The OxG Amazon hybrid: a new alternative for areas affected by bud rot disorder

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Abstract

The etiology of bud rot disorders in oil palm is complex and tolerance to these conditions has been considered a major breeding objective in this crop in tropical America. During the past 30 years, the use of interspecific hybrids, *E. oleifera* x *Elaeis guineensis* (OxG), appeared to be an alternative for achieving this purpose. Although there is field evidence that seems to confirm that OxG hybrids are indeed bud rot tolerant, no commercial varieties have been consolidated as alternatives to *guineensis* materials, mainly because of their rather low oil to bunch ratios, vigorous growth and deficient natural pollination.

Breeding for bud rot tolerance involves the use of two sources of genetic variation: the *oleifera* population as mother-palms and the *guineensis* population as pollen-pisifera sources. ASD Costa Rica[2] assembled a wide collection of *E. oleifera*, comprising accessions from all Central and South American countries where this specie is naturally distributed. Since 1970, a series of OxG hybrid combinations were evaluated in Costa Rica, and a particular hybrid stood out due to its high FFB production (>300 kg/palm/year), reduced trunk growth and short leaves (*guineensis*-like), allowing planting at the standard density of 143 palms/ha. It also has many fewer inflorescence-bunch cover-sheaths in comparison with other hybrids, and pollen auto-compatibility. This unique combination was named Amazon; its genetic background involves mother-palms from Manaus, Brazil, crossed to compact composite pisiferas and also carrying *oleifera* genes (6-12%). This combination makes the Amazon hybrid peculiar, because it carries 53 to 56% *oleifera* genes in its genome, compared with other OxG hybrids that carry only 50%.

Amazon seeds were released in 2009 for commercial plantations in Tumaco (Colombia), Brazil, Peru, Ecuador and Nicaragua. Despite the rather low oil/bunch ratio (18%) in the first Amazon planted in 1993, early field results showed that the new Amazon hybrid has high bud rot tolerance (<1% of cases) and a low proportion of palms (<10%) with a short androgynous cycle. As expected, leaves of this hybrid are shorter than for other OxG of the same age: 364 cm vs. 417 cm (31 months). Early yields are very promising (9.8 to 12.3 t/ha/year from 30 to 42 months of age) and there are also good expectations for increasing the oil to bunch content in the second generation, as seen in the first commercial data.

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Introduction

The bud rot complex has limited oil palm cultivation in several regions of tropical America, as indicated by several authors (Le Guen et al., 1991; Franquevillle, 2001; Chinchilla, 2010). In addition to improving agronomic practices in an attempt to prevent or at least attenuate the effects of bud rot, the use of OxG hybrids has been considered a basic alternative for addressing this disorder. However, Barba et al. (2010a) reported that resistance to bud rot in OxG hybrids is not total, since planting materials originating from *oleifera* mother-palms from the Amazon region and from Central America showed variable incidence after 5-10 years in the field, ranging from 0 to 81%.

Disease resistance, particularly to bud rot, has been considered a major objective in oil palm breeding in tropical America. During the past 30 years the use of interspecific hybrids, *E. oleifera* x *E. guineensis* (OxG), appeared to be a basic alternative for seeking this resistance. In spite of this, no commercial varieties have been consolidated as alternatives to *guineensis* materials, mainly because of their rather low oil to bunch ratios, vigorous growth and deficient natural pollination.

This paper summarizes the characteristics of a peculiar OxG combination selected from a group of crosses between *oleifera* mother-palms native to Manaus, Brazil, mated with composite compact pisiferas, which also carry *oleifera* genes. This combination was named Amazon and it has favorable characteristics compared to other OxG hybrids, in terms of its shorter leaves (*guineensis*-like), fewer inflorescence-bunch cover-sheaths, pollen auto-compatibility and good bunch development under natural pollination.

The use of OxG hybrids in tropical America

In some regions of Colombia and Ecuador, where commercial exploitation of the traditional African oil palm (*Elaeis guineensis*) is restricted by the presence of bud rot, a gradual expansion of OxG hybrid plantings has occurred. To date, a total of approximately 33,000 ha have been established: 12,000 ha in Colombia and 21,000 ha in Ecuador. Regarding the area planted with the Amazon hybrid, nearly 2,700 ha and 600 ha were planted in each country respectively.

