



Laidlaw Scholars Undergraduate Leadership and Research Programme
Research Proposal

Pollination Networks of Cacao Agroforestry Systems in Colombia

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Abstract

Cacao agroforestry systems are being increasingly used in Latin America as smallholder systems that restore unprotected human-impacted forest areas. A great limiting factor to this initiative is the lack of understanding on pollination dynamics in these agroecosystems, particularly since in Andean countries significant low fruit set rates have been reported. Hence, the effect of insect pollinators in fruit set of cacao trees in a Colombian agroforestry system will be observed. This will be done through 24-hour recordings of different clusters of flowers to observe visitation rate and visitor identity. Other clusters of flowers will be manually pollinated as a positive control, and others will be bagged as a negative control. Stigma squashes (for pollen penetration observation) and fruit set rate observations for these clusters will be performed, to allow relating pollination success with visitation rate and visitor identity. Further, collection of visitors will be done with insect vacuums and sticky traps, pollen presence on captured insects will be recorded and pollen load analyses will be performed on them. Species identification will be done through DNA barcoding. The field work will take place in “Granja Luker”, a 22-hectare research centre of cacao agroforestry systems. It is in the department of Caldas, Colombia, about a one-hour flight from Colombia’s capital city, Bogotá.

Introduction

I have worked on pollination since first grade, studying for over 11 years the role of urban gardens in promoting pollinator biodiversity at my school. This got me into biology: I was amazed by the beauty of the interaction between plants and insects that is the result of millions of years of evolution; a mutualism that allows the reproduction of the plants that sustain ecosystems and feed us.

Along with this passion, I have always wanted my research to have a positive impact on the most vulnerable sectors of the environment and society. As such, I want to use science to protect the unparalleled biodiversity of my home country, Colombia, in a way that serves the rural communities affected by poverty and armed conflict. I found in cacao agroecosystems the perfect opportunity to do so, since they are being increasingly used in Latin America as smallholder systems that restore unprotected human-impacted forest areas (Vansynghel, et. al., 2022; Vandromme, et. al., 2023). A great limiting factor to this initiative is the lack of understanding of pollination dynamics in these agroforestry systems, particularly since in Andean countries significant low fruit set rates have been reported (Vansynghel, et. al., 2022). Thus, fulfilling these knowledge gaps is crucial to effectively restore ecosystems such as the Amazon through projects that also improve the livelihoods of rural smallholders (Vansynghel, et. al., 2022; Vandromme, et. al., 2023).

Research Objectives & Questions

Main question: what is the effect of insect pollinators in fruit set of cacao trees from a Colombian agroforestry system?

Secondary objectives

- Identify insect pollinators of cacao.
- Determine the effectiveness of insect-mediated pollination.
- Measure pollen loads of cacao’s insect pollinators.

Background

Despite literature on pollination networks of cacao agroforestry systems in Latin America being scarce, there are two main references that guided this proposal. The paper by Vansynghel, et. al. (2022) consists of a study in Peruvian cacao agroforestry systems, where visitation rates were observed, pollen deposition was quantified and related to fruit set rate, and manual pollination was done for comparison with natural pollination. A great variety of visitors were identified, and both natural and manual pollination resulted in remarkably low fruit set rates. This article is a great guide to this research both as a theoretical framework and as methodological basis. Further, it highlights that determining efficient pollinator species and other causes of low fruit set rates is crucial to define management strategies that improve productivity in Latin American cacao agroecosystems, which are key goals of this project.

The second reference is Vandromme, et. al. (2023), who used DNA barcoding to determine the identity and breeding habitats of flies that visit cacao flowers in a Nicaraguan agroforestry system. They identified a higher diversity of pollinating flies than previously assumed, associated with several different breeding habitats such as decaying cacao pods or understory weeds. This allowed them to emphasize the importance of barcoding as a potent cost-effective tool for identification of cacao pollinators, given that morphological identification requires expert knowledge and is time-consuming, while barcoding is increasingly accessible in the Global South.

In addition to the previous, the research is nested within Professor MacIvor's lab, where extensive work in pollination ecology has been done with the methods that will be used in this project. This includes the use of camera traps for the recording of pollinator visits and the subsequent analysis using automated algorithms, as well as the use of stigma squashes as an indicator of pollination success.

