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Pruning and Fertilization of *Theobroma Cacao* L. and of Shadow Trees Affect the Flowering and Fruiting of Cacao

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Abstract

Cacao (*Theobroma cacao* L.) crop faces problems worldwide associated with low productivity due to diseases, lack of fertilization and absence of pruning. The aim of this study was to evaluate the effect of sanitary pruning of cacao, pruning of shade trees and fertilization on cacao flowering and fruiting. The study was carried out in a 40-year old cacao plantation in the Experimental Field of the Colegio de Postgraduados, Campus Tabasco (17˚ 59' 23.98'' N and 93˚ 37' 10.24'' W), at 24 masl, in Cardenas, Tabasco, Mexico. Shade tree and cacao tree pruning (both with and without pruning) and fertilization (without fertilizer and with K and NPK) were the evaluated factors and levels. There were 12 treatments with three repetitions including four trees per repetition. The assessed variables were the number of healthy, diseased and damaged fruits; number of healthy and diseased developing cacao fruits (chilillos); and number of flowers per tree. Analysis of variance indicated a significant effect of the interaction among the three evaluated factors on healthy fruits at 24 months after applying the treatment; on damaged fruits and healthy chilillos at 18 months; and on diseased chilillos at 18 and 24 months. Regarding the number of flowers, the interaction among the three factors had an effect at 6, 12 and 18 months. The best interaction of factors for increasing the number of healthy fruits was pruning of shade trees, no pruning of cacao trees and fertilization with K. The interaction of K with cacao tree pruning and with or without shade tree pruning decreased the number of diseased chilillos. The conclusion is that cacao pruning combined with K fertilization increased the number of flowers and decreased the number of diseased chilillos.

Keywords

Cacao Tree Nutrition, *Theobroma cacao*, Shadow Regulation, Flowering and

Fruiting

1. Introduction

The cultivation of cacao (*Theobroma cacao* L.) faces problems worldwide associated with low productivity caused mainly by diseases, lack of fertilization and absence of cacao and shade tree pruning [1] [2]. Among the diseases that affect cacao trees, moniliasis, caused by the fungus *Moniliophthora roreri*, can reduce the production of cacao beans by up to 100% [3] [4]. Disease damage coupled with low yields and low prices has decreased the willingness to properly cultivate cacao by producers [5], who often reduce management activities to a minimum, which leads to even lower yields. In the state of Tabasco, Mexico, such activities have caused reductions of 30% in the cultivated area and more than 50% in the production of cacao beans in the last 15 years. Thus, these practices have led to the loss of a production system that is more than 5300 years old [6], provides multiple forest and non-forest resources [7] and maintains a diversified tree cover [8], since cacao is cultivated as an agroforestry system.

Cacao cultivation is an Olmec heritage [9]. In the 1930s, Mexico transitioned from cultivating high-quality criollo cacao [10] to growing lower-quality, yet more productive, varieties. However, these varieties have now experienced a decline in productivity due to aging plantations, disease prevalence, and notably, inadequate agronomic practices [11], including insufficient pruning and fertilization.

The fertilization of shade cacao produces only small increases in yield [12], whereas the fertilization of shade-free cacao leads to a significant increase in yield, which is because photosynthesis is stronger in shade-free plantations and increases the response to fertilization [13]. However, if all shade is suppressed and the plants are not fertilized, then the yield will decrease over time and cacao trees will enter early senescence [14].

In Mexico, cacao is grown under shade. In Malaysia, Peru, Colombia, Ecuador and some African countries, production systems have been implemented in which cacao is grown in full sun [15] [16]. Although these systems can triple the output of cacao beans [17], the maintenance costs are high due to the requirements for high fertilizers rates and irrigation systems. In addition, cacao chilillos (*i.e.* developing cacao fruits) soon deteriorate due to the attack of pests and diseases. In contrast, shade cultivation mitigates some adverse environmental effects while the reduction in photosynthetic activity is offset by the greater amount of foliage [18]. The design and management of a shade canopy varies according to the tree species used. The importance of shade canopy is associated with its role in preserving biodiversity and ameliorating climatic conditions [8] [16] [19] that favor disease development and reduce crop yields.

Cacao requires a shade canopy that provides 30% shadow to have a beneficial effect on yields [20]. Shade trees contribute to maintaining the soil nutrient balance and proper humidity, provide wind protection and promote weed, pest and plant disease control [15].

Diseases such as moniliasis can be controlled by regulating the microenvironment of plantations. This microenvironment is formed, and modified by the density, diversity and age of shade and cacao trees. Pruning to remove excessive shade slows the development of moniliasis, which thrives in the humid environments of cacao plantations [21]. Such environments emerge in the absence of pruning, which leads to the development of large cacao trees with multiple trunks and dense crowns. In addition to favour high humidity, these conditions make it difficult to detect and remove diseased fruits. Excessive amounts of unpruned shade trees impede light penetration and air circulation, resulting in the plantation being kept in dark and humid conditions for most of the day, thus favoring the emergence and dissemination of fungal diseases.

The main objective of pruning is to induce the formation of new terminal buds and thereby increase flowering and fruit production [22] [23]. Pruning cacao trees also promotes the formation of straight trunks, regulation of tree growth, regulation of light needed for normal development, and control of weeds and insect pests; moreover, pruning facilitates harvest and disease management by regulating the light that enters the center of the tree and spraying areas [22] [23] [24]. Thus, the aim of this study was to evaluate the effect of the sanitary pruning of cacao trees, the pruning of shade trees and application of fertilizer on the production of cacao flowers, fruits and chilillos.

