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Research Report

Pollination Networks of Cacao Agroforestry Systems in Colombia

Felipe Sarmiento Gómez

Research Advisor: Dr. Scott J. MacIvor

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UNIVERSITY OF TORONTO

Abstract

Cacao agroforestry systems (CAS) have a great potential as a sustainable economic activity for smallholders that restores unprotected human-impacted forest areas, particularly in Colombia. The viability of this initiative is limited by low fruit set rates: there are several knowledge gaps of cacao's pollination networks. Thus, we aimed to answer what is the effect of flower visitors in fruit set of cacao trees from a Colombian agroforestry system? To do so, flowers of nine trees were isolated, left open, or manually pollinated, and fruit set was monitored. Additionally, cameras were set up to take photos every 10 seconds of some flowers for visitation observation. In parallel, visitor collection was done during sunrise, afternoon, and sunset. A total of 330 flowers were observed and 57 visitors were collected. Preliminary results show manual pollination results in higher fruit set rates than natural pollination, and a likely effect of bee colony proximity and shade cover on fruit set. Photo analysis of flower visits, along with DNA barcoding and pollen load analyses on collected visitors, will allow the proposal of management strategies at the farm level that increase pollination success.

Keywords: cacao, agroforestry systems, pollination, fruit set, Colombia

Introduction

Agroforestry systems are gaining importance in the agrifood sector as a sustainable alternative to conventional crop systems, as the integration of crops with trees results in multi-faceted benefits. These systems can reach 50 to 80% of the biodiversity of natural forests, while providing important ecosystem services and improving the livelihood resilience and food security of farmers (Food and Agriculture Organization [FAO], n.d.). An important instance of this approach are cacao agroforestry systems (CAS). In particular, given that the tree is native to

the Amazon, these systems are rapidly gaining importance in Latin America (Vandromme et. al., 2023). Since in this region most cacao producers are smallholders, CAS are a sustainable way of living for them, as they restore human-impacted forest areas that are unprotected with a productive activity (Vansynghel et. al., 2022).

Colombia stands out as a relevant country in the region, since cacao is a solid alternative to economic activities that require deforestation of jungle areas, contributing to the consolidation of peace. After the 2016 Peace Accords with the world's oldest guerrilla at the time, the Colombian Revolutionary Armed Forces – People's Army (FARC-EP in Spanish), deforestation in the country has risen due to illegal crops, extensive ranching, and land hoarding (Wight, 2023). Further, areas in the country that are affected by armed conflict, poverty, institutional weakness, and illegal crops, usually coincide with areas of dense forest cover and cacao production. (Arenas et. al., 2023). CAS then represent an important strategy in restoration of Colombian forests and ecosystem services, while they replace illegal crops and unproductive agricultural systems (Arenas et. al., 2023).

A great limiting factor to this initiative is the lack of understanding of pollination dynamics in cacao. Less than 10% of total flowers in a tree are successfully pollinated in natural conditions (Toledo-Hernández et. al., 2017). In particular, Andean countries report significant low fruit set rates (Vansynghel et. al., 2022). For Colombia, there are reports of a single tree bearing 15.000 to 120.000 flowers per year, and the production target is reached when harvesting 40 fruits per year, which translates into 2000 kilograms produced per hectare in a year; however, the national production average is less than 500 kilograms per hectare per year (J. P. Gil, personal communication, July 10, 2024). Thus, fulfilling knowledge gaps in pollination and fruit set of cacao trees is crucial for the viability of projects that improve the livelihoods of rural

smallholders, while restoring valuable ecosystems such as the Amazon (Vansynghel et. al., 2022; Vandromme et. al., 2023).

These knowledge gaps include the identity of cacao pollinators, which was thought to be midges, but a great variation shown in studies of visitation patterns in different cacao-growing countries suggest there are several other arthropod visitors that contribute to pollination (Vansynghel et. al., 2022). This is why studying patterns of flower visitors across different geographies is crucial to resolve the pollination potential of different insects and consequently improve pollination services. Furthermore, there is no research that has recorded pollination success dependent on species' identity, for which tools such as videorecording have great potential (Toledo-Hernández et. al., 2017). Moreover, it is important to determine how landscape, environmental conditions, and agroforestry management impact pollinators (Toledo-Hernández et. al., 2017).