Methodology

Study Location

The field work will take place in "[Granja Luker](#)", a 22-hectare research centre of cacao agroforestry systems in the department of Caldas, Colombia. At the site, three study plots will be defined across gradients (high or low) of shade and nearby plant diversity. In each site, stigma squashes and fruit set rate surveys will be done for insect and manual pollination.

Experimental work

For insect pollination, six specialized field cameras will be placed at 6 AM to photograph for 24 hours (the entire lifetime of the flowers) clusters of cacao flowers that are about to open, with two cameras per study plot. From these, one cluster will correspond to stigma squashes, for which after the 24-hour period, flowers that have not abscised will be collected and stored in ethanol. The other cluster will correspond to fruit set observation, for which seven days after the recording, flowers that have developed into young fruit will be registered. In both cases, the flower outcome (abscised or not, fruit set success or failure) will be registered for each flower, using individual identification codes.

For manual pollination, the same process outlined above will be followed in six other different clusters, but without the placement of cameras. Hand pollination will be done in accordance with the outlines of the centre. Additionally, a negative control observation will be set up by covering flowers with bags, to avoid insect visitation. These will not be manually pollinated, and the same observations will be done.

Further, insect collection will be done at 8 AM and at 8 PM with an aspirator, as well as with sticky traps near the clusters. The experimental work will be done over the six-weeks on weekdays.

Data Analysis

Insect visitation photos will be analyzed using a machine-learning algorithm that identifies type of visitor and their visitation rate per flower. Stigma squashes will be done by isolating the stigmas under a dissecting microscope, staining them, and counting observed pollen grains. Collected insects will be preserved in ethanol, tagged and photographed. Insect pollinators will be determined by observing presence of cacao pollen in their bodies through pollen load analyses, where the amount of carried pollen will be estimated too. Further, individuals will be identified with DNA barcoding, which will be done in Bogotá, Colombia, at the private research centre CorpoGen.

Training/ Certifications Needed

Stigma squash training: the student has undergone a day of training on how to do stigma squashes with small flowers (as cacao flowers are small too). He will complete another day of training to learn how to set up the stain and slides to perform the squashes. Also, he will bring extra supplies to practice this set up prior to starting the research. He has access to a compound microscope which is the only equipment needed for training.

Camera training: the student has received training on the usage of cameras for visit observations and has participated in the testing of cameras to select which ones to use in the research. Once these are defined, he will have several days offsite and onsite to test the positioning of cameras and the processing of the files.

Machine-learning algorithm training: the student will train himself in the programming of the machine-learning algorithm to identify visitors and record visits from the recordings. For this, he will use his previous experience with coding and the online sources on the topic (for which there are plenty of topic-specific guides). He will train the month prior to the research, with the option to ask for help to the supervisor and to multiple members of his lab that have worked with this method.

Research Location

The research will take place in [Granja Luker](#), located at the municipality of Palestina, department of Caldas, Colombia.

Timeline

The experimental work will be done over the six-week period on weekdays, on all three study plots. Therefore, there will be thirty observation days per plot with stigma squashes and insect collection observations (along with their associated manual pollination and control observations), and twenty-five fruit set observation days per plot (since in the first week, pollination has not happened and there will be no fruit set).

The following are tasks to be completed in a workday: setting cameras for recording at 6 AM, manually pollinating the other clusters of flowers, bagging of flowers in control clusters, registration and collection of flowers from all pollination treatments that have not abscised from previous observation day (for stigma squashes), registration of fruit set from previous week, an initial insect collection at 8 AM, observation of pollen presence in collected insects (from the previous night and from the morning), pollen load analysis, labelling and storage of captured insects, stigma squashes and pollen penetration counts from collected flowers, watching of the recordings from the previous day, setting of the automation algorithm, and a second insect collection at 8 PM.

Potential Impact

The key achievement of this research will be identifying the factors that cacao farmers can modify in their crops to increase pollination success and make more productive agroecosystems. As such, they will be able to better compete in the market, being motivated to keep developing a sustainable way of living, as an alternative to the abandonment and violence that condemns them to poverty or illegality.

Likewise, valuable scientific information on cacao will be obtained, particularly regarding barcoding of pollinators and their day and night dynamics, as well as comparisons between manual and natural pollination, which can be used in cacao-producing regions globally. Particularly, the DNA barcoding data will be submitted to data bases such as the Barcode of Life Data System, where researchers across the globe can access the information not only for cacao-related research, but for any insect diversity research in the tropics.

References

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., Tschardtke, 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>