites 9 and 10, consistent with the pod count trends of diseased pods in these sites.

A dip in values in monoculture sites 9 and 10 across all pod categories indicates abnormally low total pod counts in these two sites.





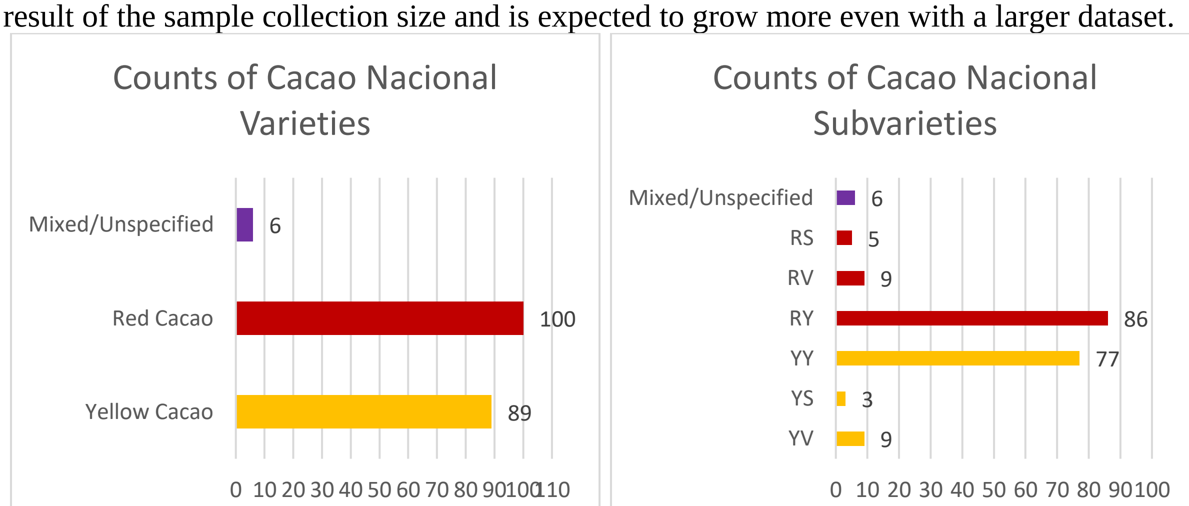




Figures 8.1, 8.2, 8.3, 8.4, 8.5: Scatterplots of tree infections within monocultures for each recorded disease.

Chakra-specific Analysis

The two principal cacao *nacional* types present in the *chakras* were called Cacao Rojo and Cacao Amarillo, among other names, by farmers and guides. While three subtypes existed within each, classified with a secondary Y, V, or S, there was too little data for a comparison between subvarieties. **Figure 9.2** shows that both red and yellow cacao varieties were dominated by the presence of the Yambumuyu subtype, denoted with a secondary letter of Y. Overall, presence of red cacao was 12% higher than yellow when accounting for all sites, but both were prevalent in large numbers in every site. The slight difference in primary variety ratios is likely a result of the sample collection size and is expected to grow more even with a larger dataset.



Figures 9.1, 9.2: Charts of varieties and subvarieties of cacao nacional in chakra systems

While both varieties appeared significantly affected by all three major fungal diseases, **figure 10** shows that slight differences existed in the pod composition of red and yellow varieties. Red cacao was slightly more impacted (35%) than yellow (32%) by black pod rot, while frosty pod rot impacted yellow cacao slightly more at 28%, compared to the 25% loss of red cacao to frosty pod. Yellow cacao was also impacted more by witches' broom (20%), while red cacao indicated a loss of 18% of its total pod count due to the disease. The two slightly lower impacts of frosty pod and witches' broom on red cacao gave it a slightly higher clean pod percentage of 22%. Yellow cacao had 20% of its total pods classified as clean.