2. Methods

2.1. Experimental Site

The present study was carried out between February 2014 and September 2016 in a 40-year old cacao plantation located in the municipality of Cardenas in the Experimental Field of the Colegio de Postgraduados, Campus Tabasco (17˚ 59' 23.98" N and 93˚ 37' 10.24" W), at 24 masl. Tabasco contributes 64% of the total cacao production in Mexico, and Cardenas is the second largest municipality in the state in terms of cacao production [25].

The climate of the study area is hot-humid, with abundant rain falling in summer [26]. The average annual temperature is 26˚C, with a monthly average maximum of 30.3˚C in May and an average minimum of 20˚C in December and January. The absolute maximum and minimum temperatures reach 40˚C and 10˚C. The average annual rainfall is 2643 mm, with a monthly maximum average of 335 mm in September and a monthly average minimum of 10 mm in April [27]. The annual average relative humidity is 83%, with a maximum of 86% in January and February and a minimum of 77% in May. The highest wind speed (30 km h^{-1}) was recorded in November and December, while the lowest (20 km h^{-1}) was recorded in June [26].

The chemical analysis of the soil in the plantation showed the following results:

pH 5.61; EC, 53.40 μS cm^{-1}; organic matter, 3.25%; total, N 0.14%; Olsen P, 12.96 mg/kg; K, 0.36 Cmol/kg; Ca, 9.13 Cmol/kg; Mg, 2.19 Cmol/kg; Na, 0.08 Cmol/kg; CIC, 15.64 Cmol/kg; Fe, 192.16 mg/kg; Cu, 5.31 mg/kg; Zn, 2.49 mg/kg; Mn, 15.00 mg/kg; clay, 42.20%; silt, 16.24% and sand, 41.56%. The soil is classified as a texturally clayey and silty soil.

The management of the plantation consisted of two weeding during the year. The density of the plantation was 625 trees per hectare, which is the typical plan tation density in the region. The shade trees associated with the plantation under study were mahogany (*Swietenia macrophylla* King), Spanish cedar (*Cedrela odorata* L.), beechwood (*Gmelina arborea* Roxb. Ex Sm.), teak (*Tectona grandis* L.F.), chipilcohite (*Diphysa robinioides* Benth.) and jinicuil (*Inga jinicuil* Schltdl. & Cham. Ex G. Don), which are typical of cacao agroforestry systems in Cárdenas, Tabasco, Mexico [8]. Such trees are established without any arrangement. The plantation was surrounded by other plantations of coconut (*Cocos nucifera* L.), bananas (*Musa paradisiaca* L.), cacao and an orchard with different tropical fruits, such as caimito (*Chrysophyllum cainito* L.), mamey sapote (*Pouteria sapota* Jacq. H.E. Moore & Stearn), jinicuil (*I. jinicuil*), soursop (*Annona muricata* L.) and Malay apple (*Syzygium malaccense* (L.) Merr. & L. M. Perry).

The present study evaluated the effect of sanitary pruning of cacao and shade trees, and the application of fertilizer with K and NPK. Before the experiment was established, we measured the intensity of sunlight that passed through the shade tree canopy to the cacao tree canopy to ensure the correct pruning shade trees and adjust the light intensity of the plantation to 60%. The instrument used for such measurements was a LI-190SA QUANTUM SENSOR from LI-COR Bioscience™ (Nebraska, U.S.A.). The pruning of cacao trees consisted of eliminating crossed, dry or damaged branches and removing diseased or damaged fruits and chilillos. The fertilizer was applied in a band at a distance of 1.5 m and a depth of 5 - 10 cm around the cacao trees. The fertilizer sources were urea (46% N), triple calcium superphosphate (46% P_2O_5) and potassium chloride (20% K_2Cl). The dose of K applied was 300 g per tree, and that of NPK was 600 g per tree.

2.2. Experimental Design and Data Analysis

The experimental design was split-plot in a factorial arrangement of treatments $2^2 \times 3$. The large plot was shade trees pruning and small plot was cacao trees pruning. The evaluated factors were: pruning of shade trees (with and without pruning), cacao pruning (with and without pruning) and fertilization (without fertilizer, with K, with NPK). There were a total of 12 treatments with three repetitions and four trees per repetition (**Figure 1**).

The variables number of healthy, diseased and damaged fruits, number of healthy and diseased chilillos, and number of flowers per tree were assessed every 15 days. Healthy fruits were those without the presence of moniliasis and/or black pod (*Phytophthora spp.*) and ready for harvest. Diseased fruits were those with presence of moniliasis and/or black pod. Damaged fruits were those attacked by squirrels

(*Sciurus aureogaster* F. Cuvier) or birds (*Melanerpes aurifrons* Wagler). Healthy chilillos were cacao fruits up to two months old without the typical hump caused by the presence of *M. roreri*. Diseased chilillos were those with the aforementioned hump. The number of flowers was assessed from the base of the stem up to a height of 1.5 m.

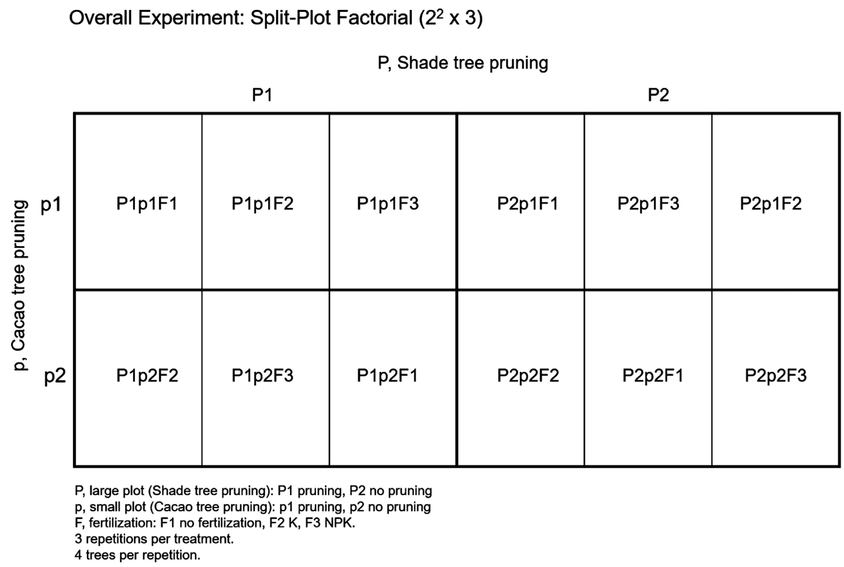

Figure 1. Schematic of the experimental design to evaluate the effect of the pruning of cacao trees, the pruning of shade trees and fertilization on the production of cacao flowers, fruits and chilillos.