Most plantations with OxG hybrids in South America were developed using OxG planting materials originating from Brazilian *oleiferas*; namely Coarí (the most used), Manicoré, Tefé and Manaus. The difference among them is the source of pisifera; Coarí and Manicoré: La Mé, and Manaus: composite compact. The majority of plantings with OxG hybrids were established at 116 palms/ha (10 x 10 m), while the Amazon hybrid was planted at 128 and 143 palms/ha (9.5 x 9.5 m, and 9 x 9 m respectively). Recently, new hybrids using *oleifera* from the Ecuadorian region of Taisha and also from other lesser known origins are coming into the picture.

Early *E. oleifera* germplasm evaluation

During the period of 1967-70, a large *oleifera* collection with 326 accessions was established in Costa Rica, with samples from most of the wild groves of Costa Rica, Panama and Colombia. The main information concerning this collection was the fact that the *oleiferas* had low oil to bunch (O/B) content, ranging from 4.3 to 7.5% (Escobar, 1980). Mohd Din et al. (2000) also reported the performance of *E. oleifera* collected in these countries, and they recorded O/B values similar to the ones found in the ASD collection.

The manipulation of *oleifera* germplasm at ASD began in 1978 with the layout of a progeny-test consisting of 236 OxG combinations, involving 105 *oleifera* palms representing 13 regions of Central America and Colombia, and 6 different *guineensis* pollen-sources (AVROS, Ekona, Ulu Remis, Yangambi, WAIFOR and composite Deli-Yangambi). The results of this work showed variation in fresh fruit bunch (FFB) production of 46-216 kg/palm/year. The oil to bunch content

varied from 13-21%, and growth was more vigorous in the OxG hybrids, with leaves 7.7-8.5 m long vs. 6.9 m for the tester Deli x AVROS at 9 years of age (Sterling et al., 1999).

In Ecuador, Barba et al. (2010b) studied OxG hybrids with different *oleifera* genetic backgrounds. The results showed that the Brazilian Manicoré induced the highest O/B content of 23.4%, while the highest FFB production of 30.6 t/ha/year was obtained from hybrids with Armuelles (Panama) *oleifera* origin. The highest O/B contents were obtained when the AVROS pollen-source was used. The same authors recommended planting OxG hybrids not exceeding the density of 128 palms/ha, due to the vigorous growth showed by the evaluated progenies.

A group of 36 OxG hybrids[3] (869 palms) were planted by ASD in the North Atlantic of Panama in 1993, to evaluate bud rot tolerance. The hybrids showed only 1.4% cases compared with the tester Deli x AVROS with 17.7% of palms affected. Based on this preliminary experience, two Brazilian *oleiferas* of Manaus origin, which showed the highest tolerance[4] to bud rot, were crossed to compact pisiferas of the first backcross generation (BC_1). This gave rise to a particular hybrid with shorter leaves and high FFB production, as described in the following paragraphs.

Characteristics of the first generation of the Amazon hybrid

Background

The oil palm breeding program of ASD Costa Rica focused on *guineensis* varieties, so the improvement of the interspecific OxG hybrid was not a priority, since these types of palms are less attractive from a commercial point of view. Typically, OxG hybrids have excessive vegetative development, require assisted pollination and have lower oil productivity per hectare. Nevertheless, they produce unsaturated oil of better quality than *guineensis* and in general better tolerate the different variations of the disorder known as bud rot.

Responding to the increasing demand for improved seeds of the OxG hybrid for the zones affected by bud rot, ASD decided to launch a new OxG hybrid named Amazon, which has a larger proportion of *Elaeis oleifera* genes than the traditional OxG hybrids. It is accepted that the tolerance of the OxG hybrid to bud rot is probably the result of the action of inherited genes of *Elaeis oleifera*; consequently, the new OxG Amazon hybrid promises to have good tolerance due to a greater concentration of *oleifera* genes in its genome (more than 50%). This point will be emphasized in the following paragraphs.

