Report on the

Coconut Lethal Yellowing Mission in Mozambique

CIRAD–DNSA

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CONTENTS

Summary

Coconut Lethal Yellowing (LY) and the *Oryctes* beetle are continuing their devastation in Zambezia, in the Quelimane region. The Madal Group has embarked upon the eradication of coconut palms affected by LY, in order to reduce *Oryctes* attacks. The variety performance trials (seednuts from Ivory Coast) testing reactions to LY were planted in March-April 2007. At the time of the visit, some seedlings were suffering from drought and scale attacks. An improvement in the watering system would not go amiss and would doubtless prevent losses. Analyses carried out by CIRAD in Montpellier show that it is doubtless still the same strain of phytoplasmas as in 2003 that is rife in Quelimane.

In Cabo Delgado province, the oldest plantations are suffering from senility exacerbated by the lack of upkeep, nutritional deficiencies and *Oryctes* attacks. The coconut palms on smallholdings usually look much better. Sporadic Lethal Yellowing syndromes were identified from the South up to the Tanzanian border, but no disease foci as severe as those in Zambezia were ever seen. Analyses carried out in Montpellier show that there are two strains of phytoplasmas associated with these syndromes in Cabo Delgado. One corresponds to the phytoplasmas identified in Zambezia, associated with what is locally known as Lethal Yellowing. The other corresponds to the phytoplasmas known in Tanzania and Kenya associated with the Lethal Decline Tanzania syndrome (LDT). The different results recorded over the last 20 years on the performance of coconut varieties in relation to these syndromes, and the sequence differences for the rRNA gene between the two "strains" or "species" of phytoplasmas mean that there could be two diseases with different epidemiologies, and in particular different vectors. A pentatomid bug has been found to carry LY phytoplasmas. That bug might be the vector of LY in Cabo Delgado. It needs to be checked whether the bug is regularly associated with cases of LY and LDT. It would also seem important to have more exhaustive data on the distribution of the 2 syndromes in Cabo Delgado, or even in Nampula province, so as to more effectively define research strategies. Lastly, we recommend getting smallholders in the North of the province to abandon the practice of making holes in stems. Under the drought conditions persisting for the last 4-5 years in the North of the country, such large holes in the stem seem to considerably weaken the palms.

Preamble

Throughout this mission to Mozambique, I was accompanied by Mrs Antonia Vaz, from the DNSA (Direcçao Nacional Servicios Agrarias) Sanidad Vegetal service of the Ministry of Agriculture.

People met

Mr B. Leclerc and Mrs A. Feirrera, Agence française pour le Développement, Maputo
Mr M. Nuisenga and Mrs G. Kessler from the Millennium Challenge Corporation
Mr Emanuel Lourenço, Madal Estate Manager at Quelimane
Mr Luis Tomo, Agronomist from the Quelimane Provincial Directorate for Agriculture
Mr M. Rafik Valá, Director of the Provincial Service of Agriculture, Zambezia
Mr O. Amimo, Director of the Provincial Service of Agriculture, Cabo Delgado
Mr J. Gemuce, Agronomist from the Provincial Service of Agriculture, Cabo Delgado.
Mrs F. Macome, Provincial Supervisor, Agronomist, Instituto Fomento Caju
Mrs Vasco Quetero Mesa, Extension Service manager, Palma District
Mr S. Eden Green, independent consultant for MCC

