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Ceratopogonidae: their role in pollination and fertilization at various technological levels of *Theobroma cacao* L. production

Ceratopogonidae: su rol en la polinización y fecundación en varios niveles tecnológicos de producción de *Theobroma cacao* L.

Ceratopogonidae: seu papel na polinização e fertilização em diversos níveis tecnológicos de produção de *Theobroma cacao* L.

Silvia Lorena Montero-Cedeño[1*]
Ernesto Gonzalo Cañarte-Bermudez[2]
José Bernardo Navarrete-Cedeño[2]
Antonio Pinargote-Borrero[2]
Pedro Sanchez-Hernández[3]

[1]Escuela Superior Politécnica Agropecuaria de Manabí. Av. 10 de agosto y Granda Centeno. Calceta, Ecuador.
[2]Instituto Nacional de Investigaciones Agropecuarias. Estación Experimental Portoviejo, Manabí, Ecuador.
[3]Instituto Nacional de Investigaciones Agrícolas, Caracas-Venezuela.

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Abstract

The goal of this study was to evaluate the role of pollinators on the pollination, fertilization and fruit set process in three technological production levels and three reproduction substrates, determinants in cocoa yield. Two pollination techniques (natural and assisted) were compared and as a control, flowers were isolated with antiaphid. Twenty-seven observation units were formed, with three biological replicates. A known number of flowers were evaluated 6, 14, 21 and 36 days after the opening of each flower on marked branches. The number of active flowers, pollinated, fertilized and fruits formed was recorded. Pollinators present in experimental units were collected using yellow sticky cards, then taken to the lab and identified. Pollination occurred up to three days after opening, the flower that remained with the ovary swollen and attached to the branch/stem was considered pollinated. Fertilization was recorded fourteen days after flower opening, fruit set begins after fertilization, an event that was quantified twenty-one days after flower opening. The systems studied did not influence the percentage of pollination, fertilization and fruit formation. However, the substrates did influence pollination, fertilization and fruit set. The assisted pollination technique is significantly superior to natural pollination. The same species of Ceratopogonidae were reported at all three levels and substrates, with genera *Forcipomyia* and *Dasyhelea* being the most abundant.

*Corresponding author: smontero@espam.edu.ec

Resumen

A objeto de valorar el rol de los polinizadores sobre el proceso de la polinización, fecundación y formación de frutos en tres niveles tecnológicos de producción y tres sustratos de reproducción, determinantes en el rendimiento de cacao. Se compararon dos técnicas de polinización (natural y asistida) y como control, se aislaron flores con mangas antiáfido. Se conformaron 27 unidades de observación, con tres réplicas biológicas. Se partió de un número conocido de flores y se evaluó a los 6, 14, 21 y 36 días después de la apertura de cada flor en ramas marcadas. Se registró el número de flores activas, polinizadas, fecundadas y frutos formados. Usando tarjetas pegantes amarillas, se colectó e identificó los polinizadores presentes en unidades experimentales. La polinización, ocurre hasta los tres días de la apertura, considerándose polinizada la flor que permaneció con el ovario hinchado, adherida a la rama/tronco. La fecundación, se registró a los catorce días de la apertura de la flor, la formación de frutos comienza desde la fecundación, evento que se cuantificó a los veintiún días después de la apertura de las flores. Los sistemas estudiados no influyeron en el porcentaje de polinización, fecundación y formación de frutos. Sin embargo, los sustratos sí influyeron en la polinización, fecundación y formación de frutos. La técnica de polinización asistida es significativamente superior a la polinización natural. Se reportaron las mismas especies de Ceratopogonidae en los tres niveles y sustratos, destacando los géneros *Forcipomyia* y *Dasyhelea.*

Palabras clave: polinizadores, cacao, fecundación, polinización natural, polinización asistida, sustrato alimenticio.

