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Pollinator conservation through nutrient substrates: A strategy for sustainable cocoa production

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ABSTRACT

The purpose of this research was to evaluate the impact of the use of nutritious substrates on the conservation of cocoa pollinators, particularly of the Ceratopogonidae family, as a strategy to improve the productivity and sustainability of the crop. The research was carried out in a 20-year-old cocoa plantation located in Manabí, Ecuador, during an annual cycle. A randomized complete block design (RCBD) with a 2 × 3 factorial arrangement was used, considering two types of cocoa (Nacional and CCN-51 clones) and three types of substrates (cocoa husk, plantain/banana pseudostem and leaf litter). Pyramid traps were used to capture pollinators and parameters of flowering, pollination, fertilization and fruit formation were recorded in 75 trees per cocoa type. During the study, 7876 individuals belonging to the Ceratopogonidae family were captured, predominantly the genus *Dasyhelea*. The substrate composed of plantain/banana pseudostems attracted the highest number of pollinators, highlighting its potential as a favorable medium for conservation. In terms of clone yield, CCN-51 showed higher flowering (up to 165 flowers per tree) and higher dry yield (2326 kg/ha). On the other hand, the Nacional clones showed better pollination (20.56 %), fertilization (7.31 %) and fruit formation (3.34 %) rates, highlighting their biological efficiency in production. One of the main limitations of the study was its development in a single area and annual cycle, which restricts the generalizability of the results. However, the practical value of the research lies in demonstrating that the management of agricultural residues can enhance pollination ecosystem services and, consequently, improve cocoa productivity. This strategy represents a sustainable, original and relevant alternative to strengthen cocoa production through the conservation of its pollinators.

Keywords: ecosystem services, pollinators, substrates, cocoa yield.

INTRODUCTION

Cocoa (*Theobroma cacao*) is a crop of great importance worldwide, especially in tropical countries such as Ecuador (Abad et al., 2020), where, in addition to its economic relevance, it has a significant impact on the local communities that depend on its production and marketing (Vargas Pérez et al., 2021). Ecuador stands out for its production and predominance of fine and aroma cocoa. However, other materials, mainly the CCN-51 clone, are also widely cultivated, preferably for their yield (Cedeño and Dilas-Jiménez, 2022). Despite its relevance, cocoa has low productivity due to several factors, including a low fertility rate, since only about 2% of the flowers are fertilized and become fruit, mainly due to its self-incompatibility and low pollinator population (Alvim, 1984; Avendaño-Arrazate et al., 2021).

Pollination in cocoa is entomophilous (Armijos Vásquez et al., 2020), and is therefore an essential ecosystem service that contributes directly to crop productivity. Pollinators are particularly insects of the order Diptera, of the Ceratopogonidae family, especially the genera *Forcipomyia* and *Dasyhelea*. These organisms transfer pollen from one flower to another, facilitating fertilization and subsequent fruit formation (Cañarte et al., 2021; García ad Guzmán-Cedeño, 2022; Montero et al., 2022). This service not only has a positive impact on the quantity and quality of the fruits, but also has a significant economic value for cocoa producers; since cocoa has low natural fertility, the efficiency of pollination significantly influences its yield (Lander et al., 2025), which means that pollinators play a fundamental ecosystemic role in the production chain of this crop.

In this context, the conservation of pollinator diversity and the proper management of their habitat are crucial to ensure that this ecosystem service continues to benefit both the environment and the economy of agricultural communities. In such circumstances, the objective of this research was to evaluate the impact of pollination as an ecosystem service in two types of cocoa (Nacional and CCN-51), analysing the diversity and abundance of pollinators, as well as their effect on pollination and fruit production, in order to promote management strategies that optimize pollination and improve crop productivity.

MATERIALS AND METHODS

Location, experimental area, and experiment management

This research was developed during twelve months in the period July 2022-June 2023, on the campus of the Escuela Superior Politécnica Agropecuaria de Manabí ESPAM MFL, located in Bolívar-Manabí, Ecuador; under the climatic conditions of 21–30°, annual rainfall of 1000 mm and average relative humidity of 82% (Valdivieso et al. 2021). An experimental area of 1350 m^2 was set up within a 20-year-old monoculture cocoa plantation, with 150 trees with the two types of cocoa. The area was divided into two experimental plots of 675 m^2 each (45×15 m), in which 75 trees of Nacional cocoa clones (EET-103, EET-510, EET-544, EET-559, EET-575, EET-576) and 75 trees of the CCN-51 clone were evaluated, established at a distance of 3 x 3 m between trees, with a population of 1111 pl. ha^{-1}. Drip irrigation was used, with monthly irrigation during the dry season (June-December). Fertilization was inorganic and was carried out at the beginning and end of the rainy season. A phytosanitary pruning was performed, and on two occasions, the removal of pods affected by Frosty Pod Rot (‘monilla’) and Witches’ Broom (‘escoba’) was carried out.

Total number of flowers per tree/month

Within the experimental area, 15 trees per plot were randomly selected, with 15 from each cocoa type (Nacional and CCN-51), totalling 30 observation units. Each tree was identified with a blue ribbon (Figure 1) for tracking purposes. Observations were made once a month for the 12 months of the experiment, and the total number of flowers on the trunk and main branches of each tree was recorded through direct counting.

Percentage of pollination, fertilization and fruit formation

In the study of flower pollination, flower fertilization and fruit formation, the type of cocoa: Nacional and CCN-51, was considered as a response factor. The same 15 trees per experimental plot were used as in the previous variable, and each tree was considered as a unit of observation. At the beginning of each of the 12 months of evaluation, a branch or section of the trunk approximately 60 cm long was selected, making sure that it contained mature flower buds (ready to open) (Figure 2). This section was marked with masking tape for tracking throughout the study.

Flower buds were counted at the start, and open, dried or detached flowers were removed. Thereafter, pollinated flowers were recorded by observing and counting open flowers 3 and 6 days after the initial marking. Fertilization was assessed by counting visibly fertilized flowers (yellow or brown) at 14 days, and finally, developing fruits were recorded at 21 and 28 days. Based on these data, the corresponding percentages were calculated using Equation 1: for pollination, the count at 6 days was taken; for fertilization, the count at 14 days was used, and for fruit formation, the count at 21 days was used.

$$\%\text{Pollination/Fertilization/Fruit Formation} = \frac{\#\ Pollinated\ flowers, fertilized\ flowers, or\ formed\ fruits}{\#\ Mature\ flower\ buds} \times 100 \quad (1)$$

Figure 1. Cocoa trees marked with blue ribbon (the yellow ribbon corresponded to the trees selected for evaluating pod production and dry cocoa yield)

Pollinators of the Ceratopogonidae family

For the monitoring of pollinators of the Ceratopogonidae family, the type of cocoa (Nacional and CCN-51) and three types of food substrates for pollinators (cocoa husk, plantain/banana pseudostem and leaf litter) were established as response factors (Table 1). The experimental area was divided into six plots (three with Nacional and three with CCN-51), each with 25 trees. Within each plot, three 'truncated pyramid' type traps were placed, which were constructed of wood with a dimension of 0.70 m in height and a base of 0.50 × 0.50 m (Figure 3A and 3B). These traps have an upper and a lateral opening, where a transparent hose approximately 10 cm in length is inserted. At the end of the hose, a 9 cm glass jar containing 70% alcohol is attached, which serves as a collector for the pollinators (Montero et al., 2019)

Figure 2. Section of tree branch marked with tape for assessment of floral biology (flowering, pollination, fertilization and fruit formation)

Each trap corresponded to one of the three substrates and a total of 18 traps were randomly distributed among the six plots. The traps were placed during one year, evaluated monthly and coincided with the harvest dates. This was done in order to take advantage of the cocoa husks. On each date, two mounds of each substrate were placed; one to place the trap and the other as a pollinator attractant. Once the substrate was placed under the trap, it was ensured that the trap was hermetically sealed, using the surrounding leaf litter to ensure the seal. Samples were collected monthly in individual jars with 70% alcohol, duly identified. The identification and counting of pollinator diversity was carried out in the Entomology Laboratory of the Portoviejo Experimental Station of the National Institute of Agricultural Research (INIAP), using the reference collection maintained by this laboratory (Marino & Spinelli, 2008).

A randomized complete block design (RCBD) was used, with a 2 × 3 factorial arrangement, considering factor A the Nacional cocoa monoculture system (a1) and the CCN-51 cocoa monoculture system (a2), factor B the substrate, where cocoa husk (b1), plantain/banana pseudostem (b2) and leaf litter (b3).

Total pod production and dry cocoa yield

Pod production and dry cocoa weight were evaluated monthly on each of the 75 trees per plot (Nacional and CCN-51), considering each of the

Figure 3. Truncated pyramid traps (a), placement of the alcohol glass jars (b)

150 trees as an individual unit of analysis. Harvesting was carried out monthly, and the data were consolidated on an annual basis. To calculate cocoa yield, the dry weight in kg per tree was multiplied by the planting density (1111 plants ha^{-1}).

Statistical analysis

The data were subjected to the Shapiro–Wilk normality test and Bartlett's test for homogeneity of variances. Since the assumptions required for parametric analysis were not met, the non-parametric Kruskal–Wallis test was used to evaluate differences between treatments. Subsequently, a post hoc median comparison analysis was conducted to group treatments using shared letters based on the significance level ($\alpha = 0.05$). All analyses were performed using the 'Agricolae' package in the R statistical software (R Core Team, 2024)

Table 1. Treatments for the counting of Ceratopogonidae pollinators

Treatment	Combination
T1	Nacional+Cocoa husk
T2	Nacional+Pseudostem
T3	Nacional+Leaf litter
T4	CCN-51+Cocoa husk
T5	CCN-51+Pseudostem
T6	CCN-51+Leaf litter

RESULTS AND DISCUSSION

Total number of flowers per tree/month

Kruskal-Wallis test revealed significant statistical differences ($P < 0.05$) in nine of the 12 months evaluated, the exceptions being the months of July and August 2022, as well as January 2023, between the two types of cocoa under study (Table 2). Clone CCN-51 showed a significant difference compared to the Nacional clones, standing out for its higher flower production throughout the year. The maximum flower production was recorded in February with an average of 165.07 flowers/tree in CCN-51, while Nacional reached only 64.07 flowers/tree in the same month. In terms of average annual production, CCN-51 recorded a total of 756 flowers/tree, far exceeding both the Nacional, which had 317 flowers/tree, and the overall average of all the trees evaluated (537 flowers/tree) (Table 3).

