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Annular spot and chlorotic ring spot in *Elaeis guineensis* and OxG hybrids at the nursery stage in Tumaco, Colombia

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Abstract

The response of 14 *tenera* crosses of oil palm (*Elaeis guineensis*) and two interspecific hybrids OxG (*E. oleifera x E. guineensis*) to 'mancha anular' (annular spot) and 'anillo clorótico (chlorotic ring spot) diseases was documented at a nursery planted in Tumaco, Colombia. 'Mancha anular' symptoms were observed in plants of all the *guineensis* crosses evaluated, but there were differences in incidence between crosses. Only one of the OxG hybrids showed affected palms. The presence of the virus AOPRV (African oil palm ring spot virus) associated with this disease was confirmed using the RT-PC molecular tests on affected leaf tissue. The presence of the potyvirus associated with chlorotic ring spot symptoms was confirmed using the ELISA test. The simultaneous presence of both viruses in the same plant as found in both *guineensis* and the OxG hybrid had not been reported before. This is also the first report on the presence of chlorotic ring spot in OxG in Colombia.
The differences in incidence found between crosses of *E. guineensis* and the two OxG hybrids may indicate that it is possible to select for tolerance or even resistance within *guineensis* and *oleifera*.

Introduction

There are about 316,000 ha already planted with oil palm in Colombia, and there are governmental plans to increase this area to 443,000 ha by 2010 and to around one million hectares by 2020 (FEDEPALMA 2000, Liebovich 2005, MADR 2006). To accomplish these goals, it would be necessary to plant about 67,000 ha in the region of Tumaco (Romero et al. 1999, Owen 1992), but there are important phytosanitary problems that could impede this, particularly spear rots (PC). In the recent past, annular spot (mancha anular) and chlorotic ring spot (anillo clorótico) had also reached high incidences on some plantations and so they are also considered important threats to the industry.

Annular spot was first found in South America in Peru in 1969, later in Ecuador (1974) and then in Colombia in 1985 (Dzido et al. 1978, Peña et al. 1991, Morales et al. 2002, Caicedo 2003). This disease has not yet been found in Central America (Chinchilla 2001). In Africa (Ivory Coast), there is a disease with similar symptoms known as 'dry basal rot', which is thought to be

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transmitted by *Sogatella kolophon* and *S. cubana* (Homoptera:Delphacidae) (Renard y Quillec 1984).

Annular spot has been observed in both *Elaeis guineensis* and *E. oleifera* and hybrids of the two species (OxG hybrids). Some of the symptoms are a rapid drying of the youngest leaves and spears and the development of a dry rot in the area of the whorl (Dzido et al. 1978, Renard and Franqueville 1989). These symptoms have been associated with the presence of a virus (Foveavirus) designated as AOPRV (African oil palm ringspot virus) (Morales et al. 2002b).

So far in Colombia, the disease has been only found in Tumaco, where incidence has varied from 2-45% (Jiménez 1988, Sánchez 1990, Nieto 1996, Morales et al. 2002). However, in certain nurseries and some young plantings (below three years of age), all palms have been affected (Morales et al. 2002). It seems that some tolerance develops with age, as symptoms are rare in palms older than five years.

A higher incidence has been associated with poor drainage and the presence of some tall grasses, such as *Panicum* sp. (Peña et al. 1991, Morales et al. 2002). Symptom expression seems to vary with environmental conditions. So far all attempts to reproduce symptoms mechanically or by using insects have failed (Morales et al. 2002).

Chlorotic ring spot affects palms mainly during the nursery stage, but symptoms persist in young palms in the field. This disease was first found in Ecuador in 1995 and later in Colombia in 1996 at Tumaco (Genty 1996, Chinchilla 2001, Morales et al. 2002). Symptoms are associated with the presence of a Potyvirus that is similar to sugarcane mosaic virus (SCMV) (Chinchilla 1996, 2001; Morales et al. 2002). Affected palms do not die, but they are poor yielders, so they are normally eradicated at the nursery stage. Incidence at this stage may reach 80% or higher (Genty 1996, Chinchilla, 2001, Morales et al. 2002).

The proper management of these two diseases is fundamental, and the search for resistance/tolerance is an important part of the strategy. Since there is no proven method for inducing symptoms, the only way available to select promising materials is to expose them to conditions known to be favorable to the diseases. This work summarizes the results of one such test where 14 *guineensis* crosses and two OxG hybrids (*E. guineensis x E. oleifera*) were planted in an area where the previous *guineensis* plantation had been severely affected by annular spot and was eradicated.