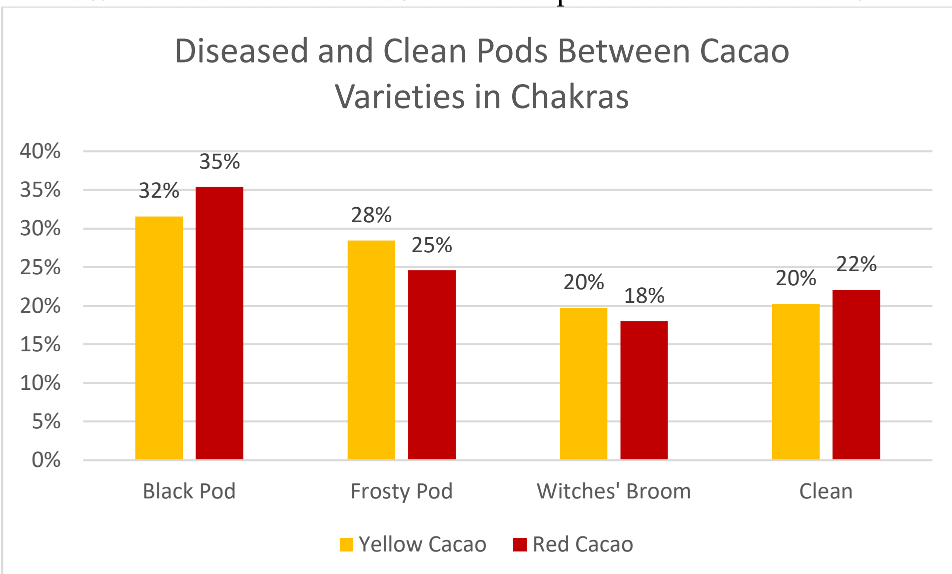


Figure 10: Chart of pod status compared between the two main varieties of cacao nacional in the *chakra* systems.

Chakra systems were found to contain trees of many ages both within and among individual farms. **Table 3** shows the average pod counts for trees of four different approximate age groups covering a 20-year range. Trees approximately 30 years old were excluded due to lack of sufficient data. Trees of approximately 20 and 25 years had the highest count of clean pods and as such the highest potential yield of the four age groups. All age groups shared high infection rates, with black pod always having the highest pod count, followed by frosty pod and witches' broom. Trees of 15, 20, and 25 years had very similar pod totals, while trees of 35 years had a substantial reduction in total pod count. Witches' broom counts decreased with tree age, with the most significant drop corresponding to the overall drop in pod count between 25- and 30-year-old trees, while other diseases rose and fell irrespective of age patterns. Disease trends as percentages can be seen in **Figures 11 and 12.**

Table 3: Average pod counts per tree within different age groups in *chakras*. The oldest age group showed a sizeable decrease in the average pod count.

Age	Black Pod Average	Frosty Pod Average	Witches' Broom Average	Clean Average	Average Total Pod Count
15	12	8.55	7.23	6.10	33.87
20	10.69	8.84	6.89	7.90	34.31
25	10.96	10.25	5.83	7.33	34.37

35	9.03	6.63	4.23	6.60	26.50

Total pod loss fell, rose, and fell again - all by small margins - as tree ages rose. The small differences, small datasets of age groups, and lack of any age-related pattern suggest that no significant difference in overall infection rates between trees of different ages can be ascertained from **figure 11.** A larger dataset would be needed to confirm any potential trends.

