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Controlled pollination for interspecific hybrid American Oil Palm vs Oil Palm seed production

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ABSTRACT: The interspecific hybrid American Oil Palm vs Oil Palm has superior characteristics that can be exploited commercially. This work aimed to evaluate the effect of the amount of pollen, reapplication and climatic periods in the production of hybrid seeds of these species. The variables considered in this experiment were climatic season (dry and wet), pollen dosage (15, 25, 40 and 62.5 mg) and repeatability of pollen application (one and two repetitions) in a factorial design with four replications of each combination. There was a significant difference in the amount of seed produced in climatic seasons as an effect of the amount of pollen applied and reapplication. In the rainy season, it is recommended the use of 15 mg of pollen without repetition and in the dry season, 40 mg of pollen with repetition.

Keywords: *Elaeis guineensis*, *Elaeis oleifera*, palm trees, pollen.

Polinização controlada para produção de sementes híbridas interespecíficas de caiaué vs dendê

RESUMO: O híbrido interespecífico caiaué vs dendê possui características superiores as quais podem ser exploradas comercialmente. Assim, este trabalho teve como objetivo avaliar o efeito da quantidade de pólen, repetição de aplicação e períodos climáticos na produção de sementes híbridas entre essas espécies. Foram considerados neste experimento as variáveis período climático (seco e chuvoso), dosagem de pólen (15, 25, 40 e 62,5 mg) e repetibilidade da aplicação de pólen (uma e duas) em esquema fatorial com quatro repetições cada combinação. Observou-se diferença significativa na quantidade de sementes produzidas nos períodos climáticos efeito da quantidade de pólen aplicada e repetição da aplicação. Na estação chuvosa recomenda-se a aplicação de 15 mg de pólen sem repetição e na estação seca 40 mg de pólen com repetição da aplicação.

Palavras-chave: *Elaeis guineensis*, *Elaeis oleifera*, palmeiras, pólen.

1. INTRODUCTION

Palm oil known as Oil Palm in Brazil (*Elaeis guineensis*) stands out among the oleaginous species for its high capacity of oil production per unit area (RIOS et al., 2012), what makes it the world's largest producer of palm oil, with 62.8 million tonnes (United States Department of Agriculture - USDA, 2014).

Currently, phytosanitary factors threaten the expansion of Oil Palm cultivation in Brazil. The disease known as Lethal Yellowing (LY) of Oil Palm is a major problem for agricultural development of Latin American countries that grow this crop, particularly Brazil. This disease has caused heavy losses since 1984 and has become spread in the country since the first report (RAMALHO FILHO et al., 2010). In the state of Pará, which is the largest Oil Palm producer in the country, thousands of Oil Palm trees have been eradicated because of this problem (BOARI, 2008).

The interspecific hybrid (ISH OxG) of Oil Palm with the American species, American Oil Palm (*E. oleifera*) has emerged as a viable alternative to give continuity to Oil Palm culture in areas affected by the LY because the ISH OxG shows to be resistant to this disease (LOPES et al., 2012). Thus, hybrid seeds are obtained through controlled crossing of American Oil Palm, female parent, with Oil Palm, as male parent (CUNHA & LOPES, 2010).

Controlled pollination applied to produce ISH OxG seeds is an adaptation of the procedure used for production Oil Palm seeds (CUNHA et al., 2007). Studies are needed to better understand the process of interspecific hybridization, as for example, the amount of pollen, frequency of application and best time for applying pollen. According to Cunha et al. (2007), 62.5 mg of pollen should be used for each female inflorescence, but the authors do not describe how many times this application should be performed. Tinker & Corley (2015), in the other hand, do not recommend an optimal amount of pollen, but report the importance of repeating the application due to the irregularity of the sensitive period of female flowers, either in the case of Oil Palm or American Oil Palm trees. These authors also make no considerations with regard to climatic conditions under which controlled pollination should be carried out and if these affect the results of the procedure.

Given the above, this study aimed to optimize the production of ISH OxG seeds by assessing the effect of controlled pollination under different weather conditions and quantities of pollen.