For each of the variables evaluated, an analysis of variance (ANOVA) was performed to test statistical differences between the factors studied: pruning of shade trees, pruning of cacao trees, fertilization, and the interactions. The ANOVA was performed on the data at 6, 12, 18 and 24 months after applying the treatments (months). The significance of the interactions and the factors evaluated was recorded at $p \leq 0.05$. For significant interactions, a least squares mean test was performed to determine statistically different interactions ($p \leq 0.05$). For non-significant interactions, a multiple comparison of means of the factors was carried out using the Tukey test ($p \leq 0.05$). The assumption of normality of the ANOVA errors was verified using the Shapiro-Wilk test ($p > 0.05$); while the assumption of homoscedasticity was verified using the graphs of the predicted values vs. the residuals, whose points presented a random distribution in the plane, which meant homogeneity of variances in the groups. The information was processed using the Proc Mixed Procedure and the slice instruction of the SAS software version 9.4 [28].

3. Results and Discussion

3.1. Number of Healthy Cacao Fruits

The ANOVA results indicated a significant effect ($F = 2.85$, $p > F = 0.060$) of the interaction among the three evaluated factors on the number of healthy fruits at 24 months (**Table 1**). The best interaction in terms of its effect on the number of healthy fruits was shade tree pruning, no cacao pruning and K fertilization (**Figure 2**). These results contrast with those obtained by other authors, such as [12] and [29], who reported a lack of response of cacao trees to fertilization treatments in agroforestry systems. This discrepancy likely stems from competition, primarily among cacao trees, given the higher plant density in the agroforestry systems reported by those authors (1242 plants) compared to our study (625 plants). Furthermore, the age of the cacao trees and the fertilizer dosage and formulation may also contribute.

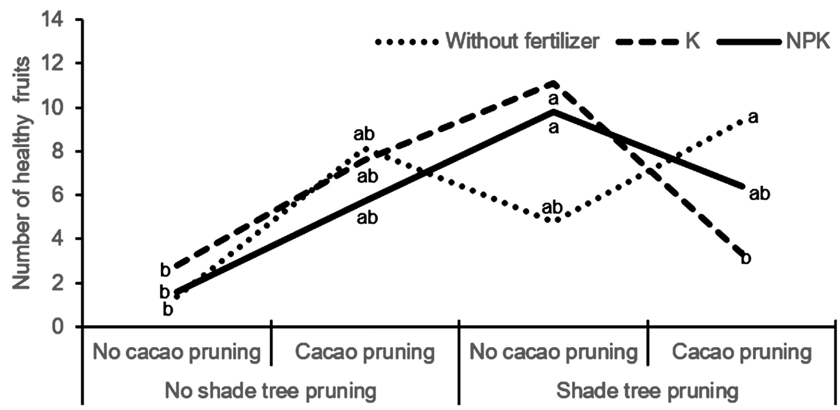

Figure 2. Effect of the combination of shade tree pruning, cacao pruning and fertilization on the number of healthy cacao fruits 24 months after applying the treatments. Equal letters in the same line indicate statistically equal values (Tukey, p ≤ 0.05). n = 12.

Table 1. Main effects and interaction among shade trees pruning, cacao pruning and fertilization on cacao (*Theobroma cacao* L.) flowering and fruiting at 6, 12, 18 and 24 months after applying the treatments.

Tested variables		6 MAAT		12 MAAT		18 MAAT		24 MAAT	
Variation model and factor	df	F value	Pr > F	F value	Pr > F	F value	Pr > F	F value	Pr > F
Number of fruits		$3.54_{(11,180)}$	0.0002	$4.21_{(11,163)}$	<0.0001	$3.71_{(11,167)}$	<0.0001	$4.51_{(11,167)}$	<0.0001
LP	1	2.57	0.1107	21.58	<0.0001	16.67	<0.0001	10.66	0.0013
SP	1	0.02	0.8913	9.90	0.0020	7.08	0.0085	2.76	0.0983
FERT	2	4.09	0.0184	1.86	0.1585	0.57	0.5674	0.05	0.9505
LP*SP	1	5.87	0.0164	2.52	0.1146	12.57	0.0005	16.65	<0.0001
LP*FERT	2	0.83	0.4365	0.63	0.5341	0.36	0.6984	0.68	0.5068
SP*FERT	2	9.67	0.0001	2.75	0.0672	0.54	0.5843	5.72	0.0040
LP*SP*FERT	2	0.67	0.5147	0.07	0.9353	1.45	0.2372	2.85	0.0608
Diseased fruits		$3.18_{(11,180)}$	0.0006	$3.8_{(11,163)}$	<0.0001	$2.08_{(11,167)}$	0.0242	$8.16_{(11,167)}$	<0.0001
LP	1	2.41	0.122	11.62	0.0008	6.27	0.0132	50.95	<0.0001
SP	1	8.89	0.0033	0.13	0.7223	2.64	0.1061	0.09	0.7668
FERT	2	1.47	0.2333	7.8	0.0006	0.05	0.9496	0.95	0.3882