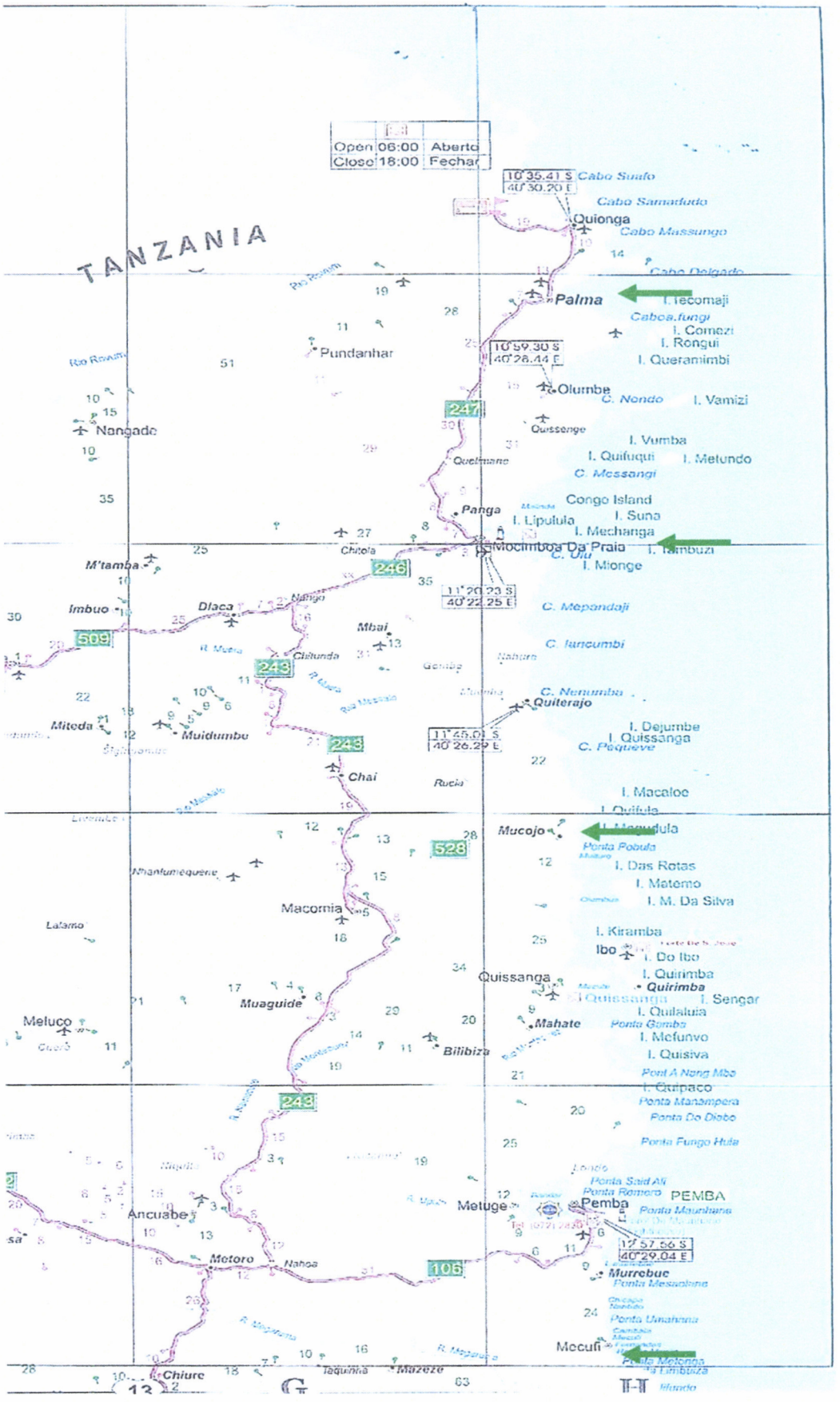
Acknowledgements

Thanks to Emanuel Lourenço and Luis Tomo for their warm welcome at Quelimane and the time they gave up to accompany me in the field.
In Cabo Delgado province, thanks to the Provincial Service of Agriculture for providing a car. Thanks also to Feliza Macome who guided me throughout the visit. Thanks to Vasco Quetero Mesa for his hospitality in Palma.

Schedule

05.09: Flight Montpellier- Paris- Johannesburg
06.09: Flight Johannesburg- Maputo.
Visit to AFD
07.09: Flight Maputo-Quelimane
Meeting with M. Nuissenga and G. Kessler from the Millennium Challenge Corporation (MCC).
08.09: Field visit MADAL estates.
09.09: Visit to the Provincial Service of Agriculture.
Flight Quelimane-Maputo
10.09: Flight Maputo – Pemba
Visit to Direcçao da Agricultura.
Field visit, Mecufi region
11.09: By road, Pemba-Palma
Field visit, Palma
Travel Palma-Mocimboa da Praia
12.09: Field visit, Cabo de Ulo
Field visit, Chimbango
Halt for 2 hours in Chai (burst tyre)
Field visit, Mucujo, Nambo, Nanjaba
Halt for 90 minutes (low pressure in spare tyre)
Return to Pemba
13.09: Preparation of samples
Meeting with the Director of Agriculture, Cabo Delgado province
14.09: Flight Pemba-Maputo
Meeting with S. Eden Green, consultant to MCC.
15.09: Flight Maputo-Johannesburg-Paris
16.09: Flight Paris – Montpellier

Map 1: provinces visited



Map 2: Sites surveyed in Cabo Delgado

1. ZAMBEZIA

1.1 Field visit

In addition to the Lethal Yellowing (LY) type syndrome, two major problems affect coconut palms around Quelimane:

- *Oryctes* beetle attacks
- The drought existing for 4 to 5 years which, in addition to its direct effects, appears to encourage outbreaks of nut mites (*Aceria* type)

Oryctes damage is a consequence of Lethal Yellowing. In fact, smallholders do not fell coconut palms affected by LY, and the crownless stems become *Oryctes* reservoirs.