In this study, we aimed to answer what is the effect of flower visitors in fruit set of cacao trees from a Colombian agroforestry system? To do so, three specific objectives where formulated: identify cacao's flower visitors, establish visitation patterns (in relation to weather, shade, time of day, etc.), and measure pollen loads of cacao's visitors. Here we present the preliminary findings of our field work which, along with subsequent analysis, will allow us to propose management strategies and research avenues that increase pollination success and make more productive agroforestry systems.

Materials & Methods

Study site

Field work took place in Granja Luker, a 22-hectare research centre of cacao agroforestry systems near the town of Santágueda, in the department of Caldas, Colombia (5°04'13.6"N 75°41'07.6"W, 1.050 m.a.s.l.). The area is part of the tropical dry forest of the Cauca River zonobiome, whose rainfall oscillates between 1.500 and 1.800 millimeters per year, split between two rainy seasons: from April to May and from October to November, in which 70% of the total annual rainfall occurs (World Wildlife Fund – Colombia [WWF – Colombia], 2013). The flowering seasons of cacao usually occur during and after the rainy seasons.

At the research centre, all observations took place in a single straight furrow of the clonal nursery, corresponding to the Imperial College Selection 1 clone (ICS-1), which is auto-compatible (Federación Nacional de Cacaoteros [Fedecacao], 2022). The clonal nursery entrance is in the south, and the plot extends northward. Trees were numbered starting from the entrance towards the north, and they were 2.6 meters apart from each other. Observed trees were numbers 1, 2, 4, 6, 8, 10, 11, 13, and 15. To the west, there was a furrow with the clone FEAR-5, then a furrow with FSA-13, and then timber-yielding trees that started perpendicular to trees number 9 and 10, going all the way until tree 16. To the east there was a furrow with the clone TCS-01, then a furrow with TCS-06 and another one with SCC-19, and then timber-yielding trees that started perpendicular to tree number 4 and went all the way until tree 16 too.

Distances between furrows was 3.2 meters, and further east and west of the timber-yielding trees the clonal nursery extended with more cacao clones. South of the clonal nursery there was a plot with plantain, and northwards of the clonal nursery there is a plot with old cacao trees (>50 years old) and other fruit trees. At the beginning of the studied furrow, there was a natural colony of *Tetragonisca angustula* stingless bees in a fence of *guadua* bamboo.

Data collection

The experimental work was done from July 8th to July 26th, 2024. For each of the nine observed trees, at the beginning of every week, 10 flower buds that were just about to open were selected and labelled. This was done before sunset as flowers usually open during the night (Jaramillo et. al., 2024). Randomly, three flowers were assigned to three distinct categories, and the tenth one was observed with a camera. The categories were: closed observation (negative control), for which the flowers were isolated from visitors with a plastic tube and plasticine; open observation, for which the flowers were untreated; and manual observation (positive control), for which the flowers were manually pollinated.

Two distinct camera types were used: 5 GardePro T5CF cameras that recorded day and night, and 4 Wingscapes TimelapseCam Pro cameras that recorded only during daytime (but had a better image of a single flower). They took photos of the observed flower every 10 seconds. The flowers observed with cameras were left untreated. Individual observations of flowers finalized once they abscised or set fruit. Given that the flower lifetime of a cacao flower varies between 36 hours and 72 hours, some flowers observed with cameras finalized their observation earlier and a second set of 10 flowers could selected for a new observation in the same tree in a single week. Additionally, data from the research centre's weather station will be obtained, which will contain meteorological data of the site every 30 seconds during the entire period.

In parallel, visitor collection was done in the same time period on Tuesdays, Wednesdays and Thursdays. It consisted of 50-minute surveys at sunrise (between ~5:40 a.m. and ~6:40 a.m.), afternoon (between ~2:00 p.m. and ~3:00 p.m.), and sunset (between 5:40 p.m. and 6:40 p.m.), in the same studied furrow. A frontal red-light was used in low-light conditions. The researcher looked for visitors that were at or near reproductive flower parts (i.e. stigma, anther, petal, or staminode) all along the trees of the furrow, so that the collection was narrowed to

potential pollinators. They were collected with a modified Mini Cordless Car Vacuum aspirator. Time, tree, and part of the flower in which a visitor was collected were registered. Collected visitors were placed in a portable freezer for immobilization, and subsequently stored in 98% ethanol.