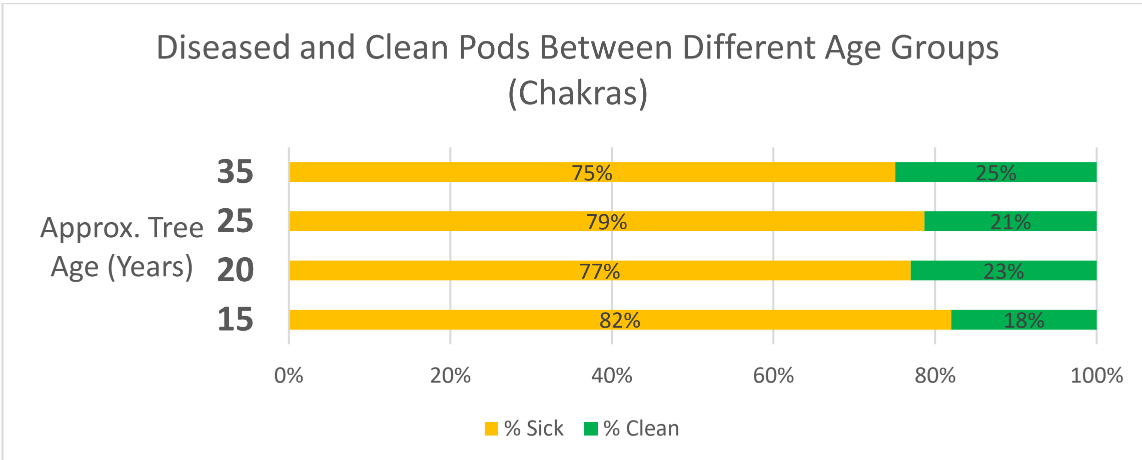


Figure 11: Bar chart comparing percentages infected pods to clean pods among different tree age groups in *chakras*.

Black pod exhibited slight decreases as a percent of overall diseased pods up until the oldest tree category of 35 years. Frosty pod exhibited a slow and then substantial increase (31% to 33%, 33% to 38%) between the first three categories before dropping again in 35-year-old trees. Both these trends follow no apparent pattern. Witches' broom was the only one of the diseases to show a consistent but slight downward trend with tree age. Differences in witches' broom between 15- to 20-year-old trees and 25- to 35- were significantly smaller than the jump between 20 year and 25-year trees, indicating an inconsistent rate of change for the downward trend. Because of this inconsistency, a larger dataset would be necessary to determine if this trend indicates a true relationship between tree age and vulnerability to witches' broom.

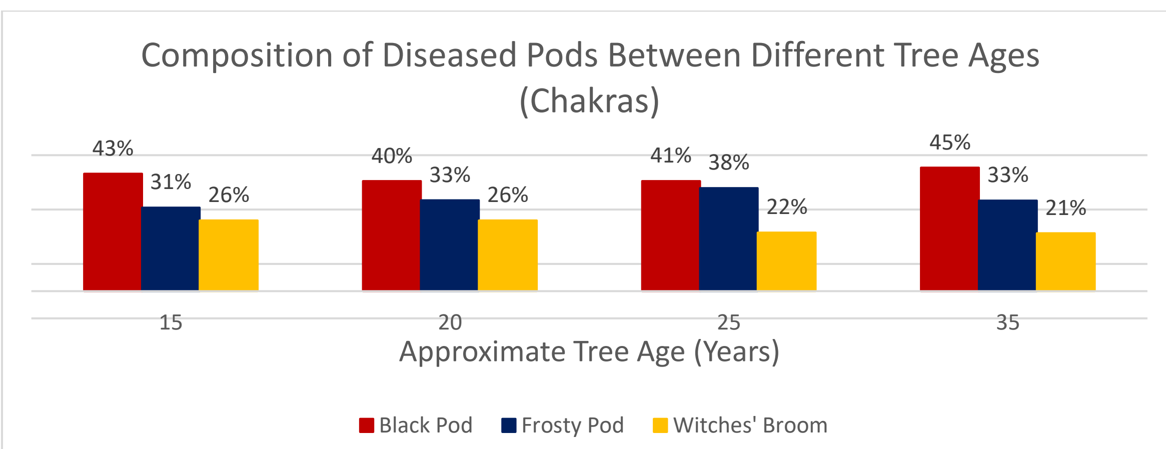


Figure 12: This chart shows the overall disease composition of different age groups of cacao trees in *chakra* systems.

Analysis

Comparative Analysis

Overall, *chakra* systems had both a substantially higher pod count as well as substantially higher rate of infection, such that monocultures outperformed *chakras* in terms of potential yield, in which all recorded clean pods are assumed to have the potential to develop fully into a mature fruit. The size of the difference suggests that multiple factors are responsible for the overall decreased yield.

While increased shade cover in agroforests compared to monocultures are often mentioned by conventional farmers to increase fungal disease incidence by creating favorable microclimatic conditions, literature suggests that agroforests can have equal or lower rates of disease (Armengot et al. 2020, Andres et al. 2016) for reasons such as improved tree health and increased competitiveness of fungal disease antagonists.