Continued

LP*SP	1	6.56	0.0112	0.09	0.7623	7.01	0.0089	11.21	0.0010
LP*FERT	2	2.38	0.0957	3.99	0.0203	1.31	0.2737	4.70	0.0103
SP*FERT	2	2.57	0.0795	1.76	0.1746	0.5	0.6081	4.10	0.0183
LP*SP*FERT	2	2.17	0.1176	0.84	0.4349	2.04	0.1333	1.93	0.1485
Damaged fruits		$4.66_{(11,180)}$	<.0001	$3.29_{(11,163)}$	0.0004	$2.01_{(11,167)}$	0.0306	$3.21_{(11,167)}$	0.0006
LP	1	5.18	0.024	6.23	0.0135	1.92	0.1682	1.68	0.1964
SP	1	5.71	0.0179	10.55	0.0014	2.43	0.121	27.95	<0.0001
FERT	2	8.53	0.0003	2.76	0.0662	3.38	0.0364	0.21	0.8112
LP*SP	1	4.93	0.0276	3.85	0.0516	1.78	0.1838	2.84	0.0936
LP*FERT	2	1.39	0.2508	4.38	0.014	0.42	0.6550	0.16	0.8526
SP*FERT	2	5.82	0.0035	0.02	0.9756	0.03	0.9727	0.63	0.5316
LP*SP*FERT	2	1.80	0.1675	0.40	0.6724	4.32	0.0148	0.21	0.8114
Healthy chilillos		$3.48_{(11,180)}$	0.0002	$3.44_{(11,163)}$	0.0003	$2.4_{(11,167)}$	0.0086	$2.74_{(11,167)}$	0.0028
LP	1	10.8	0.0012	6.57	0.0113	0.25	0.6170	1.28	0.2603
SP	1	9.34	0.0026	4.44	0.0367	3.72	0.0555	4.79	0.0300
FERT	2	5.44	0.0051	0.94	0.3944	5.92	0.0033	0.81	0.4449
LP*SP	1	3.62	0.0587	14.72	0.0002	0.28	0.5989	2.97	0.0865
LP*FERT	2	1.13	0.3268	1.17	0.314	2.00	0.1390	0.69	0.5037
SP*FERT	2	0.26	0.7711	2.19	0.1153	0.07	0.9370	7.41	0.0008
LP*SP*FERT	2	0.46	0.6305	1.75	0.1762	3.05	0.0500	1.45	0.2363
Diseased chilillos		$5.89_{(11,180)}$	<.0001	$5.14_{(11,163)}$	<.0001	$7.64_{(11,167)}$	<0.0001	$2.96_{(11,167)}$	0.0013
LP	1	14.75	0.0002	18.24	<.0001	0.27	0.6061	6.63	0.0109
SP	1	11.58	0.0008	2.15	0.1445	31.76	<.0001	11.68	0.0008
FERT	2	8.93	0.0002	5.41	0.0053	1.6	0.206	1.61	0.2040
LP*SP	1	6.77	0.01	2.2	0.1404	1.44	0.2321	0.11	0.7352
LP*FERT	2	3.22	0.0421	6.78	0.0015	12.22	<.0001	0.00	0.9995
SP*FERT	2	1.63	0.1988	3.54	0.0313	1.55	0.2145	2.65	0.0739
LP*SP*FERT	2	2.03	0.1346	2.16	0.1183	9.51	0.0001	2.89	0.0584
Number of flowers		$2.99_{(11,180)}$	0.0011	$4.33_{(11,163)}$	<0.0001	$7.58_{(11,167)}$	<0.0001	$2.22_{(11,170)}$	0.0154
LP	1	0.01	0.9352	8.23	0.0047	19.40	<0.0001	0.54	0.4616
SP	1	0.47	0.4946	8.38	0.0043	22.96	<0.0001	0.81	0.3680
FERT	2	1.09	0.3368	2.91	0.0574	2.85	0.0605	1.08	0.3408
LP*SP	1	0.40	0.5259	8.37	0.0043	1.37	0.2430	7.53	0.0067
LP*FERT	2	2.82	0.0621	3.02	0.0516	8.69	0.0003	2.57	0.0795
SP*FERT	2	9.48	0.0001	2.96	0.0546	1.48	0.2315	2.64	0.0744
LP*SP*FERT	2	2.60	0.0768	2.96	0.0544	7.83	0.0006	1.00	0.3702

MAAT, Months after applying the treatments. LP, Large plot (shade trees pruning). SP, Small plot (cocoa pruning). FERT, Fertilization.

At 24 months, the number of healthy fruits was also significantly affected by shade tree pruning, the interaction between cacao and shade tree pruning, and the interaction between cacao pruning and fertilization (**Table 1**). The mean comparison test indicated that shade tree pruning significantly increased this variable, and that the effects of NPK fertilization and the control treatment without fertilizer were statistically equals (**Table 2**). This can be attributed to the fact that nutrient content in the soil was enough for the number of fruits that can be produced by cacao trees.

3.2. Number of Diseased Cacao Fruits

The interaction among the three factors did not have a significant effect on the number of diseased fruits at any of the evaluation dates (**Table 1**). The factors that had the greatest effect on this variable were cacao pruning at 6 months, shade tree pruning and fertilization at 12 months and shade tree pruning at 18 and 24 months. The greatest effect of the interaction between cacao and shade tree pruning was observed at 6, 18 and 24 months. With the exception of the first evaluation date, shade tree pruning significantly increased the number of diseased fruits on all evaluation dates.