1.1.1. Eradication

In the Madal estates, a great effort has been made to eradicate diseased and dead palms. The Madal Group has acquired 20 chainsaws to cut down coconut palms affected by LY as quickly as possible. Unfortunately they come up against the tricky problem of a shortage of labour. They not only eliminate those palms with LY symptoms, but also the neighbouring palms. The stems are cut into several pieces, piled up, covered with fronds and burnt (Figs. 1 and 2).

Figs.1 and 2.
Burning of coconut palms affected by LY in the Madal estates at Quelimane.

1.1.2. Lethal Yellowing

The LY type syndrome continues to be the main problem for these plantations in Zambezia around Quelimane. There are numerous continuously active foci.
All stages of the disease can be seen in a focus, from the start of yellowing to the final stage (Fig. 3).

Some green hybrid coconut palms were found on several occasions to subsist in the middle of certain very active foci (Fig.4). Whilst green coconut palms might initially seem more numerous, some unattacked yellow palms can also be seen in developing foci (Fig. 5).

Fig. 3 LY focus in the Madal estates at Quelimane.

Fig.4 Green coconut hybrid subsisting in a LY focus.

Fig.5 Yellow and green coconut palms in a LY focus.

1.1.3. New variety performance trial at Inhangule (D3)

Planting of this Lethal Yellowing performance trial was completed in April 2007. The seedlings of numerous varieties were still small, and they are now suffering more than others. There have been some substantial losses. Despite some rainfall in June-July, drought is the major problem in this trial of 8 blocks. Watering facilities are very limited (Fig. 6), and the wells sunk in this trial are virtually dry. There are severe scale attacks followed by sooty mould development, which needs treating (Fig. 7), and various cases of mortality (Fig. 8). In addition, it seems that some of the labelling is wrong. More generally, this trial is highly heterogeneous; the largest palms at the time of planting will no doubt have a better chance of surviving than the smallest (Fig. 9).

1.1.4. New performance trial at Machimbui

The seedlings were planted in March 2007. On average, they are in better condition than at Inhangule (Fig. 10). However, there too, there are scale attacks and sooty mould. If the seedlings manage to resist the drought and various attacks while immature, it can be considered that they will provide some interesting information in terms of varietal resistance. Indeed, this trial is next to coconut palms attacked by LY (Fig. 11), though it should be noted that the wind blows from the performance trial towards the LY foci.

1.1.5. Miscellaneous

Some very severe *Oryctes* attacks were seen on some Mozambique Tall palms planted in 2005 (Fig. 12).

Some coconut-pineapple intercropping trials seem to be giving some very good results (Fig. 13).

1.2. Meeting with the Director of the Provincial Service of Agriculture

According to Mr Rafik Valá, plant diseases are a major constraint in Zambezia, particularly as available resources are limited. Research is lengthy and costly. It took 6 to 8 years to diagnose an *Oidium* on cashew nuts and 5-6 years for a cassava mosaic study. *Citrus* plants (there have been some imports from Brazil) have suffered heavy losses of unknown origin (though it seems that Tristeza can be ruled out).

Coconut provides a living for 15% of the population of Zambezia and accounts for 30% of GDP. LY development is therefore a major worry for that province, which lacks expertise in that field. LY is all the more serious in that smallholders are very attached to this crop and refuse, for example, to replace dead coconut palms with cashew trees. The Service of Agriculture hopes to receive support from IIAM (Instituto de Investigacion Agraria de Mozambique) of the Ministry of Agriculture.

However, it would also like support from CIRAD, for all aspects of agronomy and crop protection.
Mr Amimo would like to:

- **have an updated inventory of the disease carried out in Zambezia, with diagnosis training,**
- **undertake research on disease transmission (search for the vector),**
- **be able to have the results of variety performance trials in order to distribute seednuts of resistant or tolerant varieties,**
- **set up an extension service capable of getting information and research results across to farmers.**

Fig.6 Manual watering in the Inhangule performance trial

Fig.7 Scale attack with sooty mould

Fig.8 Mortality of unknown origin.

Fig.9 Inhangule performance trial.