2. MATERIAL AND METHODS

The study was conducted at the Experimental Field Urubu River (CERU), Embrapa Western Amazon, in Rio Preto da Eva municipality, AM (02° 35'S, 59° 28'W, 200 m asl). The temperature, humidity and precipitation for the period of application of treatments are presented in Table 1. Female parent lines of American Oil Palm from Manicoré and Oil Palm palm from La mé, were used in the production of hybrid seeds.

Treatments consisted in combinations of the amount of pollen (four dosages: 15, 25, 40 and 62.5 mg), frequency of pollen application (one controlled application of pollen and two controlled applications with 24-hour intervals between them) and climatic season at the moment of application (dry and rainy season). There were four replicates for each combination of factors, and each inflorescence artificially pollinated was considered a sample unit.

Pollen used in the experiment was selected from Oil Palm matrix area of psífera type. After harvesting the male inflorescence, pollen was prepared, purified and vacuum packed in Castellani glass. Before application, pollen was analyzed in the lab in order to determine the viability, moisture and vacuo of the vials which was 82.0%, 7.23% and 4 ml of ejection of the plunger of a syringe, respectively, following the methodology proposed by Cunha et al. (2007).

The methodology proposed by Donough et al (1993) was used to carry out pollinations. 1993). The leaves near the female inflorescence were cut, the sheaths were removed and the female inflorescence was covered with canvas bag (# 8) before anthesis to avoid contamination with pollen from other plants. To control the pollination process, daily inspections of inflorescences were carried out from the tenth day to anthesis, when they received the controlled pollination. Pollination consisted of pollen plus four grams of inert material (neutral talc), which acts as an excipient also favoring pollen dispersal preview in the bagged inflorescence (CUNHA et al., 2007).

Collection of clusters was performed approximately 150 days after the application of pollen, when they were in the period of maturation (three to five fruits naturally dispersed). After harvesting bunches and separating normal and partencarpic fruits, seeds were counted.

A completely randomized 2 × 4 × 2 factorial design consisting of two applications of pollen, four dosages and two climatic periods, was used in the study. The experimental model corresponds to the equation: $Y = x + Di + Aj + Cz + DAij + DCiz + ACjz + DACijz + Eijz$, where: Y, Number of normal fruits; x, mean; Di, Effect of pollen dosage; Aj, Effect of number of applications; Cz, Effect of climatic season; DAij, DCiz, ACjz and DACijz, Effects due to interactions and Eijz, experimental error. Data were converted into $(x+0,5)^{0,5}$ and subjected to analysis of variance, observing the assumptions of parametric tests, and the means were compared by Tukey test ($p < 0.05$) using the program Sisvar 5.3 (FERREIRA, 2008).

Table 1. Temperature (mean, maximum and minimum), humidity and precipitation observed in the months in which the experiment of artificial pollination was carried out, Experimental Field Urubu River, Rio Preto da Eva - AM.
Tabela 1. Temperatura (média, máxima e mínima), umidade e precipitação observadas nos meses em que foi realizada a aplicação das polinizações artificiais do experimento, no Campo Experimental do Rio Urubu, Rio Preto da Eva - AM.

Month/ Year	Temperature (°C)			Humidity (%)	Rain (mm)
	Mean	Maximum	Minimum		
06/2012	25.6	26.1	24.8	86.9	120.8
07/2012	26.0	26.5	24.4	89.0	195.8
08/2012	26.3	26.6	25.0	84.5	64.4
09/2012	26.0	26.7	25.4	85.1	140.0
04/2013	25.5	26.2	25.1	90.1	213.2
05/2013	25.3	25.9	24.9	91.4	295.2
06/2013	25.9	25.6	25.3	87.4	120.0

3. RESULTS AND DISCUSSION

Seed production under application of different amounts of pollen ranged from 124 to 1299 fruits. When considering all applications, we observed a gradual increase in pollinated fruits where the largest amount of seeds produced occurred with application of 40 mg of pollen per inflorescence, decreasing in pollen amounts above this value (Figure 1). Similar results were observed in part by Harun & Noor (2002), with increasing fruiting in *E. guineensis* when applying from 0.01 mg to 10 mg of pollen. For values beyond this amount, no statistical differences were found, even testing large pollen concentrations (5000 mg).