Cacao pruning increased the number of diseased fruits at 6 months, decreased it at 12 and made no significant difference at 18 and 24 months (**Table 2**). This can be attributed to the fact that cacao pruning is usually an annual practice, although this was not done in the plantation under study. As indicated [30] the damage to fruits due to disease is a direct function of the age of the cacao plantation. The plantation under study was 40 years-old, and the age of maximum production is 28 years [31]. However, the trees responded to fertilization.

3.3. Number of Damaged Cacao Fruits

The ANOVA results showed a significant effect ($F = 4.32$, $p > F = 0.0148$) of the interaction among the three evaluated factors on the number of damaged fruits at 18 months. The main effect of fertilization on this variable was also recorded at this time (**Table 1**). The mean comparisons indicated that K fertilization significantly decreased the number of damaged fruits (**Table 2**). Independent of fertilization, the combination of cacao pruning and shade tree pruning decreased the number of damaged fruits (**Figure 3**). The best interactions to reduce the number of damaged fruits were K fertilization, no shade tree pruning and cacao pruning as well as shade tree pruning, cacao pruning and no fertilization. The above two interaction groups contrasted with the interaction among NPK fertilization, cacao pruning and no shade tree pruning, which had the highest number of damaged fruits (**Figure 3**).

The damage to cacao fruits caused by squirrels and birds was similar to that by moniliasis [1]. Pruning can thus be used to reduce the effect of the above problems by decreasing the number of rest locations for squirrels and birds, improving the aeration and regulating the humidity in the plantation. Another way to reduce the damage by these animals is to avoid cultivating shade trees that attract the animals [29], which was not the case in the plantation under study and surrounding plantations.

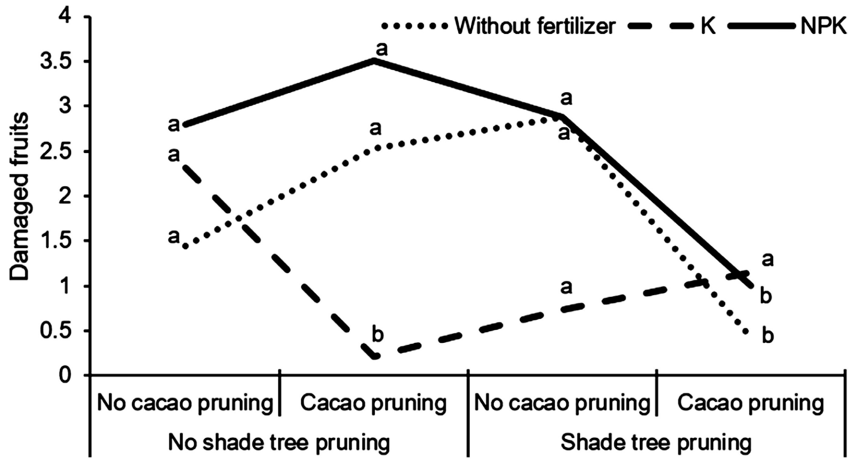

Figure 3. Effect of the combination of shade tree pruning, cacao pruning and fertilization on the number of damaged cacao fruits 18 months after applying the treatments. Equal letters in the same line indicate statistically equal values (Tukey, $p \leq 0.05$). n = 12.

Table 2. Effect of the shade tree pruning, cacao pruning and fertilization on cacao (*Theobroma cacao* L.) yield components at 6, 12, 18 and 24 months after applying the treatments.

	6 MAAT			12 MAAT			18 MAAT			24 MAAT		
	STP	CP	FERT	STP	CP	FERT	STP	CP	FERT	STP	CP	FERT
NHF	1) 7.7a 2) 6.0a	1) 6.9a 2) 6.8a	1) 7.4a 2) 4.8b 3) 8.4a	1) 5.4b 2) 9.8a	1) 9.1a 2) 6.2b	1) 8.2a 2) 6.4a 3) 8.4a	1) 4.4b 2) 7.9a	1) 5.0b 2) 7.3a	1) 5.5a 2) 6.6a 3) 6.3a	1) 4.4b 2) 7.6a	1) 5.2a 2) 6.8a	1) 5.8a 2) 6.2a 3) 5.9a
DF	1) 1.2a 2) 0.8a	1) 0.6b 2) 1.4a	1) 1.2a 2) 0.7a 3) 1.1a	1) 1.6b 2) 3.4a	1) 2.6a 2) 2.5b	1) 3.7a 2) 1.4b 3) 2.5b	1) 0.6b 2) 1.1a	1) 0.7a 2) 1.0a	1) 0.9a 2) 0.8a 3) 0.9a	1) 2.4b 2) 7.2a	1) 4.7a 2) 4.9a	1) 4.9a 2) 4.1a 3) 5.4a
DF	1) 1.9b 2) 2.8a	1) 2.8a 2) 1.9b	1) 3.3a 2) 1.4c 3) 2.3b	1) 1.2b 2) 2.0a	1) 2.1a 2) 1.1b	1) 1.7ab 2) 1.1b 3) 1.9a	1) 2.1a 2) 1.6a	1) 2.2a 2) 1.5a	1) 1.8a,b 2) 1.1b 3) 2.6a	1) 1.7a 2) 1.3a	1) 2.2a 2) 0.7b	1) 1.3a 2) 1.5a 3) 1.6a
HCh	1) 5.2b 2) 8.2a	1) 8.1a 2) 5.3b	1) 7.0a 2) 4.7b 3) 8.3a	1) 2.7a 2) 1.7b	1) 1.7b 2) 2.7a	1) 2.6a 2) 1.9a 3) 2.0a	1) 6.5a 2) 7.0a	1) 7.6a 2) 5.8a	1) 5.5b 2) 5.7b 3) 9.1a	1) 2.6a 2) 3.2a	1) 3.4a 2) 2.4b	1) 2.4a 2) 3.1a 3) 3.3a
DCh	1) 2.6b 2) 4.8a	1) 4.7a 2) 2.7b	1) 5.1a 2) 2.2b 3) 3.8a	1) 3.4b 2) 11.3a	1) 6.a 2) 8.8a	1) 11.4a 2) 4.7b 3) 6.1b	1) 4.8a 2) 4.6a	1) 6.4a 2) 2.8b	1) 4.3a 2) 4.2a 3) 5.5a	1) 5.2b 2) 6.7a	1) 6.9a 2) 4.9b	1) 5.5a 2)5.6a 3) 6.7a
NFl	1) 8.1a 2) 8.2a	1) 7.7a 2) 8.6a	1) 7.6a 2) 7.4a 3) 9.5a	1) 2.5a 2) 0.0b	1) 0.0b 2) 2.5a	1) 0.3b 2) 0.7a,b 3) 2.7a	1) 4.7b 2) 7.7a	1)4.7b 2) 7.9a	1) 5.1b 2) 6.9a 3)6.7a,b	1) 1.1a 2) 0.73a	1) 0.6a 2) 1.1a	1) 1.4a 2) 0.7a 3) 0.5a