Fig.10 Machimbui performance trial

Fig.11 Case of LY near the Machimbui trial

Fig.12 *Oryctes* on an immature Mozambique Tall palm

Fig.13 Coconut-pineapple intercropping in the Madal estates at Quelimane

2. CABO DELGADO

The visit to Cabo Delgado province revealed the very advanced age of the coconut palms in some plantings. Many of the palms are certainly over 40 to 50 years old, and some plantations have been more or less abandoned. It has to be said that the majority of those old coconut palms are in extremely desperate physiological condition. The palms, of the "local Tall" or "East African Tall", or even "Mozambique Tall" type, are very tall and have probably never been given a single gram of fertilizer, or it was a very long time ago when they were first planted. Consequently, most of the old coconut palms display "pencil point" type symptoms, with a gradual decrease in stem diameter from bottom to top (Fig. 14, 15 and 16), evolving irreversibly into the death of the palm in one way or another.

This pencil point symptom, which is synonymous with basic deficiencies (N, P, K, etc.), and with trace element deficiencies (Mg, Mn, Fe, B, S...), goes hand in hand with a very impressive reduction in frond size. The result is a sort of "tuft" of short, more or less yellow fronds at the top of a stem with a very small diameter, which is therefore very weak and will snap in the event of strong winds or beetle attacks, leading to these "telegraph poles", frondless stems, which are a true disaster for neighbouring coconut palms or plantings. Indeed, those crownless stems will very rapidly become a source of *Oryctes* beetle multiplication and those beetles will, in turn, weaken nearby coconut palms whether they are in good physiological condition or already sickly. That is why traces of numerous *Oryctes* attacks can be seen on most of the palms in the province (Fig. 14). If the coconut palm is not too weak, it might resist a while but it will be less productive; if it is spindly, it will die and itself become a new source of *Oryctes.*

Fig.14

Fig.15

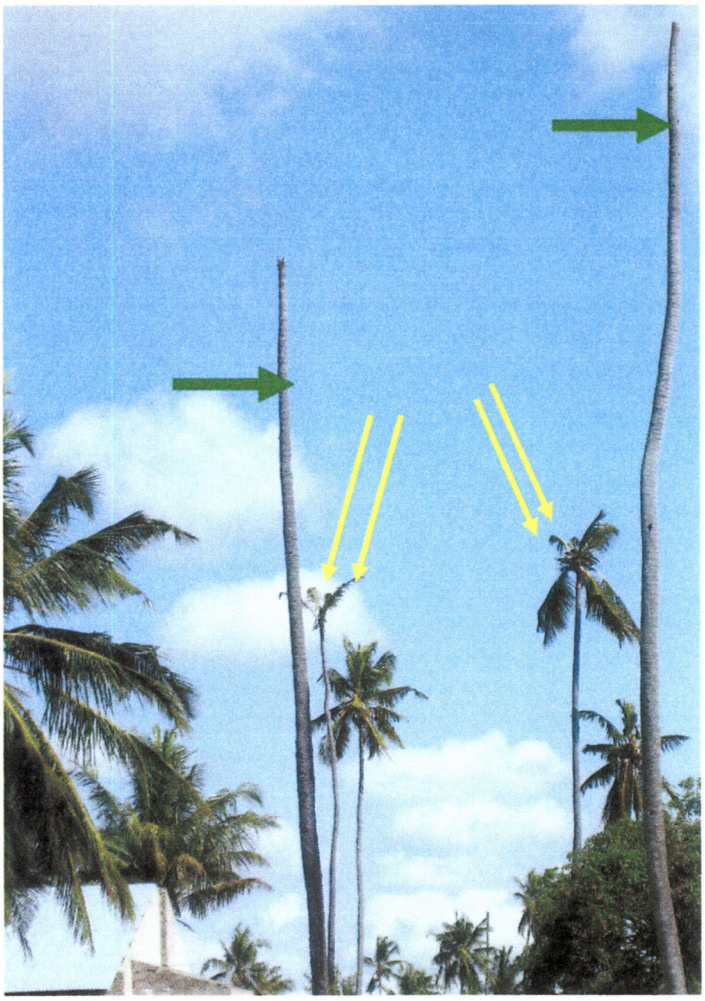


Fig. 16

Figs. 14, 15 and 16. Old coconut palms with the "pencil point" symptom (green arrows), small yellow fronds, and *Oryctes* attacks (red arrows) at Cabo de Ulo (or Ulu). The double arrows indicate palms dying via the meristem zone, a case different from LY.