Origin of first Amazon generation

The compact trait or "compact gene" (trunk and short leaves), was fixed after three backcrossing cycles originated from a palm with exceptional characteristics of short leaves and slow vertical growth, known as the original compact palm (OCP). Unfortunately, the OCP had a poor bunch composition and low oil productivity. For the fixation of the compact trait, more than 35 years of field tests were needed (Sterling et al., 1987; Escobar and Alvarado, 2004; Alvarado et al., 2006).

The OCP proportionally had 25% of *oleifera* genes in its genome. To increase the oil productivity potential, diverse and improved *guineensis* populations were used. This process generated different recombinant populations mixing genes of *oleifera* and *guineensis* in several proportions, as described by Escobar and Alvarado 2004 (Table 1). The Amazon OxG first generation hybrid, which is characterized in this paper, has 56.25% of *oleifera* genes: 50% from the mother palm and 6.25% from the compact male of the first backcross cycle BC_1 (Table 1).

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[3]Oleiferas origins used: Surinam, Manaus-Brazil, Penonome-Panama, composite Quepos/Palmar Costa Rica, composite Palmar/unknown pollen, and Palmar; crossed to guineensis AVROS, Ghana, La Me and Ekona pisiferas

[4]One showed no bud-rot symptoms and the other had only 8% of palms affected.

The rest of the genes (43.75%) are of *guineensis* origin, from various genetic advanced populations coming from the compact population.

Table 1. Concentration (theoretical proportion) of *E.oleifera* genes in the different compact populations

Population	*E. oleifera* genes
OxG hybrid (open pollination)	**50%**
Original Compact palm (OCP)[5]	**25%**
Backcross First Cycle (BC_1)	**12.5%**
Sub population: BC_1F_1	12.5%
Sub population: BC_1F_2	12.5%
Sub population: BC_1F_1 x E. *guineensis*	6.25%
Backcross Second Cycle (BC_2)	**6.25%**
Sub population: BC_2F_1	6.25%
Backcross Third Cycle (BC_3)	**3.125%**

Growth and yield characteristics

For the characterization of the first generation Amazon OxG hybrids, 42 palms were evaluated in an observation plot planted in 1993 in Coto, Costa Rica. As a reference, this information was compared with the average data of the *guineensis* variety Deli x AVROS (Alvarado et al., 2010).

Amazon hybrids have leaves with lengths similar to or shorter than the conventional *guineensis* varieties; this characteristic allows them to be planted at the standard density of 143 palms/ha (9 x 9 m triangular layout). Indeed, in the conditions at Coto[6], Costa Rica, their leaf length (LL = petiole + rachis) at 14 years after planting was 697 cm on average, similar to the *guineensis* variety Deli x AVROS of the same age (Table 2). This characteristic is an advantage compared to the traditional OxG hybrids, whose leaves are longer than 9 m. Another relevant vegetative characteristic is that the petioles of Amazon hybrids are thinner than the conventional *guineensis* variety. The petiole cross section was smaller: 22.0 cm^2 versus 26.7 cm^2, thereby facilitating harvest and pruning work (Table 2).

Table 2. Vegetative characteristics of first generation Amazon OxG hybrids

Variety	LL (cm)	PxS (cm^2)	TH (m)
Amazon OxG hybrid	697	22.0	2.6
Deli x AVROS	704	26.7	6.9

LL: leaf length; PxS: petiole cross section; TH: trunk height

It is well known that potential fresh fruit bunch (FFB) production of OxG hybrids is often similar or superior to that of *guineensis* varieties (Le Guen et al., 1991; Sterling et al., 1999 and references therein). The first generation Amazon OxG hybrid showed FFB productivity of 227.5 kg/palm/year (32.5 t/ha/year, adult phase) without assisted pollination; it was highly superior to that of the *guineensis* variety Deli x AVROS that yielded only 174.8 kg/palm/year of FFB (24.9 t/ha/year) (Table 3). In spite of the comparative advantage of greater production of the OxG

[5]Originated from open pollinated seeds.