This result can be explained, according to Tandon et al. (2001), be the fact that the Oil Palm female inflorescence has approximately an average of 900 flowers and this sets a limit to the fruitting on clusters, regardless the increase of pollen availability.

It can also be inferred that fruitting of bunches varied according to climatic conditions. For the rainy season, an average of 671 seeds per bunch were obtained, while the average seed number per bunch in the dry season was 523, a statistically significant difference (Table 2).

This difference between climatic periods could justify the use of controlled pollinations with larger amounts of pollen, corroborating in part Cunha et al. (2007) who recommend the use of 62.5 mg of pollen, regardless of weather conditions. Using the proper amount of pollen ensures greater amount of seeds

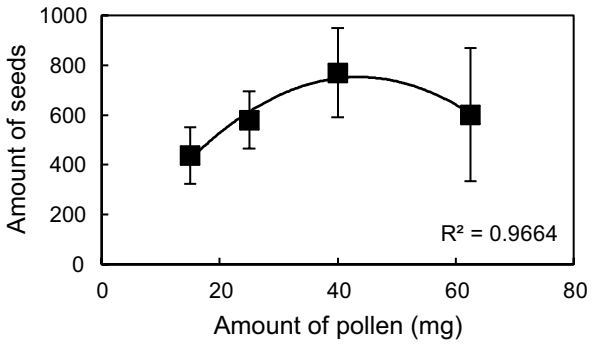


Figure 1. Amount of seeds in function of the applied amount of pollen (mg). Each point represents the mean of 16 observations. Vertical lines indicate the standard deviation.
Figura 1. Quantidade de sementes em função da quantidade de pólen aplicada (mg). Cada ponto representa a média de 16 observações. As linhas verticais indicam o desvio padrão.

Table 2. Summary of the analysis of variance for Climatic Season, Pollen count, frequency of application and interactions between these variables.
Tabela 2. Resumo da análise de variância para Período Climático, Quantidade de pólen, frequência de aplicação e para as interações entre estas variáveis.

Source of variation	DF	MS	F
Climate period (F1)	1	105.811838	5.869*
Amount (F2)	3	135.407997	7.511**
Frequency application (F3)	1	113.022690	6.269*
Interaction F1xF2	3	15.344089	0.851 NS
Interaction F1xF3	1	164.577265	9.129**
Interaction F2xF3	3	16.839849	0.713 NS
Interaction F1XF2XF3	3	39.604512	2.197 NS
Error	48	18.027580	

ns - not significant ($p \geq 0.05$); ** significant at 1% probability according to F test; * significant at 5% probability according to F test; DF - Degrees of freedom; MS - Medium Square.

per bunch. According to Harun & Noor (2002), low quantity of pollen can cause poor fruit set and reduced production of seeds per bunch.

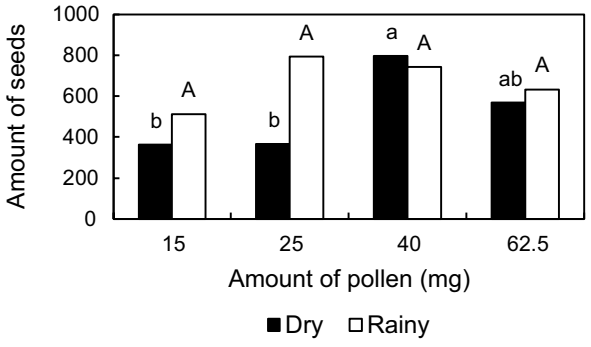
As for the fruitting of bunches (Figure 2), there was statistically significant difference in the amount of seeds in the dry season only. In this season, at least 40 mg of pollen needs to applied per each inflorescence; for values above this amount, there is no significant difference in the amount of formed seeds, and this amount has statistically different outcomes when contrasted with other quantities. In turn, during the rainy season, the application of 15 mg of pollen is sufficient. It should be noted that, in Brazilian Amazon conditions, the dry season is characterized by increased temperature due mainly to lower rainfall, which can directly influence the formation of seeds.

