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High-Oleic Palm Oil (HOPO) Production from Parthenocarpic Fruits in Oil Palm Interspecific Hybrids Using Naphthalene Acetic Acid

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Abstract: Interspecific OxG hybrids of African palm *Elaeis guineensis* Jacq. and the American palm *Elaeis oleifera* Cortes produce high-oleic palm oil (HOPO) with low saturated fatty acid content. OxG hybrids are highly productive, grow slowly, and are resistant to bud rot disease. However, OxG hybrid pollen presents low viability and germinability, so assisted pollination is a must. Hybrids can produce parthenocarpic or seedless fruits, with the exogenous application of plant growth regulators. Thus, naphthalene acetic acid (NAA) effects on parthenocarpic fruits induction, bunch formation, and oil quality were evaluated. The OxG hybrid Coari x La Mé was used. NAA doses, frequency, number of applications, and the phenological stages for the treatments were defined. A total dose of 1200 mg L^{-1} NAA applied three or four times produced bunches with better fruit set, similar average bunch weight, and oil to dry mesocarp than those obtained with assisted pollination. At a semi-commercial scale, 1200 mg L^{-1} NAA induced bunches that consisted of 93% or more of seedless fruits. Bunch number (2208 $\pm$ 84 versus 1690 $\pm$ 129) and oil to bunch (32.2 $\pm$ 0.7 versus 25.3 $\pm$ 0.8) were higher in the NAA induced bunches than in the assisted pollination. However, the average bunch weight was lower (12.2 $\pm$ 0.4 versus 14.9 $\pm$ 0.6). NAA increased oil to bunch in 36% (8.7 $\pm$ 0.1 versus 6.4 $\pm$ 0.3). Thus, with this technology, it is plausible to reach more than 10 tons per hectare per year of HOPO. Potentially, without increasing the planted oil palm area, OxG hybrids and NAA applications could alone meet the world's fats and oil demands.

Keywords: HOPO; pollination; plant growth regulators; auxins; bunch components; fatty acid profile

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1. Introduction

Oilseed production has been increasing due to demands for traditional uses (food, animal feed) and industrial uses (biofuels). Between 1991 and 2018, edible oils' production almost tripled from 84 million tons to 231 million tons [1]. By the year 2030, edible oil consumption will be more than 300 million tons and more than 500 million tons by 2045 [2]. Palm oil has been responsible for supplying a large part of the increased demands of edible oils. In 1990, it accounted for less than 14% of the consumed oils, 32% in 2018, and more than 50% by 2050. However, consumer habits and health policies have moved the market toward consuming specialized oils, mostly highly unsaturated and oleic acid-rich.

The most important oils are not naturally rich in oleic acid. To cope with the demands of the markets, research efforts have been placed to obtain high oleic soy, rapeseed, and camelina oils [3], in most cases, through genetic modifications and, more recently, gene editing. In the oil palm, the interspecific OxG hybrids were developed through conventional breeding to respond to the bud rot disease. Some of the obtained cultivars were high oleic genotypes. As a result, interspecific OxG hybrids have been developed, which produce oil with more than 55% oleic acid content and 33% saturated acid content [4,5].

The OxG interspecific hybrids result from the crossing of the American oil palm *Elaeis oleifera* and the African oil palm *Elaeis guineensis*. They account for 12% of the total area planted with oil palm in Colombia [1]. The OxG hybrids are highly productive. In one region of Colombia, the average production of the whole producers is 38 t ha^{-1} $year^{-1}$ fresh fruit bunches (FFB), with some plantations producing close to 45 t ha^{-1} $year^{-1}$ [6]; moreover, the OxG hybrids have a slow growth rate besides the named characteristics of the oil quality. However, the OxG hybrids have several features that lead to the obligation to perform assisted pollination using pollen derived from *E. guineensis* palms as a prerequisite to obtained well-conformed bunches with oil content close to that of the *E. guineensis* cultivars [7]. The required assisted pollination is an expensive and labor-intensive process, with an estimated annual expense of approximately 18% of the crop's total cost [8], negatively impacting the crop's competitiveness.

The natural pollination of the OXG hybrids is limited because of fertility problems, most likely derived from some type of sexual incompatibility between the two species resulting in very low pollen viability and germinability [9]. Moreover, the anemophilous and entomophilous pollination are affected by female inflorescence characteristics such as a long peduncular bract and a high number of bracteoles that reduce the access of pollinators such as *Elaeidobius subvittatus* and *Mystrops costaricensis* to the flowers [10]. Furthermore, the flower attractiveness (volatiles emitted) of *E. guineensis* and the OxG is different, and the number of pollinators visiting OxG is meager compared with *E. guineensis*.

In the OxG hybrids, the final accumulated oil is produced by fertile (normal) fruits and parthenocarpic (seedless) fruits [5]. In many plant species, it is possible to induce parthenocarpic fruit formation by exogenously applying plant growth regulators [11–17]. Therefore, using plant growth regulators to replace assisted pollination in the OxG hybrids can become an essential technology for oil palm production.

The parthenocarpy is a strategy to induce fruit formation when the conditions are not propitious. For example, when functional pollen is limited, the number or activity of pollinators is reduced, or the pollen dispersal is low [18]. The fruit set and fruit development regulation are complex processes in which several plant hormones are involved, with auxins, gibberellins, and cytokinins acting as positive regulators, while abscisic acid and ethylene most commonly working as negative regulators [19].

Parthenocarpic fruit induction has been achieved in African oil palm cultivars through auxins [20,21]. However, the parthenocarpic fruits of those cultivars were smaller and had less mesocarp than normal fruits. Furthermore, the fruit's oil synthesis was impaired, resulting in low oil yield [22], which is different from the oil production observed in the parthenocarpic fruits of the OxG hybrids [7]. Thus, a recent study has shown that bunches entirely composed of oily parthenocarpic fruits can be obtained with auxin applications [23].

The auxin activity of naphthaleneacetic acid (NAA) has been recognized for a long time [24]. Several studies have shown that NAA parthenocarpic fruit induction is superior to other auxin type substances or other plant hormones [25–27]. Thus, this study aimed to develop a technology to overcome the required assisted pollination of interspecific OxG oil palm hybrids by inducing parthenocarpic fruits using NAA, without affecting the oil's quality as a mean to competitively produce the high oleic oil that the markets are demanding. Furthermore, the study involved the standardization of doses, time, frequency, and mode of application.

2. Materials and Methods

2.1. Location and Plant Material

The research was carried out in plantations located in the central oil palm growing region of Colombia. The average temperature is 26 °C and the average annual rainfall is 2200 mm/year. Five- and six-year-old interspecific OxG hybrids, cultivar "Coari x La Me" obtained from the cross of *E. oleifera* ecotype Coari (Brazil) and *E. guineensis* from La Mé station (Ivory Coast) were used. At this age, the characteristics of floral development

and fruit growth have been stabilized [7]. The palms had been planted at a density of 115 palms per hectare (experiments 1 and 2) or 128 palms per hectare (experiment 3). The treatments were applied to inflorescences in specific phenological stages (Figure 1) according to the BBCH ("Biologische Bundesanstalt, Bundessortenamt and CHemische Industrie") phenology scale [7].