Data analysis

Flower visitor images will be analyzed using a software with a machine-learning algorithm that will be trained through the observation of a fraction of the photos. The algorithm allows determining visitation rate per flower and type of visitor (whenever the image quality allows so) at the different times of the recording.

A pollen load analysis will be performed on the collected visitors to confirm presence of cacao pollen. Further, DNA barcoding will be performed on the individuals for identification, which will be done at the Unit of Ecology and Systematics (UNESIS) of the Pontificia Universidad Javeriana in Bogotá, Colombia.

Results and Next Steps

A total of 329 flowers were observed, of which 13.98% (46 flowers) set fruit. Preliminary reports show that only 4 of those flowers (i.e., 1.21% of the total) are still growing fruits; all others have been aborted. None of the isolated flowers set fruit, while manually pollinated flowers had more than double (32.3%) the fruit set rate of naturally pollinated flowers (14.1%) (Figure 1). The second week of observation presented the highest fruit set percentage (17.27%), while the first and last week had a fruit set rate of 10% and 14%, respectively (Figure 2). Fruit set rate per tree had a great variation, ranging from 6.6% to 25%, with trees located towards the

center of the furrow having relatively less fruit set than those on the south and north ends (Figure 3).

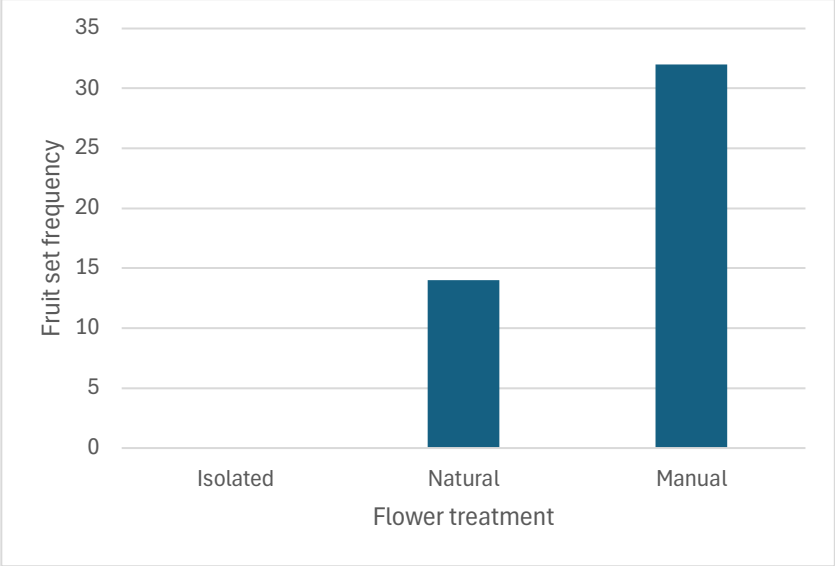

Figure 1. Frequency of fruit set in cacao trees per flower treatment for the entire observation period (July 8th to July 27th, 2024). All treatments had a total of 99 observed flowers.

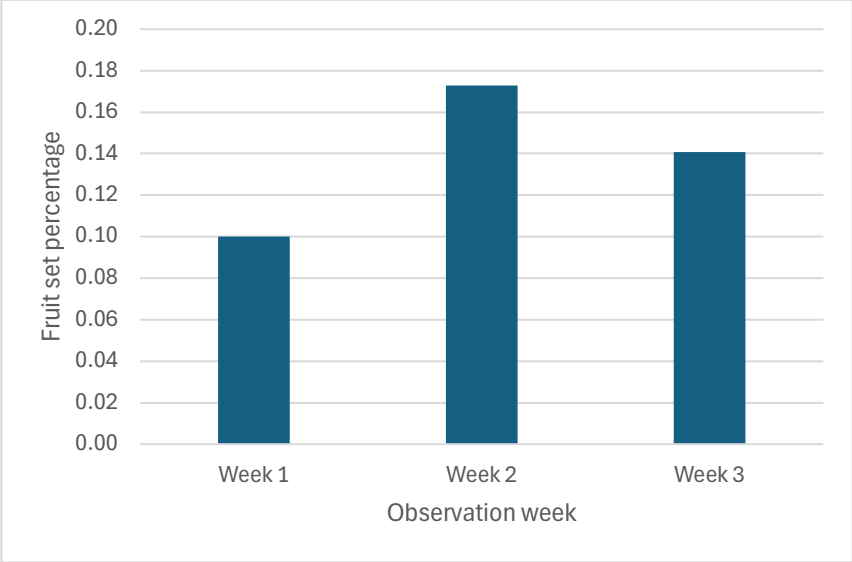

Figure 2. Percentage of fruit set among flowers of cacao trees from each observation week (Week 1: July 8th to 12th, Week 2: July 15th to 19th, Week 3: July 22nd to 27th).