MAAT, Months after applying the treatments. Variation factors: STP, Shade tree pruning [1) no pruning, 2) pruning]. CP, Cacao pruning [1) no pruning, 2) pruning]. FERT, Fertilization [1) no fertilizer, 2) K, 3) NPK]. NHF, Number of healthy fruits; DF, Diseased fruits; Df, Damaged fruits; HCh, Healthy chilillos; DCh, Diseased chilillos; NFl, Number of flowers. Values with the same letters by date, variation factor and variable are statistically equal (Tukey, $p \leq 0.05$).

3.4. Number of Healthy Cacao Chilillos

The ANOVA results showed a significant effect ($F = 3.05$, $p > F = 0.05$) of the interaction among the three evaluated factors on the number of healthy chilillos at 18 months. The main effect of cacao pruning and fertilization on this variable was also recorded at that time (Table 1). The mean comparisons did not show statistically significant differences between the cacao pruning treatments or between the shade tree pruning treatments. Significant differences were observed between fertilization treatments. Fertilization with NPK significantly increased the number of healthy chilillos (Table 2). The best interaction to increase the number of healthy chilillos was NPK fertilization, no cacao pruning and no shade tree pruning (Figure 4).

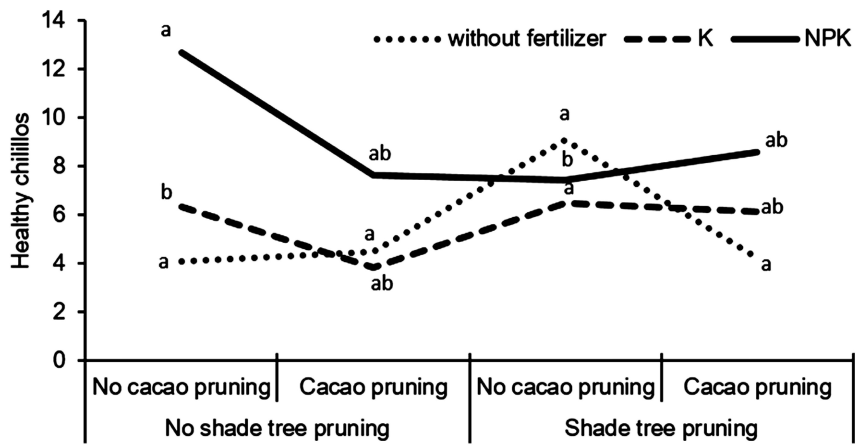

Figure 4. Effect of the combination of shade tree pruning, cacao pruning and fertilization on the number of healthy cacao chilillos 18 months after applying the treatments. Equal letters in the same line indicate statistically equal values (Tukey, $p \leq 0.05$). n = 12.

3.5. Number of Diseased Cacao Chilillos

The ANOVA results showed a significant effect of the interaction among the three evaluated factors on the number of diseased chilillos at 18 months ($F = 9.51$, $p > F = 0.0001$) and at 24 months ($F = 2.89$, $p > F = 0.0584$). On those dates, the main effect of cacao pruning on the number of diseased chilillos was also observed (Table 1) and it led to a significant decrease in this variable (Table 2). The best interactions to decrease the number of diseased chilillos were shade tree pruning, cacao pruning and NPK or K fertilization at 18 months (Figure 5). At 24 months, the best interact tree pruning and NPK or K fertilization (Figure 5).

The absence of an effect of the evaluated treatments on the number of healthy and diseased chilillos during the first year can be attributed to the 9.3 months that the moniliasis epidemic lasts in cacao plantations [4]). Thus, cacao fruits remain infected throughout the entire cacao production cycle. In other words, eliminating possible inocula (diseased chilillos) in a plantation has a positive effect one year after the activity has ceased.

The reasons why K fertilization reduced diseased chilillos could be several. Potassium deficiency can increase the accumulation of soluble sugars and amino acids, which are nutrients for pathogens. An adequate supply of potassium reduces this accumulation, decreasing the plant's susceptibility to disease [32]. Potassium strengthens the plant's structural and biochemical defenses, improving its ability to resist pathogen attack. Also, K contributes to the synthesis of lignin and cellulose, essential components of cell walls. Strong cell walls act as a physical barrier, making it difficult for pathogens to penetrate [32] [33].