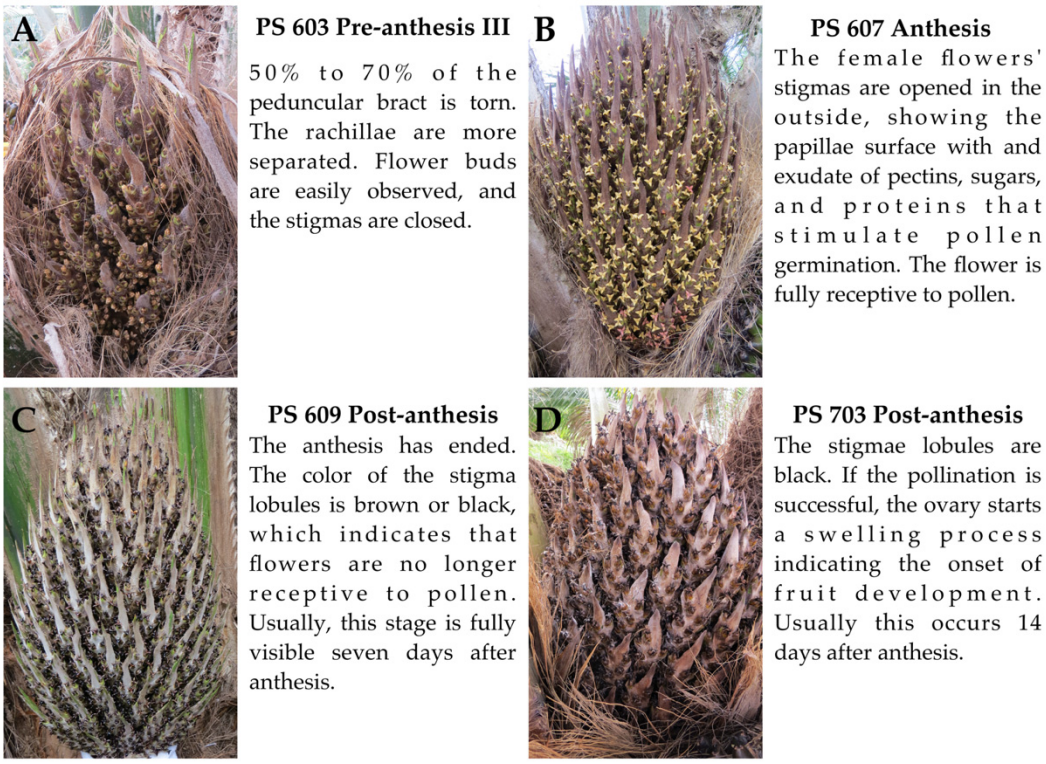

Figure 1. Phenological stages (PS) used in the different experiments. (**A**) PS 603 (Pre-anthesis III). (**B**) PS 607 (anthesis). (**C**) PS 609 (post-anthesis, seven days after anthesis). (**D**) PS 703 (post-anthesis 14 days after anthesis). The phenological stages were defined according to the BBCH phenological scale developed by [7].

2.2. Treatments

Two experiments were performed in which inflorescences in phenological stage pre-anthesis I (PS 601) [7] were identified, the peduncular bracts removed, and the inflorescences isolated using a polyester bag (PBS International, Eastfield, UK) that blocked the entry of pollen transported by wind or by pollinating insects. In a third experiment, semi-commercial conditions were used in which the inflorescences were treated as before but without isolation with the polyester bags. The treatments consisted of a mixture of NAA (product number N0640 Sigma Aldrich, Merck KGaA, Darmstadt, Germany) with 0.25% adjuvant, 0.2% Tween 80, and 2.5% ethanol in water in all the experiments. A total dose of 200 mL was sprayed per inflorescence.

Experiment 1 was designed to define the phenological stage at which parthenocarpic fruit formation was triggered. In addition, the most effective NAA concentration for inducing the parthenocarpic fruits in a single application was determined. Different NAA solutions were applied when the isolated inflorescences reached the phenological stages indicated in Table 1. The full description of the phenological stages used is shown in Figure 1.

Table 1. Treatments for the definition of phenological stage and naphthalene acetic acid (NAA) concentration for triggering parthenocarpic fruit formation in OxG hybrids (Coari x La Mé). NAA solutions were applied once at different phenological stages. Assisted pollination treatment was performed only in inflorescences in anthesis (phenological stage 607).

NAA mg L^{-1}	Phenological Stage of the Application			
	PS 603	**PS 607**	**PS 609**	**PS 703**
50	X			
100	X			
200	X			
300	X			
600	X			
1200	X			
50		X		
100		X		
200		X		
300		X		
600		X		
1200		X		
50			X	
100			X	
200			X	
300			X	
600			X	
1200			X	
50				X
100				X
200				X
300				X
600				X
1200				X
AP		X		

NAA, 1-naphthalene acetic acid; AP, assisted pollination.

Experiment 2 was performed to establish the best moment (phenological stage) and the frequency of application using the plant growth regulator concentrations defined in the previous experiment (Table 2).

Experiment 3 was set up to test the effectiveness of the technology at a semi-commercial scale. For this, plots of 2 ha were used (three plots per treatment). The treatment consisted of the plant growth regulator applied at the dose and frequency defined as the best in the previous experiments (1200 mg L^{-1} NAA applied three times). The first NAA application was made to inflorescences in phenological stages PS 603, PS 607, or PS 609. Then the NAA solution was applied two more times on the same inflorescences at seven-day intervals. Inflorescences in PS 607 were assisted pollinated with a mixture of talc: pollen 9:1 as a control. In all the experiments, the bunches were harvested at the optimal harvest point [5], about 5.5 to 6 months after the NAA applications.

Experiment 3 was carried out applying the treatments on every inflorescence of the experimental plots for 12 continuous months with a total duration of the experiment of around 18 months.

Table 2. Treatments for the definition of best naphthalene acetic acid (NAA) concentration and application frequency to induce parthenocarpy in OxG hybrids (Coari x La Mé). NAA solutions were applied one, two, three, or four times at different phenological stages. Assisted pollination treatment was performed only in inflorescences in anthesis (phenological stage 607).

Treatment	NAA mg L^{-1}	Phenological Stage of the Application			
		1 PS 603	2 PS 607	3 PS 609	4 PS 703
1	600	X			
2			X		
3				X	
4					X
5		X	X		
6		X		X	
7		X			X
8			X	X	
9			X		X
10				X	X
11		X	X	X	
12		X	X		X
13		X		X	X
14			X	X	X
15		X	X	X	X
16	1200	X			
17			X		
18				X	
19					X
20		X	X		
21		X		X	
22		X			X
23			X	X	
24			X		X
25				X	X
26		X	X	X	
27		X	X		X
28		X		X	X
29			X	X	X
30		X	X	X	X
31	AP		X		

NAA = 1-naphthalene acetic acid; AP = assisted pollination.

2.3. Bunch and Oil Quality Analysis

Bunches were harvested at the optimal harvest point [5] to maximize the oil potential. The bunch component analyses were performed according to [28] to record bunch weight (BW), oil-to-bunch (O/B), oil content per bunch (OC), oil-to-dry mesocarp (O/DM), average fruit weight of parthenocarpic fruits (AFWpf), and fruit set. To test the effect of NAA on oil quality parameters, the fatty acid profile was measured according to [23], vitamin E, carotenoids, and free fatty acids were determined according to [29] in the harvested bunches of experiment 2.

2.4. Experimental Design and Statistical Analysis

A randomized complete block design with 20 replications per treatment was used in experiments 1 and 2. The experimental unit was defined as one female inflorescence of different palms. Thus, in each experiment, 20 different palms per treatment were selected, and, in each palm, only one inflorescence was used to avoid any interaction between inflorescences and palms. In experiment 3, 2-ha plots were used, three plots per treatment. The variables were measured in the bunches produced by all the palms in the plot. The

response variables were subjected to one-way analysis of variance (ANOVA) and mean comparison tests using the least significant difference (LSD) test ($p \leq 0.05$) for experiment 1 and the Dunnett's test for experiment 2 using the statistical software SAS® version 9.1.3. (SAS Institute Inc., Cary, NC, USA) In experiment 3, the NAA treatment was compared with the assisted pollination by the independent samples *t*-test ($p \leq 0.05$) using the statistical software Jamovi® version 1.2.27.0.

3. Results

3.1. Experiment 1. Definition of Phenological Stage and NAA Concentration to Trigger Parthenocarpic Fruit Formation in OxG Interspecific Hybrids

3.1.1. Effect of Single Applications of Different NAA Concentrations on Bunch Formation

In Experiment 1, single applications of NAA solutions of different concentrations triggered the formation of bunches of parthenocarpic fruits when applied at different phenological stages. However, there were differences depending on the NAA dose. The lowest percentage of formed bunches was obtained with 50 mg L^{-1} NAA (30% to 55%) (Table 3). Doses of 600 mg L^{-1} NAA and 1200 mg L^{-1} NAA induced 90% and 100% of a bunch formation similar to the percentage of bunches formed when assisted pollination was used. Furthermore, these two last NAA treatments were effective in inducing a high percentage of bunch formation in all the phenological stages, PS 603 (pre-anthesis), PS 607 (anthesis), PS 609 (7 days after anthesis), and PS 703 (14 days after anthesis).

Table 3. Effect of single NAA applications on the proportion of bunches formed. Different NAA concentrations were applied to female inflorescences at four phenological stages to induce parthenocarpy in the OxG hybrids (Coari x La Mé).