Fig. 17

Fig. 18

Figs. 17 et 18. Coconut palms in the village of "upper" Palma (18) and Palma beach (17)

The existence of these yellowing symptoms on the old coconut palms and the presence of crownless stems have suggested to some the problem of coconut Lethal Yellowing. That is most probably not the case in the great majority of the old plantations visited (at Cabo de Ulo, Chimbango, Mucojo and Nambo, Mocimboa de Praïa, Macomia and vicinity, Pemba, etc.). There may be some cases of LY, but most of the palms are dying from senility or their desperate physiological condition.

In the villages, the coconut palms look much better, with greener foliage, longer fronds and bunches of handsome nuts (Figs. 17 and 18).
Younger coconut palms are usually found there. It can easily be imagined that those palms receive much more attention from the villagers than those in the old isolated plantations.

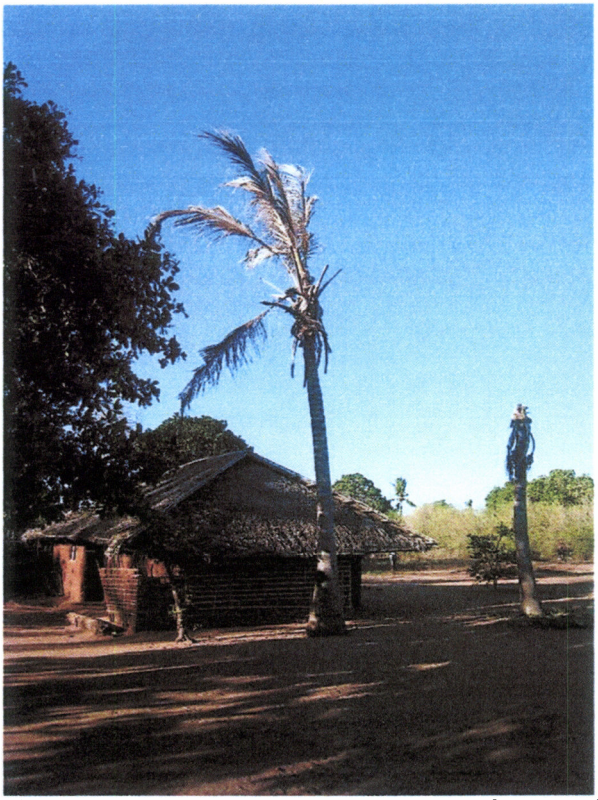
2.1. Field visits

2.1.1. Mecufi, Pemba district.

On the road between Pemba and Mecufi (in the South) there are only very few coconut palms, except in the villages. The palms are fairly green and no cases of mortality were seen, apart from the odd isolated case.
At Mecufi, the small coconut grove on the shoreline is disease-free. However, in a hamlet south of the village a few crownless stems can be seen, along with some cases clearly resembling various stages of lethal yellowing (Figs. 19 and 20). Around the same house, it was possible to see 4 palms affected by more or less advanced LY symptoms. One palm at the beginning of the yellowing stage was dissected to collect samples (see later, sampling section).

2.1.2. Palma (near the border with Tanzania)

In the lower part of Palma (Palma beach) the coconut palms of all ages are very handsome, green with long fronds (Fig. 17 and 18), but a highly likely pocket of LY was found with 3 crownless stems and 2 cases of terminal symptoms (Fig. 21) next to each other. However, no cases in the initial stages were seen. The other coconut palms in the vicinity had some fine-looking nuts and the fronds were green.
In the upper part of the village, more or less the same kind of situation is found. Most of the coconut palms are really green and bearing nuts. However, some isolated cases of LY type symptoms were found (Figs. 22 and 23). A palm at the beginning of the yellowing stage was dissected to collect samples (see later, sampling section), and an inflorescence was taken from another palm displaying slight yellowing. That inflorescence, seen from below, had necrosis on some rachillas, suggesting a LY symptom. In fact, once the inflorescence had been collected, the necrosis was found to be lateral and seemed to begin at the base of the petiole, probably due to an attack by *Oryctes* or another insect (Fig. 24). It was kept all the same, but the sample was completely rotten by the time it reached the laboratory.




Figs. 19 and 20. Isolated coconut palms in a hamlet near Mecufi, with LY symptoms.

Fig. 21. Probable small LY focus at Palma beach.

Fig. 22: Probable case of LY at "upper" Palma: advanced symptoms alongside a possible earlier case of LY.