Despite the differences between the two types of cocoa trees, a marked parallelism was observed in the temporal pattern of flowering. It was also evident that the highest flowering load was concentrated in the first half of the year, which corresponds to the rainy season (Figure 4). These results confirm a strong influence of environmental factors, mainly rainfall, on cocoa flowering, as pointed out by Ríos Moyano et al. (2023). This superiority of the CCN-51 clone is associated with its adaptability and genetic stability of its flowering, depending on the environmental conditions of the production area, as supported by studies carried

Table 2. Floral production (number of flowers per tree) in two cocoa types: Nacional and CCN-51

Month	Overall Mean	Mean – CCN-51 (flowers/tree)	Mean – Nacional (flowers/tree)	CV (%)	Normality Test (Shapiro-Wilk: W; p value)	Homogeneity Test (Bartlett: K²; p value)	Kruskal-Wallis Test (Chi²; p value)	MSD
July 2022	14.5	18.53a	10.47a	79.22	W = 0.809; p < 0.0001	K² = 8.57; p = 0.0034	χ² = 3.50; p = 0.0614	6.27
August 2022	18.37	23a	13.73a	77.67	W = 0.88; p = 0.0026	K² = 11.43; p = 0.0007	χ² = 1.26; p = 0.2619	6.54
September 2022	25.2	34.87a	15.53b	73.7	W = 0.91; p = 0.01502	K² = 10.94; p = 0.00094	χ² = 7.64; p=0.00570	5.74
October 2022	29.5	39.80a	19.20b	74.7	W= 0.9; p = 0.0063	K² = 4.26, p = 0.0389	χ² = 7.06; p = 0.0079	5.83
November 2022	38	57.80a	18.20b	62.02	W= 0.93; p= 0.04913	K² = 7.63, p = 0.0057	χ² =14.58; p = 0.0001	4.72
December 2022	36.23	48.53a	23.93b	59.49	W = 0.98; p = 0.6943	K² = 9.94, p = 0.0016	χ² = 6.30; p = 0.0120	5.93
January 2023	70.27	83.27a	57.27a	79.43	W = 0.83, p < 0.001	K² = 2.57, p = 0.12	χ² = 1.87; p = 0.171	6.47
February 2023	114.57	165.07a	64.07b	121.78	W = 0.75, p = <0.001	K² = 3.83, p = 0.05	χ² = 7.85; p = 0.005	5.72
March 2023	47.93	75.53a	20.33b	191.33	W = 0.52, p < 0.001	K² = 31.33, p < 0.001	χ² =5.40; p = 0.02	6.04
April 2023	52.97	82.20a	23.73b	146.69	W = 0.46, p < 0.001	K² = 29.49, p < 0.001	χ² =11.58; p = 0.0006	5.19
May 2023	44.53	66.27a	22.80b	121.41	W = 0.47, p < 0.001	K² = 31.78, p < 0.001	χ² =14.57; p < 0.001	4.72
June 2023	44.63	61.53a	27.73b	148	W = 0.45, p < 0.001	K² = 26.7, p < 0.001	χ² =5.7; p = 0.02	6

Note: W = Shapiro-Wilk statistic (normality of residuals); K² = Bartlett's test statistic (homogeneity of variances); χ² = Kruskal-Wallis test statistic (non-parametric comparison); MSD = Minimum Significant Difference. Different letters indicate statistically significant differences (p < 0.05). Raw data are provided in Annex 1 (Supplementary Material).

Table 3. Summary of statistical results for monthly and annual flower production (number of flowers per tree) in two cocoa types: Nacional and CCN-51

Parameters	Overall Mean (# of flowers/ tree)	Mean – CCN-51 (# of flowers/tree)	Mean – Nacional (# of flowers/tree)	CV (%)	Normality Test (Shapiro-Wilk: W; p value)	Homogeneity Test (Bartlett: K²; p value)	Kruskal-Wallis Test (Chi²; p value)	MSD
# total flowers/tree/ year	537	756 a	317 b	89.95	W = 0.64, p < 0.001	K² =14.90; p < 0.001	χ² =12; p < 0.001	5.13
# mean flowers/tree/ month	45	63 a	26 b	89.86	W = 0.64, p < 0.001	K² =14.89; p < 0.001	χ² =12; p < 0.001	5.13

Note: W = Shapiro-Wilk statistic (normality of residuals); K² = Bartlett's test statistic (homogeneity of variances); χ² = Kruskal-Wallis test statistic (non-parametric comparison); MSD = Minimum Significant Difference. Different letters indicate statistically significant differences (p < 0.05). Raw data are provided in Annex 2 (supplementary material).

out by Gil-Restrepo et al. (2017) and Torres de La Cruz et al. (2023), who, when evaluating several cocoa clones, including CCN-51, determined that it always produced the highest number of flowers/tree, strongly associating this behaviour with rainfall, but that it also requires greater nutritional demand (Puentes-Páramo et al. 2014).

Percentage of flowers pollinated

When analysing the pollination of flowers in the two cocoa types (Nacional and CCN-51), the Kruskal-Wallis test revealed significant statistical differences (P < 0.05), in the months of August, October, November 2022, as well as in January, February, March and June 2023 (Table 4). During this period, with the exception of August, the National cocoa stood out significantly from CCN-51, by presenting the highest pollination percentages, reaching peak values in the months of November (32.13%), May (31.19%), March (22.04%) and August (19.73%). The CCN-51, on the other hand, showed peaks in August (30.60%), May (25.75%), November (18.40%) and March

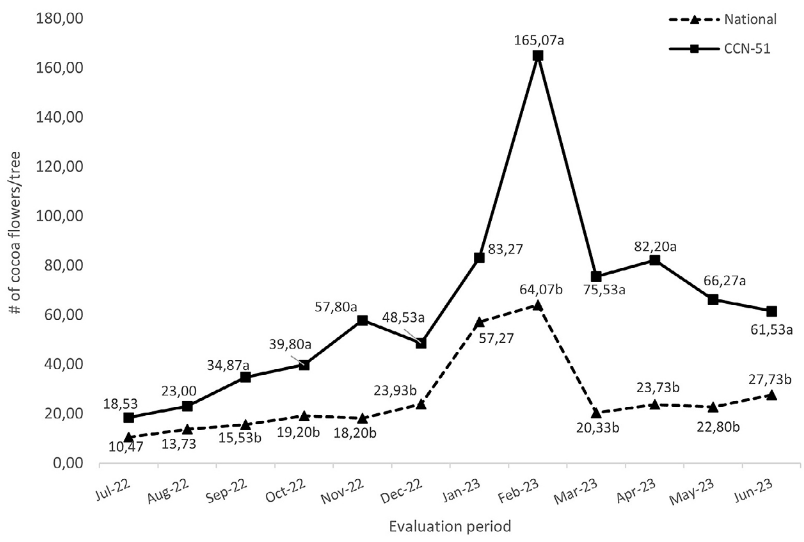

Figure 4. Fluctuation of the average flower/tree production of two types of cocoa. Averages with different letters show significant differences ($p < 0.05$).

(14.27%). Despite these differences, both cocoa types exhibited a very similar fluctuation in pollination over time, with coinciding peak periods, such as the one observed in May, results similar to those reported by Córdoba et al. (2013).

Although there was no marked seasonality in the percentage of pollination of flowers, a slight increase was observed in the rainy season in the Nacional type, while in CCN-51 this increase occurred during the dry season of the year (Figure 5). Ríos-Moyano et al. (2023) state that pollination is more effective during the rainy season, which is associated with the greater abundance of pollinator populations at this time of year. Studies by Vansynghel et al. (2022) in Peru found that climatic factors may influence the absence of a clear pattern in pollination, suggesting that the region may face a significant pollination deficit in cocoa cultivation. Finally, another interesting and under-researched aspect is presented by Arnold et al. (2019), who explored the role of flower odour on pollinators in an attempt to understand the plant-insect interaction.

Percentage of fertilized flowers

In reference to the fertilization of flowers (Table 5), Kruskal-Wallis established significant statistical differences ($p < 0.05$) between the two cocoa types, during the months of August 2022 and January, February, March, April, May 2023. The results obtained for this variable were consistent with the pollination patterns previously described, since, once again, the Nacional cocoa type stood out over the CCN-51, by presenting higher fertilization percentages, mainly in the months of August (dry season) and January, February, March, April, May and June (rainy season).

A synchrony was observed in both cocoa types throughout the year; coinciding in the months of greatest productive activity, March (12.26 and 5.04%) and May (18.38 and 6.81%), respectively. It was also determined that in the Nacional type, fertilization was more intense in the rainy season, which suggests a positive influence of rainfall on the reproductive process. This situation is not marked in the CCN-51 clone (Figure 6). Cañarte et al. (2021), in their annual evaluation, obtained an increase in fertilization from March to December, as well as a significant drop between pollination and fertilization, from 5.88% to 2.74% of fertilized flowers. Córdoba et al. (2013) obtained similar fertilization percentages, in their case from 2 to 4%. The percentages of this research coincide with the literature, since a decline in cocoa fertilization could be related to fluctuations in climatic conditions or the availability of pollinators during these months (Vansynghel et al., 2022; Jaramillo et al., 2024).

Percentage of fruit formation

When analysing the response of cocoa type on the percentage of cocoa fruit formation (Table 6), Kruskal-Wallis detected significant statistical differences ($P < 0.05$) in the months of August and

Table 4. Percentage of pollinated flowers in two cocoa types: Nacional and CCN-51

Month	Overall Mean (% flower pollination)	Mean – CCN-51 (% flower pollination)	Mean – Nacional (% flower pollination)	CV (%)	Normality Test (Shapiro-Wilk: W; p value)	Homogeneity Test (Bartlett: K²; p value)	Kruskal-Wallis Test (Chi²; p value)	Minimum significant difference (MSD)
July 2022	13.57	15.57a	11.47a	76.64	W = 0.9652, p= 0.4175	K² = 2.3603e-07; p = 0.99	χ² = 1.79; p = 0.18	6.44
August 2022	25.17	30.60 a	19.73 b	33.95	W= 0.898, p = 0.007561	K² = 0.04; p = 0.842	χ² = 10.4; p = 0.001	5.35
September 2022	12.6	12a	13.2a	48.09	W = 0.986, p = 0.9622	K² = 0.89; p=0.345	χ² = 0.53; p = 0.466	6.61
October 2022	16.87	12.47 b	21.27 a	53.79	W = 0.985, p = 0.9302	K² = 5.47, p = 0.019	χ² = 5.74; p = 0.017	5.97
November 2022	25.27	18.4 b	32.13 a	51.31	W = 0.956, p = 0.247	K² = 12.36, p=0.0004	χ² = 7.42; p = 0.006	5.77
December 2022	20.77	18.4a	23.13a	65.83	W= 0.78, p = 0.00001	K² = 0.7, p=0.4	χ² = 1.8; p = 0.18	6.5
January 2023	13.23	7.65 b	18.81 a	80.24	W=0.95, p=0.23	K² = 8.6, p=0.003	χ² = 4.6; p = 0.03	6.12
February 2023	15.75	10.55 b	20.95 a	75	W = 0.946, p= 0.133	K² = 9.12, p = 0.0025	χ² = 4.32; p = 0.038	6.16
March 2023	18.15	14.27 b	22.04 a	71.45	W = 0.891, p = 0.0051	K² = 0.217, p = 0.65	χ² = 4.67; p = 0.031	6.13
April 2023	11.8	8.53a	15.07a	99	W = 0.882, p= 0.0031	K² = 9.02, p = 0.002	χ² =1.27; p = 0.26	6.52
May 2023	28.47	25.75a	31.19a	62	W = 0.95, p = 0.2	K² = 0.22, p = 0.63	χ² =0.92; p = 0.33	6.58
June 2023	12.73	7.74 b	17.73 a	100.64	W = 0.72, p < 0.001	K² = 7.44, p = 0.006	χ² =6.12; p = 0.01	5.93

Note: W = Shapiro-Wilk statistic (normality of residuals); K² = Bartlett's test statistic (homogeneity of variances); χ² = Kruskal-Wallis test statistic (non-parametric comparison); MSD = Minimum Significant Difference. Different letters indicate statistically significant differences ($p < 0.05$). Raw data are provided in Annex 3 (Supplementary Material).