[6] This location has agro-climatic conditions favoring vigorous growth, due to its more than 4,000 mm/year rainfall and high fertility alluvial soils.

hybrid, its oil extraction was lower than the *guineensis* variety. The Amazon OxG hybrid showed an oil to bunch content (O/B) of 18.5% in the laboratory. This corresponds to an expected mill extraction of around 16.1%, quite inferior to a *guineensis* variety, with an industrial extraction of 24.3% (Table 3). In terms of potential oil production per hectare, the hybrid has the possibility of producing 5.2 tons of oil/ha/year; versus 6.2 t/oil/ha/year for the *guineensis* variety (Table 3).

Table 3. Bunch composition and potential FFB productivity per hectare for first generation Amazon OxG hybrids, Coto, Costa Rica (average of 42 palms)

Variety	FFB	BW	BN	FF/B	PF/B	F/B	M/F	O/MF	OER	O/ha
Amazon OxG	227.5	18.5	12.3	42.2	15.5	57.7	67.2	43.0	16.1	5.2
Deli x AVROS	174.8	21.3	8.2	68.2	2.5	70.7	87.9	47.8	24.3	6.2

FFB: fresh fruit bunch in kg/palm/year (adult phase); BW: average bunch weight kg; BN: bunch number/palm/year; FF/B: fertile fruits to bunch %; PF/B: parthenocarpic fruits to bunch %; F/B: fruits to bunch %; M/F: mesocarp to fruit %; O/MF: oil to mesocarp fertile fruits %; OER: oil to bunch x 0,855 (estimated industrial extraction rate); O/ha: oil to hectare (calculated using the estimated OER)

Coverage of the female inflorescences and pollen compatibility

One important aspect in OxG hybrids is the degree of coverage by sheaths of the female inflorescence while in anthesis, which restrains natural pollination. The majority of *oleifera* populations used in OxG seed production have excessive bunch cover-sheaths, except for the Taisha population from Ecuador. However, in the hybrids there is evidence of a paternal effect on the degree of bunch sheath coverage. In the case of the Amazon hybrid, where compact pisiferas are used, the coverage of the bunch is around 50%; thus natural pollination is facilitated.

Preliminary pollen compatibility evaluation of mature Amazon palms (15-17 years old) using controlled pollination showed that a pollen mix of their own (from several individuals) used to pollinate 16 inflorescences gave a lower proportion of fertile fruits in the bunch, as compared with pollen *guineensis*, used in 18 inflorescences: 31% vs. 49% respectively. Although the inflorescences pollinated with the pollen mix developed normal bunches, and the parthenocarpic fruits proportion was higher (16% vs. 7%), total oil content was lower: 11.8% vs. 15.3%. On the other hand, naturally pollinated Amazon inflorescences had 17.9% oil content in the bunch (Table 4).

Table 4. Bunch analysis results in a preliminary pollen compatibility evaluation of a sample of 15-17 years old Amazon palms, Coto, Costa Rica

Pollen Source	BA	BWt	FF	PF	F/B	M/F	O/MF	O/MP	O/B
Amazon mix	16	17.4	31.0	16.0	41.2	65.2	39.2	37.7	11.8
E. guineensis mix	18	23.1	49.0	7.4	55.6	63.3	41.7	34.7	15.3
Open pollinated	21	19.8	41.0	15.8	56.0	67.0	41.8	38.9	17.9

BA: bunch analysis number; BWt: bunch weight kg; FF: fertile fruits %; PF: parthenocarpic fruits %; F/B: fruits to bunch % (fertile + parthenocarpic); M/F: mesocarp to fruit %; O/MF: oil to mesocarp fertile fruits %; O/MP: oil to mesocarp parthenocarpic fruits %; O/B: oil to bunch % (fertile + parthenocarpic fruits)

Apparently, Amazon pollen is not totally auto-compatible, and its quantity per inflorescence is much lower than for *guineensis* (3-10 g vs. 15-30 g), thus, a more abundant pollen source is required, naturally or by assisted pollination, especially considering that the average viability of pollen is only 15 to 20%.