Resumo

Com o objetivo de avaliar o papel dos polinizadores no processo de polinização, fertilização e formação dos frutos em três níveis tecnológicos de produção e três substratos de reprodução, fatores determinantes na produtividade do cacau. Duas técnicas de polinização (natural e assistida) foram comparadas e como controle, flores com mangas anti-afídeos foram isoladas. Foram formadas 27 unidades de observação, com três réplicas biológicas. Foi iniciada a partir de um número conhecido de flores e avaliada aos 6, 14, 21 e 36 dias após a abertura de cada flor em ramos marcados. Foi registrado o número de flores ativas, polinizadas, fertilizadas e frutos formados. Por meio de cartões amarelos adesivos, os polinizadores presentes nas unidades experimentais foram coletados e identificados. A polinização ocorre até três dias após a abertura, considerando-se polinizada a flor que permaneceu com o ovário inchado, aderido ao galho / tronco. A fecundação foi registrada quatorze dias após a abertura da flor, a formação dos frutos começa a partir da fecundação, evento que foi quantificado em vinte e um dia após a abertura das flores. Os sistemas estudados não influenciaram na porcentagem de polinização, fertilização e formação de frutos. No entanto, os substratos influenciaram a polinização, fertilização e formação dos frutos. A técnica de polinização assistida é significativamente superior à polinização natural. As mesmas espécies de Ceratopogonidae foram relatadas nos três níveis e substratos, com destaque para os gêneros *Forcipomyia* e *Dasyhelea*.

Palavras-chave: cacau, fertilização, polinização natural, polinização assistida, substrato alimentar.

Introduction

Theobroma cacao L. is an important crop worldwide and its production depends on the activity of pollinating insects. Ninety percent of the crop requires adequate pollination (FAO, 2008). This is basically entomophilous, due to the size, arrangement of the perianth and the condition of incompatibility. Therefore, the presence and activity of pollinators is essential, especially Diptera of the Ceratopogonidae family, mostly species of the genus *Forcipomyia.* These diptera transfer pollen from a flower of a donor tree (father) to a flower of a recipient tree (mother), thus effecting pollination, fertilization and subsequent fruit formation (Córdoba *et al*., 2013). In cocoa, fruit production is affected by the reduction of pollinator populations in their ecosystems (Adjaloo *et al*., 2012; FAO, 2008; Salazar-Díaz and Torres-Coto, 2017).

The virtual conservation of the biodiversity of the original forest is a benefit offered by traditional cocoa agroforestry systems at the ecological level, due to the high diversity and complex structure of the shade canopy, in which farmers incorporate various tree species, especially forest and fruit trees, from which they obtain different products that support the economy of the production units (Gebauer *et al*., 2002).

The diversity and abundance of pollinating insects in cocoa agroforestry systems can be influenced by several biotic and abiotic factors, as well as vegetation cover, organic matter, spacing of shade trees and shade tree flowering (Adjaloo *et al*., 2012; Ramos-Serrano, 2011; Schroth and Harvey, 2007). Habitat suitability for Ceratopogonidae species require shaded conditions, balanced humidity, and rich organic matter to develop and thrive (Kaufmann, 1975; Toledo-Hernández *et al*., 2017; Young, 1982, 1986).

The environmental crisis of the world is observed in threats to environmental goods and services, loss of natural vegetation, soil degradation, ecosystem degradation, environmental pollution, as well as climate change and the accelerated disappearance of species (Plan Nacional de Desarrollo, 2017). Hence, this research aims to assess the activity of pollinators on pollination, fertilization and fruit formation of cocoa at different technological production levels and three reproduction substrates determinants in the yield of fine cocoa.

Materials and methods

This research was carried out at the Portoviejo Experimental Station of INIAP, located in Santa Ana-Manabí, Ecuador. Geographically located (1°07'25.7 "S and 80°24'50.9 "W) and conditions (81% RH; 851.57 mm and 26.4 °C).

Experimental management

The work was carried out at three technological production levels and three reproduction substrates (cocoa husks, leaf litter and musaceae pseudostem). The monoculture level corresponded to a 25-year-old plantation with three known clones (EEP 95, EEP 96 and EPP 103); the cocoa/fruit level consisted of a 25-year-old cocoa plantation associated with three citrus species (*Citrus reshni* Hort. ex Tan*, Citrus reticulata* Blanco, *Citrus sinensis* L. Osbeck). The cocoa/forest level consisted of cocoa with timber trees of the Guayaquil yellow species *Handroanthus chrysanthus* (Jacq.) (Bignoniaceae) y *Cedrus atlantica* (Endl.) Manetti ex Carrière (Pinaceae), 25 years old. For the study, a known number of cocoa flowers in each of the plots (27 observation units) was used as the starting point. Three biological replicates were carried out. An average of 8.3, 7.6 and 10.7

flowers were used for biological replicates I, II and III, respectively. Evaluations were made at 3, 6, 14, 14, 21 and 36 days after flower opening. Two pollination techniques (natural and assisted) were compared and isolated flowers in sleeves made with anti-aphid mesh free from pollinator interference were used as controls.