PS	NAA mg L^{-1}	Number of Bunches			Formed Bunches (%)
		Formed	Bunch Failures	Total	
PS 603	50	9	11	20	45%
	100	15	5	20	75%
	200	18	2	20	90%
	300	14	6	20	70%
	600	20	0	20	100%
	1200	19	1	20	95%
PS 607	50	6	14	20	30%
	100	9	11	20	45%
	200	17	3	20	85%
	300	18	2	20	90%
	600	20	0	20	100%
	1200	20	0	20	100%
PS 609	50	9	11	20	45%
	100	12	8	20	60%
	200	16	4	20	80%
	300	16	4	20	80%
	600	16	3	20	90%
	1200	19	1	20	95%
PS 703	50	11	9	20	55%
	100	14	6	20	70%
	200	12	8	20	60%
	300	16	4	20	80%
	600	18	2	20	90%
	1200	19	1	20	95%
PS 607	AP	20	0	20	100%

NAA = naphthalene acetic acid; AP = assisted pollination; PS = phenological stages for application.

3.1.2. Effect of Single Applications of Different NAA Concentrations on Bunch Components

Bunch weight increased in response to the NAA applications with values between 11.5 ± 0.8 kg and 12.7 ± 0.7 kg for 600 mg L^{-1} NAA and between 13.1 ± 0.7 kg and 15 ± 0.8 kg for 1200 mg L^{-1} NAA. However, compared to assisted pollination (18.9 ± 1.0 kg), a single NAA application resulted in a significant BW reduction regardless of the phenological stage in which the application was made (Figure 2A).

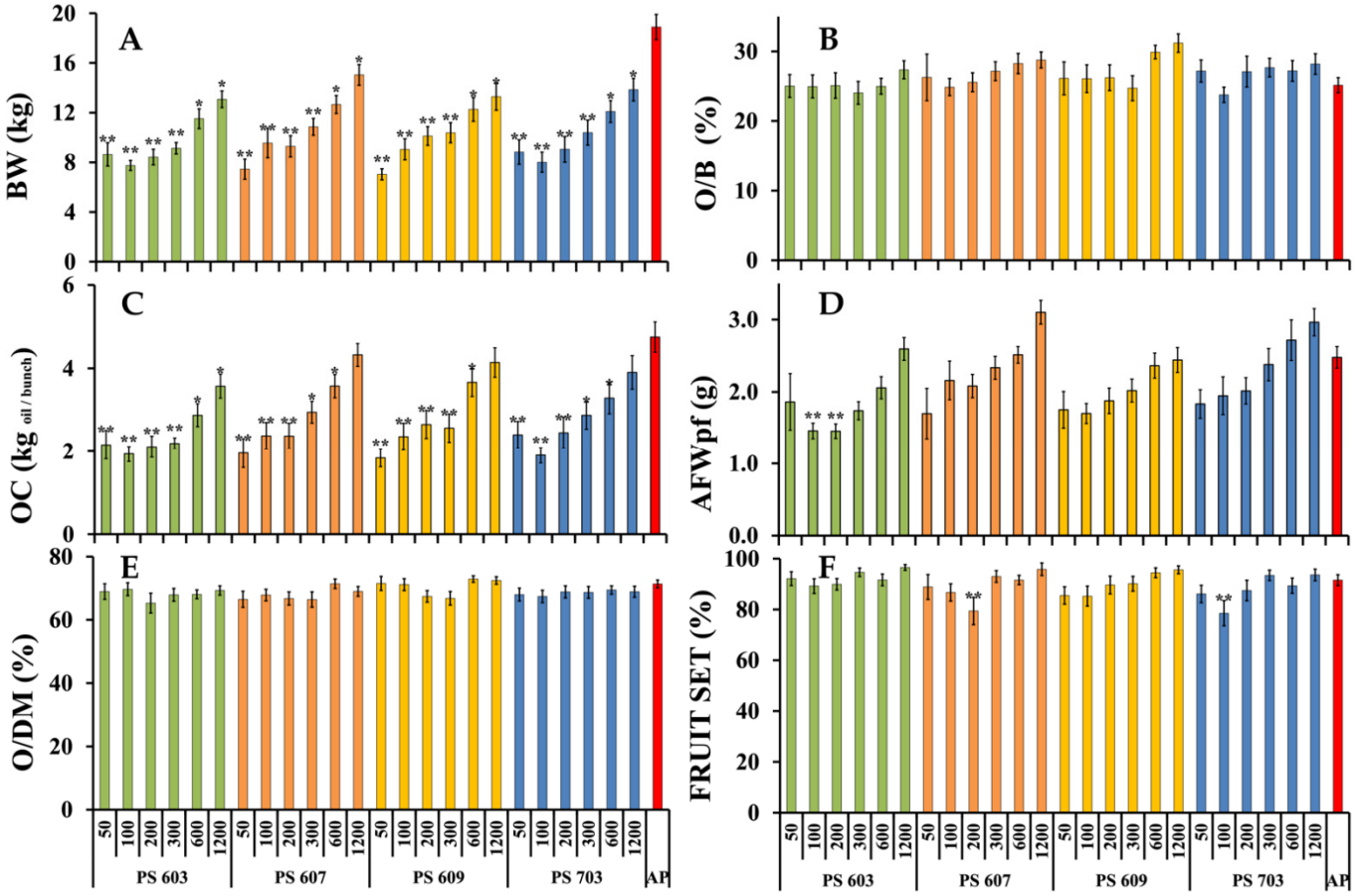

Figure 2. Effect of single applications of different NAA concentrations on the bunch components in OxG hybrids (Coari x La Mé). The NAA solutions were applied once at different phenological stages (PS) (603, 607, 609, 703), represented by the different colors. Assisted pollination treatment was performed only in inflorescences in anthesis (phenological stage 607), represented by the red bar. (**A**) Average bunch weight (BW). (**B**) Oil to bunch (O/B). (**C**) Oil content per bunch (OC). (**D**) Average fruit weight of parthenocarpic fruits (AFWpf). (**E**) Oil to dry mesocarp (O/DM). (**F**) Fruit set. Statistically significant differences between assisted pollination (AP) as commercial control and the NAA treatments across dosages/PS are indicated by asterisks (One-Way ANOVA, Dunnett's test, * = $p < 0.05$, ** = $p < 0.01$).

NAA treatments strongly impacted the oil to bunch (O/B), with increments as high as six percentage points in NAA-induced bunches compared to those produced by assisted pollination. The best results were obtained with 600 mg L^{-1} NAA, and 1200 mg L^{-1} NAA applied at PS 609, with 30 ± 1.0% and 31 ± 1.3% O/B. O/B for assisted pollination was 25 ± 1.1% (Figure 2B). O/B increased with NAA concentration, an effect that was sustained before anthesis (PS 603) and up to 14 days after anthesis (PS 703).

NAA in doses 600 mg L^{-1} and 1200 mg L^{-1} induced the highest oil content per bunch (OC) among the plant regulator treatments, with values that fluctuated between 2.9 ± 0.3 kg and 3.7 ± 0.3 kg with 600 mg L^{-1} NAA, and between 3.6 ± 0.3 kg and 4.3 ± 0.3 kg with 1200 mg L^{-1} NAA. However, assisted pollination presented the highest OC

with 5.2 ± 0.4 kg of oil per bunch, a difference that was related mostly to the higher bunch weight of the assisted pollination treatment (Figure 2C).

The average fruit weight of parthenocarpic fruits (AFWpf) increased with the NAA concentration. It was significantly higher than assisted pollination only in treatments with 1200 mg L^{-1} NAA when applied at PS 607 or PS 703. However, from 600 mg L^{-1} NAA, the AFWpf was similar or higher than in bunches obtained by assisted pollination (Figure 2D). On the other hand, NAA did not change the oil to dry mesocarp ratio (O/DM) in comparison to assisted pollination, with an average value of 70% (Figure 2E).

Regarding the fruit set, at a general level, a high proportion of formed fruits (>90%) was observed with most of the evaluated NAA concentrations. Bunches induced by one application of 1200 mg L^{-1} NAA showed the best conformation, with fruit set values that fluctuated between 94 ± 1.9% and 97 ± 1.1% depending on the phenological stage at which the first NAA application was performed. In the assisted pollination treatment fruit set was 92% (Figure 2F).

3.2. Experiment 2: Determination of the Frequency of Application of NAA Solutions to Induce Parthenocarpic Fruits

3.2.1. Effect of Multiple Applications of 600 mg L^{-1} NAA and 1200 mg L^{-1} NAA on Bunch Formation

Most NAA treatments induced a high proportion of bunches, with values between 80% and 100%, similar to the assisted pollination (Table 4). When a single dose of 600 mg L^{-1} NAA was used, 100% of bunches were formed applying at PS 603 and PS 607 (treatments 1 and 2). The single applications at PS 609 (treatment 3) and PS 703 (treatment 4) produced the lowest bunch formation with 80% and 90%, respectively. Single applications of 1200 mg L^{-1} NAA induced between 95% and 100% bunch formation (treatments 16 to 19). For multiple 600 mg L^{-1} NAA applications, treatment 7 (two applications at PS 603 and PS 703) induced the fewest bunches (85%). In comparison, with 1200 mg L^{-1} NAA, the lowest values were obtained with two applications at PS 607 and PS 703 (treatment 24), and with three applications at PS 603, PS 607, and PS 703 (treatment 27) with a value of 90% in both cases.