Based on the results above, a more systematic test was laid out to evaluate the effect of assisted pollination on the composition of Amazon bunches. Two six-palm groups of Amazon were randomly selected, one with young palms (2 years, young new Amazon hybrid) and another with old palms (18 years). Later, four inflorescences of each palm were also randomly chosen: two were isolated for assisted pollination (pollen applied once), and two were left for natural (open) pollination. The pollen for assisted pollination was collected from tenera palms of the Deli x AVROS variety, to mimic the standard practice in many hybrid plantations in tropical America. The results showed no differences in bunch composition between assisted and natural pollination in Amazon palms that were 2 and 18 years old (t_{005}), except in the oil to bunch ratios of the older palms (Table 5). Differences in oil contents were probably due to the higher fruit to bunch ratios in the natural pollinated bunches. These results also denote that Amazon inflorescence sheaths do not restrict natural pollination.

Table 5. Composition of assisted and naturally pollinated Amazon bunches of 2 and 18 years of age, Coto, Costa Rica

Age	Pollination	BWt	FF	PF	F/B	M/F	O/MF	O/B
2 yrs.	Assisted	3.0	27.3	3.6	30.9	76.6	33.3	6.3
	Natural	3.4	32.2	4.3	36.5	75.4	36.6	9.2
		ns	ns	ns	ns	ns	ns	ns
18 yrs.	Assisted	24.9	35.3	10.8	45.6	67.2	42.8	14.2
	Natural	23.9	32.3	17.4	49.7	65.5	42.8	15.7
		ns	ns	ns	ns	ns	ns	**

BWt: bunch weight kg; FF: fertile fruits %; PF: parthenocarpic fruits %; F/B: fruits to bunch % (fertile + parthenocarpic); M/F: mesocarp to fruit %; O/MF: oil to mesocarp fertile fruits %; O/B: oil to bunch % (fertile + parthenocarpic fruits)

Amazon Second Generation

In breeding OxG hybrids, the contribution of *oleifera* mother-palms is crucial, since their ability to combine with several sources of pisiferas will allow selecting a combination with the desired characteristics, particularly oil content. The second Amazon generation refers to the use of F_1 recombinants of Manaus *oleiferas* with advanced compact pisiferas of the first and second compact backcross cycles: BC_1F_2 and BC_2F_1 respectively (Table 1).

The current F_1 population of 133 Manaus *oleiferas* planted in 2003, which are being used for seed production, refers to the recombination of 14 palms selected from the original 350 palms introduced from Brazil in 1979. Oil content was improved from 5.5% O/B in the original population to 9.0% in the F_1 population.

Performance in commercial plantings

ASD has commercialized Amazon seeds of the second generation since 2008, mainly in Costa Rica, Nicaragua, Colombia, Brazil and Peru, representing about 5,000 ha planted. The oldest Amazon plots are in Tumaco, Colombia (2,700 ha), and the observations on initial production, bunch development, growth and bud rot incidence of the Amazon hybrid compared with other hybrids are summarized in the following paragraphs.

In general, young Amazon palms started producing mainly male inflorescences, which eventually may favor the proliferation of pollinating insects (*E. kamerunicus* or other species) in new extensive areas. Depending on field condition and management practices, this stage could be short, not allowing good establishment of pollinators. The majority of Amazon palms started inflorescence production after 18 months in the field. Approximately less than 10% of the palms

showed a relatively short androgynous cycle, which may be related to one or both parental palms (genetic expression). On the other hand, between 2 to 5% of the palms showed totally spathe-covered female inflorescences; this condition was observed more frequently in areas where palms were under-developed or showed nutritional deficiencies. Prolonged androgynous cycle and heavy spathe-coverage of the female inflorescence are apparently associated with the origin of the *guineensis* pisifera. In experimental plots in Costa Rica, hybrids obtained from Taisha *oleiferas* crossed to Yangambi, Ekona and Ghana pisiferas showed strong and prolonged androgyny while this effect was much lower in crosses of the same *oleiferas* to compact pisiferas.