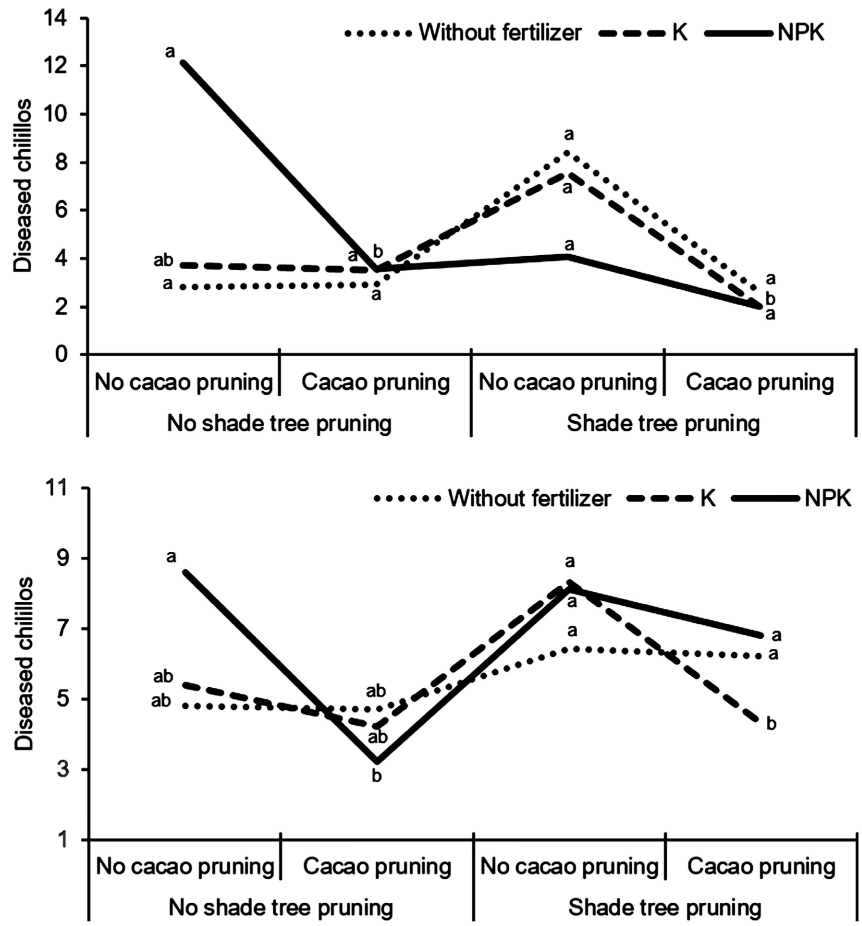

Figure 5. Effect of the combination of shade tree pruning, cacao pruning and fertilization on the number of diseased cacao chilillos at 18 and 24 (up and down) months after applying the treatments. Equal letters in the same line indicate statistically equal values (Tukey, $p \leq 0.05$). n = 12.

3.6. Number of Flowers Per Tree

The ANOVA results showed a significant effect of the interaction among the three evaluated factors on the number of flowers at 6 months ($F = 2.6$, $p > F = 0.0768$), 12 months ($F = 2.96$, $p > F = 0.0544$) and at 18 months ($F = 7.83$, $p > F = 0.0006$). An effect of the interaction between fertilization and cacao or fertilization and shade tree pruning on the number of flowers was also observed at 6 months (**Table 1**). The best three-way interaction to increase the number of flowers was cacao pruning, no shade tree pruning and no fertilization. This interaction significantly increased this variable compared to all other interactions (**Figure 6**). This finding can be attributed to the fact that the nutrient content of the soil, which was determined according to the analysis performed here and in Aikpokpodion [34], was sufficient to meet the needs of the flowering process. Another explanation is that 60% of incident light on cacao trees was enough to stimulate the flowering process, as indicated by Tezara *et al.* [35].

At 12 months, the ANOVA results showed a significant effect of pruning, their interactions, and the interaction between fertilization and each of the pruning