Materials and Methods

The study was conducted in Tumaco (Nariño, Colombia) from July 2007 to July 2008 at the 'Hacienda Aguas Lindas' belonging to the Palmar Santa Elena, S. A. plantation on the Tumaco-Pasto road. The place was characterized by recurrent attacks of annular spot, where incidence reached 10-25% in previous plantings (1987 and 1989).

A total of 16 crosses were evaluated (Table 1). Eleven of these came from the CORPOICA experimental station (Colombia), and five were from ASD de Costa Rica (Costa Rica). A three-month prenursery was initially established at CORPOICA's El Mira Experimental Station located

30 km from the final planting site. Management at the prenursery stage was standard, but plants received three sprays with monocrotophos (5 cc.c.p./l) at monthly intervals.

Table 1. *Elaeis guineensis* crosses and two OxG hybrids (*E. guineensis* x *E. oleifera*) planted to evaluate their response to 'annular spot' and 'ring spot' diseases in Tumaco, Colombia

Cross	Genotype	Origin
D 1 x P, Yangambi	*guineensis*	Colombia
D 1 x P, La Mé	*guineensis*	Colombia
D 1 x P, Pobé	*guineensis*	Colombia
D 2 x P, Yangambi	*guineensis*	Colombia
D 2 x P, La Mé	*guineensis*	Colombia
D 2 x P, Pobé	*guineensis*	Colombia
D 3 x P, Yangambi	*guineensis*	Colombia
D 3 x P, La Mé	*guineensis*	Colombia
D 3 x P, Pobé	*guineensis*	Colombia
D x AVROS	*guineensis*	Costa Rica
D x Ekona	*guineensis*	Costa Rica
Compacta x Ekona	*guineensis*	Costa Rica
Bamenda x Ekona	*guineensis*	Costa Rica
Tanzania x Ekona	*guineensis*	Costa Rica
Elaeis oleifera 1 x D	OxG	Colombia
E. oleifera 2 x D	OxG	Colombia

D= Deli; P= *pisifera*

After standard selection of the best plants from the nursery, these were taken to the experimental site and planted at a main nursery using 30 x 40 cm plastic bags, filled with soil from the same site. Bags were spaced 1.0 m apart on the corners of equilateral triangles. The planting site was close to a commercial lot where annular spot reached 20% in 1987. A total of 3,200 palms were planted following a completely randomized design with 16 treatments (crosses) and 200 replications per treatment, where the experimental unit was a particular palm.

The palms remained at the main nursery for 56 weeks, where they were fertilized with the formula 17-6-18-2. Mechanical control of weeds was only done every four months in order to allow the presence of potential insect vectors and other conditions that could favor disease appearance and dissemination. Monitoring for disease presence was done three times every week, when every individual palm was closely examined for the presence of the characteristic

symptoms of both annular and ring spot diseases: yellow leaves, typical yellow streaks on leaflets and rachis of youngest leaves, ring spots, etc. (Peña et al. 1991, Morales et al. 2002). Additionally, the presence of other diseases, such as spear and whorl rots, was documented following descriptions given by Peña et al. (1991), Nieto (1996) and Morales et al. (2002).

Tissue with symptoms from a sample of 54 affected palms was taken to the Virology laboratory at the CIAT (International Centre of Tropical Agriculture) in Palmira, where molecular tests (RT-PCR and ELISA) were conducted following Morales et al. (2002b).

The resident insect population was followed weekly by collecting them with a sweep net between 9:00 and 10:00 am, and later between 3:00 and 4:00 pm.

General incidence (referring to total number of plants in the nursery) and incidence per cross of annular spot were determined from the records of number of palms with symptoms in each cross. Homoptera insects captured were initially classified to species level at the ICN (Institute of Natural Sciences) of the National University of Colombia in Bogotá.

Data on the number of affected palms per genotype were analyzed using the FREQ procedure on SAS ver. 9.1.3., by using the CHISQ (Chi-square) option to test the hypothesis (a=0.05) of the dependency of the presence of the disease on the different genotypes and its precedence.

Results and Discussion

The symptoms observed in the field (Fig. 1) corresponded to those described in the literature as annular leaf spot (Peña et al., 1991, Morales et al., 2002, Chinchilla 2001). The first plant with symptoms appeared 121 days after planting at the nursery, and the last one at 328 days. Considering that the prenursery was completed in an area free of the disease, the information allowed estimation of an incubation period of four months or less.