For the implementation of pollination techniques in each of the production levels, the methodology described by Córdoba *et al.* (2013) was used as a reference. In each plot, those trees with more flowers were considered, and in each selected tree, three branches (observations) were chosen for each technique. First, in each of the selected branches, all open, dry flowers and small buds were eliminated, leaving only closed mature flower buds, which were counted. From that date, the number of pollinated flowers, fertilized flowers and number of fruits formed were recorded.

For the free or natural pollination technique, the section of the branch on each marked branch (figure 1A) containing the selected flower buds, ready to be opened the following morning, was delimited with tape and set free to be visited by the pollinating midges.

For the manual technique, the flower buds that were close to opening were selected on each marked branch 24 hours before. These were covered with cotton to prevent the arrival of pollinators. Recently opened flowers were selected the following morning and used as pollen donors. Subsequently, the stamens were removed with fine forceps from each of the selected flowers (covered with the vial) (figure 1B). Finally, pollen from the anthers of the male donor is gently rubbed onto the surface of the style and stigma of the recipient flower (Vera and Mogrovejo, 2010).

In order to have a control as in the previous techniques, three branches per tree (3 trees) containing flower buds were selected, which were individualized with 60 cm long sleeves, constructed with anti-aphid mesh. The sleeves were hermetically sealed at the ends with tape, thus preventing the entrance of any floral visitor (figure 1C). For the sampling and identification of pollinators of the Ceratopogonidae family present in the three levels of cocoa production, yellow plastic traps (5 x 5 cm) with light oil were used (figure 1D). A total of 27 traps were placed for each production level, distributed in nine traps for each pollination technique, located next to each branch containing the marked flowers. Three days later, they were removed and taken to the Entomology laboratory of the Portoviejo Station of INIAP for identification and counting of pollinators, using the reference collection maintained by the laboratory.

Figure 1. Pollination technique and pollinators: free or natural pollination (A); assisted pollination (B); mesh sleeve control (C); yellow sticky traps (D).

Statistical analysis

Variables were analyzed in a variance analysis corresponding to a split-plot design with three replications. The Wilcoxon and Barlett tests were used to test normality and homogeneity of variances, respectively. Tukey's test at 0.05 was used for dividing means. The Agricolae package of R Studio software (Mendiburu and Yaseen, 2020) was used for these analyses.

Results and discussion

In the variable percentage of pollinated flowers between the technological levels and reproduction substrates, the ADEVA did not establish significant statistical differences (P>0.05) between treatments in each of the pollination techniques (natural and assisted), while assisted pollination had higher values in the monoculture of cocoa (31%) of replicate III and the leaf litter substrate with 27% of pollinated flowers in replicate I. While, in natural pollination, a maximum of 12% of pollinated flowers were obtained in the fruiting cocoa technological level in replicate II and 15% in the substrate with pseudostem of musaceae (plantain/banana) in replicate III. Cocoa flowers that were in the sleeves (control) always presented zero values in the three biological replicates, both in the technological levels and in the substrates. The sleeves prevented the access of floral visitors to the cocoa flower, preventing pollination. These results confirm the role of Ceratopogonidae on the natural pollination of cocoa flowers. The highest percentage of pollinated flowers in monoculture cocoa with leaf litter in the assisted technique is due to the technique and not to the action of pollinators, which occurs in lower population in this substrate. Consequently, the assisted technique was responsible for the increase in pollinated flowers.

These results agree with studies conducted by Forbes and Northfield (2017), in Australia, who manipulated the habitat in a monoculture cocoa plantation with artificial treatment and pollination (manual pollination) to evaluate the addition effects of cocoa shells on pollination, obtaining that cocoa shells increased the presence of pollinators in the plantations, thus improving the efficiency of pollination and fruit set in cocoa.

The results of the variables percentage of fertilized flowers and average percentage of cocoa fruits formed in the three biological replicates at 14 and 21 days after flower opening recorded in the two factors under study, show equal average values in both variables, i.e., 100% of fertilized flowers yielded cocoa fruits. The ADEVA did not establish significant statistical differences (P>0.05) for the technological levels factor in any of the two pollination techniques (natural and assisted) in the three biological replicates carried out. While the substrate factor did register significant differences in the natural pollination of replicate III (F2.18 = 16.32; P<0.01), where the substrate with musacea (plantain/banana), stands out significantly from cocoa husk and leaf litter (Tukey), for its higher percentage of fertilized flowers and fruits formed (12%) (figure 2). The higher fertilization percentage and fruit formation may be influenced by the presence of pollinators in these plots, where the humidity and decomposing material provided by the musaceae favors pollinator populations.