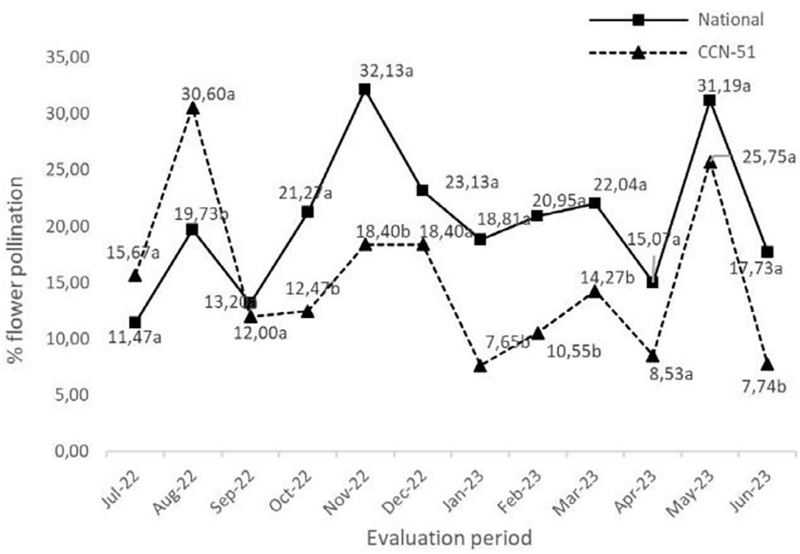

Figure 5. Fluctuation of pollination percentage. Means with different letters represent significant differences ($p < 0.05$)

May. It was observed that, even without statistical differences, Nacional numerically outperformed CCN-51 in seven months of the year. The highest percentages of fruits formed (6.18, 6.69, 3.51, 4.83, 7.37 and 4.35%), belonging to the Nacional, were reported in the months of January to June, respectively, which correspond to the rainy period, indicating a seasonality. On the other hand,

Table 5. Monthly percentage of fertilized flowers in two cocoa types: Nacional and CCN-51

Month	Overall Mean (% fertilized flowers)	Mean – CCN-51 (% fertilized flowers)	Mean – Nacional (% fertilized flowers)	CV (%)	Normality Test (Shapiro-Wilk: W; p value)	Homogeneity Test (Bartlett: K²; p value)	Kruskal-Wallis Test (Chi²; p value)	MSD
July 2022	2.87	3.27a	2.47a	109.25	W = 0.864, p = 0.00126	K² = 0.0001; p = 0.992	χ² = 0.6; p = 0.44	6.41
August 2022	3.87	2.07 b	5.67 a	87.47	W = 0.95, p = 0.162	K² =2.45; p = 0.118	χ² =6.54; p = 0.0105	5.69
September 2022	3.17	3.33a	3a	82.97	W = 0.93, p = 0.0255	K² = 3.71; p = 0.054	χ² =0.11; p = 0.735	6.56
October 2022	3	3.47a	3.4a	79.64	W = 0.92, p = 0.0224	K² = 0.93; p = 0.336	χ² =0.028; p = 0.867	6.62
November 2022	3.6	3.87a	3.33a	67.45	W = 0.95, p = 0.1584	K² = 3.38; p = 0.066	χ² =0.394; p = 0.53	6.59
December 2022	3.4	3.33a	3.47a	93.51	W = 0.84, p = 0.00031	K² = 1.02; p=0.312	χ² =0.056; p = 0.81	6.45
January 2023	6.56	2.64 b	10.48 a	123	W = 0.80, p < 0.001	K² = 15.9; p<0.001	χ² =4.97; p = 0.0258	5.9
February 2023	6.08	2.32 b	9.85 a	128	W = 0.81, p < 0.001	K² = 20.43; p<0.001	χ² =4.97; p = 0.0258	5.9
March 2023	8.65	5.04 b	12.26 a	101.42	W = 0.83, p = 0.00024	K² =11.52; p = 0.00069	χ² =4.90; p = 0.026	6.07
April 2023	5.97	2.68 b	9.27 a	140.18	W = 0.735, p-value <0.0001	K² = 14.27; p < 0.001	χ² =2.8; p = 0.051	6.03
May 2023	12.6	6.81 b	18.38 a	84	W = 0.893, p = 0.0058	K² = 3.29; p = 0.07	χ² =8.43; p = 0.004	5.6
June 2023	4	1.9a	6.09a	127.8	W = 0.7, p < 0.001	K² =16.13; p < 0.001	χ² =2.01; p = 0.156	5.95

Note: W = Shapiro-Wilk statistic (normality of residuals); K^2 = Bartlett's test statistic (homogeneity of variances); χ^2 = Kruskal-Wallis test statistic (non-parametric comparison); MSD = Minimum Significant Difference. Different letters indicate statistically significant differences ($p < 0.05$). Raw data are provided in Annex 4 (Supplementary Material).

CCN-51 reached a maximum of 3.33% of fruits formed in the month of December. These results are consistent with the higher pollination and fertilization values described above.

In contrast to pollination and fertilization, there was no synchronism in fruit formation between the two cocoa types throughout the year. On the contrary, there was a differentiated erratic fluctuation between the two types of cocoa. With regard to the seasonality of CCN-51, as with pollination and fertilization, a slight increase in fruit formation was observed during the dry period of

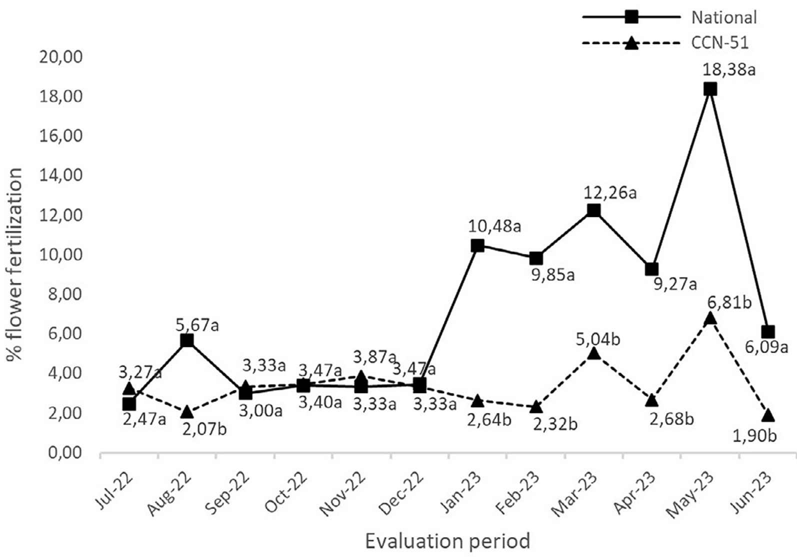

Figure 6. Fluctuation of the percentage of fertilized flowers. Means with different letters show significant differences ($p < 0.05$)

Table 6. Percentage of fruit formation in two cocoa types

Month	Overall Mean (% fruit formation)	Mean – CCN-51 (% fruit formation)	Mean – Nacional (% fruit formation)	CV (%)	Normality Test (Shapiro-Wilk: W; p value)	Homogeneity Test (Bartlett: K²; p value)	Kruskal-Wallis Test (Chi²; p value)	MSD
July 2022	1.83	2.33 a	1.33 a	117.83	W = 0.87, p = 0.00198	K² = 1.22; p = 0.249	χ² = 1.25; p = 0.2629	6.19
August 2022	2.9	1.53 b	4.27 a	96.37	W = 0.93, p = 0.065	K² = 2.48; p = 0.092	χ² =5.72; p = 0.0168	5.73
September 2022	2.5	2.47 a	2.53 a	96.05	W = 0.84, p = 0.00037	K²=0.61; p = 0.434	χ² = 0.007; p = 0.931	6.45
October 2022	2.9	2.73 a	3.07 a	76.51	W = 0.94, p= 0.092	K²=3.77; p = 0.052	χ² = 0.075; p = 0.785	6.6
November 2022	2.57	3 a	2.13 a	67.37	W = 0.96, p= 0.33	K²= 0.27; p = 0.605	χ² = 1.56; p = 0.211	6.38
December 2022	2.57	3.33 a	1.8 a	99.26	W = 0.93, p = 0.043	K²=0.26; p = 0.612	χ² = 2.73; p = 0.099	6.08
January 2023	3.99	1.81 a	6.18 a	137.7	W = 0.864, p = 0.00125	K² = 15.83; p < 0.001	χ² =1.72; p = 0.189	6.16
February 2023	4.16	1.63 a	6.69 a	138	W = 0.861, p = 0.00108	K² = 21.04; p < 0.001	χ² = 1.96; p = 0.161	6.13
March 2023	3.27	3.03 a	3.51 a	136.3	W = 0.79, p < 0.001	K² = 6.59; p = 0.10	χ² = 0.32; p = 0.573	6.14
April 2023	3.38	1.93 a	4.83 a	126.45	W =0.896, p = 0.0067	K² = 7.05; p = 0.008	χ² = 1.2; p = 0.27	6.22
May 2023	4.71	2.05 b	7.37 a	121	W = 0.874, p = 0.002	K² = 6.58; p = 0.01	χ² = 4.35; p = 0.037	5.77
June 2023	3.02	1.68 a	4.35 a	193.17	W = 0.7, p < 0.001	K² = 14.48; p < 0.001	χ² = 0.34; p = 0.558	5.9

Note: W = Shapiro-Wilk statistic (normality of residuals); K² = Bartlett's test statistic (homogeneity of variances); χ² = Kruskal-Wallis test statistic (non-parametric comparison); MSD = Minimum Significant Difference. Different letters indicate statistically significant differences (p < 0.05). Raw data are provided in Annex 5 (Supplementary Material).

the year (Figure 7A). Finally, Figure 7B shows the annual mean values of the three variables presented above, depending on the type of cocoa (Nacional and CCN-51). The results show that, in general, Nacional outperforms CCN-51 in the annual mean percentages of pollination (20.56%), fertilization (7.31%) and fruit formation (3.34%) (Figure 8A, 8B and Table 7).