Table 4. Effect of multiple NAA applications on the proportion of bunches formed. Doses of 600 mg L^{-1} or 1200 mg L^{-1} NAA were applied from one to four times to female inflorescences at different phenological stages (PS) to induce parthenocarpy in the OxG hybrids (Coari x La Mé).

Treatment	NAA mg L^{-1}	PS	Number of Bunches			Formed Bunches (%)
			Formed	Failures	Total	
1	600	1	20	0	20	100
2		2	20	0	20	100
3		3	16	4	20	80
4		4	18	2	20	90
5		1, 2	18	2	20	90
6		1, 3	19	1	20	95
7		1, 4	17	3	20	85
8		2, 3	20	0	20	100
9		2, 4	20	0	20	100
10		3, 4	18	2	20	90
11		1, 2, 3	20	0	20	100
12		1, 2, 4	18	2	20	90
13		1, 3, 4	20	0	20	100
14		2, 3, 4	19	1	20	95
15		1, 2, 3, 4	20	0	20	100

Table 4. *Cont.*

Treatment	NAA mg L^{-1}	PS	Number of Bunches			Formed Bunches (%)
			Formed	Failures	Total	
16	1200	1	19	1	20	95
17		2	20	0	20	10
18		3	19	1	20	95
19		4	19	1	20	95
20		1, 2	20	0	20	100
21		1, 3	20	0	20	100
22		1, 4	19	1	20	95
23		2, 3	20	0	20	100
24		2, 4	18	2	20	90
25		3, 4	19	1	20	95
26		1, 2, 3	20	0	20	100
27		1, 2, 4	18	2	20	90
28		1, 3, 4	19	1	20	95
29		2, 3, 4	20	0	20	100
30		1, 2, 3, 4	20	0	20	100
31	AP	2	20	0	20	100

NAA = naphthalene acetic acid; AP= assisted pollination; PS = phenological stages for application: 1 = PS 603; 2 = PS 607; 3 = PS 609; 4 = PS 703.

3.2.2. Effect of Multiple Applications of 600 mg L^{-1} and 1200 mg L^{-1} NAA on Bunch Components

The assisted pollination bunches (treatment 31) showed 91.5 ± 2.2% of fruit set (Table 5), similar to that obtained in the previous experiment (Figure 2F). Only when 600 mg L^{-1} NAA was applied once at PS 703 (treatment 4), the fruit set (89.4 ± 3.1%) was lowered than with AP. The same NAA concentration applied once at PS 603 or PS 607 induced a similar fruit set than AP (91.6%). The rest of the NAA treatments yielded a higher fruit set than AP. The best fruit set was reached when 1200 mg L^{-1} NAA was applied three or four times; thus, treatment 27 (applications at PS 603, PS 607, and PS 703) and treatment 30 (applications at PS 603, PS 607, PS 609, and PS 703) were the best with fruit set values close to 99%. One of the bunch components negatively impacted when the NAA treatments replaced AP was the BW (Figure 3, Table 5). Single applications of the plant growth regulator resulted in bunches 5 kg (1200 mg L^{-1} NAA) or 6 kg (600 mg L^{-1} NAA) lighter than those produced when assisted pollination was used. Three applications (treatments 11, 12, and 14) or four applications (treatment 15) of 600 mg L^{-1} NAA induced bunches 3 kg lighter than AP. However, when 1200 mg L^{-1} NAA was applied, the BW was positively correlated with the number of applications. The more times the plant regulator was used, the more massive the bunches were. Thus, the weight of bunches induced with three (treatments 26 to 29) or four (treatment 30) NAA applications was not significantly different than the BW obtained when AP was used. Furthermore, BW of treatments 28 (NAA applications at PS 603, PS 607, and PS 703) and 29 (NAA applications at PS 607, PS 609, and PS 703) were very close to the BW of AP (18.6 ± 0.8 kg, 18.5 ± 1.1 kg, and 18.9 ± 1.0 kg, respectively).

Table 5. Bunch components in OxG hybrids (Coari x La Mé) treated with 600 mg L^{-1} or 1200 mg L^{-1} NAA for the induction of parthenocarpy. The NAA solutions were applied one, two, three, or four times at different phenological stages (603, 607, 609, 703). Assisted pollination treatment was performed only in inflorescences in anthesis (phenological stage 607).