It is known that the immature instars of Ceratopogonidae develop mainly in moist and decaying plant matter, such as cocoa cobs, pseudostem of musaceae that offer an excellent substrate for feeding, since the larvae of these insects feed mainly on bacteria and fungi that develop in these substrates; as well as habitat and sources of reproduction and protection of the immature stages of these pollinators (Adjaloo *et al.,* 2012). This is also stated by Shackelford *et al.* (2013), who mention that some pollinators and predators have shown responses to habitat manipulation and reduced pesticide use (Bianchi *et al*., 2006), with positive effects on fruit set and agricultural productivity. Also, Bianchi *et al*. (2006) and Wratten *et al*. (2012) mention that habitat manipulation promotes pollinator populations and provides additional benefits such as the availability of habitats and food resources for pollinators and other organisms present in the agroecosystem, favoring agricultural production and biological conservation. As for the cocoa flowers kept inside the sleeves (control control), they presented zero fertilized flowers in the three biological replicates, both in the technological levels and between substrates, because pollination was interrupted by the non-intervention of the Ceratopogonidae in pollination.

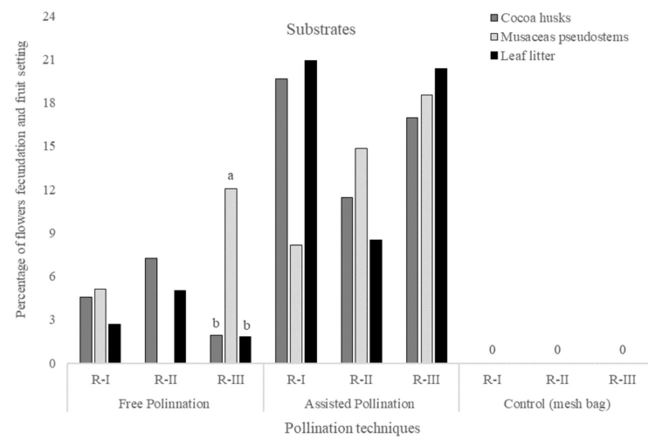

Figure 2. Percentage of cocoa flower fertilization and fruits formed.

When comparing the percentage of pollinated and fertilized flowers and fruits formed, between the two pollination techniques (natural and assisted), in the three biological replicates, significant statistical differences were found in the variables studied in most of the biological replicates (P<0.05). The percentage of pollinated flowers, fertilized flowers and fruits formed was always significantly higher in the assisted pollination technique (figure 3), with an overall average of 20% of pollinated flowers, 16% of fertilized flowers and 15% of fruits formed. In contrast with the natural pollination technique, where 9% of flowers were pollinated, 4% fertilized and 4% of fruits formed.

These results agree with those reported by Córdoba *et al.* (2013), who state that flower fertilization and fruit formation of up to 25% can be achieved with manual pollination in cocoa agroforestry systems in Indonesia, while only 4% of flowers are fertilized with natural pollination. Likewise, Vera and Mogrovejo (2010) determined that better results are obtained with the assisted pollination technique, since the number of fruits can be doubled and therefore productivity can be increased. In addition, the application of this technique facilitates the management of fruit diseases that increase during the rainy season. However, Wessel and Quist (2015), argue that although pollination is improved, the pollination technique is not sufficient to guarantee fertilization and potential yield of cocoa. Thus, there are factors such as self-incompatibility, crop management and tree aging (>25 years) that can considerably decrease these yields (Claus *et al.,* 2018). Other studies conducted by Vera and Vera (2018), indicate that despite not having found significant differences between manual and natural technique in Criollo cocoa plantations, they reported differences between natural and assisted pollination in CCN-51 cocoa plantations, being higher in assisted pollination. In addition, Toledo-Hernández *et al*. (2020) report that manual pollination in Indonesia increases production up to 51% if 13% of the tree's flowers are pollinated, and could even increase up to 161% if 100% of the tree's flowers are pollinated, which, for the farmer, would be equivalent to a gain of up to 69%.