The most likely causes of such significant differences in infection rate between *chakra* sites and monoculture sites are precipitation levels, variety differences, and intensity of management. Average monthly rainfall in Tena, the nearest city to *chakra* sites, is about 450 and 490mm in April and May respectively, the months during which this study was conducted. Rainfall in March averages at a slightly lower 370mm. Chonta Punta averages 217, 231, and 151mm in March, April, and May respectively, with peak rainfall occurring about a month earlier than in Tena. Deberdt et al. 2008 found a strong positive correlation between rainfall and incidence of black pod rot when adjusted for a lag period of 1 week. Similar results were found by Leandro Muñoz et al. in 2017 when examining frosty pod rot, which requires a wet surface to germinate and subsequently penetrate the substrate. Witches' broom is also known to rely on moisture for the fulfilment of its disease cycle (Purdy et Schmidt 1996). Both studies are consistent with the disease incidence and rainfall patterns of the two cultivation systems.

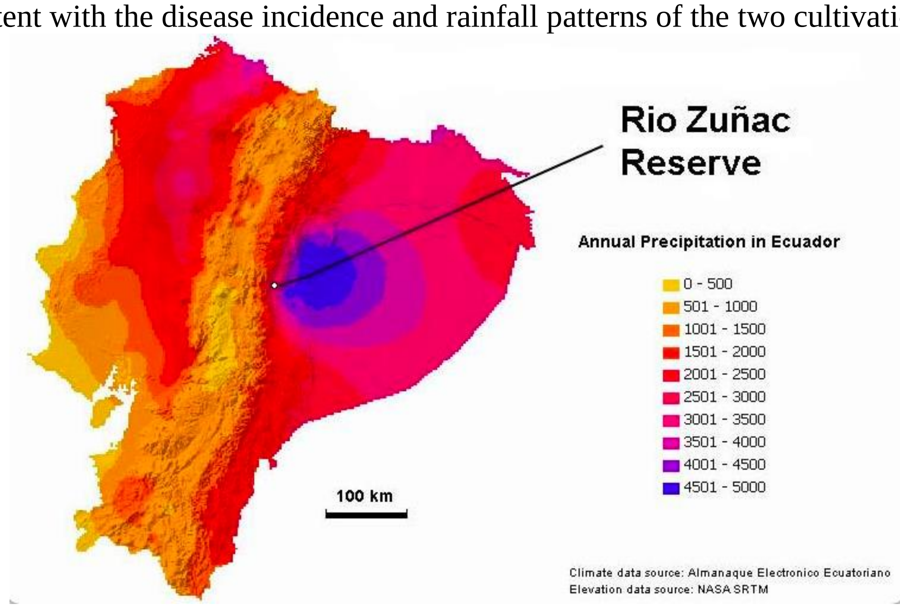


Figure 13: Average annual precipitation in Ecuador (Jost, n.d.). Tena rests in the area of highest rainfall, indicated in dark blue. Chonta Punta lies East in light purple.

Cacao *nacional* has been said to be highly vulnerable to disease, but no scientific sources could be found to confirm this theory. However, the common practice in *chakras* of sowing new cacao by seed does compromise the efficiency of developing and distributing more resistant subtypes of cacao *nacional* due to extensive cross-pollination between cacao trees. Practices such as grafting of more resistant cacao *nacional* varieties onto existing tree stumps would more readily distribute more resistant cacao throughout farms at the price of reduced overall genetic diversity. Breeding programs crossing *nacional* with non-*nacional* varieties can increase resistance, but also have the potential to diminish the fine-flavor quality of pure cacao *nacional* which commands a premium market price (Lerceteau et al. 1997).

Intensity of management could also play a significant role in magnifying observed differences in disease presence. Monocultures were sprayed with herbicide and insecticide and conventional fertilizer applied to trees. All cacao trees were pruned extensively. Cacao was furthermore the only market crop present on-farm, meaning labor was concentrated towards a primary goal of increasing cacao yield. Armengot et al. 2016 found that in terms of pure cacao yield, monocultures were 41% more productive than diversified agroforests, but had diminished returns on labor. Both the agroforests of the Armengot et al. study and this study provided additional non-cacao yields. Pruning and understory management of *chakras* occurred at significantly longer time intervals, and farmers in *chakra* systems divided their time between a diversity of other crops such as yucca and plantain for subsistence and guayusa and vanilla as additional market crops. These same farmers also sometimes split their time further between multiple small farms of varying distances from their house.