Treatment	NAA (mg L^{-1})	PS	N	Fruit Set (%)	BW (kg)			O/DM (%)	O/B (%)		OC (kg)		AFWpf (g)	
1		1	20	91.6 ± 2.3	cdef	11.5 ± 0.8	n	68.1 ± 1.4	25.0 ± 1.1	f	2.9 ± 0.3	m	2.1 ± 0.2	ml
2		2	20	91.6 ± 1.8	cdef	12.7 ± 0.7	lmn	71.4 ± 1.5	28.3 ± 1.5	bcde	3.4 ± 0.3	lm	2.5 ± 0.1	klm
3		3	16	94.5 ± 1.9	abcde	12.3 ± 1.0	mn	72.9 ± 1.0	29.9 ± 1.0	abcde	3.7 ± 0.3	jklm	2.4 ± 0.2	lm
4		4	15	89.4 ± 3.1	f	12.1 ± 0.9	mn	69.4 ± 1.3	27.2 ± 1.5	def	3.5 ± 0.4	klm	2.7 ± 0.3	hijkl
5		1, 2	18	94.3 ± 2.6	abcde	13.4 ± 0.8	ijklmn	69.3 ± 1.5	28.3 ± 1.2	bcde	3.8 ± 0.3	ijklm	2.7 ± 0.2	hijkl
6		1, 3	19	93.6 ± 2.2	bcdef	13.6 ± 0.9	ijklmn	69.2 ± 1.6	28.6 ± 1.1	abcde	4.0 ± 0.4	hijkl	2.9 ± 0.2	efghijkl
7		1, 4	17	96.8 ± 1.0	ab	14.0 ± 0.8	ghijklm	70.5 ± 1.5	29.5 ± 1.2	abcde	4.2 ± 0.4	ghijkl	2.6 ± 0.2	ijkl
8	600	2, 3	19	97.6 ± 0.7	ab	16.0 ± 0.5	cdefgh	70.5 ± 1.4	31.3 ± 0.9	ab	5.0 ± 0.2	bcdeg	3.1 ± 0.1	cdefghij
9		2, 4	19	90.3 ± 2.7	ef	13.2 ± 0.8	jklmn	69.2 ± 1.3	27.3 ± 1.0	cdef	3.6 ± 0.3	jklm	2.8 ± 0.2	fghijkl
10		3, 4	17	96.8 ± 1.8	ab	13.4 ± 0.6	ijklmn	67.1 ± 1.5	29.9 ± 1.1	abcde	4.0 ± 0.3	hijkl	2.9 ± 0.2	defghijk
11		1, 2, 3	19	97.3 ± 1.0	ab	15.1 ± 1.0	efghijk	71.1 ± 0.7	29.4 ± 1.1	abcde	4.2 ± 0.3	ghijkl	3.1 ± 0.2	abcdefghi
12		1, 2, 4	18	96.1 ± 1.2	abcd	15.2 ± 0.5	efghijk	69.7 ± 1.5	29.0 ± 0.7	abcde	4.4 ± 0.2	deghijk	3.0 ± 0.2	cdefghij
13		1, 3, 4	20	94.0 ± 1.9	abcdef	13.9 ± 1.2	hijklm	69.1 ± 1.0	26.8 ± 1.1	ef	3.9 ± 0.4	hijkl	2.7 ± 0.2	hijkl
14		2, 3, 4	19	95.5 ± 1.7	abcd	15.5 ± 0.8	defghijk	71.2 ± 1.1	31.1 ± 1.0	ab	4.8 ± 0.3	bcdegh	3.2 ± 0.2	abcdefg
15		1, 2, 3, 4	20	96.8 ± 2.0	ab	15.6 ± 0.9	cdefghij	69.6 ± 1.2	29.9 ± 1.2	abcde	4.7 ± 0.4	bcdeghi	3.2 ± 0.1	abcdefg
16		1	19	96.5 ± 1.1	abc	13.1 ± 0.7	klmn	69.3 ± 1.5	27.3 ± 1.3	cdef	3.6 ± 0.3	klm	2.6 ± 0.2	jkl
17		2	19	95.8 ± 2.5	abcd	15.0 ± 0.8	efghijkl	69.0 ± 1.5	28.8 ± 1.1	abcde	4.3 ± 0.3	eghijkl	3.1 ± 0.2	cdefghi
18		3	17	95.6 ± 1.5	abcd	13.3 ± 1.1	jklmn	72.4 ± 1.2	31.2 ± 1.3	ab	4.1 ± 0.4	ghijkl	2.4 ± 0.2	lm
19		4	17	93.6 ± 2.3	bcdef	13.9 ± 0.9	hijklmn	68.9 ± 1.8	28.2 ± 1.5	bcdef	4.0 ± 0.4	hijkl	3.0 ± 0.2	cdefghijk
20		1, 2	18	97.1 ± 1.8	ab	16.8 ± 1.0	abcdef	69.2 ± 1.7	28.6 ± 1.7	abcde	4.8 ± 0.4	bcdegh	3.1 ± 0.2	abcdefgh
21		1, 3	20	95.5 ± 1.6	abcd	17.2 ± 0.9	abcdef	69.3 ± 1.6	29.1 ± 1.2	abcde	5.1 ± 0.4	bcde	3.1 ± 0.2	abcdefghi
22		1, 4	18	97.6 ± 1.9	ab	14.9 ± 0.7	fghijkl	71.0 ± 1.4	29.3 ± 1.0	abcde	4.4 ± 0.3	deghijk	2.7 ± 0.2	ghijkl
23	1200	2, 3	20	96.9 ± 1.8	ab	15.1 ± 0.8	efghijk	72.6 ± 1.3	31.0 ± 1.3	ab	4.8 ± 0.4	bcdegh	3.1 ± 0.2	bcdefghi
24		2, 4	17	96.3 ± 2.2	abcd	15.7 ± 0.9	cdefghi	67.3 ± 1.7	28.3 ± 1.1	bcde	4.5 ± 0.4	cdeghij	3.6 ± 0.2	ab
25		3, 4	19	97.4 ± 1.3	ab	16.3 ± 0.9	bcdefg	71.2 ± 1.4	31.8 ± 1.2	a	5.3 ± 0.4	abcd	3.4 ± 0.2	abcd
26		1, 2, 3	19	97.9 ± 1.1	ab	17.4 ± 0.8	abcde	70.4 ± 1.4	28.9 ± 1.0	abcde	5.1 ± 0.3	bcde	3.4 ± 0.2	abcd
27		1, 2, 4	17	98.8 ± 0.4	a	17.9 ± 1.0	abc	71.1 ± 1.9	29.3 ± 1.4	abcde	5.2 ± 0.3	abcde	3.3 ± 0.1	abcde
28		1, 3, 4	19	97.5 ± 1.5	ab	18.6 ± 0.8	ab	71.0 ± 1.2	30.2 ± 1.0	abcd	5.6 ± 0.3	ab	3.4 ± 0.2	abc
29		2, 3, 4	19	96.3 ± 1.7	abcd	18.5 ± 1.1	ab	70.5 ± 1.2	30.7 ± 1.2	ab	6.1 ± 0.4	a	3.6 ± 0.1	a
30		1, 2, 3, 4	19	98.9 ± 0.5	a	17.8 ± 0.8	abcd	71.6 ± 1.4	30.5 ± 0.8	abc	5.4 ± 0.3	abc	3.2 ± 0.2	abcdef
31	AP	2	19	91.5 ± 2.2	def	18.9 ± 1.0	a	71.4 ± 1.3	25.2 ± 1.1	def	5.2 ± 0.4	abcde	2.5 ± 0.2	klm
LSD/Significance		-		5.0	**	2.4	***	ns	3.2	***	1.0	***	0.5	***

NAA = naphthalene acetic acid; AP = assisted pollination; PS = phenological stages for application: 1 = PS 603; 2 = PS 607; 3 = PS 609; 4 = PS 703; BW = mean bunch weight; O/B = percentage of oil to bunch; O/DM = percentage of oil in the dry mesocarp; OC = oil content per bunch; AFWpf = average fruit weight of parthenocarpic fruits. The values correspond to the average ± standard error of at least 15 bunches per treatment. Values with the same letter are not statistically different according to the least significant difference (LSD) test ($p < 0.05$); ns: not significant, ** = $p < 0.01$, *** = $p < 0.001$.

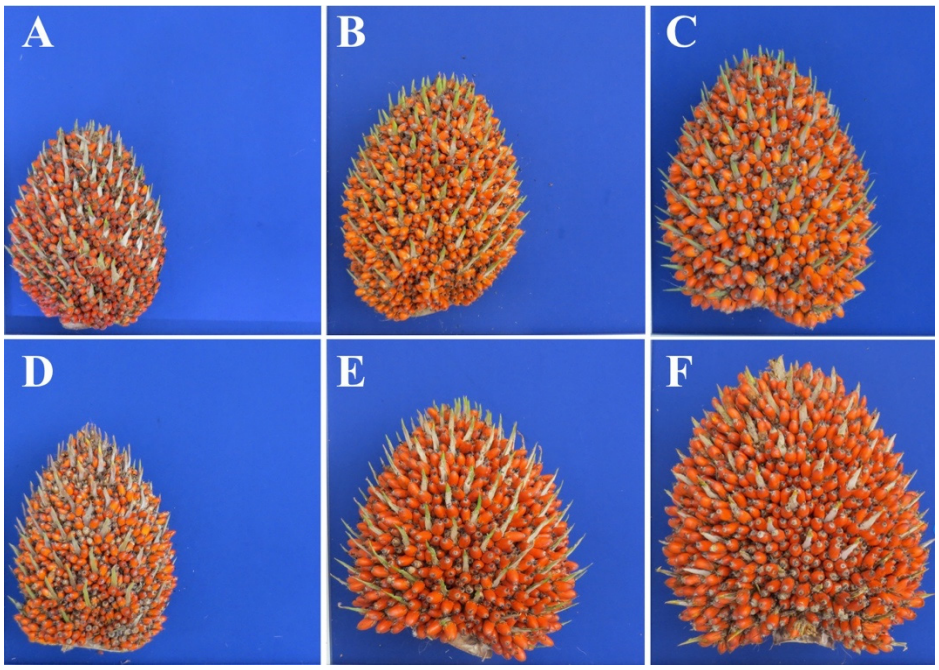

Figure 3. Bunches formed by 600 mg L^{-1} or 1200 mg L^{-1} NAA applications in OxG hybrids (Coari x La Mé). Bunch conformation improved with multiple hormone applications. Bunches were composed only of oily parthenocarpic fruits. The pictures are from representative bunches selected from treatments started at different phenological stages. (**A**) A dose of 600 mg L^{-1} NAA applied once. (**B**) A dose of 600 mg L^{-1} NAA applied twice. (**C**) A dose of 600 mg L^{-1} NAA applied three times. (**D**) A dose of 1200 mg L^{-1} NAA applied once. (**E**) A dose of 1200 mg L^{-1} NAA applied twice. (**F**) A dose of 1200 mg L^{-1} NAA applied three times.

NAA applications consistently increased the O/B compared to AP (Table 5). O/B in the AP treatment (25.2 ± 1.1%) was similar to that obtained in the previous experiment (25%), and only treatment 1 (600 mg L^{-1} NAA applied once) resulted in a slightly lower O/B (25 ± 1.1%). Most of the NAA treatments resulted in O/B statistically higher than in the AP treatment. Twelve of the NAA treatments accumulated four or five percentage points more oil than AP. Moreover, two of the 600 mg L^{-1} NAA treatments (8, 14) and three of the 1200 mg L^{-1} NAA treatments (18, 23, 25) accumulated six percentage points more oil than the AP treatment with O/B values higher than 31%. (Table 3).

In the NAA treatments, the bunch oil content (OC) was statistically similar or higher than assisted pollination (4.4 ± 0.3 kg). Only three treatments with 600 mg L^{-1} NAA (1, 2, and 4) were significantly lower than AP. On the other hand, three of the 1200 mg L^{-1} NAA treatments (28, 29, and 30) were significantly higher than AP, with oil contents of 5.6 ± 0.3 kg, 6.1 ± 0.4 kg, and 5.4 ± 0.3 kg per bunch, respectively (Table 5).