Despite the better performance of Nacional in these variables, fruit production in *Theobroma cacao* remains extremely low, as evidenced by Vansynghel et al. (2022), where they obtained a 2% success rate for natural pollination compared to 7% for hand pollination in Peru. Likewise, Weinstein et al. (2024) showed that nutrient availability increases the percentage of natural fruit set, especially in terms of nitrogen, which highlights the importance of considering nutritional management in future research. The drastic reduction in fruit set is also related to environmental and biological factors, as supported by studies by Groeneveld et al. (2010). Lahive et al. (2019) and Ramírez-Argueta et al. (2022) agree that agroecological management of cocoa plantations could favourably influence their productivity.

Finally, the results on the seasonality of pollination, fertilization and fruit formation in this research suggest that Nacional cocoa is more adaptable to the environmental conditions of the rainy season, in contrast to CCN-51, which showed a slightly more favourable response during the dry season.

Pollinators of the family Ceratopogonidae on two cocoa types and three food substrates

During the research, 11 species of Diptera: Ceratopogonidae were identified, grouped into five species of the genus *Forcipomyia* (*F. quatei, F. uramensis, F. youngi, F. genualis* and *F. pictoni*), three species of the genus *Dasyhelea* (*D. borgmeiri, D. cacaoi* and *D. winderi*), as well as three species of the genus *Culicoides* (*C. pusillus, C. paraensis* and *Culicoides* sp.1). A total of 7876 specimens of Ceratopogonidae collected during the period July 2022 to June 2023 were counted, grouped into 1550 specimens of the genus *Forcipomyia*; 6067 specimens of the genus *Dasyhelea* and 259 specimens of the genus *Culicoides* (Figure 9).

This predominance of individuals of the genus *Dasyhelea* is consistent with reports by Montero et al. (2019), Cañarte et al. (2021) and Montero et al. (2022) in similar areas. In contrast, studies by Salazar-Díaz and Torres-Coto (2017) in Costa Rica reported that the Ceratopogonidae family was the least abundant among all pollinators captured, with only 9% of the population. In Nicaragua, Vandromme et al. (2023) captured pollinators in the reproductive part of cocoa flowers, finding that 95% of dipterans belonged to the family Cecidomyiidae (86%) and 9.4% to Ceratopogonidae. The latter study was carried out in habitats with similar substrates to those evaluated in the present investigation, and highlights that the relevance of the Ceratopogonidae family has been underestimated in the literature, despite its crucial role in cocoa pollination.

Population fluctuation of Ceratopogonidae pollinators in relation to the two cocoa types

When the population fluctuation values of the specimens of the Ceratopogonidae family, present in the two cocoa types (Nacional and CCN-51) were analysed, the Kruskal-Wallis test did not establish a significant statistical response of the populations (P > 0.05), to the types of cocoa, in any of the 12 months of evaluation (Table 8). The populations had a very similar fluctuation throughout the year of study, coinciding in several population peaks, mainly in August and December, being in the latter month when the average maximum was reached for both cocoa types (Figure 10A).

Population fluctuation of Ceratopogonidae pollinators in relation to the three food substrates

With regard to the analysis according to the three food substrates (cocoa husk, plantain/banana pseudostem and leaf litter), the Kruskal-Wallis test reported significant statistical difference (P < 0.05) in all months of evaluation, except March and May (Table 9). The pseudostem

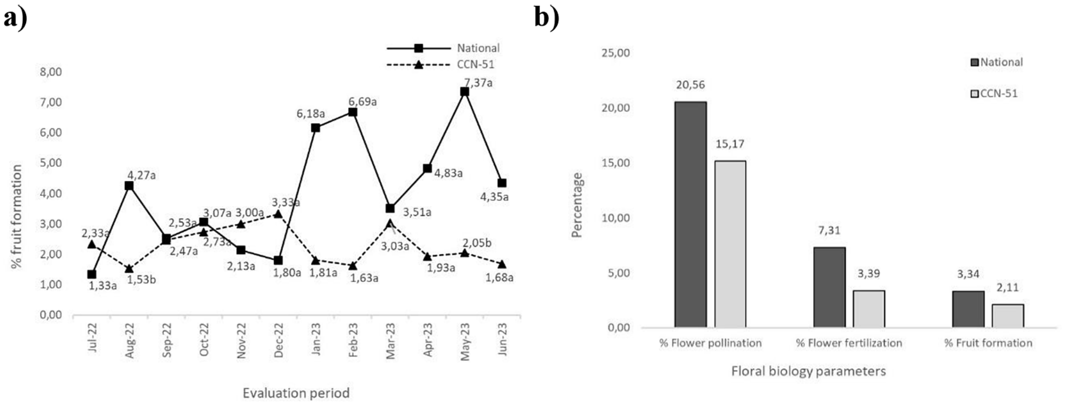

Figure 7. A. Fluctuation of fruit formation, B. Annual average values of floral biology parameters of two cocoa types. Averages with different letters show significant differences (p < 0.05)

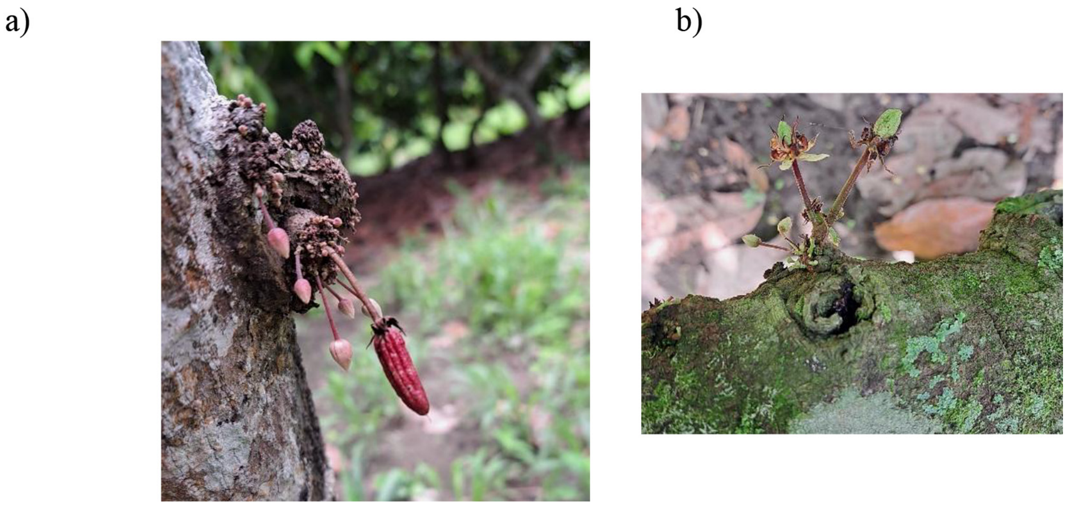

Figure 8. A. Fruit development on a CCN-51 cocoa tree, B. Fruit development on a Nacional cocoa tree

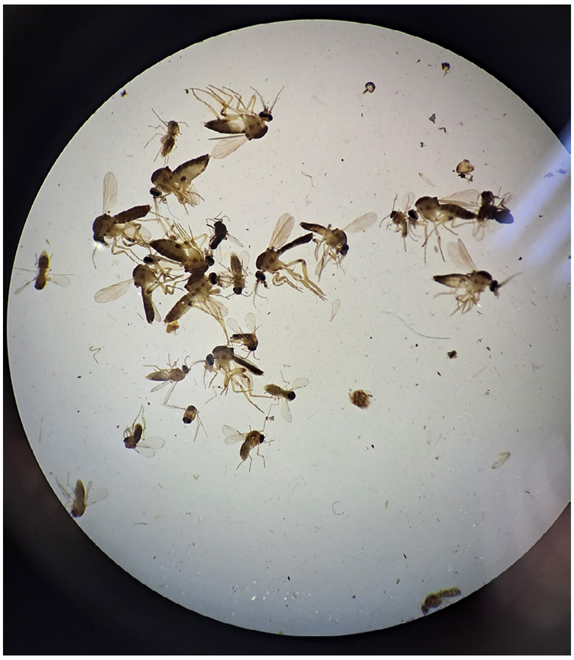

Figure 9. Sample of pollinators collected in one of the traps

substrate stood out as always having the highest populations of pollinators of the Ceratopogonidae family. There was a very consistent population fluctuation throughout the year of evaluation in the pseudostem and cocoa husk substrates; in the pseudostem substrate there were two very distinct population peaks in August and December. In the cocoa husk substrate, there was a population peak in December (Figure 10B). The leaf litter substrate showed the worst performance. These results are supported by findings reported by Kaufmann (1975), Young (1983), and Montero et al. (2019), who state that the plantain/banana pseudostem, when cut into pieces and left within the plantation, facilitates water retention in its compartments, providing suitable sites for pollinators to lay their eggs.

Population fluctuation of Ceratopogonidae pollinators in the interaction between two cocoa types and three nutritional substrates

The Kruskal–Wallis analysis applied to the interaction between the two cocoa types and the three food substrates revealed significant differences ($P < 0.05$) in pollinator populations among treatments during nine months of the evaluation period: July, August, September, October, November, December, January, April, and June (Table 10). Treatment T2 (Nacional + plantain/banana pseudostem substrate) showed significantly higher populations of these organisms in the aforementioned months, followed by T5 (CCN-51 + plantain/banana pseudostem substrate). In contrast, the leaf litter substrate (T3 and T6) showed the lowest pollinator populations across all months of evaluation in both cocoa types. It was also observed that Ceratopogonidae pollinator populations increased during the dry season (July–December), while population levels

Table 7. Summary of annual averages of pollination, fertilization and fruit set parameters

Parameters	Overall Mean	Mean – CCN-51	Mean – Nacional	CV (%)	Normality Test (Shapiro-Wilk: W; p value)	Homogeneity Test (Bartlett: K^2; p value)	Kruskal-Wallis Test (Chi²; p value)	Minimum Significant Difference (MSD)
% flower pollination (3days)	48.2	45.61 b	50.78 a	11.9	W = 0.96 p = 0.355	K^2 = 1.19; p = 0.276	χ^2 = 4.74; p = 0.029	6.13
% flower pollination (6days)	17.86	15.17 b	20.56 a	17.86	W = 0.97, p = 0.537	K^2 = 1.08; p = 0.299	χ^2 = 6.09; p =0.014	5.96
% fertilization (14days)	5.35	3.39 b	7.31 a	40.84	W = 0.96, p = 0.304	K^2 = 6.1; p = 0.014	χ^2 = 14.72; p < 0.001	4.7
% fruit formation (21days)	3.15	2.29 b	4.01 a	50	W = 0.933, p= 0.059	K^2 = 6; p = 0.0145	χ^2 = 6.3; p = 0.012	5.93
% fruit formation (28days)	2.73	2.11 b	3.34 a	46.69	W = 0.96, p = 0.254	K^2 = 3.83; p = 0.05	χ^2 = 5.11; p = 0.0238	6.08

Note: W = Shapiro-Wilk statistic (normality of residuals); K^2 = Bartlett's test statistic (homogeneity of variances); χ^2 = Kruskal-Wallis test statistic (non-parametric comparison); MSD = Minimum Significant Difference. Different letters indicate statistically significant differences ($p < 0.05$). Raw data are provided in Annex 6 (Supplementary Material).