Rainfall differences are insufficient to explain the different proportions of black pod and frosty pod between systems, since both are positively affected by increased precipitation. Black pod – particularly in cherelles – was more prevalent than frosty pod in *chakras*.

Higher total pod count in *chakra* systems, alongside only a small associated increase in cherelle wilt when compared to monocultures, suggests that without the presence of fungal diseases or insect pests overall productivity per tree has the potential to be higher in *chakras* than cacao trees investigated in monoculture. This potential for productivity in agroforests on par with monoculture plots is supported by other studies but contradicted by others (Blaser et al. 2018). Andres et al. 2016 found in a comparative study between monocultures and dynamic agroforests that productivity could reach similar or higher levels in agroforests compared to monocultures through a reduction in disease and soil problems. Preliminary pod counts in the *chakras* and monocultures of this study suggest the same result is – at least theoretically – possible, but the complexity of ecological management can work hard against making this a reality. Blaser et al. 2018 found that moderate- to low-shade agroforests were not detrimental to pod production as compared to monocultures and emphasized that agroforestry systems inherently compromise on high short-term productivity in exchange for providing other services such as extended productive lifespan, diversified income, utility of a diversity species in a small piece of land, and biodiversity and climate benefits.

Witches' broom was unique among the observed cacao diseases due to its highly uneven distribution between trees. In both systems the disease ranks as the least significant in terms of damaged cacao pods, and even this significance has the potential to be artificially inflated by the overcounting of severely affected trees in the random sampling process. Purdy and Schmidt 1996 found accurate quantification of infection intensity in witches' broom to be a difficult task, due also to the degree the fungus affects more than just the cacao pods. The very low presence of infected pods on most trees in both systems, however, continues to suggest the greater significance of black pod and frosty pod rot in limiting the development of clean, mature cacao pods.

Unknown and unidentified cherelles in cacao monocultures were classified as such due to interior damage, most often caused by insects such as ants and worms. However, it is impossible to say whether these observed insects were the causal agent of pod death, as insects have been found to be responsible for only a small proportion of cherelle deaths (Melnick 2016). The higher pod-eating insect presence and diversity seems a possible explanation for why the primary form of pest control in sampled monocultures was insecticide rather than fungicide. Fewer insect pests both within and outside of pods were observed during data collection in *chakras.* A study concentrated in insect pest presence between *chakras* and monocultures would yield more definitive results around this topic.

Chakra Systems Analysis

Differences in disease composition between cacao nacional of different varieties are difficult to assess due to genetic variation resulting from sowing cacao by seed, the most common practice of Kallari's *chakra* systems for developing new cacao agroforest. If a correlation exists between the observed slight differences in disease pattern between yellow and red cacao nacional sown by associates of Kallari, it cannot be confirmed without the collection of more data.

Farmers communicated that increased tree age in *chakras* was positively associated with disease resistance and negatively associated with pod production. Results from a wide variety of cacao tree age groups communicate a degree of truth to this belief, with the oldest age group of 35 years having both the lowest infection rate (75%) and the lowest total average pod count (26.5). However, fluctuations of infection rates with age reduced the strength of this hypothesis and suggests that a larger dataset is necessary to determine if older cacao trees in *chakra* systems are truly more resistant to disease in a way which compensates for their lower total pod procuction. The observation of reduced pod count in 35-year-old trees is consistent with some of the literature comparing cacao yield to tree age, but it is important to note the extraordinary differences in productive lifespan across cacao varieties and cultivation systems. Studies have shown cacao under shade conditions to have a longer productive lifespan (Obiri et al. 2007), and literature points to yield declines after 30 years in good soil conditions (Somarriba et al. 2021), consistent with observed trends.

Chakra System Management Suggestions

Fungal disease analysis of *chakra* systems in the region covered by Kallari suggests that cacao yields are severely limited by the presence of black pod rot and frosty pod rot, primarily. This leads to reduced farmer income and limits the growth of the organization by directly affecting the quantity of chocolate it can produce and sell in local and international markets. Effective disease management is imperative to protect the livelihoods of Kallari's associates. Moreover, these management practices must align appropriately with the mission of Kallari and the cultivation standard of Kallari associates, who must produce their product without chemicals, conserve forest on their property, and produce using the *chakra* system. Cultural management practices – mainly consistent removal of infected pods and construction of drainage systems – have been proven to strongly reduce the intensity of cacao diseases regardless of the cultivation method (Armengot et al. 2020). Drainage systems were not evident in the sampled *chakras* and heavy rains resulted in temporary inundation of some sites during the rapid assessment phase of the project, suggesting that some if not all sites could benefit from the construction of simple drainage systems to reroute standing water.