The AFWpf in the NAA treatments was similar to or higher than AP (Table 5). In general, the AFWpf increased with the number of applications and was higher in the 1200 mg L^{-1} NAA treatments than in the 600 mg L^{-1} NAA treatments. Thus, the AFWpf in the 600 mg L^{-1} NAA treatments of a single application (treatments 1 to 4) was between 2.1 ± 0.2 g and 2.7 ± 0.3 g per fruit; with two applications (treatments 5 to 10), it was between 2.6 ± 0.2 g and 3.1 ± 0.1 g per fruit, and with three applications (treatments 11 to 14), it was between 2.7 ± 0.2 g and 3.2 ± 0.2 g per fruit. With four 600 mg L^{-1} NAA applications, the AFWpf (treatment 15) reached 3.2 ± 0.1 g. When 1200 mg L^{-1} NAA was applied, the same tendency was observed, in which AFWpf increased with the number of applications, with Values that ranged between 2.4 ± 0.2 g and 3.1 ± 0.2 g per fruit with a single application (treatments 16 to 19), 2.7 ± 0.2 g and 3.6 ± 0.2 g per fruit with two applications (treatments 20 to 25), and 3.2 ± 0.2 g and 3.6 ± 0.1 g per fruit with three applications (treatments 26 to 29). In the case of four applications, the AFWpf did not

further increase but dropped to 3.2 ± 0.2 g per fruit, which is a value similar to the one obtained with four 600 mg L^{-1} NAA applications but, in any case, significantly higher than the 2.5 ± 0.2 g per fruit obtained with AP (Table 5).

Finally, as in the previous experiment, the O/DM was not affected by the NAA applications. No statistical differences were observed between the NAA treatments and the AP, with values close to 70% in all cases (Table 5).

3.2.3. Effect of Multiple Applications of 600 mg L^{-1} and 1200 mg L^{-1} NAA on Oil Quality

NAA did not change the fatty acid profile compared to assisted pollination (Table 6). There were no statistical differences between the treatments in the different saturated and unsaturated fatty acids. Thus, in the AP treatment, palmitic acid content was 28.9 ± 0.5%, and in the NAA treatments, this fatty acid fluctuated between 24.7 ± 1.0% and 31.6 ± 1.4%. Oleic acid in the AP bunches was 54.4 ± 1.0%, while in the NAA treatments, it ranged between 51.6 ± 1.2% and 58.8 ± 0.7%. Stearic acid content was between 2.2 ± 0.1% and 2.9 ± 0.5%, while linoleic acid content fluctuated between 8.7 ± 2.2% and 11.4 ± 0.8%. The total saturated fatty acids ranged between 28.4 ± 0.8% and 35.0 ± 1.4%, with a value of 31.1 ± 0.2% for AP. The total unsaturated fatty acids ranged between 65.0 ± 1.4% and 71.6 ± 0.8% in the NAA treatments, while in the assisted pollination accounted for 68.9 ± 0.2% of the fatty acids (Table 6).

Table 6. Fatty acid profile in OxG hybrids (Coari x La Mé) treated with 600 mg L^{-1} or 1200 mg L^{-1} NAA for the induction of parthenocarpy. The NAA solutions were applied one, two, three, or four times at different phenological stages (603, 607, 609, 703). Assisted pollination treatment was performed only in inflorescences in anthesis (phenological stage 607).

Treatment	NAA (mg L^{-1})	PS	Palmitic Acid C16:0 (%)	Oleic Acid C18:1 (%)	Stearic Acid C18:0 (%)	Linoleic Acid C18:2 (%)	∑SFA	∑UFA
1		1	26.4 ± 1.7	57.9 ± 1.5	2.7 ± 0.4	10.3 ± 0.2	29.9 ± 1.5	70.1 ± 1.5
2		2	27.7 ± 0.4	55.7 ± 0.3	2.6 ± 0.4	10.9 ± 0.3	31.1 ± 0.3	68.9 ± 0.3
3		3	31.6 ± 1.4	51.9 ± 1.3	2.4 ± 0.2	10.9 ± 0.4	35.0 ± 1.4	65.0 ± 1.4
4		4	29.6 ± 1.0	54.5 ± 1.1	2.2 ± 0.1	10.5 ± 0.3	32.6 ± 1.1	67.4 ± 1.1
5		1.2	28.2 ± 1.2	55.7 ± 2.0	2.4 ± 0.1	10.7 ± 0.7	31.4 ± 1.3	68.6 ± 1.3
6		1.3	24.7 ± 1.0	58.8 ± 0.7	2.3 ± 0.6	8.7 ± 2.2	28.4 ± 0.8	71.6 ± 0.8
7		1.4	27.3 ± 1.7	57.7 ± 2.0	2.9 ± 0.2	9.4 ± 0.4	31.0 ± 1.7	69.0 ± 1.7
8	600	2.3	28.1 ± 0.9	55.6 ± 1.4	2.4 ± 0.2	10.8 ± 0.7	31.3 ± 0.8	68.7 ± 0.8
9		2.4	29.8 ± 1.2	54.4 ± 1.5	2.4 ± 0.2	10.2 ± 0.5	33.0 ± 1.2	67.0 ± 1.2
10		3.4	28.6 ± 1.3	55.1 ± 1.1	2.9 ± 0.5	10.3 ± 0.5	32.3 ± 1.0	67.7 ± 1.0
11		1,2,3	27.0 ± 1.2	57.6 ± 1.7	2.4 ± 0.2	9.9 ± 0.8	30.3 ± 1.3	69.7 ± 1.3
12		1,2,4	28.5 ± 0.6	56.2 ± 0.6	2.4 ± 0.2	10.0 ± 0.2	31.6 ± 0.8	68.4 ± 0.8
13		1,3,4	27.9 ± 0.9	55.4 ± 1.3	2.7 ± 0.2	11.0 ± 0.6	31.4 ± 1.1	68.6 ± 1.1
14		2,3,4	28.9 ± 1.2	55.0 ± 1.4	2.6 ± 0.2	10.2 ± 0.2	32.5 ± 1.4	67.5 ± 1.4
15		1,2,3,4	28.3 ± 1.3	56.1 ± 1.2	2.4 ± 0.2	10.2 ± 0.3	31.5 ± 1.4	68.5 ± 1.4
16		1	27.8 ± 0.6	55.9 ± 0.8	2.3 ± 0.2	10.9 ± 0.4	30.9 ± 0.7	69.1 ± 0.7
17		2	27.1 ± 0.8	57.0 ± 0.6	2.5 ± 0.1	10.8 ± 0.7	30.5 ± 0.8	69.5 ± 0.8
18		3	28.4 ± 1.3	55.0 ± 2.1	2.3 ± 0.1	10.6 ± 0.7	31.5 ± 1.3	67.9 ± 1.3
19		4	30.3 ± 0.7	52.7 ± 0.8	2.4 ± 0.3	11.3 ± 0.6	32.3 ± 1.3	67.7 ± 1.3
20		1.2	28.2 ± 1.3	55.5 ± 1.8	2.5 ± 0.2	10.7 ± 0.4	31.6 ± 1.3	68.4 ± 1.3
21		1.3	27.4 ± 0.8	56.3 ± 1.5	2.6 ± 0.3	10.5 ± 0.8	30.9 ± 1.0	69.1 ± 1.0
22		1.4	28.9 ± 1.4	55.3 ± 1.2	2.3 ± 0.2	10.3 ± 0.5	32.0 ± 1.4	68.0 ± 1.4
23	1200	2.3	29.5 ± 0.6	55.3 ± 0.8	2.3 ± 0.1	9.5 ± 0.4	32.6 ± 0.6	67.4 ± 0.6
24		2.4	28.6 ± 0.6	55.1 ± 0.7	2.4 ± 0.1	10.8 ± 0.3	31.9 ± 0.6	68.1 ± 0.6
25		3.4	28.2 ± 1.0	56.0 ± 1.1	2.7 ± 0.2	10.2 ± 0.4	31.7 ± 1.0	68.3 ± 1.0
26		1,2,3	29.3 ± 1.8	54.7 ± 2.1	2.4 ± 0.2	10.4 ± 0.4	32.6 ± 1.8	67.4 ± 1.8
27		1,2,4	29.4 ± 1.2	53.4 ± 1.5	2.6 ± 0.4	11.4 ± 0.8	33.0 ± 1.6	67.0 ± 1.6
28		1,3,4	30.1 ± 1.0	53.4 ± 1.1	2.2 ± 0.1	11.0 ± 0.4	33.2 ± 1.1	66.8 ± 1.1
29		2,3,4	31.0 ± 1.4	51.6 ± 1.2	2.5 ± 0.2	11.4 ± 1.0	34.5 ± 1.4	65.5 ± 1.4
30		1,2,3,4	29.4 ± 1.6	55.0 ± 1.2	2.7 ± 0.4	10.0 ± 0.4	32.9 ± 1.5	67.1 ± 1.5
31	AP	-	28.9 ± 0.5	54.4 ± 1.0	2.8 ± 0.3	10.9 ± 0.4	31.1 ± 0.2	68.9 ± 0.2
Significance		-	ns	ns	ns	ns	ns	ns

NAA = naphthalene acetic acid; AP = assisted pollination; PS = phenological stages for application: 1 = PS 603; 2 = PS 607; 3 = PS 609; 4 = PS 703. ∑SFA, total saturated fatty acids; ∑UFA, total unsaturated fatty acids. The values correspond to the average ± standard error of at least 15 bunches per treatment. Values with the same letter are not statistically different according to the least significant difference (LSD) test ($p < 0.05$), ns: not significant.