Table 8. Summary statistics, monthly Kruskal-Wallis test of Ceratopogonidae pollinators between two types of cocoa: Nacional and CCN-51

Month	Overall Mean (# of Ceratopogonidae pollinators)	Mean – CCN-51 (# of Ceratopogonidae pollinators)	Mean – Nacional (# of Ceratopogonidae pollinators)	Kruskal-Wallis Test (Chi²; p value)	MSD
July 2022	49.89	35.44 a	64.33 a	$\chi^2 = 0.57$; $p = 0.45$	5.39
August 2022	62.17	62.67 a	61.67 a	$\chi^2 = 0.24$; $p = 0.625$	5.43
September 2022	29.06	24.89 a	33.22 a	$\chi^2 = 0.38$; $p = 0.535$	5.42
October 2022	36.72	39.00 a	34.44 a	$\chi^2 = 0.05$; $p = 0.825$	5.48
November 2022	29.56	43.44 a	15.67 a	$\chi^2 = 1.65$; $p = 0.199$	5.215
December 2022	102.72	96.89 a	108.56 a	$\chi^2 = 0.24$; $p = 0.627$	5.46
January 2023	35.5	42.00 a	29.00 a	$\chi^2 = 0.44$; $p = 0.51$	5.43
February 2023	25.67	29.67 a	21.67 a	$\chi^2 = 0.44$; $p = 0.51$	5.43
March 2023	27	17.89 a	36.11 a	$\chi^2 = 0.071$; $p = 0.79$	5.46
April 2023	19.94	19.11 a	20.78 a	$\chi^2 = 0.2$; $p = 0.66$	5.46
May 2023	10.17	11.22 a	9.11 a	$\chi^2 = 0.39$; $p = 0.534$	5.4
June 2023	9.17	8.67 a	9.67 a	$\chi^2 = 0.05$; $p = 0.825$	5.48

Note: χ^2 = Kruskal-Wallis test statistic (non-parametric comparison). Different letters indicate statistically significant differences ($p < 0.05$). Raw data are provided in Annex 7 (Supplementary Material).

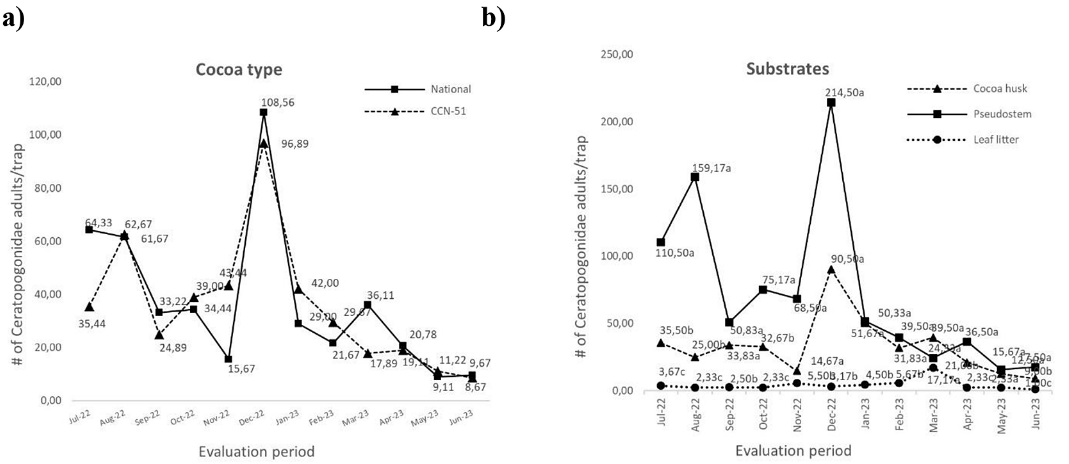

Figure 10. A. Comparison of the fluctuation of pollinators of the Ceratopogonidae family recorded on two cocoa types B. Comparison of the fluctuation of pollinators of the Ceratopogonidae family recorded on three substrates. Means with different letters present significant differences ($p < 0.05$).

declined across all treatments during the rainy season (January–June).

Seasonal dynamics of Ceratopogonidae populations during the dry season (2022) and rainy season (2023)

The dry season obtained the best results, with T2 being the best performing, followed by T5 (Table 11). The Kruskal Wallis test found no significant differences in relation to the type of cocoa, although in the average annual population, the Nacional type was 13 individuals higher than the CCN-51 (Figure 11A). In relation to the substrates, significant differences were found, with the pseudostem in the dry season being the best compared to the others, presenting the highest average population of Ceratopogonidae, with 679 specimens, in contrast to the 185 individuals in the rainy season (Figure 11B). These results coincide with Armijos et al., (2020) where the highest populations of insects captured were recorded in November and December, while the lowest were observed in March. Salazar-Díaz &

Table 9. Monthly summary statistics of the number of Ceratopogonidae pollinators in relation to the three food substrates

Month	Overall Mean (# of Ceratopogonidae pollinators)	Mean – Cocoa Husk (# of Ceratopogonidae pollinators)	Mean – Plantain/ banana pseudostem (# of Ceratopogonidae pollinators)	Mean – Leaf litter (# of Ceratopogonidae pollinators)	Kruskal-Wallis Test (Chi²; p value)	(MSD)
July 2022	49.89	35.50 b	110.50 a	3.67 c	$\chi^2 = 12.1$; $p = 0.002$	3.75
August 2022	62.17	25.00 b	159.17 a	2.33 c	$\chi^2 = 13.48$; $p = 0.001$	3.17
September 2022	29.06	33.83 a	50.83 a	2.50 b	$\chi^2 = 11.95$; $p = 0.0025$	3.8
October 2022	36.72	32.67 b	75.17 a	2.33 c	$\chi^2 = 12.96$; $p = 0.0015$	3.41
November 2022	29.56	14.67 a	68.50 a	5.50 b	$\chi^2 = 9.91$; $p = 0.007$	4.51
December 2022	102.72	90.50 a	214.50 a	3.17 b	$\chi^2 = 12.56$; $p = 0.0018$	3.57
January 2023	35.5	50.33 a	51.67 a	4.50 b	$\chi^2 = 10.16$; $p = 0.006$	4.43
February 2023	25.67	31.83 a	39.50 a	5.67 b	$\chi^2 = 9.63$; $p = 0.008$	4.6
March 2023	27	39.5 a	24.33 a	17.17 a	$\chi^2 = 2.75$; $p = 0.253$	6.37
April 2023	19.94	21.00 b	36.50 a	2.33 c	$\chi^2 = 13.4$; $p = 0.001$	3.21
May 2023	10.17	12.50 a	15.67 a	2.33 a	$\chi^2 = 4.06$; $p = 0.131$	6.06
June 2023	9.17	9.00 b	17.50 a	1.00 c	$\chi^2 = 13.7$; $p = 0.001$	3.1

Note: χ^2 = Kruskal-Wallis test statistic (non-parametric comparison). Different letters indicate statistically significant differences ($p < 0.05$). Raw data are provided in Annex 7 (supplementary material).

Torres (2017) state that the decrease in the Ceratopogonidae population may be attributed to environmental conditions or to the influence of some external agent.

Production of total cocoa

In reference to the average monthly production ha^{-1} of total cocoa pods (large, medium and small), Kruskal Wallis established significant statistical differences ($p < 0.05$), in the months of July, August, September and November 2022 (Table 12). When analysing the fluctuation during the year, and as a function of the two cocoa types, it was consistently observed that CCN-51 outperformed Nacional (Figure 12). This superior performance of CCN-51 could be attributed to its higher flower production throughout the year, which could compensate for its lower pollination, fertilization and fruit set rates compared to Nacional. This finding is in agreement with that reported by Ramos-Remache et al. (2023), who identified CCN-51 as the material with the highest yield in pod production, and who also attributed this to the fact that the Nacional clones produce only 42% of the total flowers produced by CCN-51.

Seasonality of total cocoa

CCN-51 stood out with an average of 41344 pods ha^{-1}/year (monthly average of 3445), much higher than the 26768 pods ha^{-1}/year (monthly average of 2231) achieved by the Nacional clone. As for the dry and rainy season, Kruskal-Wallis, determined statistical differences ($P < 0.05$) between them, in the dry period of the year, with the CCN-51 clone standing out significantly, presenting the highest average number of pods ha^{-1} (30752), in contrast to the Nacional clones, which reached 15598 pods ha^{-1}. Production decreased in the rainy period, a pattern also reported by Bacca et al. (2023), who recorded peak yields towards the latest months of the year.

Dry cocoa yield

The summary of the results is presented in Table 13, and Figure 13 shows the fluctuation of the average monthly dry cocoa yield in kg ha^{-1},

Table 10. Statistical summary of the number of Ceratopogonidae pollinators in relation to the interaction between two coca types and three food substrates.

Month	Overall Mean (# of Ceratopogonidae pollinators)	Mean-T1 (Nacional + Cocoa husk)	Mean-T2 (Nacio-nal + Pseudo-stem)	Mean T3 (Nacional + Leaf litter)	Mean-T4 (CCN-51 + Cocoa husk)	Mean-T5 (CCN-51 + Pseudostem)	Mean-T6 (CCN-51+Leaf litter)	CV (%)	Normality Test (Shapiro-Wilk: W; p value)	Homogeneity Test (Bartlett: K²; p value)	Kruskal-Wallis Test (Chi²; p value)	MSD
July 2022	49.89	51.67 abc	136.67 a	4.67 cd	19.33 bc	84.33 ab	2.67 d	49.88	W = 0.79, p = 0.001	K² = 34.33; p < 0.0001	χ² = 12.85; p = 0.025	5.57
August 2022	62.17	17.33 bc	165.67 a	2.00 c	32.67 ab	152.67 a	2.67 c	155.17	W = 0.70, p < 0.001	K² =29.33; p < 0.001	χ² = 14; p = 0.02	4.73
September 2022	29.06	41.67 a	56.00 a	2.00 b	26.00 a	45.67 a	3.00 b	79.81	W = 0.93, p = 0.212	K² =12.82; p = 0.03	χ² = 12.8; p = 0.03	5.63
October 2022	36.72	33.33 a	68.00 a	2.00 b	32.00 a	82.33 a	2.67 b	105	W = 0.96, p = 0.585	K² =19.63; p = 0.0015	χ² = 13.11; p = 0.022	5.4
November 2022	29.56	16.00 b	29.67 ab	1.33 c	13.33 b	107.33 a	9.67 bc	73.15	W = 0.92, p = 0.13	K² =15.138; p = 0.009	χ² = 12.6; p = 0.027	5.73
December 2022	102.72	85.33 a	236.67 a	3.67 b	95.67 a	192.33 a	2.67 b	100.77	W = 0.9, p = 0.05	K² =32.32; p < 0.001	χ² = 12.82; p = 0.025	5.6
January 2023	35.5	45.33 a	41.33 ab	0.33 c	55.33 ab	62.00 a	8.67 bc	106	W = 0.91, p = 0.1	K² =18.53; p = 0.002	χ² = 11.21; p = 0.047	6.6
February 2023	25.67	27.67 ab	35.33 ab	2 c	36 a	43.67 a	9.33 bc	69	W = 0.96, p = 0.54	K² =10.85; p = 0.054	χ² = 10.1; p = 0.07	7.2
March 2023	27	41.33 a	34.33 a	32.67 a	37.67 a	14.33 a	1.67 a	135	W = 0.91, p = 0.09	K² =14.18; p = 0.015	χ² = 3.4; p = 0.64	10.07
April 2023	19.94	26.00 ab	33.33 a	3.00 cd	16.00 bc	39.67 a	1.67 d	38.78	W = 0.96, p = 0.52	K² = 8.4; p = 0.14	χ² = 14.28; p = 0.014	4.51
May 2023	10.17	11.67 a	13.33 a	2.33 a	13.33 a	18.00 a	2.33 a	144	W = 0.89, p = 0.035	K² = 14.3; p = 0.014	χ² = 5; p = 0.42	9.45
June 2023	9.17	9.33 b	19.00 a	0.67 c	8.67 b	16.00 ab	1.33 c	52	W = 0.9, p = 0.078	K² = 10.3; p = 0.07	χ² = 14.12; p = 0.014	4.64