Infected pod removal is already conducted alongside harvests, but the benefits conferred by this method have been found to change significantly with the frequency of pod removal. Armengot et al. 2020 found that harvest and diseased pod removal every 15 days led to increased productivity compared to harvests and removals every 25 days. A study conducted in Peru found furthermore that weekly infected pod removals to confer significantly more benefit than fortnightly pod removal, and that the resulting yield increase more than compensated for the extra labor (Soberanis et al. 1999). Pod removal in *chakras* is often done alongside harvests, which are done one to three days prior to being purchased by Kallari. These purchases occur every 15 days for the duration of the harvest season, February to June, for *chakras* of cacao *nacional*. Promotion of weekly harvests by having Kallari purchase less cacao more frequently could, if technically and financially feasible, offer an effective system-wide improvement to disease management by associates of Kallari. The simple promotion of more frequent pod removal by Kallari alongside the organization's existing suggestions for associates is also necessary for expanding disease management practices.

Biocontrol of major cacao pathogens using strains of endophytic bacteria and fungi have been researched extensively but is far more difficult than cultural control to use successfully. Farmers must have the capital to invest in biocontrol agents and the equipment to facilitate inoculation of the host plant. In the case of frosty pod rot, multiple strains of the endophytic fungi genus *Trichoderma* native to the Amazon Basin have been tested as successful inhibitors of *Moniliopthera roreri* (Leiva et al. 2020). Another strain of *Trichoderma, T. martiale,* was observed to be successful in reducing black pod rot in inoculated cacao pods (Hanada et al. 2009). Another promising study on black pod rot biocontrol points to the related tree *Theobroma grandiflorum* as a potential host of antagonistic endophytic fungi (Hanada et al. 2010). *T. grandiflorum*, commonly called copoazú, cacao blanco, or pata, is currently being developed by Kallari as a market crop. These studies conducted their research using the *Phytophthera palmivora* strain of black pod, a strain common in the Amazon and the possible culprit of infections in *chakra* systems (Zentmyer 1988). The greatest logistic difficulty of applying biocontrol in the context of a smallholder agricultural cooperative like Kallari is obtaining enough culture for practical use in field application. If biological control through the dispersal of beneficial inoculum on cacao trees is to become a practical disease management measure in this context, a consistent and economic source of inoculum is required, be it purchasing inoculum externally or developing infrastructure to produce it on-site. Either option will be costly to implement, especially in the initial phase, and pilot studies are necessary to determine the actual effectiveness of these biocontrol agents when applied to the farms of Kallari associates.

Conclusion

Main Findings

Overall, it was found that *chakra* systems belonging to associates of Kallari were highly vulnerable to fungal disease, resulting in most pods being lost mainly to black pod and frosty pod rot. Compared with monocultures, about half as many of the total produced pods produced by sampled trees were observed to be clean. The significant difference in disease susceptibility is most likely explained by the 3 factors of rainfall differences, management intensity, and differences in cacao variety. Increasing management intensity while maintaining organic farming practices in *chakra* systems is a conceptually simple solution but must consider the physical cost of increased labor in relation to the overall yield increases management practices such as drainage construction and more frequent pod removal can provide. Given that *chakras* preserve both biodiversity and indigenous culture, disease management practices which align with both is an important and underexplored field. Biocontrol agents originally isolated from the Amazon provide a promising source for reducing pod susceptibility to disease, but inoculating trees is dependent on access to biocontrol agents and distribution tools, both of which are a significant investment unachievable by individual farmers.

Chakras and monocultures also differed in the disease responsible for the greatest pod loss, with few answers able to be found given the similar physiology of both black pod and frosty pod, the most damaging diseases for *chakras* and monocultures respectively.

Differences in pod count point to the potential for cacao *nacional* grown in *chakra* systems to be immensely productive if fungal disease can be sufficiently and consistently contained. This productive potential can only be realized through the continued experimental application of organic disease control methods, the forms of which could be many.