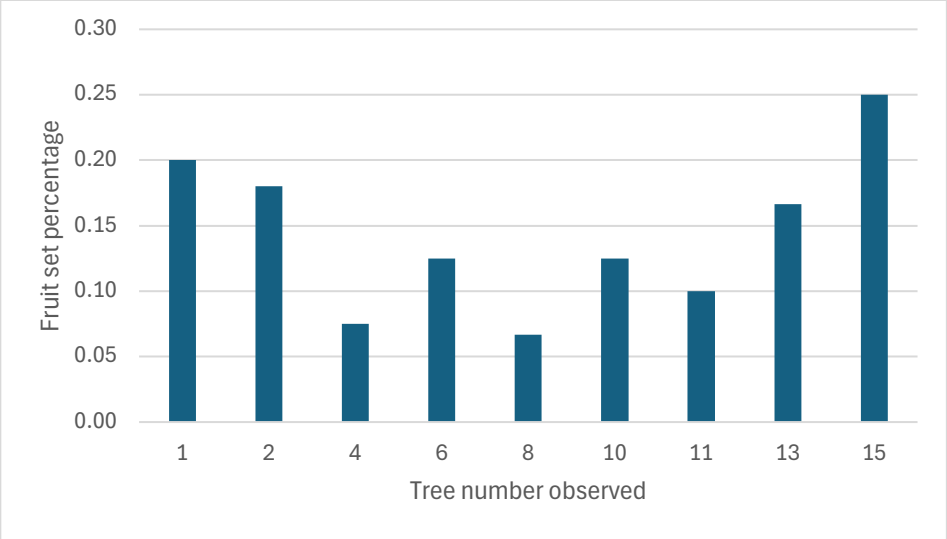

Figure 3. Percentage of fruit set among flowers of each observed cacao tree in the entire observation period (July 8th to July 27th, 2024). Trees were numbered from south to north.

Additionally, 57 flower visitors were collected in the observation period. The afternoon and sunset intervals showed similar frequencies of collected visitors, while the morning interval time had significantly less visitors (Figure 4). The first week had the most collected visitors, followed by the third week and lastly the second week (Figure 5). Regarding flower parts, most visitors were found on staminodes, followed by petals, anthers, and lastly a single visitor collected at the stigma (Figure 6). Finally, there was no definite pattern in the visitors collected per individual cacao tree, with trees 11, 10 and 15 having the highest frequency of captured visitors, and trees 1, 6, and 14 having the lowest (Figure 7).