Fig. 23: Likely case of LY at "upper" Palma: in the centre background, a case in the early/middle stages with browning lower fronds and yellowing fronds in the upper crown; in the right-hand background, the beginnings of a LY case at the yellowing lower frond stage. The two palms have lost all their nuts.

Fig. 24. Lateral necrosis of an inflorescence that has just opened and might be the result of insect attacks.

2.1.3. Cabo de Ulo (or Ulu) near Mocimboa de Praia.

A visit was made to the Lusoma plantation. This 300-ha coconut plantation appears on the 1975 land register, but the palms seem to be more than 50 years old. It has been abandoned for the last 7 years. No nuts have been harvested since 1997. The palms are in desperate physiological condition (Figs. 14, 15 and 16). Many have a small number of fronds (4 to 6). Those fronds are small and often more or less yellow. The stems often terminate in pencil points, and there are many *Oryctes* attacks.

There are many cases of terminal rot: the spear and the most recent fronds have disappeared, whilst the lower fronds remain, as in the case of budrot disease, caused by the fungus *Phytophthora.* However, in view of the drought affecting the zone for several years, it can be imagined that those cases of mortality are due more to *Oryctes* attacks. The existence of numerous crownless stems and the yellowish appearance of the small fronds do not automatically mean LY. Leaf yellowing may have various causes: other diseases (e.g. in Latin America and the Caribbean, another disease caused by a trypanosomatid displays exactly the same syndrome), various deficiencies, drought, and... senility combined with deficiencies as here. No chainsaw or axe was available at the time to fell one of the coconut palms to dissect it and take samples.

2.1.4. Chimbango (11 km west of Mocimboa de Praia)

In this village, some "garden palms" were seen, particularly red and yellow Dwarf palms. A case was seen that had been reported as LY.

However, during the visit, the advanced stage of the case prevented any diagnosis from being made (Fig. 25). The main problems of the province can be found in this village: *Oryctes* attacks – though not lethal, only foliar damage – and drought. The mean annual rainfall in this region is 1,000 mm, with very unequal distribution, including a long dry period from February-March to November. Figure 26 gives an idea of the likely effect of the drought: twenty to thirty green, lower fronds can be seen hanging down the stems, with upper fronds that are still erect but the leaflets are very limp. All the coconut palms in this village, and in many others in the province, are suffering all the more from the drought because the villagers make quite a large hole in the base of the stem for superstitious reasons (Fig. 27). It is clear, that given the size of the hole, the number of functional vascular bundles is considerably reduced, sometimes by nearly ¾, and the lack of water for the palm due to the drought is all the more exacerbated.

2.1.5. Macomia

The coconut palms visible along the main roads of the town seem to be healthy with green fronds and handsome bunches of nuts.

Near Macomia on the Mucojo track, there is a small coconut grove of around a hundred palms that are apparently free of any disease.

2.1.6. Nambo (Mucojo shoreline)

There are several small coconut plantings on the shoreline, apparently of different ages. In the oldest plots, numerous crownless stems can be seen. What was the cause of those deaths? It can be seen that most of the stems culminate in a "pencil point".

In addition, among the palms that are still alive, many are spindly with only a few small chlorotic, more or less yellow fronds, and do not produce any nuts (Fig. 28). It is therefore likely that those coconut palms died from senility and malnutrition.

In the youngest, neighbouring coconut plantings, the palms are greener, with more fronds, and there are no LY symptoms (Fig. 29). However, all the coconut palms at Nambo seem to suffer from strong sea breezes and deficiencies due to a lack of fertilization and the sandy nature of the soil. The existence of small, chlorotic, more or less yellow fronds on the old palms, and rust-coloured lower fronds on the younger palms, is probably due to a nitrogen deficiency, and a sulphur or potassium deficiency respectively, the two combining as the palms grow older. This could easily be checked by leaf analyses, but it is doubtful that the owners can afford to correct these deficiencies.

2.1.7. Nanjaba

Between Nambo and Macomia, attention was drawn to the existence of one stem without fronds, and two palms with numerous brown or yellow fronds alongside the track in the village of Nanjaba (Figs. 30 and 31). Close observation revealed that it was a case of ascending yellowing, that recently opened inflorescences were necrotic and that there were no longer any nuts. According to the villagers, the palms lost all their nuts 1 to 2 months earlier. These three cases clearly resemble what happens in a developing LY focus.
The most surprising thing in such a situation is the isolation of these coconut palms: there are very few coconut palms in this village (twenty at the most?), they are far apart from each other, far from any other coconut plantation (more than 20 km to the East, 10 km to the West) and no other LY focus has been identified between Macomia and Nambo. Some samples were therefore taken (see below).