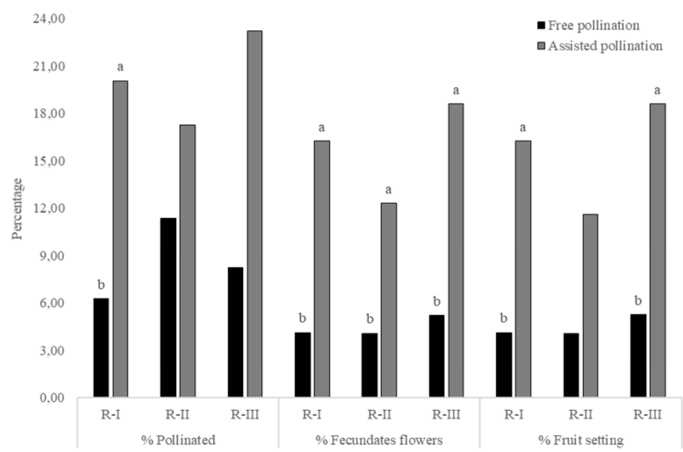

Figure 3. Percentage of pollinated flowers, fertilized flowers and formed cocoa fruits.

Regarding the presence of Ceratopogonidae, five species were identified: *Forcipomyia* (Forcipomyia) *quatei* Wirth*, Forcipomyia* (Forcipomyia) *youngi* Wirth*, Dasyhelea cacaoi* Wirth & Waugh*, Dasyhelea borgmeieri* Wirth & Waugh*, Culicoides pusillus* Lutz*,* which were present in the three technological production levels and substrates in the three biological replicates. The genera *Forcipomyia* and *Dasihelea* are widely known as cocoa pollinators (Cañarte-Bermúdez *et al*., 2021; Kaufmann, 1975; Montero *et al*., 2019). Likewise, these genera found in this research agree with some groups found by Young (1982), in studies conducted in cocoa plantations, reporting 22 species of Ceratopogonidae distributed in six genera, among which were the groups present in our research. Azhar and Wahi (1984) also reported the species *Forcipomyia* sp., *Dasyhelea* sp., *Brachypogon* sp. and *Atrichopogon* sp., coinciding with several species found in our work. Also, Sánchez *et al*. (2001), cite species of *Forcipomyia*. Martinez *et al*. (2000) also reported the presence of *Forcipomyia brachyrhynchus*.

Out of the five identified species of adult specimens of the Ceratopogonidae family, yellow.trap^{-1} observed among the technological levels, ADEVA established significant statistical differences for the species *F. quatei* only in replicate III ($F_{2.18}$ = 33.26; P<0.001), registering the highest average number of specimens/trap in the cocoa + forest technological level, significantly different according to Tukey to the other two technological levels. These results differ from those reported by Young (1986), who found that there were different groups of pollinators in two farms evaluated, indicating that the greater presence of groups of pollinators in one farm was due to the greater diversity of plant species that provide heterogeneity in the substrate, as opposed to the other, which was a simple agroecosystem.

In relation to the breeding substrate, significant statistical differences were recorded in three of the five Ceratopogonidae species identified (*F. quatei*, *F. youngi* and *D. borgmeieri*). Regarding *F. quatei,* analysis of variance reported highly significant differences in replicates I and III ($F_{2,18}$ = 22.75; P<0.01 and $F_{2,18}$ = 33.26; P<0.01), respectively. In both replicates, the highest average number of specimens/trap was recorded in the substrate pseudostem of musaceae (plantain/banana), while the lowest average number of adult specimens was recorded in the plots with leaf litter substrate. yellow trap^{-1}. In the pollinator species *F. youngi*, the substrate with pseudostem of Musaceae showed a higher average value of adult specimens/trap of this species, confirming the leaf litter as the substrate that contributes the least to the presence of pollinators. In the species *D. cacaoi*, the analysis of variance found significant statistical differences in replicate I ($F_{2.18}$ = 8.00; P<0.05), presenting the highest average values of the number of adult specimens/trap in musaceae pseudostem substrate, while the population was zero in substrate with leaf litter.

These results are supported by a previous prognosis, where substrates (plantain pseudostem) for the reproduction of Ceratopogonidae midges in cocoa plantations increased the presence of pollinators (Montero *et al*., 2019). Likewise, Bravo *et al*. (2010) state that the suitable variables in the behavior of mosquito populations are mostly related to the substrate and not to the abundance of the different species present in the plantations. Kaufmann (1975) and Young (1982) showed that pollinators frequently oviposit and complete their cycle in the soil (cocoa leaf litter). In addition, Forbes and Northfield (2017), stated that decomposing cocoa shells influenced the increase of cocoa-pollinating mosquito populations through increased habitat availability for oviposition and successful larval development, compared to the control without cocoa shells.