Note: W = Shapiro-Wilk statistic (normality of residuals); K² = Bartlett's test statistic (homogeneity of variances); χ² = Kruskal-Wallis test statistic (non-parametric comparison); MSD = Minimum Significant Difference. Different letters indicate statistically significant differences (p < 0.05). Raw data are provided in Annex 7 (Supplementary Material).

recorded between the two cocoa types under study. Kruskal-Wallis recorded significant statistical differences ($p < 0.05$) between the cocoa types in July, August, October, November, February and June. CCN-51 had the highest average dry cocoa yields in kg ha^{-1}. Despite the statistical difference between the two types of cocoa, they maintain a very similar production rate over time, even coinciding in several production peaks in July, October, January and May. In the case of CCN-51, July is reported as the month of highest production, while in the case of Nacional it was January.

According to studies carried out in Ecuador, by Pérez-Garcia and Freile-Almeida (2017), CCN-51 reaches its highest production in coastal areas, where climatic conditions differ from those of the Amazon region, favouring better crop development. In Colombia, research by Bacca et al. (2023) reiterates the superiority of genotypes with red-coloured fruit (CCN-51), as they obtained results of more than 460 kg ha^{-1}, exceeding regional averages.

Seasonal variation in dry cocoa yield

When analysing the cumulative annual dry cocoa yield in kg ha^{-1}, obtained between the two cocoa types, the Kruskal-Wallis analysis determined significant statistical differences ($p < 0.05$), with CCN-51 standing out, which reached an average of 2326 kg ha^{-1} of dry cocoa (Table 13) (Figure 14A). It was observed that for CCN-51 the highest yields occurred during the dry season, while the Nacional clones produced more during the rainy season. When the yield in kg ha^{-1} was analysed according to cocoa type, Kruskal-Wallis determined statistical differences ($p < 0.05$) between them, during the dry and rainy periods of the year, with CCN-51 standing out significantly, as it had the highest average yield in the dry season (1311 kg ha^{-1}) and 1009 kg ha^{-1} in the rainy season (Figure 14B).

This higher yield reported by the CCN-51 clone is consistent with what is expressed in the literature, as its genetic improvement is intended to increase production values compared to other Nacional type varieties (Pérez-García and Freile-Almeida, 2017; Abad-Sánchez et al. 2018). However, in general, cocoa yield is limited by an important factor, such as incompatibility, which may depend on the genetic origin of the pollen grains, and as it has a high variability, it is recommended to carry out studies locally first, as stated by Akoa et al. (2021).

Table 11. Summary of statistical results of Ceratopogonidae pollinators as a function of seasonality in two cocoa types (Nacional and CCN-51) and three nutritional substrates

Parameters		Total 2022 (dry season)	Total 2023 (rainy season)	Annual total
Overall Mean (# of Ceratopogonidae pollinators)		310.11	127.44	437.56
Cocoa type	Mean-Nacional (# of Ceratopogonidae pollinators)	317.89 a	126.33 a	444.22 a
	Mean-CCN-51 (# of Ceratopogonidae pollinators)	302.33 a	128.56 a	430.89 a
	Kruskal-Wallis Test (Chi²; p value)	$\chi^2 = 0.008$; $p = 0.92$	$\chi^2 = 0.018$; $p = 0.89$	$\chi^2 = 0.05$; $p = 0.82$
Substrates	Mean – Cocoa Husk (# of Ceratopogonidae pollinators)	232.17 b	164.17 a	396.33 b
	Mean – Plantain/banana pseudostem (# of Ceratopogonidae pollinators)	678.67 a	185.17 a	863.83 a
	Mean – Leaf litter (# of Ceratopogonidae pollinators)	19.50 c	33.00 b	52.50 c
	Kruskal-Wallis Test (Chi²; p value)	$\chi^2 = 14.76$; $p < 0.001$	$\chi^2 = 11.24$; $p = 0.003$	$\chi^2 = 15.16$; $p < 0.001$
Treatments combination	Mean-T1 (Nacional+Cocoa husk) (# of Ceratopogonidae pollinators)	245.33 b	161.33 a	406.67 b
	Mean-T2 (Nacional+Pseudostem) (# of Ceratopogonidae pollinators)	692.67 a	176.67 a	869.33 a
	Mean-T3 (Nacional+Leaf litter)	15.67 c	41.00 b	56.67 c
	Mean-T4 (CCN-51+Cocoa husk) (# of Ceratopogonidae pollinators)	219.00 b	167.00 a	386.00 b
	Mean-T5 (CCN-51+Pseudostem) (# of Ceratopogonidae pollinators)	664.67 a	193.67 a	858.33 a
	Mean-T6 (CCN-51+Leaf litter) (# of Ceratopogonidae pollinators)	23.33 c	25.00 b	48.33 c
	Kruskal-Wallis Test (Chi²; p value)	$\chi^2 = 14.79$; $p = 0.01$	$\chi^2 = 11.29$; $p = 0.046$	$\chi^2 = 15.22$; $p = 0.009$
CV (%)		59.94	40.64	39.67
Normality Test (Shapiro-Wilk: W; p value)		$W = 0.94$; $p = 0.27$	$W = 0.98$; $p = 0.94$	$W = 0.94$; $p = 0.29$
Homogeneity Test (Bartlett: K²; p value)		$K^2 = 18.7$; $p = 0.002$	$K^2 = 5.18$; $p = 0.4$	$K^2 = 18.7$; $p = 0.004$

Note: W = Shapiro-Wilk statistic (normality of residuals); K^2 = Bartlett's test statistic (homogeneity of variances); χ^2 = Kruskal-Wallis test statistic (non-parametric comparison). Different letters indicate statistically significant differences ($p < 0.05$). Raw data are provided in Annex 7 (Supplementary Material).

a) **b)**

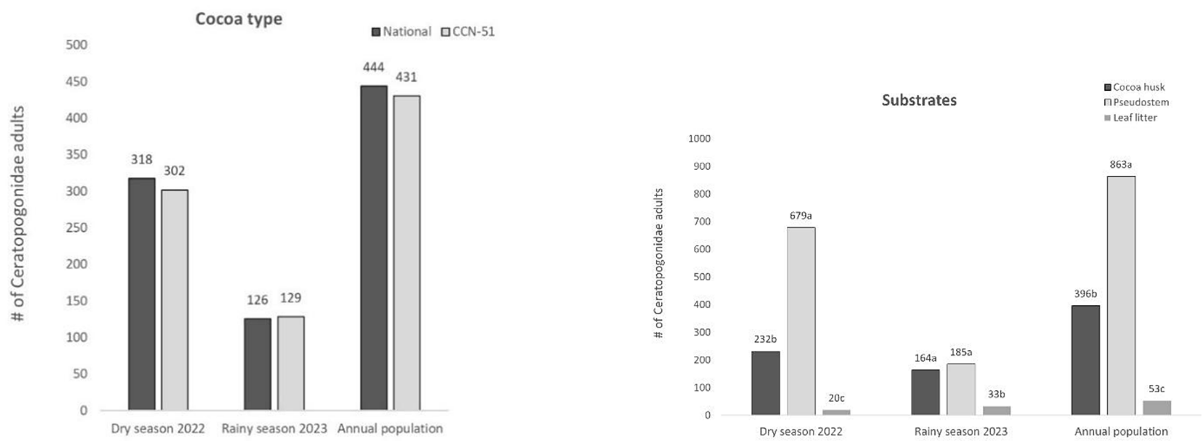

Figure 11. A. Population seasonality of pollinators of the Ceratopogonidae family recorded on two cocoa types B. Population seasonality of pollinators of the Ceratopogonidae family recorded on three substrates. Means with different letters present significant differences ($p < 0.05$).