Fig. 25

Fig. 26

Fig. 27

Fig. 25. Mortality of unknown origin (LY?) in the village of Chimbango, Cabo Delgado province.

Fig. 26. Drought symptoms on a Dwarf coconut palm in Chimbango.

Fig. 27. Holes made in the base of coconut stems – for superstitious reasons – in some villages of Cabo Delgado province, here in Chimbango.

Fig. 28. Old coconut planting at Nambo (near Mucojo), Cabo Delgado province. The red arrow indicates the "pencil point" symptom. The yellow arrow indicates a spindly palm with small, chlorotic fronds, probably due to nutritional deficiencies.

Fig. 29 Another, younger coconut planting at Nambo


Fig. 30 and 31. Case of Lethal Yellowing at Nanjaba, Cabo Delgado province.

3. Sampling for variability analyses of Lethal Yellowing phytoplasmas

3.1. Zambezia

3.1.1. Palm 1. Inhangule (Fig. 32)

The first palm felled, in a LY focus, no longer had any nuts. It had 3 brown lower fronds and 4 to 5 yellowing fronds higher up. It was only possible to take one piece of very young inflorescence stem as all the others had rotted, and the rot descended far into the meristem zone.

3.1.2. Palm 2. Inhangule

This palm did not display any highly visible LY symptoms (no proven yellowing). It bore a few nuts. Some inflorescences were sampled (Nos. 9, 8, 7, 6) that looked healthy. According to E. Lourenço, this was just the beginning of LY, before the initial yellowing. On arrival at the laboratory, only one inflorescence was usable.

3.2. Cabo Delgado

3.2.1. Palm 3. Mecufi, near Pemba, Cabo Delgado province (Fig. 33)

This palm no longer had any nuts (nut-fall recorded by the owner 2 months earlier) and revealed yellowing of the lower fronds. It was in a group of 3 palms near a dwelling, one was dead the other displayed advanced symptoms. Dissection revealed rotting of the young fronds descending well into the meristem zone, and rotting of virtually all the inflorescences. Consequently, only portions of rachis were taken from some very young fronds near to the meristem zone.

Dissection revealed the existence of a very large number of predominantly green pentatomid bugs on the sheathes surrounding the spears. One might wonder whether those bugs play a role in disease transmission. Indeed, it should be remembered that the phloem-restricted trypanosomes associated with coconut hartrot disease are transmitted by pentatomid bugs. It can therefore be imagined that some of the bugs might be able to transmit phytoplasmas inside coconut sieve tubes. Some bug specimens were therefore collected for analysis to check that hypothesis.

3.2.2. Palm 4. Palma, Cabo Delgado province (Fig. 34)

This coconut palm no longer had any nuts and displayed yellowing on the tips of certain lower fronds. The inflorescence, which was in the process of opening (No. 9) was completely necrotic. Dissection revealed necrosis of the unopened inflorescences (Nos. 8, 7, 6, 5).

Samples were taken from the base of some very young spears just above the meristem zone, along with the petioles of 3 very young inflorescences.

3.2.3. Palm 5. Nanjaba, Cabo Delgado province

Unfortunately, it was only possible to deal with one palm (2 hours late due to a burst tyre, felling with an axe as the chainsaw was faulty, then nightfall). This palm had already advanced symptoms: lower fronds already fallen, brown fronds, and yellowing of upper fronds (Fig. 31). Dissection revealed unopened inflorescences Nos. 8 and 7 to be rotten, but the following were white and healthy looking. The petioles of inflorescences 6 and 5 were brought back.

Fig. 32. Palm 1 felled at Inhangule (Quelimane), Zambezia province.
The palm was in a LY yellowing focus with all stages of the disease represented. There were no longer any nuts and the palm revealed yellowing of the lower fronds. The spear and young fronds were dry, and rotting of the spear sheathes descended to the upper level of the meristem zone.

Fig. 33. Palm 3 felled at Mecufi, Cabo Delgado province.
Coconut palm without nuts (nut-fall recorded by the owner 2 months earlier) with yellowing of the lower fronds. Dissection revealed rotting of the young fronds descending way down towards the meristem zone, with the presence of numerous bugs (red arrows).

Fig. 34. Palm 4 felled in Palma, Cabo Delgado province, near the Tanzanian border.
The palm no longer had any nuts and showed yellowing on the tips of certain lower fronds. The opening inflorescence (no. 9) was completely necrotic. Dissection revealed the unopened inflorescences (Nos. 8, 7, 6, 5) to be necrotic.