Conclusions

The technological production levels did not influence pollination, fertilization and fruit formation in the two pollination techniques. While the substrates of musaceae pseudostems and cocoa husks significantly influenced the increase pollination, fertilization and fruit formation in the two pollination techniques. The percentage of pollinated and fertilized flowers and fruits formed, obtained with the assisted technique was higher than with natural pollination. The sleeving technique made it possible to verify the role of floral visitors on the pollination and fertilization of cocoa. The Ceratopogonidae species identified in this study agree with the diversity of pollinators cited in the literature, associated with the technological levels of cocoa production, where the genera *Forcipomyia* and *Dasyhelea* stand out.

Literture cited

Adjaloo, M., Oduro, W., & Banful, B. (2012). Floral phenology of upper Amazon cocoa trees: Implications for reproduction and productivity of cocoa. *International Scholarly Research Notices 2012,* 1-8. https://cutt.ly/4Z2xxXf

Azhar, I. and Wahi, M. (1984). Pollination of cocoa in Malaysia: identification of taxonomic composition and breeding sites, ecology and pollinating activities, and seasonal abundance. *Incorporated Society of Planters*, 77-91. https://cutt.ly/RZ2xSor

Bianchi, F. Booij, C. & Tscharntke, T. (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of The Royal Society B 273,* 1715-1727. https://royalsocietypublishing.org/doi/pdf/10.1098/rspb.2006.3530

Bravo, J., Somarriba, E. & Arteaga, G. (2010). Factores que afectan la abundancia de insectos polinizadores del cacao en sistemas agroforestales. *Revista de Ciencias Agrícolas XXVIII* (1), 119-131. https://revistas.udenar.edu.co/index.php/rfacia/article/view/36

Cañarte-Bermúdez, E., Montero-Cedeño, S. & Navarrete-Cedeño, B. (2021). *Reconocimiento, importancia y cuidado de los polinizadores en los sistemas de producción del cacao*. Instituto Nacional de Investigaciones Agropecuarias. Guía No. 177. República del Ecuador. 38 p. http://repositorio.iniap.gob.ec/handle/41000/5749

Claus, G., Vanhove, W., Van Damme, P., Smagghe, G., & Mokwala, P. 2018. Challenges in cocoa pollination: the case of Cˆote d'Ivoire. *In* Mokwala P. (Ed.) *Pollination in Plants* (pp. 39-58). IntechOpen, London, UK. https://sci-hub.se/http://dx.doi.org/10.5772/intechopen.75361

Córdoba, C., Cerda, R., Deheuvels, O., Hidalgo, E. & Declerck, F. (2013). Polinizadores, Polinización y Producción Potencial de Cacao en Sistemas Agroforestales de Bocas del Toro, Panamá. *Agroforesteria en las Americas 49*, 26-32. https://repositorio.catie.ac.cr/handle/11554/6677

Forbes, S. and Northfield, T. (2017). Increased pollinator habitat enhances cacao fruit set and predator conservation. *Ecological Applications 27*(3):887-89. https://sci-hub.se/10.1002/eap.1491

Gebauer, J., Kamal, E. & Georg, E. (October 9-11, 2002). *The potential of underutilized fruit trees in Central Sudan* [Abstract]. Conference on International Agricultural Research for Development. Witzenhausen, Germany. https://www.tropentag.de/2002/abstracts/full/42.pdf

Kaufmann, T. (1975). Ecology and behavior of cocoa pollinating Ceratoponidae in Ghana, W. Africa. Environmental Entomology *4*(2), 347-351. https://sci-hub.se/10.1093/ee/4.2.347

Martínez, A, Narváez Z. & Spinelli, G. (2000). Midge pollinators (Diptera: Ceratopogonidae) of the cocoa plant collected in Piaroa Amerindian Communities in Amazons State, Venezuela. *Boletín de Entomología Venezolana 15*(2), 249-253. https://ri.conicet.gov.ar/handle/11336/35304

Mendiburu, F. & Yaseen M. (2020). Agricolae: Statistical Procedures for Agricultural Research. R package versión 1.4.0. https://github.com/myaseen208/agricolae