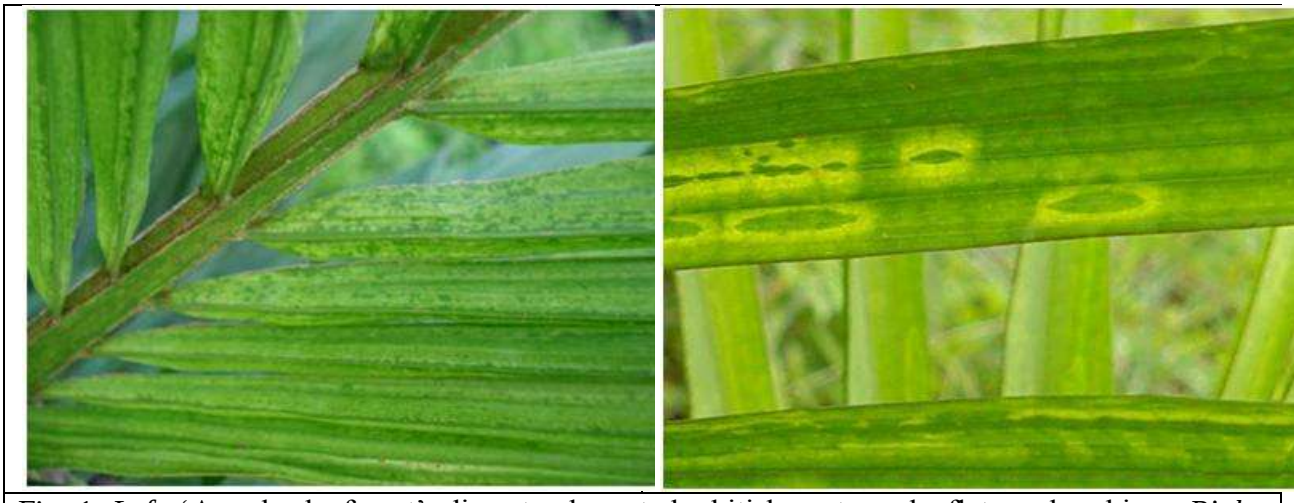


Fig. 1. *Left*. 'Annular leaf spot': discrete elongated whitish spots on leaflets and rachises. *Right*. Chlorotic ring spot. Symptoms in an oil palm nursery in Tumaco, Colombia.

At the end of the nursery period, the total number of plants showing symptoms of annular spot was 110 (3.44%, Table 2), which is within the normal range reported in the area (2-45%, Morales et al. 2002a).

Table 2. Incidence (%)[1] of 'annular leaf spot' at the nursery stage in 14 *guineensis* crosses and two OxG (*Elaeis oleifera* x *E. guineensis*) hybrids). Tumaco, Colombia

Cross	General incidence[1]	Incidence per cross[2]
Deli 1 x *Pisifera*, Yangambi	0.38	6.00
Deli 1 x *Pisifera*, La Mé	0.34	5.50
Deli 1 x *Pisifera*, Pobé	0.03	0.50
Deli 2 x *Pisifera*, Yangambi	0.22	3.50
Deli 2 x *Pisifera*, La Mé	0.34	5.50
Deli 2 x *Pisifera*, Pobé	0.13	2.00
Deli 3 x *Pisifera*, Yangambi	0.06	1.00
Deli 3 x *Pisifera*, La Mé	0.38	6.00
Deli 3 x *Pisifera*, Pobé	0.28	4.50
Deli x AVROS	0.50	8.00
Deli x Ekona	0.25	4.00
Compacta x Ghana	0.03	0.50
Bamenda x Ekona	0.19	3.00
Tanzania x Ekona	0.13	2.00
Nolí 1 (*Elaeis oleifera*) x Deli	0.00	0.00
Nolí 2 x Deli	0.19	3.00
General incidence	3.44	

[1] 3,200 palms at the nursery
[2] 200 palms in each cross

All *guineensis* crosses tested showed symptoms, but one of the OxG hybrids did not show affected palms. The *guineensis* crosses with the highest incidence were Deli x AVROS (8.0 %), Deli 1 x Yangambi (6.0 %), Deli 3 x La Mé (6.0 %), Deli 1 x La Mé (5.5 %) and Deli 2 x La Mé (5.5 %). The lowest incidence was obtained in Compact x Ghana (0.5 %).

The absence of symptoms in one of the hybrids could indicate resistance or an escape situation, which has to be verified in another experiment. On the other hand, the presence of symptoms in

the other hybrid confirms the susceptibility of these materials to annular leaf spot, as had been noted by Dzido et al (1978). The Chi square test ($a < 0.0001$) indicated that there were differences between crosses, but not between origins (Colombia vs. Costa Rica).