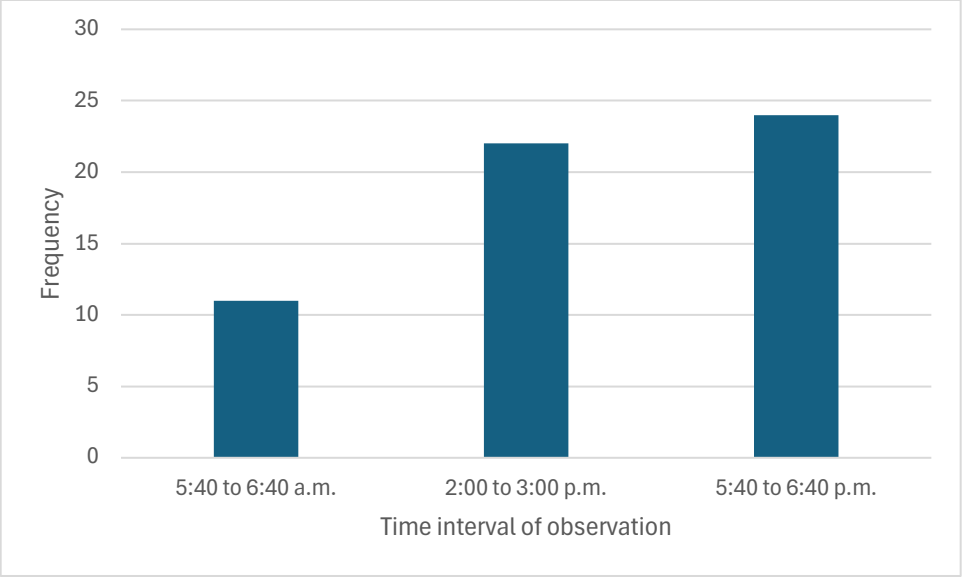

Figure 4. Frequency of collected flower visitors of cacao per time interval in the entire observation period (July 8th to July 27th, 2024).

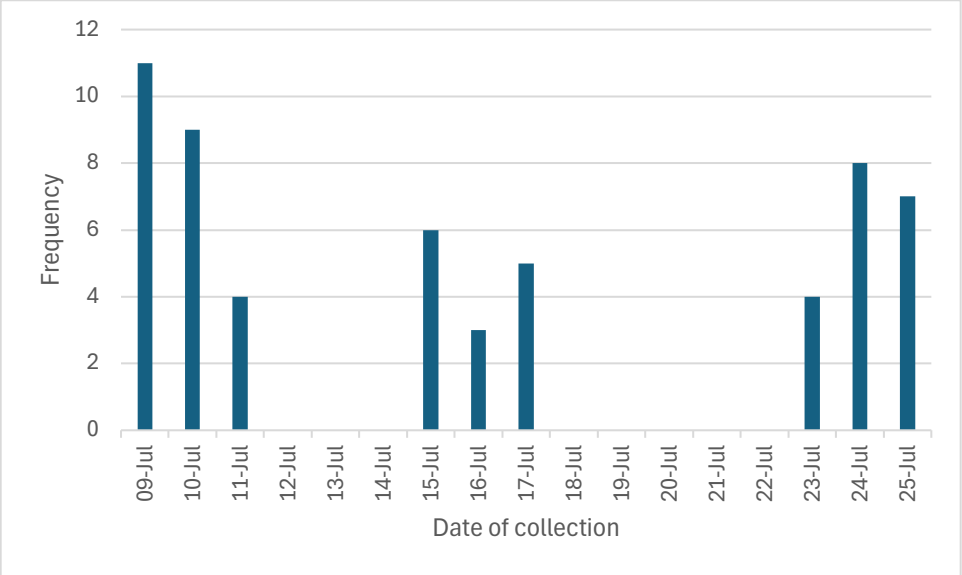

Figure 5. Frequency of collected flower visitors of cacao per date of collection in the entire observation period (July 8th to July 27th, 2024). Surveys were only done Tuesdays (July 9th, 10th, and 11th), Wednesdays (July 15th, 16th, and 17th), and Thursdays (July 23rd, 24th, and 25th).

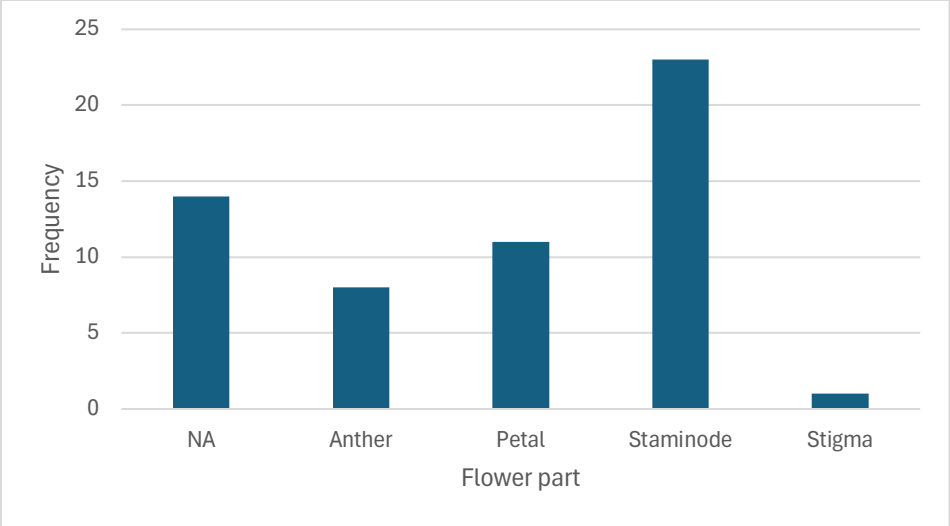

Figure 6. Frequency of collected flower visitors per cacao flower parts at or near reproductive parts for the entire observation period (July 8th to July 27th, 2024). "NA" refers to instances where there was no record of the flower part in which the visitor was collected.