Montero-Cedeño, S., Sánchez, P., Solórzano, R., Pinargote, A. & Cañarte, E. (2019). Floración y Diversidad de Insectos Polinizadores en un Sistema Monocultivo de Cacao. *ESPAMCIENCIA. 10*(1): 1-7. https://cutt.ly/PZ2cfP8

Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO). (2008). Polinización, un servicio del ecosistema. https://cutt.ly/rZ2cvpx

Plan Nacional de Desarrollo. (22 de septiembre 2017). Plan Nacional de Desarrollo. https://cutt.ly/ZZ2cTHH

Ramos-Serrano, R. (2011). *Estudio de la Diversidad de Insectos Polinizadores en Sistemas Agroforestales de Cacao y su Relación con la Productividad y Diversidad de Especies del Dosel*. [Tesis de Licenciatura]. Universidad de San Pedro. Honduras. 73 p. https://cutt.ly/ZZ2cFyX

Salazar-Díaz, R., and Torres-Coto, V. (2017). Estudio de la dinámica de polinizadores del cultivo de cacao (*Theobroma cacao*) en tres niveles tecnológicos de producción. *Tecnología en Marcha 30*(1), 90-100. https://www.scielo.sa.cr/pdf/tem/v30n1/0379-3982-tem-30-01-90.pdf

Sánchez P., Morillo F., Munóz W., Soria S. & Marín, C. (2001). Las especies de *Forcipomyia, Meigen* (Diptera: Ceratopogonidae) polinizadoras del cacao (*Theobroma cacao L*.) en la Colección de la Estación Experimental del INIA-Miranda, Venezuela. *Entomotropica 16*(2),147-148. http://www.bioline.org.br/pdf?em01013

Schroth, G. and Harvey, C. (2007). Biodiversity conservation in cocoa production landscapes: an overview. *Biodiversity and Conservation 16*(8), 2237–2244. https://doi.org/10.1007/s10531-007-9195-1

Shackelford, G., Steward, P., Benton, T., Kunin, W., Potts S., Biesmeijer J. & Sait, S. (2013). Comparison of pollinators and natural enemies: A meta-analysis of landscape and local effects on abundance and richness in crops. *Biological Reviews 88*, 1002-1021. https://doi.org/10.1111/brv.12040

Toledo-Hernández, M., Wanger, T. & Tscharntke, T., (2017). Neglected pollinators: ¿can enhanced pollination services improve cocoa yields? A review. *Agriculture, ecosystems & environment 247*, 137–148. https://doi.org/10.1016/j.agee.2017.05.021

Toledo-Hernández, M., Tscharntke, T., Tjoa, A., Anshary, A., Cyio, B. & Wanger, T. (2020). Hand pollination, not pesticides or fertilizers, increases cocoa yields and farmer income. *Agriculture, ecosystems & environment 304*, 107160. https://doi.org/10.1016/j.agee.2020.107160

Vera, J., and Mogrovejo, E. (2010). Aumente la producción de sus cacaotales haciendo polinizacion manual suplementaria. INIAP. https://cutt.ly/WZ2c1XP

Vera, J., and Vera, J. (2018). *Polinización artificial para incrementar la productividad en huertas y la obtención de cruces internacionales de cacao (Theobroma cacao L.),* Editorial Grupo Compás, Guayaquil Ecuador, 53 p. http://142.93.18.15:8080/jspui/bitstream/123456789/272/3/libro%20listo3.pdf

Wessel, M. and Quist-Wessel P. (2015). Cocoa production in West Africa, a review and análisis of recent developments. *Wageningen Journal of Life Sciences 1-7* (Supplement C), 74-75. https://www.tandfonline.com/doi/full/10.1016/j.njas.2015.09.001

Wratten, S., Gillespie, M., Decourtye, A., Mader, E. & Desneux, N. (2012). Pollinator hábitat enhancement: Benefits to other ecosystem services. *Agriculture, Ecosystems & Environment 159*, 112-122. https://www.sciencedirect.com/science/article/abs/pii/S0167880912002460

Young, A. (1982). Effects of shade cover and availability of midge breeding sites on pollinating midge population and fruit set in two cocoa farm. *Journal of Applied Ecology 19*, 47-63. https://www.jstor.org/stable/2402990

Young, A. (1986). Hábitat differences in cocoa tree flowering, fruit-set and pollinator availability in Costa Rica. *Journal of Tropical Ecology 2*, 163–186. https://cutt.ly/2Z2vwSW