Melo et al. (2002), who worked with *atemóia (Annona squamosa* x *A*. cherimola), observed that pollination is not favored when performed in periods of high temperatures, so that there is a reduction in the percentage of valid fruits. This may also take place in the formation of ISH OxG seeds, which would explain the statistically significant outcome of different amounts of pollen applied in the dry season. As female inflorescences at the time of pollen application were isolated inside canvas bags and the anthesis of flowers occurs gradually over three days, Tandon et al. (2001) state that,in this environment, a microclimate with unfavorable temperatures to pollen grains can develop before full opening of female flowers and this may lead these grains to lose viability and that is why there is the need to increase the amount of pollen applied.

As for interactions, there was statistically significant interaction between climatic season vs frequency of application. In general, with respect to repetition (frequency) of pollen application, it was observed that two pollen applications in female inflorescences were needed only during the dry season, as a significant difference of 202 seeds was observed when comparing to other pollen application frequencies (Figure 3). In turn, there was no statistical difference in the amount of seeds produced during the rainy season.

Corley & Tinker (2015) justify the reapplication of artificial pollination in the genus *Elaeis* sp., since the Oil Palm female flowers present irregular sensitive period, which ranges from 36 to 48 hours. In turn, American Oil Palm the anthesis is even more irregular and may last up to four weeks. Tandon et al. (2001) further complement this recommendation, noting that from 150 to 250 female flowers come in anthesis per day. As the pollen grain only loses its total viability after seven days in ambient temperatures (TANDON et al., 2007), there is no need to repeat the application during the rainy season because there are no unfavorable conditions for viability of pollen. Nevertheless, in dry periods, high temperatures can be lethal to pollen grains before they fertilize the female flowers. Thus, a positive effect of repetition is observed in this season.

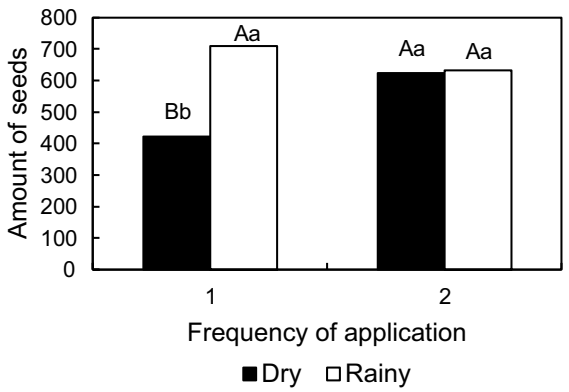
Therefore, these results reinforce that the management of pollen quantity and frequency of application in controlled pollination should be different in the in the rainy and dry seasons; the latter requires larger amounts of pollen. Harun & Noor (2002) observed a better fruit set in open pollinated bunches due to the constant visits of pollinating insects when compared to bunches pollinated artificially and these authors linked this difference to the absence of repetition in the controlled application.



CV (%) - 17.87. Columns followed by the same capital letters for the rainy season and the same lowercase letters for the dry season do not differ statistically according to Tukey test ($P < 0.05$).

Figure 2. Amount of seeds produced for each amount of pollen applied (mg) in the female inflorescence of caiaué matrices for the production of ISH OxG seeds in two different climatic conditions.

Figura 2. Quantidade de sementes produzidas referentes a cada quantidade de pólen aplicada (mg) na inflorescência feminina de matrizes de caiaué para produção de sementes HIE OxG, em duas condições climáticas diferentes.



CV (%) - 17.87. Columns followed by the same capital letters (unfolding of climatic season within each level of frequency of application) and lowercase letters (unfolding of each level of frequency of application within climatic season) did not differ statistically according to Tukey test ($P < 0.05$).

Figure 3. Amount of seeds produced by the number of applications in different climatic conditions.

Figura 3. Quantidade de sementes produzidas ao considerar a quantidade de aplicações em diferentes condições climáticas.

4. CONCLUSIONS

The application of 15 and 40 mg of pollen in the rainy and dry season, respectively, is recommended for the production of interspecific hybrid seeds of American Oil Palm vs Oil Palm in the Amazon. Furthermore, in order to ensure a greater fruit set of bunches, pollen reapplication in the dry season is recommended.

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