Relationships between cacao tree age and variety in *chakra* systems could not be fully extrapolated on due to smaller groupings of data, and there is a wealth of possibility for future studies which could shine light on these relationships as well as on other existing cultural management practices which have the potential to reduce incidence of fungal disease in *chakras*.

Sources of Error

Counting pods from a proportion of branches on each tree came with the risk of improperly multiplying this count, especially in the highly heterogeneous *chakra* systems. Effort was made in instances of highly heterogeneous composition to count every pod or only multiply appropriate pod categories. Distinguishing black pod rot and frosty pod rot grew difficult when observing very young small pods too high up to be touched, as the two often look very similar in pods that are just starting to develop. Furthermore, while counting pod infections is quick and effective in determining disease trends in cacao trees, black pod rot and witches' broom are both diseases which extend beyond just cacao pods, and as such their negative effects might also manifest through reduced levels of flowering and fruit bearing, indirectly contributing to cherelle wilt and subsequent fungal infection. Differences in average rainfall, a significant factor in the propagation of fungal disease, added to the difficulty of a direct comparison between monocultures and *chakras* and unfortunately muddled the significance of other potential disease and resistance factors.

This study was limited by its time frame and its seasonal context. Data in chakra sites was collected after a major harvest of cacao in which many owners also removed many of their infected pods. This could have affected the ratio of diseases.

Future Studies

This study contains a multitude of variables within *chakra* systems of cacao nacional which must be investigated further to determine the extent of their relationships to fungal disease, cacao yields, and each other. Farmers consistently mentioned the mode and frequency of understory control to be an important factor in the management of fungal diseases, and significant variation was observed in the frequency of these understory clearings. A temporal study tracing understory herbaceous presence with disease incidence between plots of different clearing frequency would shine more light on the true relationship between the herbaceous layer and cacao disease and productivity in an Amazonian climate. There is precedent for similar studies in different environments (Deheuvels et al. 2012). Moreover, a study on herbaceous layer succession under different clearing practices, weed whacker or machete, could provide valuable information on the influence of weed management on understory diversity and the *chakra's* ability to act as a refuge for plant biodiversity.

Farmers indicated that shade trees increase the health and overall disease resistance of mature cacao nacional. These statements were contradicted by a farmer at the monoculture sites, but shade conditions have been shown to reduce disease incidence in several previously mentioned studies (Armengot et al. 2020, Andres et al. 2016). The variable levels of shade cover in *chakras* of cacao *nacional* are conducive to a study analyzing disease presence and overall cacao yield between shade levels in agroforests.

Data on tree age, when split into age groups of 5-year intervals, proved insufficient to confirm or deny potential relationships between tree age and disease vulnerability, even while pointing to decreased pod production after 30 years. As such, a study dedicated to discovering the nature of these relationships for cacao nacional grown in *chakra* systems would be a useful tool for determining the optimal productive lifespan of these agroforestry systems.

This study's data collection was limited to 2.5 weeks, and as such was unable to cover the duration of the growing season and contained no information on disease presence during the dry season in Tena, in which rainfall levels are still very high. A similar study to this, conducted on a longer timescale and covering more sample sites, could track disease presence temporally to better understand how fungal diseases respond to the climatic and microclimatic variables present in *chakras* located near Tena.

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Appendix A: Pod Disease Photo Gallery

Damaged cherelles, though lost primarily to cherelle wilt, were nearly always infected by fungal disease, and could consequently be classified as both. No photos of ants in pods were taken due to their aggressive nature after being disturbed.

Black Pod Rot (Phytophthera spp.)

Frosty Pod Rot (Moniliopthera roreri)

Witches' Broom (Moniliopthera Perniciosa)

Unknown/Unidentified

Cherelle Wilt

Appendix B: Tree Varieties and Subvarieties

CCN-51

Nacional YY (Amarillo Yambumuyu)


Nacional YV (Amarillo Volamuyu)


Nacional YS (Amarillo Sacumuyu)


Nacional RY (Rojo Yambumuyu)


Nacional RS (Rojo Sacumuyu)

Nacional RV (Rojo Volamuyu)