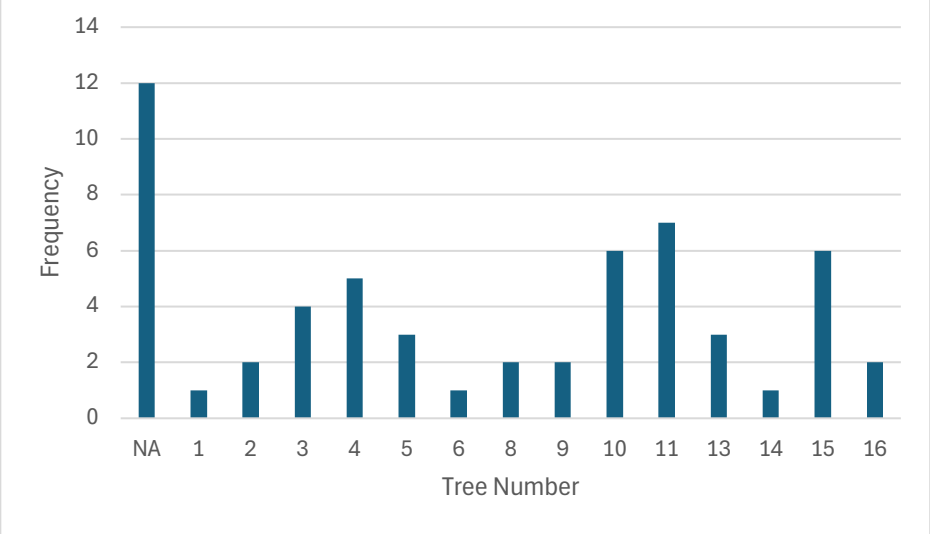

Figure 7. Frequency of collected flower visitors per individual cacao tree in the entire observation period (July 8th to July 27th, 2024). "NA" refers to instances where there was no record of the tree number in which the visitor was collected. Trees were numbered from south to north.

Next steps in the analysis of the data will be running the images through the software for the establishement of visitation rates and identification of visitors (Figure 8 & 9). This information will be then processed along with meteorological data, to establish patterns throughout the day. Further, visitors will be identified taxonomically with DNA barcoding and their pollen loads will be observed to confirm cacao presence. Finally, the trajectory of the fruits up until harvest will be analyzed and related to collected pollination data.

Figure 8. Example of a day photo of a cacao flower taken with a Wingscapes TimelapseCam Pro at Granja Luker research centre.

Figure 9. Example of a night photo of a cacao flower taken with a GardePro T5CF camera at Granja Luker research centre.

Discussion

The observed fruit set rates for the entire observation are within what has been reported in the literature, with natural pollination surrounding 10% and significant increases when doing manual pollination (Jaramillo et. al., 2024). Nonetheless, studies on manual pollination as a way to increase yields are not conclusive (Vansynghel et. al., 2022), which is supported in this research given that a month after pollination, only four fruits have not been aborted. Future research should aim to do large-scale manual pollination experiments in different geographical locations, and examining the factors related to fruit abortion in manually pollinated flowers. Lastly, the fact that none of the isolated flowers set fruit confirms the need for a biotic agent in cacao pollination.

The patterns showed in the fruit set rate per tree most probably obey to the two gradients presented along the furrow: distance from stingless bee colony and canopy cover. *T. angustula* bees have been proposed as potential pollinators of cacao in Colombia due to large cacao pollen loads and high mobility between flowers and trees (Jaramillo et. al., 2024). Also, there is consensus that shade canopy contributes to the abundance of pollinators in cacao (Toledo-Hernández et. al., 2017). Thus, it is expected that the trees closest to the stingless bees (those at the entrance) and the trees with more shade cover (those towards the end of the furrow) will have higher pollination success.