Fresh fruit bunch production

Under relatively fair field management with certain limitations (low soil fertility and poor drainage), the Amazon hybrid produced 30-50 female inflorescences per hectare during the period of 24-36 months in the field. Under good management inflorescence production increased to 100-200/ha (1.6 inflorescences/palm) in the period of 30-42 months after planting. Usually, assisted pollination is initiated at month 24 after planting, hence the expected FFB production in the first year was estimated at 9.8 -12.3 t/ha compared with 2.9-7.5 t/ha of the Coarí hybrid (Table 6). This difference in the production potential between the hybrids can be partially attributed to the higher planting density of the Amazon hybrid due to the shorter leaves it inherits from the compact pisifera. It is important to note that the Amazon hybrid was planted at 128 palms/ha despite the recommendation of 143 palms/ha, based on its vegetative characteristics (Table 2.)

Table 6. Estimates for first year (30 to 42 months after planting) production potential with assisted pollination of the Amazon and Coarí hybrids in Tumaco, Colombia *

	Amazon	**Coarí**
Palms/ha	128	116
Bunches/palm/month	1.5 - 2.5	0.7 - 2.3
Bunch weight (kg)	4.0 - 5.0	2.3- 6.0
Production potential (t/ha)	9.8– 12.3	2.9 a 7.5

* General reference after visiting several plantations in Tumaco; in Oleaginosas Salamanca it is expected to harvest 10 to 11 t/ha during this period in Amazon compared to 8 to 9 t/ha in Coarí

The industrial extraction of the first batches of Amazon bunches fluctuated between 15-16% according to Palmas de Tumaco (personal communication with the first author, April 2012).

Bud rot tolerance

Evaluation criteria for bud rot incidence among commercial plantations are not uniform. In several cases this disorder is confused with common spear rot, or else plantation management differences may also interfere with incidence data for comparison purposes.

The incidence of bud rot in the commercial plantings of Amazon in Tumaco was relatively low compared with the Coarí hybrid: 2.0-4.3% vs. 2.8-16.7% in the Agrigan plantation; and 0.1 – 0.3% vs. 0.2 – 3.7% in Salamanca (Table 7). In general, the incidence of bud rot is relatively low in OxG hybrids in both commercial plantations; however, it is important to see if this condition is maintained as they get old.

Table 7. Bud-rot incidence (%) in the Amazon and Coarí hybrids of different age, in two commercial plantings in Tumaco, Colombia.

	Agrigan		Salamanca	
Age (months)	**Amazon** (66 ha)	**Coarí** (182 ha)	**Amazon** (768 ha)	**Coarí** (272 ha)
23	2.0	-	0.1	-
24	3.6	5.9	0.2	-
25	-	6.4	0.1	-
26	4.3	-	0.1	0.2
27	2.4	-	-	-
29		6.1	0.1	-
30		-	0.2	-
31		-	0.3	0.7
32		6.8		-
34		-		1.8
37		-		3.7
41		16.7		

Conclusions

Young Amazon plantations showed certain valuable characteristics, which can make this OxG hybrid a good commercial alternative: high bud rot tolerance (lower than 1% at the age of 36 months), leaf length similar to common *guineensis* materials, low inflorescence spathe coverage and high FFB production potential. The oil content of the Amazon bunches will be properly evaluated when the commercial plantations become 48 and 60 months old and oil content reaches its maximum, but the first commercial data seems promising.

In the short-term, the nutrition requirements of the Amazon hybrid to improve fertilization should be investigated further. It is important to continue looking for alternative *guineensis* varieties tolerant to bud rot to serve as pollen donors in large Amazon plantations, in order to avoid assisted pollination. This scheme is based on the proven tolerance to bud rot of certain OxG hybrids, which can be intercalated with less tolerant *guineensis* creating a horizontal type of resistance, if the causes of bud rot are biotic agents.

Other aspects that need to be studied and understood are: i) the influence of certain field management practices on the level of spathe coverage of female hybrid inflorescences; ii) whether the length of the androgynous period is genetic or environmental; and iii) the proper way to manage insect pollinators and to attract them to the female OxG inflorescences that are almost odorless.

In ASD the selection of *E. guineensis* bud rot tolerant varieties is integrated with the program for the development of new OxG hybrids. ASD is testing and developing a new generation of Manaus mother palms (F_2) and compact pisiferas from the second back-cross cycle (BC_2F_2); this will generate a wider genetic variation, aimed at finding better combinations than the current Amazon hybrid.

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