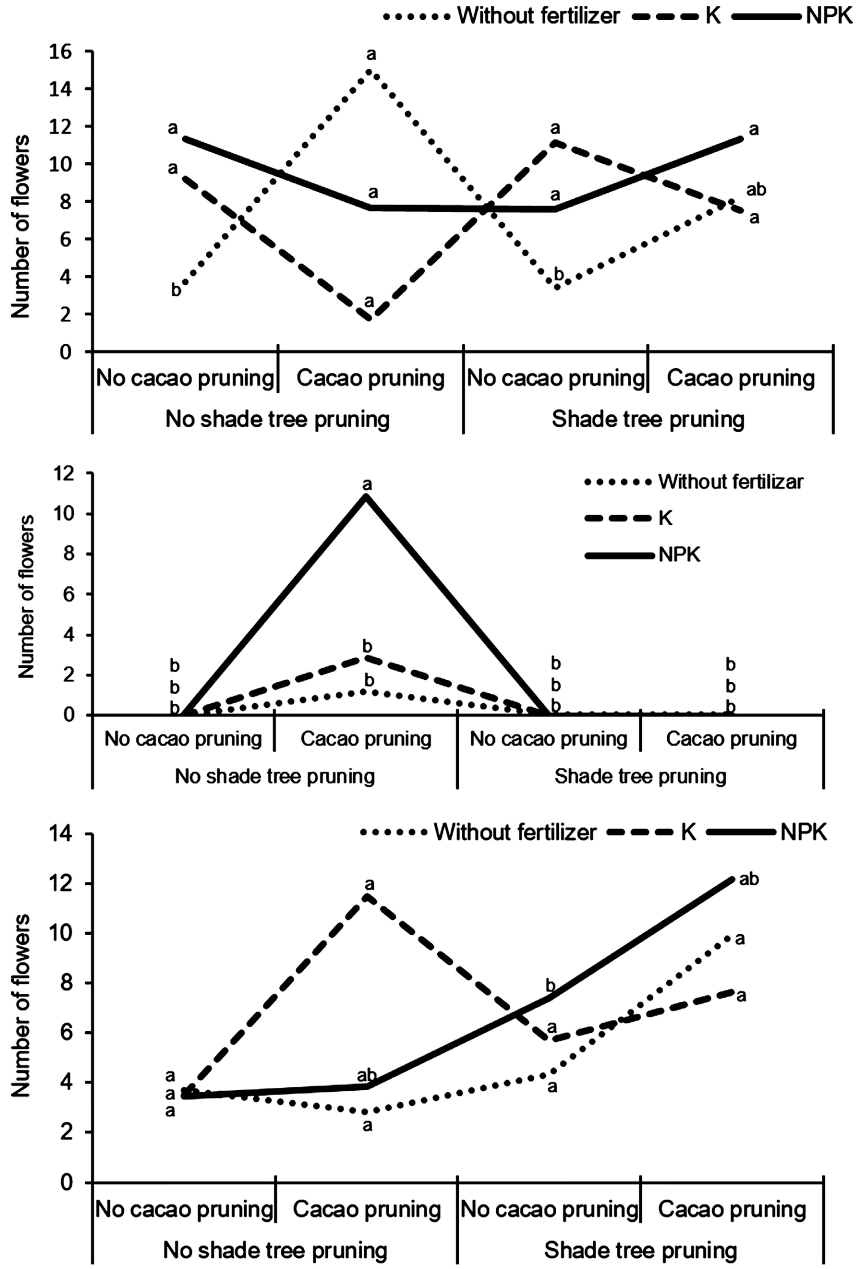

Figure 6. Effect of the combination of shade tree pruning, cacao pruning and fertilization on the number of cacao flowers 6, 12 and 18 months after applying the treatments (up, middle and down). Equal letters in the same line indicate statistically equal values (Tukey, $p \leq 0.05$). n = 12.

treatments on the number of flowers (**Table 1**). Shade tree pruning significantly decreased the number of flowers per tree, while cacao pruning and NPK fertilization increased it (**Table 2**). The best interactions to increase this variable were cacao pruning without shade tree pruning, NPK fertilization without shade tree pruning, and cacao pruning with NPK fertilization (data not tabulated). The best interaction among the three factors to increase the number of flowers was NPK fertilization, cacao pruning and no shade tree pruning (**Figure 6**). In contrast to [36], who associated the increase in flowering with increased lighting, the interaction of cacao and shade tree pruning in the present study decreased the number of flowers. Such a result could be attributed to the depletion of plant reserves or photosynthate sources. Additionally, it may be due to the increased stress experienced by the cacao trees because of the increase in incident light. This explanation is consistent with [37] and [38], who indicated that cacao trees exhibit optimal physiological behavior under low radiation conditions.

At 18 months, the ANOVA results showed a significant effect of the interaction between tree pruning, fertilization and each of the pruning treatments on the number of flowers (**Table 1**). Shade tree pruning, cacao pruning and K fertilization significantly increased this variable (**Table 2**). The best interaction between two factors to increase the number of flowers was shade tree pruning and NPK fertilization (data not tabulated). At this time, the best interactions among the three evaluated factors to increase it were the combination of K fertilization, cacao pruning and no shade tree pruning and the combination of NPK fertilization, cacao pruning and shade tree pruning (**Figure 6**).

Shade tree pruning is economically costly and dangerous [38]; in addition, the greater amount of incident light on cacao trees increases the requirement of nutrients for the flowering process. Therefore, we would advise an initial pruning, repeating the pruning after one year and fertilizing with NPK at the same time, and then fertilizing with K at 18 months after the first pruning. This advice takes into account that fertilization is not a common practice in cacao cultivation [39]-[41]; however, when applied correctly and combined with pruning and disease management, it can significantly increase the yield of cacao trees, as reported by Ortiz *et al.* [4].

4. Conclusions

The results of the present study demonstrate the effect of sanitary cacao pruning, shade tree pruning and fertilization on the production of flowers, chilillos and cacao fruits. While cacao is a shade-tolerant species with C3 metabolism, dense agroforestry systems have low cacao yields. The sanitary pruning of cacao trees, along with shade trees allows cacao plants to have the right conditions for flowering and fruiting by adjusting the microclimatic conditions of the plantation, mainly the aeration, humidity and solar radiation. Thus, pruning is an important activity in the management of cacao agroforestry systems, as it improves flowering, healthy fruit production, and production yields. To maintain yields, cacao trees should receive the proper nutrition. Fertilization is another important agricultural practice that should be part of the management of cacao agroforestry systems, especially K fertilizer.

Good production of flowers and fruits in cacao agroforestry systems requires good management for fertilizer, shade, diseases and pests. These factors are closely linked with the pruning of shade trees and the sanitary pruning of cacao. Pruning allows for the indirect control of moniliasis and damage from squirrels and birds. It also helps to reduce the use of fertilizers, mainly NPK fertilizer. However, if pruning is combined with fertilization, then only K fertilizer should be used because K accounts for constitutes 60% of cacao pod contents and is therefore the most in-demand element in cacao agroforestry systems.

In summary, the pruning of cacao trees increased the number of flowers, on which depends the formation and production of fruits. In addition, fertilization, mainly with K, decreased the damage observed in developing and mature fruits. Therefore, proper management of pruning and fertilization can increase the yield of cacao agroforestry systems.

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Authors' Contribution

Conceptualization: JPF; Methodology: JPF, JJOO; Formal analysis and investigation: JPF, JJOO, EGL; Writing—original draft preparation: JPF; Writing—review and editing: all authors; Statistical analysis: FIR; Funding acquisition: JPF, VCA; Resources: JPF, EGL; Supervision: ORR, VCA; Project administration: JPF.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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