3.2.4. Effect of Multiple Applications of 600 mg L^{-1} and 1200 mg L^{-1} NAA on Free Fatty Acids, Vitamin E, and Carotenes

Free fatty acids (FFA), vitamin E, and carotene content were highly variable within all the treatments. There were no statistical differences among the treatments in any of these parameters (Table 7). Low FFA levels were observed with values that ranged between 0.90 ± 0.90 and 1.88 ± 1.43 (as a percentage of palmitic acid). FFA in the AP was 1.17 ± 0.51, with the highest value reached with 1200 mg L^{-1} NAA applied four times at PS 603, PS 607, PS 609, and PS 703 (treatment 30), while the lowest valued was obtained when 1200 mg L^{-1} NAA was applied three times at PS 603, 607 and 703.

Table 7. Free fatty acids, Vitamin E, and carotene content in OxG hybrids (Coari x La Mé) treated with 600 mg L^{-1} or 1200 mg L^{-1} NAA for the induction of parthenocarpy. The NAA solutions were applied one, two, three, or four times at different phenological stages (603, 607, 609, 703). Assisted pollination treatment was performed only in inflorescences in anthesis (phenological stage 607).

Treatment	NAA mg L^{-1}	PS	FFA (% Palmitic Acid)	Vitamin E mg kg^{-1}	Carotenes mg kg^{-1}
1	600	1	1.43 ± 1.24	1247 ± 387	949 ± 395
2		2	1.13 ± 0.59	1259 ± 312	1355 ± 274
3		3	1.39 ± 1.06	1309 ± 292	1000 ± 176
4		4	1.14 ± 0.49	1320 ± 227	1041 ± 205
5		1,2	0.99 ± 0.73	1341 ± 259	1002 ± 250
6		1,3	1.04 ± 0.60	1140 ± 101	998 ± 168
7		1,4	1.48 ± 0.94	1158 ± 109	1048 ± 361
8		2,3	0.92 ± 0.57	906 ± 350	968 ± 184
9		2,4	1.29 ± 0.54	1319 ± 131	725 ± 441
10		3,4	0.94 ± 0.58	1026 ± 103	1007 ± 141
11		1,2,3	1.01 ± 0.39	1209 ± 245	1133 ± 120
12		1,2,4	0.99 ± 0.57	1129 ± 163	946 ± 249
13		1,3,4	1.19 ± 0.49	1299 ± 253	973 ± 426
14		2,3,4	1.45 ± 0.53	1418 ± 188	689 ± 243
15		1,2,3,4	1.45 ± 0.46	1261 ± 257	818 ± 454
16	1200	1	1.45 ± 0.36	1167 ± 213	979 ± 139
17		2	1.16 ± 0.73	1198 ± 309	1098 ± 240
18		3	1.56 ± 1.06	1361 ± 222	865 ± 183
19		4	1.20 ± 0.33	1207 ± 473	1109 ± 385
20		1,2	1.15 ± 0.42	945 ± 368	644 ± 267
21		1,3	1.54 ± 0.59	1088 ± 476	865 ± 68
22		1,4	1.32 ± 1.25	1131 ± 151	865 ± 439
23		2,3	1.11 ± 0.57	1289 ± 338	1132 ± 151
24		2,4	1.48 ± 1.06	1245 ± 470	761 ± 239
25		3,4	1.58 ± 0.58	1037 ± 240	947 ± 254
26		1,2,3	1.63 ± 0.80	1093 ± 180	554 ± 130
27		1,2,4	0.90 ± 0.90	1056 ± 285	765 ± 433
28		1,3,4	1.27 ± 0.41	1064 ± 132	719 ± 222
29		2,3,4	1.38 ± 0.61	1331 ± 511	1093 ± 285
30		1,2,3,4	1.88 ± 1.43	989 ± 103	897 ± 392
31	AP	-	1.17 ± 0.51	1272 ± 298	838 ± 512
Significance		-	ns	ns	ns

NAA = naphthalene acetic acid; AP = assisted pollination; PS = phenological stages for application: 1 = PS 603; 2 = PS 607; 3 = PS 609; 4 = PS 703. Vitamin E, sum of tocotrienols δ, β/γ, α plus tocopherols δ, β/γ, α; carotenes, sum of α plus β-carotene. The values correspond to the average ± standard error of at least 15 bunches per treatment. Values with the same letter are not statistically different according to the least significant difference (LSD) test ($p < 0.05$), ns: not significant.

The vitamin E ranged between 945 ± 368 mg kg^{-1} when 1200 mg L^{-1} NAA was applied twice at PS 603 and PS 607 (treatment 20), and 1418 ± 188 mg kg^{-1} with 600 mg L^{-1} NAA applied three times at PS 607, PS 609, and PS 703 (treatment 14). The Vitamin E was 1272 ± 298 mg kg^{-1} in the assisted pollination treatment. The carotenes ranged from 644

± 267 mg kg^{-1} (treatment 20) and 1355 ± 274 mg kg^{-1} (treatment 2). For the AP treatment, the carotene content was 838 ± 512 mg kg^{-1} (Table 7).

3.3. Experiment 3: Effect of NAA Applications under Semi-Commercial Scale Conditions

Table 8 shows the effect of continuous applications of 1200 mg L^{-1} NAA on the inflorescences of 2 ha plots. After 12 months of using the technology, the fruit set in both treatments (NAA and AP) did not show statistical differences, with 93.2 ± 1.1% in the NAA treatment and 90.8 ± 1.8% in the AP. In the case of BN, the NAA treatment induced the formation of 30% more bunches than the AP (2208 ± 84 bunches versus 1690 ± 129 bunches, respectively). However, the NAA treatment bunches were, on average, 2.7 kg lighter than the AP bunches. As a result, the FFB did not show statistical differences between the two treatments.

Table 8. Bunch components in OxG hybrids (Coari x La Mé) treated with 1200 mg L^{-1} NAA for the induction of parthenocarpy at plantation level. The NAA solutions were applied three times staring at PS607 and reapplying at PS 609 and PS 703.

Treatment	Fruit Set (%) ns	BN *	BW (kg) *	FFB (t ha^{-1} $year^{-1}$) ns	O/B (%) **	Oil Yield (t ha^{-1} $year^{-1}$) **
NAA	93.2 ± 1.1	2208 ± 84	12.2 ± 0.4	26.9 ± 1.6	32.2 ± 0.7	8.7 ± 0.1
AP	90.8 ± 1.8	1690 ± 129	14.9 ± 0.6	25.2 ± 2.2	25.3 ± 0.8	6.4 ± 0.3

NAA = naphthalene acetic acid; AP = assisted pollination; BN = bunch number; BW = mean bunch weight; FFB = fresh fruit bunches; O/B = percentage of oil to bunch. The values correspond to the average ± standard error of all the bunches formed in 2 ha per replicate, four replicates per treatment for a total of 8 ha. The means were compared with the independent samples *t*-test. ns = not significant, * $p \leq 0.05$. ** $p \leq 0.01$.

As observed in experiments 1 and 2, the oil accumulation was positively impacted by the NAA treatment. The O/B in the NAA treatment was more than seven percentage points higher than in the AP (32.2 ± 0.7% versus 25.3 ± 0.8%), resulting in nearly 36% more oil yield in the NAA treatment compare to the AP, with an extra oil production of 2.3 t ha^{-1} $year^{-1}$ in the NAA treatment compare to assisted pollination.