Table 12. Statistical analysis of monthly, seasonal, and annual total pod production (pods ha⁻¹) in two cocoa types: Nacional and CCN-51

Evaluation period	Overall Mean (# of total cocoa pods/ha)	Mean-Nacional (# of total cocoa pods/ha)	Mean-CCN-51 (# of total cocoa pods/ha)	CV (%)	Normality Test (Shapiro-Wilk: W; p value)	Homogeneity Test (Bartlett: K²; p value)	Kruskal-Wallis Test (Chi²; p value)
July 2022	8525.07	4681.01 b	12369.13 a	137.63	W = 0.61; p < 0.001	K² = 83; p < 0.001	χ² = 21.1; p < 0.001
August 2022	2288.66	1541 b	3037 a	128.46	W = 0.78; p < 0.001	K² = 6.83; p = 0.009	χ² = 11.8; p < 0.001
September 2022	3444.1	2651. 59 b	4236.61 a	107.16	W = 0.9; p < 0.001	K² = 7.05; p = 0.008	χ² = 5.4; p = 0.02
October 2022	3570.01	3229.31 a	3910.72 a	101.46	W = 0.9; p < 0.001	K² = 0.23; p = 0.63	χ² = 2.52; p = 0.113
November 2022	2947.85	1274 b	4622 a	102.16	W = 0.93; p < 0.001	K² = 67.97; p < 0.001	χ² = 30.4; p < 0.001
December 2022	2399.76	2222 a	2577.52 a	127.71	W = 0.80; p < 0.001	K² < 0.001; p = 0.989	χ² = 0.48; p = 0.49
January 2023	3392.25	3466.32 a	3318.19 a	133.17	W = 0.74; p < 0.001	K² = 11.76; p < 0.001	χ²=0.2; p=0.65
February 2023	3088.58	3140.43 a	3036.73 a	121.31	W = 0.81; p<0.001	K² = 0.09; p = 0.77	χ² = 0.25; p = 0.62
March 2023	2281.25	2222 a	2340.51 a	131.5	W = 0.78; p < 0.001	K² = 3.38; p = 0.07	χ² = 0.76; p = 0.38
April 2023	1325.79	1362.83 a	1288.76 a	152.36	W = 0.71; p < 0.001	K² = 2,05; p = 0.15	χ² = 0.08; p = 0.78
May 2023	725.85	948.05 a	503.65 a	197.32	W = 0.66; p < 0.001	K² = 9.3; p = 0.002	χ² = 3.56; p = 0.06
June 2023	66.66	29.63 a	103.69 a	585	W = 0.22; p < 0.001	K² = 28.7; p < 0.001	χ² = 1.83; p = 0.176
Dry season (2022)	23175	15598 b	30752 a	81	W = 0.81; p < 0.001	K² = 36.96; p < 0.001	χ² = 24.45; p < 0.001
Rainy season (2023)	11251	11762 a	10740 a	101	W = 0.86; p < 0.001	K² = 11.05; p < 0.001	χ² < 0.001; p = 0.99
Total	34055.85	26767.69 b	41344.01 a	66.7	W = 0.89; p < 0.001	K² = 4.09; p = 0.04	χ² = 17.43; p < 0.001

Note: W = Shapiro-Wilk statistic (normality of residuals); K² = Bartlett's test statistic (homogeneity of variances); χ² = Kruskal-Wallis test statistic (non-parametric comparison). Different letters indicate statistically significant differences ($p < 0.05$). Raw data are provided in Annex 8 (supplementary material).

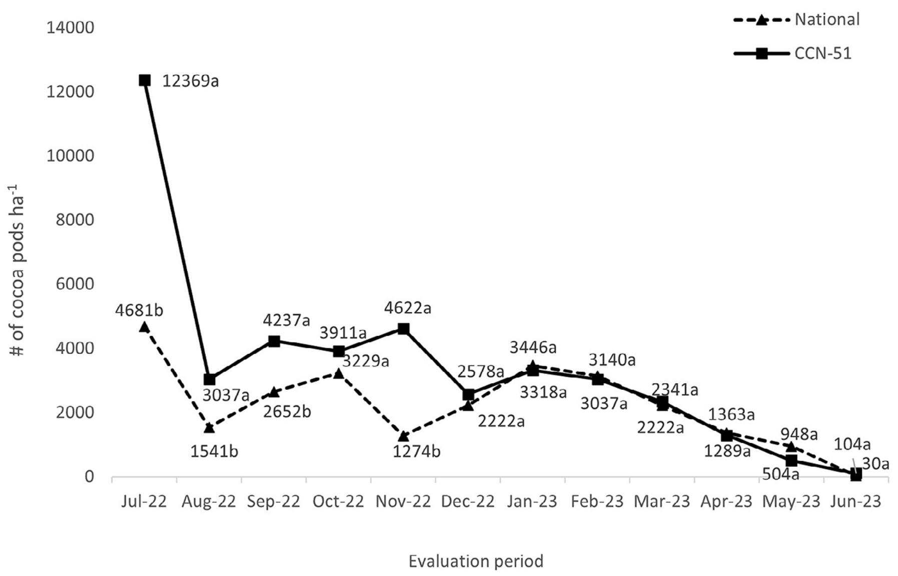

Figure 12. Fluctuation of the average monthly production ha⁻¹ of total pods in two cocoa types. Averages with different letters show significant differences ($p < 0.05$)

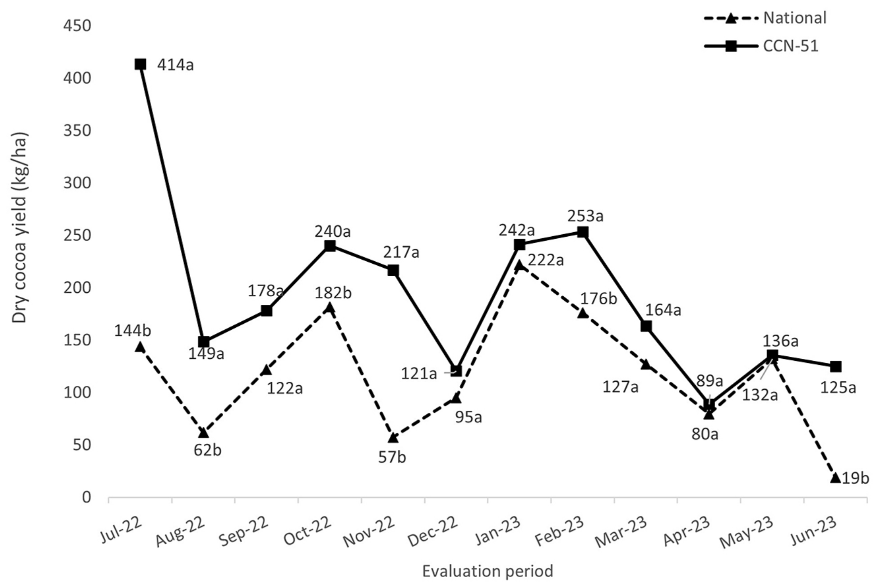

Figure 13. Fluctuation of the average monthly dry cocoa yield in kg ha^{-1}. Averages with different letters show significant differences ($p < 0.05$).

Table 13. Monthly, seasonal and annual dry cocoa yield (kg/ha) and statistical analysis.

Month	Overall Mean-Dry cocoa yield (kg/ha)	Mean-Nacional-Dry cocoa yield (kg/ha)	Mean-CCN-51- Dry cocoa yield (kg/ha)	CV (%)	Normality Test (Shapiro-Wilk: W; p value)	Homogeneity Test (Bartlett: K²; p value)	Kruskal-Wallis Test (Chi²; p value)
July 2022	279	144 b	414 a	140	W = 0.6; p < 0.001	K² = 80.7 p < 0.001	χ² = 27; p < 0.001
August 2022	105	62 b	149 a	123	W = 0.85; p < 0.001	K² = 24.13 p < 0.001	χ² = 11.88; p < 0.001
September 2022	149	122 a	178 a	115	W = 0.8; p < 0.001	K² = 8.17; p = 0.004	χ² = 3.72; p = 0.05
October 2022	210	182 b	240 a	114	W = 0.88; p < 0.001	K² = 0.9 p = 0.34	χ² = 4.77; p = 0.029
November 2022	137	57 b	217 a	104	W = 0.93; p < 0.001	K² = 69.01 p < 0.001	χ² = 33.4; p < 0.001
December 2022	108	95 a	121 a	118	W = 0.86; p < 0.001	K² = 0.16; p = 0.69	χ² = 1.12; p = 0.29
January 2023	232	222 a	242 a	91	W = 0.82; p < 0.001	K² = 8.82; p = 0.003	χ² = 2.17; p = 0.14
February 2023	215	176 b	253 a	96	W = 0.89; p < 0.001	K² = 2.37; p = 0.10	χ² = 7.93; p = 0.0048
March 2023	145	127 a	164 a	105	W = 0.88; p < 0.001	K² = 0.05 p = 0.82	χ² = 3; p = 0.083
April 2023	84	80 a	89 a	138	W = 0.75; p < 0.001	K² = 1.91; p = 0.17	χ² = 0.43; p = 0.51
May 2023	133	132 a	136 a	115	W = 0.82; p < 0.001	K² = 0.002; p = 0.96	χ² = 0.055; p = 0.81
June 2023	72	19 b	125 a	177	W = 0.74; p < 0.001	K² = 97.3; p < 0.001	χ² = 21.72; p < 0.001
Mean-monthly total	156	119 b	194 a	63	W = 0.89; p < 0.001	K² = 7.13; p < 0.001	χ² = 24.8; p < 0.001
Annual Total	1867	1423 b	2326 a	63	W = 0.89; p < 0.001	K² = 7.13; p = 0.008	χ² = 24.82; p < 0.001
Dry Season (2022)	987	663 b	1311 a	78	W = 0.89; p < 0.001	K² = 23.13 p < 0.001	χ² = 24.68; p < 0.001
Rainy season (2023)	880	751 b	1009 a	69	W = 0.88; p < 0.001	K² = 0.28; p = 0.6	χ² = 10.1; p < 0.001

Note: W = Shapiro-Wilk statistic (normality of residuals); K² = Bartlett's test statistic (homogeneity of variances); χ² = Kruskal-Wallis test statistic (non-parametric comparison). Different letters indicate statistically significant differences ($p < 0.05$). Raw data are provided in Annex 9 (supplementary material).

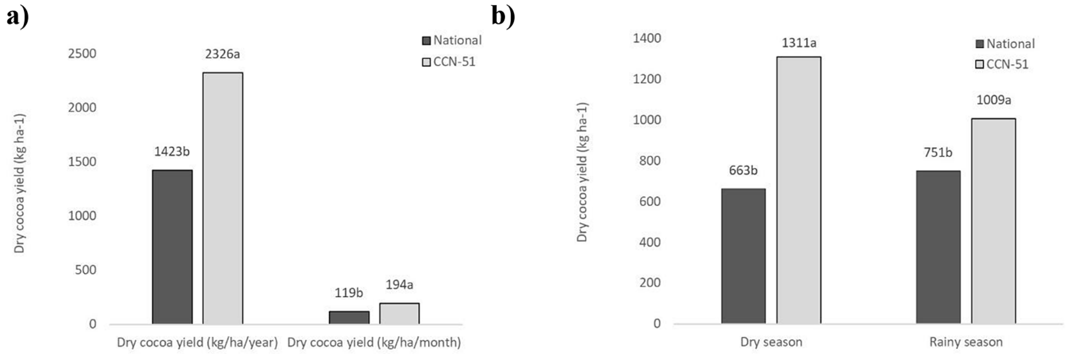

Figure 14. Annual dry cocoa yield in kg ha^{-1} (a), seasonality of dry cocoa production in kg ha^{-1} (b); averages with different letters show significant differences ($p < 0.05$).

CONCLUSIONS

The genus *Dasyhelea* of the Ceratopogonidae family predominated as a pollinator in the cocoa crop, showing a significant population fluctuation between the Nacional and CCN-51 cocoa types. The use of plantain/banana pseudostem food substrate chopped in the field favoured notably the population increase of these pollinators, especially during the dry season. The CCN-51 clone showed superior performance in terms of flower production per tree, which resulted in higher yields of cobs and dry cocoa, compared to the Nacional clones. The seasonality of production, especially during the dry season, underlines the importance of pollination activity during the rainy season, influenced by various environmental factors. The results of this research highlight the relevance of the ecosystem service of pollination in cocoa, showing how the interaction between pollinators, the specific characteristics of the clones and environmental conditions play a fundamental role in the productivity of this crop.