The obtained results with respect to visitors have similar reports in the literature too. For instance, Ceratopogonidae flies, which are usually acknowledged as cacao's pollinators, have been shown to visit flowers throughout the day, preferably around sunset, and often on staminodes (Jaramillo et. al., 2024). There is some evidence that this flower part emits olfactory cues that attract pollinators, but many flies sit on staminodes without interest in reproductive

parts (Vandromme et. al., 2023), so further research should aim to study the role of these visitors in pollination. Further, the time patterns are likely due to the size of visitors, which make them rely strongly on ambient temperature to be active: sunrise is cooler than the other observed time intervals.

On the other hand, the fact that no patterns were observed per individual tree (in contrast with fruit set rates), is most probably due to the fact that there was no standardization of time spent per tree. The main objective was collecting as many visitors (that were on the furrow) as possible, not measuring visitor abundance per tree. Moreover, the modified vacuum and portable freezer approach proved to be a good method in for cacao flower visitors, given that it is less labor intensive than manual aspirators, and it avoids the problems of methods such as glue, which may damage collected specimens or have attractive properties that alter results (Vandromme et al., 2023).

In conclusion, our subsequent data analysis should focus on finding evidence in the photos for the suggested patterns of fruit set rate in relation to shade cover and stingless bee colony. This would allow a more robust proposal of management strategies that maximize fruit set and guide more research into the effect of these strategies in yields. The previous is also true through the analysis of the meteorological data and its correlation with weekly patterns of fruit set and visitator collection. The DNA barcoding and pollen load analysis will allow the identification of potential pollinators. With this, establishing their patterns would lead to strategies that improve pollination success, such as the promotion of specific pollinator habitats or scheduling harvesting and maintenance activities outside of hours in which pollinators are most active.

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References

Arenas, W., Salgado, J., & Ponce de León, P. (2023). *2018-2022 Reporte: Cacao, Bosques & Paz*. World Cocoa Foundation. https://worldcocoafoundation.org/storage/files/1-reporte-cbp-espanol.pdf

Federación Nacional de Cacaoteros. (2022). *Matriz de Compatibilidad Sexual de los Clones de Cacao más Utilizados en Colombia* [image]. Grupo de Investigación e Innovación en Cacao. Retrieved May 21, 2024.

Food and Agriculture Organization. (n.d.). *Agroforestry*. Retrieved August 31st, 2024, from https://www.fao.org/agroforestry

Jaramillo, M. A., Reyes-Palencia, J., & Jiménez, P. (2024). Floral biology and flower visitors of cocoa (*Theobroma cacao* L.) in the upper Magdalena Valley, Colombia. *Flora*, 313, Article 152480. https://doi.org/10.1016/j.flora.2024.152480

Toledo-Hernández, M., Wanger, T. C., & Tscharntke, T. (2017). Neglected pollinators: Can enhanced pollination services improve cocoa yields? A review. *Agriculture, Ecosystems and Environment*, 247, 137-148. http://dx.doi.org/10.1016/j.agee.2017.05.021

Vandromme, M., Van de Sande, E., Pinceel, T., Vanhove, V., Trekels, H., & Vanschoenwinkel, B. (2023). Resolving the identity and breeding habitats of cryptic dipteran cacao flower

visitors in a neotropical cacao agroforestry system. *Basic and Applied Ecology*, 68, 35-45. https://doi.org/10.1016/j.baae.2023.03.002

Vansynghel, J., Ocampo-Ariza, C., Maas,B.,Martin, E.A., Thomas, E., Hanf-Dressler, T., Schumacher, N.-.C., Ulloque-Samatelo, C., Tscharntke, T., & Steffan-Dewenter, I. (2022). Cacao flower visitation: low pollen deposition, low fruit set and dominance of herbivores. *Ecological Solutions and Evidence*, 3, e12140. https://doi.org/10.1002/2688-8319.12140

Wight, A. (2023, May 18). *La agricultura sostenible está construyendo la paz en Colombia*. Alliance Biodiversity & CIAT. https://alliancebioversityciat.org/es/stories/agricultura-sostenible-construyendo-paz-colombia

World Wildlife Fund – Colombia. (2013). *Clasificación de Ecosistemas Naturales Terrestres del Eje Cafetero: Análisis de Representatividad del Sistema Regional de Áreas Protegidas*. https://wwflac.awsassets.panda.org/downloads/eje_cafetero_final_marcadores.pdf