4. Discussion

This research's primary purpose was to develop a system that could replace the assisted pollination, an essential task for obtaining fruit bunches at a commercial level in the different OxG cultivars planted worldwide [30], inducing the formation of parthenocarpic fruits. Our results show that exogenous NAA applications at specific phenological stages of the inflorescences result in oil-producing parthenocarpic fruits.

The induction of parthenocarpic fruits was achieved with NAA applications at different times before and after the anthesis. Bunches were formed when NAA was applied, regardless of the phenological stage at which the applications occurred. Thus, bunches were induced with NAA applications before anthesis (PS 603) and up to 14 days after anthesis (PS 703). On the contrary, the different models used to study parthenocarpy have shown a significant role of auxins in fruit set and parthenocarpy right before or a couple of days after anthesis. Then, the auxin concentration drops to allow the action of other hormones such as gibberellins [31]. For example, in tomato, auxin levels are low two days before anthesis and start increasing after anthesis, reaching the maximum value four days after anthesis (DAA) and dropping rapidly after that [32,33]. In African oil palm, applied auxins failed to induce oil-producing parthenocarpic fruits [21], even though parthenocarpy was achieved when the hormone was used very closed to anthesis [34]. Thus, the application moment is pivotal not only for inducing fruit formation but also for fruit growth and oil accumulation.

Oil palm fruits complete their development and maturation in approximately 160 days. They have a biphasic growth, with an initial increase in the mass and size between 30 days

and 60 days after pollination. Subsequently, there is a lag period of 40 days (60–100 DAA) where the total growth rate is reduced. Finally, the fruits show a new weight increase, particularly between 140 DAA and 160 DAA, accompanied by an increase in fruit size [35]. For this reason, the times selected for the NAA applications were the most adequate both in the phenological stages before anthesis to trigger fruit initiation and during the first 14 days after anthesis for cell division and expansion.

These results indicate a dual role for NAA. It triggers fruit initiation and then induces and stabilizes fruit cell growth preventing premature abscission and leading to more mesocarp biomass accumulation. Furthermore, NAA triggers a signal transduction cascade that releases fruit set and parthenocarpic development in the OxG hybrids during a more extended period than in the African oil palm, indicating a larger competence window in these hybrids for fruit induction than in other species. In this respect, the study of Yeap, et al. [36] showed that in terms of gene regulation during oil palm normal fruit production, there is a lag phase of mesocarp development in which auxin and gibberellin are at maximum concentration. According to their analysis, this phase could last up to 14 weeks after pollination. Potentially, the right stimulus when ovaries are still receptive could be translated in fruit set and parthenocarpic development during a longer competence window.

The induction of parthenocarpic fruits at different phenological stages contrasts with the time limitation of pollen application in the assisted pollination (AP). In AP, the pollen must be applied at anthesis (PS 607) when the flowers are receptive to pollen. When the pollen is used after anthesis, it does not trigger fruit set and development, leading to undesirable results that range from inflorescence abortion to bunches with low filling and poorly developed fruits. Therefore, using NAA, the application cycles to induce fruit formation could be longer because the inflorescence presents a favorable response to forming the fruits that are not circumscribed to the PS 607. Thus, the hormone application to induce the parthenocarpy can be made once a week compared to three times a week that AP is performed. As a result, labor could be optimized, lowering production costs and reducing losses caused by non-formed bunches typical of AP not performed at PS 607.

Plant hormones are usually produced in the cells at low concentrations, below the concentration of other compounds such as nutrients and vitamins [37]. Depending on their concentration, plant hormones stimulate or inhibit the same response [11]. Our results show that bunch formation is related to the applied NAA concentration in the different phenological stages. Thus, the success in bunch formation was higher with 600 mg L^{-1} NAA and 1200 mg L^{-1} NAA, while the lowest bunch formation was reached with 50 mg L^{-1} NAA. The 1200 mg L^{-1} NAA treatments consistently showed the most outstanding results in the bunch formation, bunch components, and oil yield.

The induction of parthenocarpic fruits with plant hormones usually leads to abnormal organ development and smaller and malformed fruits [31]. In addition, auxin or gibberellin applications for inducing fruit formation trigger premature abscission with the consequent low fruit set percentages [18]. In the OxG hybrids, 1200 mg L^{-1} NAA applied three times to the inflorescences did not show those typical drawbacks but resulted in a high fruit set (96% to 99%), with fruits that were 30% heavier than those produced with assisted pollination.

A consequence of the larger NAA-induced parthenocarpic fruits was the increased oil yield. Because there was more mesocarp per fruit, the overall oil production was higher even though the oil in the dry mesocarp did not change. Thus, the multiple NAA applications were fundamental in obtaining high oil to bunch ratios. In this respect, the low oil yield obtained in auxin-induced fruits in *E. guineensis* [21] could have resulted from not using the appropriate auxin or not applying it at the right phenological stages. Furthermore, the repeated applications were not performed in those early experiments, and the high fruit set and larger fruits were not obtained. Our results open a door for revisiting the possibility of inducing parthenocarpic fruits in *E. guineensis*. It is plausible that the results could be replicated in the African oil palm by adjusting doses and application times. This technology is appealing because the *E. guineensis* breeding programs are increasingly producing highly feminine cultivars in which natural pollination is restricted due to the lack

of male pollen-producing inflorescences. Moreover, the populations of natural pollinators have been declining with substantial consequences on bunch formation, bunch abortion, and overall oil yield [38].

It is plausible that the drawbacks attributed to the induction of fruits with hormones [18] result from not using the right concentration at the appropriate phenological stages, opening a door for further investigations to better define doses, frequencies, and application time. The result could be the commercial production of parthenocarpic fruits of different species.

Our results show that NAA could be used under commercial conditions. The hormone treatments applied for a year resulted in larger fruits, more bunches per hectare of better conformation, and a higher fruit set than assisted pollination. Under the semi-commercial conditions of experiment 3, the NAA bunches produced up to 36% more oil than the AP, with oil to bunch beyond 32%. As a result, more than two additional tons of oil could be obtained per hectare per year. However, some productivity parameters were lower at the semi-commercial conditions than in experiments 1 and 2. For example, in experiment 2, the reduction in average bunch weight (BW) was minimal with 1200 mg L^{-1} NAA applied three times compared to assisted pollination. The bunches at the semi-commercial conditions of experiment 3 were, on average, 2.7 kg lighter. In experiments 1 and 2, fruit sets as high as 99% were measured, while at semi-commercial conditions, the fruit set was close to 93%. Thus, despite the high oil to bunch obtained and high bunch number, the 36% increment in oil yield at the semi-commercial conditions of experiment 3 could be further improved with a more careful NAA application.

One of the OxG hybrids' main characteristics is their oil quality in terms of high oleic acid percentage and a considerable content of phytonutrients such as vitamin E and carotenes [39]. Our results show that the parthenocarpic fruits induced by NAA applications yield oil of the same quality as when assisted pollination is used. An oil that is considered high oleic (more than 55% of oleic acid) and rich in phytonutrients, with low levels of FFA.

Commercial use of hybrids is limited by low filling and ripening, the need for costly assisted pollination, and low oil yields [4]. By applying NAA, those limitations are overcome, leaving the oil palm industry with a crop resistant to diseases such as bud rot, which has a longer commercial life span due to its low growth rate and that produces a very high-quality oil full of antioxidants and phytonutrients.

The OxG hybrids are highly productive. Commercial plantations can produce more than 40 t ha^{-1} $year^{-1}$ FFB, which, together with the high oil yield obtained using NAA (approximately 27% oil extraction rate or more), results in more than 10 t ha^{-1} $year^{-1}$ of high oleic palm oil. If this technology is implemented in the oil palm planted area worldwide, oil palm production would be closer to 250 million tons per year, satisfying the world's fats and oil demands without using additional arable land.

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Abbreviations

NAA	1-Naphthalene acetic acid
HOPO	High oleic palm oil
OxG	Oil palm interspecific hybrids between *Elaeis oleifera* × *Elaeis guineensis*
PS	Phenological stages
AP	Assisted pollination.
BW	Bunch weight (kg)
BN	Bunch number
FFB	Fresh Fruit Bunches.
O/B	Oil-to-bunch (%)
OC	Oil content per bunch (kg of oil/bunch)
O/D	Oil-to-dry mesocarp (%)
AFWpf	Average fruit weight of parthenocarpic fruits (g)
DAA	Days after anthesis
SFA	Saturated fatty acids
UFA	Unsaturated fatty acids
FAA	Free fatty acids

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