REFERENCES

1. Abad, A., Acuña, C., Naranjo, E. (2020). Cacao in the Ecuadorian Coast: A study of its cultural and economic dimension. *Estudios de la Gestión: Revista Internacional de Administración*, *7*, Article 7. https://doi.org/10.32719/25506641.2020.7.3
2. Abad-Sánchez, M., Alvarado-Aguayo, A., Gallardo-Campoverde, A. (2018). Comparative analysis of the incidence of the three main diseases in cocoa CCN-51, in the canton of La Troncal, province of Cañar, Ecuador. *Revista Científica Ciencias Naturales y Ambientales*. *12*(1), 20–30. https://doi.org/10.53591/cna.v12i1.271
3. Akoa, S. P., Onomo, P. E., Ndjaga, J. M., Ondobo, M. L., Djocgoue, P. F. (2021). Impact of pollen genetic origin on compatibility, agronomic traits, and physicochemical quality of cocoa (*Theobroma cacao* L.) beans. *Scientia Horticulturae*, *287*, 110278. https://doi.org/10.1016/j.scienta.2021.110278
4. Alvim, P. (1984). Flowering of cocoa. Cocoa Growers' Bulletin 35: 23–31. *Cocoa Growers' Bulletin*, *25*, 23–31.
5. Armijos Vásquez, V., García Cruzatty, L. C., Castro Olaya, J., Martínez, M. (2020). Pollinizing insects in production systems of *Theobroma cacao* L. in the central zone of the ecuadorian coastal zone. *Ciencia y Tecnología*, *13*(2), Article 2. https://doi.org/10.18779/cyt.v13i2.389
6. Arnold, S. E. J., Forbes, S. J., Hall, D. R., Farman, D. I., Bridgemohan, P., Spinelli, G. R., Bray, D. P., Perry, G. B., Grey, L., Belmain, S. R., Stevenson, P. C. (2019). Floral Odors and the Interaction between Pollinating Ceratopogonid Midges and Cacao. *Journal of Chemical Ecology*, *45*(10), 869–878. https://doi.org/10.1007/s10886-019-01118-9
7. Avendaño-Arrazate, C. H., Suárez-Venero, G. M., Mendoza-López, A., Martínez-Bolaños, M., Reyes-Reyes, J., Espinosa-Zaragoza, S. (2021). Tree composition of species associated with cacao: Selva Lacandona and agroforestry systems, Chiapas, Mexico. *Agronomía Mesoamericana*, *32*(2), 365–381. https://doi.org/10.15517/am.v32i2.41630
8. Bacca, P. P., Alarcon, K. A., González, J. C., Guzmán, F. A., Coronado, R. A., Barrera, Y. R. (2023). Evaluation of four cocoa genotypes in Nariño, Colombia. *Revista Mexicana de Ciencias Agrícolas*, *14*(7), Article 7. https://doi.org/10.29312/remexca.v14i7.3331
9. Cañarte, E., Montero, S., Navarrete, B. (2021). Recognition, importance and care of pollinators in cocoa production systems. INIAP. http://repositorio.iniap.gob.ec/handle/41000/5749
10. Cedeño, E. P., Dilas-Jiménez, J. O. (2022). Production and export of Ecuadorian cocoa and the potential

of fine flavor cocoa. *Qantu Yachay*, *2*(1), Article 1. https://doi.org/10.54942/qantuyachay.v2i1.17

11. Córdoba, C., Cerda Bustillos, R., Deheuvels, O., DeClerck, F. A. J. (2013). Pollinators, pollination and potential cocoa production in agroforestry systems in Bocas del Toro, Panama. *Agroforestería en las Américas*, *49*, 26–32. https://repositorio.catie.ac.cr/handle/11554/6677
12. García, V. S., Guzmán-Cedeño, Á. M. (2022). Incidence of pollinating agents on cocoa (*Theobroma cacao* L.) flower fertilization. *Revista ESPAMCIENCIA*, *13*(2), Article 2. https://doi.org/10.51260/revista_espamciencia.v13i2.343
13. Gil Restrepo, J. P., Leiva Rojas, E. I., Pisco, R. (2017). Phenology of cocoa tree in a tropical moist forest. *Científica*, *45*(3), Article 3. https://doi.org/10.15361/1984-5529.2017v45n3p240-252
14. Groeneveld, J. H., Tscharntke, T., Moser, G., Clough, Y. (2010). Experimental evidence for stronger cacao yield limitation by pollination than by plant resources. *Perspectives in Plant Ecology, Evolution and Systematics*, *12*(3), 183–191. https://doi.org/10.1016/j.ppees.2010.02.005
15. 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*, 152480. https://doi.org/10.1016/j.flora.2024.152480
16. Kaufmann, T. (1975). Ecology and behavior of cocoa pollinating ceratopogonidae in Ghana, W. Africa. *Environmental Entomology*, *4*(2), 347–351. https://doi.org/10.1093/ee/4.2.347
17. Lahive, F., Hadley, P., Daymond, A. J. (2019). The physiological responses of cacao to the environment and the implications for climate change resilience. A review. *Agronomy for Sustainable Development*, *39*(1), 5. https://doi.org/10.1007/s13593-018-0552-0
18. Lander, T. A., Atta-Boateng, A., Toledo-Hernández, M., Wood, A., Malhi, Y., Solé, M., Tscharntke, T., Wanger, T. C. (2025). Global chocolate supply is limited by low pollination and high temperatures. *Communications Earth & Environment*, *6*(1), 97. https://doi.org/10.1038/s43247-025-02072-z
19. Marino, P., Spinelli, G. (2008). Biting Midges of the Forcipomyia (Forcipomyia) argenteola group in southern South America, with description of a new species and a key to the Neotropical species (Diptera: Ceratopogonidae). *Revista de Biología Tropical*, *56*, 789–794. https://doi.org/10.15517/rbt.v56i2.5624
20. Montero-Cedeño, S., Sánchez, P., Solórzano-Faubla, R., Pinargote-Borrero, A., Cañarte-Bermúdez, E. G. (2019). Flowering and diversity of insect pollinators in a cocoa monoculture system. *Revista ESPAMCIENCIA, 10*(1), 1-7. https://revistasespam.espam.edu.ec/index.php/Revista_ESPAMCIENCIA/article/view/178/183
21. Montero-Cedeño, S. L., Cañarte-Bermudez, E. G., Navarrete-Cedeño, J. B., Pinargote-Borrero, A., Sanchez-Hernández, P. (2022). Ceratopogonidae: Their role in pollination and fertilization at various technological levels of Theobroma cacao L. production. *Revista de La Facultad de Agronomía de La Universidad Del Zulia*, *39*(3), e223943. https://produccioncientificaluz.org/index.php/agronomia/article/view/38625
22. Pérez, G. A., & Freile, J. A. (2017). Adaptability of promising clones of national cocoa (*Theobroma cacao* L.), in the Arosemena Tola canton of Ecuador. *Centro Agrícola*, *44*(2), 44–51. http://scielo.sld.cu/scielo.php?script=sci_abstract&pid=S0253-57852017000200006&lng=es&nrm=iso&tlng=pt
23. Puentes-Páramo, Y., Menjivar-Flores, J., Aranzazu-Hernández, F. (2014). Nitrogen, phosphorus and potassium use efficiencies in cocoa (*Theobroma cacao* L.) clones. *Bioagro*, *26*(2), 99–106.
24. Ramírez-Argueta, O., Orozco-Aguilar, L., Dubón, A. D., Díaz, F. J., Sánchez, J., Casanoves, F. (2022). Timber growth, cacao yields, and financial revenues in a long-term experiment of cacao agroforestry systems in northern Honduras. *Frontiers in Sustainable Food Systems*, *6*. https://doi.org/10.3389/fsufs.2022.941743
25. Ramos-Remache, R. A., Sotomayor Cantos, K. F., Amores Puyutaxi, F. M., Rhón Dávila, F. (2023). Adaptation of cocoa clones (*Theobroma cacao* L.) Nacional Type in the Piedemonte de Guasaganda, Cotopaxi, Ecuador. *Ciencia Latina Revista Científica Multidisciplinar*, *7*(4), 2938–2957. https://doi.org/10.37811/cl_rcm.v7i4.7148
26. R Core Team. (2024). R: The R Project for Statistical Computing. R Foundation for Statistical Computing. https://www.r-project.org/
27. Ríos-Moyano, D. K., Rodríguez-Cruz, F. A., Salazar-Peña, J. A., Ramírez-Godoy, A. (2023). Factors associated with the polinización of the cocoa (*Theobroma cacao* L.) crop. *Agronomía Mesoamericana*, *34*(3), 52280. https://doi.org/10.15517/am.2023.52280
28. Salazar-Díaz, R., Torres-Coto, V. (2017). Study of cocoa (*Theobroma cacao*) pollinator dynamics in three production systems. *Revista Tecnología En Marcha*, *30*(1), 90–100. https://doi.org/10.18845/tm.v30i1.3088
29. Torres-de La Cruz, M., Ortiz-García, C. F., Lagunes-Espinoza, L. D. C., Gaspar-Génico, J. Á., Leiva-Espinoza, S. T., Oliva-Cruz, S. M. (2023). Reproductive behavior of *Theobroma cacao* in southeastern Mexico. *Revista Fitotecnia Mexicana*, *46*(2), 203. https://doi.org/10.35196/rfm.2023.2.203

30. aldivieso López, C., Cedeño García, G., Guanoluisa, A. (2021). Statistical Analysis of the historical climate data of ESPAM MFL. *Research Gate*. https://doi.org/10.13140/RG.2.2.11868.26248
31. Vandromme, M., Van de Sande, E., Pinceel, T., Vanhove, W., 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
32. 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*(2), e12140. https://doi.org/10.1002/2688-8319.12140
33. Vargas Pérez, O. A., Vite Cevallos, H. A., Quezada Campoverde, J. M. (2021). Comparative analysis of the economic impact of cocoa cultivation in Ecuador for the first half of 2019 versus the first half of 2020. *Revista Metropolitana de Ciencias Aplicadas*, *4*(2), 169–179.
34. Weinstein, M., Baram, S., Yermiyahu, U., Lieberman-Lazarovich, M., Tsehansky, L., Elmakias, A., Kumar, P., Graber, E. R. (2024). Nitrogen, phosphorus and potassium nutrition effects on flowering and reproduction in an F1 segregating population of the *Theobroma cacao* L. CCN-51 cultivar. *Scientia Horticulturae*, *337*, 113591. https://doi.org/10.1016/j.scienta.2024.113591
35. Young, A. M. (1983). Seasonal Differences in abundance and distribution of cocoa-pollinating midges in relation to flowering and fruit set between shaded and sunny habitats of the la Lola cocoa farm in Costa Rica. *Journal of Applied Ecology*, *20*(3), 801–831. JSTOR. https://doi.org/10.2307/2403127