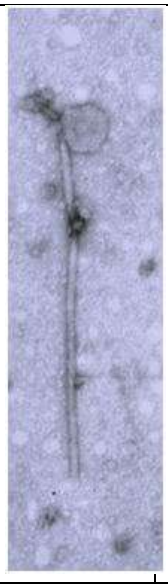
The RT-PCR analysis confirmed the presence of the annular spot virus (AOPRV) in all palms with symptoms tested (Table 3). The sequential bands obtained had an identity of 100% with the AOPRV when compared with the world bank of genetic data. Besides this, all samples tested also showed a positive serological reaction (ELISA) with the monoclonal antibody for potyvirus indicating the presence of the ringspot virus in both *guineensis* and the OxG hybrid (Figs. 2 and 3). Extraction and synthesis of DNA and complementary tests were done following Lozano et al. (2009).

Table 3. Determination of the presence of annular spot (AOPRV) and ring spot (potyvirus) viruses in samples from nursery oil palms

Cross	RT-PCR[1]	DAS ELISA[2]
Deli 1 x *Pisifera*, Yangambi	+	+
Deli 1 x *Pisifera*, La Mé	+	+
Deli 1 x *Pisifera*, Pobé	+	+
Deli 2 x *Pisifera*, Yangambi	+	+
Deli 2 x *Pisifera*, La Mé	+	+
Deli 2 x *Pisifera*, Pobé	+	+
Deli 3 x *Pisifera*, Yangambi	+	+
Deli 3 x *Pisifera*, La Mé	+	+
Deli 3 x *Pisifera*, Pobé	+	+
Deli x AVROS	+	+
Deli x Ekona	+	+
Compacta x Ghana	+	+
Bamenda x Ekona	+	+
Tanzania x Ekona	+	+
Nolí (*Elaeis oleifera*) 1 x Deli	-	-
Nolí 2 x Deli		+
Negative control (healthy bean)		+
Positive control (BCMNVNL3)		+

1. Annular spot; 2. Ring spot specific antibody

Fig. 2. Filamentous particles in the cytoplasm of cells from oil palm spear leaves with 'annular spot' symptoms. Courtesy of the Virology laboratory of CIAT, 2009



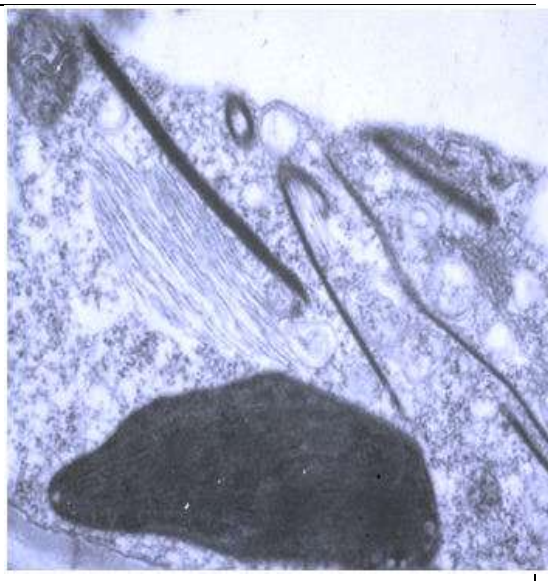


Fig. 3. *Left*. Chlorotic ring spot filamentous particle. *Right*. Filamentous particles and cytoplasmatic inclusions in cells from spear leaves of oil palm affected by both virus causing 'chlorotic ring spot' and 'annular spot'. Courtesy of the Virology Laboratory of CIAT, 2009.

It was somewhat disappointing to find susceptibility even in crosses such as Bamenda x Ekona where the mother palms are genetically distant from the more common Deli *dura* mother palms used in the rest of the crosses. In both OxG hybrids, the pollen source was of Deli origin, and so there is still a chance of finding a better response using other parents, but it is already known that *Elaeis oleifera* is susceptible to annular leaf spot (Renard and Franqueville 1989). Palms showing other symptoms, such as spear rots turned out to be negative for the presence of virus indicating no association between these elements.

A total of 13,013 adult insects of the Homoptera order were captured and classified into 21 species, illustrating the large diversity of the enthomofauna associated with the oil palm in the region, as already found by Caicedo (2003). No attempts were done during this phase of the research to associate these insects with the diseases found.

Conclusions

The presence of the potyvirus associated with chlorotic ringspot in a OxG hybrid was reported for the first time in Colombia. It was also found for the first time that both viruses associated with annular spot (AOPRV) and ringspot (potyvirus) diseases can be present in the same plant in nurseries of *E. guineensis* and the hybrid OxG (*E. guineensis x E. Oleifera*).

All *guineensis* crosses tested turned out to be susceptible to annular leaf spot (and chlorotic ringspot). However, some differences were found between crosses (percentage of infected palms), which may be an indication that resistance/tolerance could be found in other sources of genetic materials.

Acknowledgment

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