4. PCR diagnosis analyses in Montpellier

4.1. Laboratory work

4.1.1. Extraction of nucleic acids

On arrival in Montpellier, the samples were cleaned, cut up and conditioned.
Nucleic acids were extracted by the CTAB method used by Daire *et al.* (1992), with precipitation in isopropanol. The nucleic acids were recovered in water. They were then quantified on a spectrophotometer and used for PCR (Polymerase Chain Reaction) analyses.
Nucleic acids from the pentatomid bugs were extracted either with CTAB, or with the Qiagen DNeasy Blood and Tissue Kit.

4.1.2. PCR

In the search for phytoplasmas, two sets of primers were used for direct PCR analyses:

- primers P1/P7, known as "universal phytoplasma" primers, which amplify the RNA 16S gene and 5' end of the 23S of all phytoplasmas
- Rohde primers, which are considered as "specific to coconut Lethal Decline in Tanzania" (LDT) (Rohde *et al.* 1993)
- primers G813/GAKSR used for diagnosis of LY in Ghana (Dollet *et al.* 2006) for nested PCR analyses.

4.1.3. Cloning, sequencing and RFLP

The PCR products were cloned, depending on their size, using the TOPO-TA Kit (Qiagen) or the pGEM-T Vector System Kit (Promega), then sequenced.
Lastly, the different amplification products obtained by RFLP were compared using restriction enzymes Bam HI, Dra I, RsaI and TaqI.

The results are given in the following table:

	Primers P1/P7	Rohde-LDT primers	Sequencing result
Source of samples			
Quelimane	+ and -	-	LYM
Mecufi	+	-	LYM
Palma	+	-	LYM
Nanjaba	+	+	LDT

4.2. Results in Zambezia

For the 2 palms sampled at Quelimane in Zambezia, only the first gave an amplification product with P1/P7. Once cloned and sequenced, that product revealed a sequence very similar to those obtained in 2003 on other samples from Quelimane. Those sequences were also very similar (99%) to those of the phytoplasmas associated with coconut LY in Ghana, known locally as Cape Saint Paul Wilt. It was therefore indeed a case of "Lethal Yellowing", the name given to the pathological syndrome of coconut palms, found at Quelimane since the beginning of the 1990s. These sequences fall into phylogenetic group 16S rRNA IV, a group containing all the phytoplasmas associated with various pathological syndromes of coconut having frond yellowing in common. The disease, currently spreading at the end of 2007 at Quelimane, is therefore caused by the same "strain" of phytoplasmas as that which was rife in 2003, there has not been any variation.
As for the second palm, which gave negative results, either this was a case really in the very early stages and the phytoplasma concentration was too low, or it was not a case of LY. For the first hypothesis, it needs to be remembered that the incubation period for this type of disease is very long (probably 6 to 9 months). Moreover, it can be imagined that in this type of pathology with phloem-restricted microorganisms, the yellowing symptom occurs as soon as the sap has been exhausted by the parasites. It would therefore be at that moment that the parasite concentration would peak, or start to peak. That is, in any case, what happens – this has been checked – for another coconut and oil palm pathology due to another phloem-restricted parasite (hartrot of coconut and marchitez sorpresiva of the oil palm). If this coconut palm was not affected by LY, it is an alarm signal in the strategy of rapidly eradicating cases of LY that currently prevails in the Madal estates. Indeed, care must be taken not to fell a healthy coconut palm and the neighbouring palms due to wrongful assumption of LY.

4.3. Results in Cabo Delgado

4.3.1. Mecufi

- Coconut

For PCR analyses, the coconut sample from Mecufi was amplified with primers P1/P7, but not with the LDT primers. The sequence of the 16S-23S gene obtained showed that LY was involved, a sequence very similar (99%) to those obtained at Quelimane. LY cases were therefore indeed involved, as the symptoms observed in this hamlet suggested.

-Bugs

The nucleic acids of pentatomid bugs gave direct PCR amplification products with P1/P7, but in small quantities, and in Nested PCR with primers G813/GAKSR. (Figs. 35 and 36). At the time this report was drafted, no sequence had yet been obtained, but the amplification obtained in Nested PCR with the specific primers of LY in Ghana suggested that these bugs carried LY phytoplasmas, the same as those of the coconut palm on which they were found. As the palm was affected by LY, those bugs could have taken up the phytoplasmas while feeding and it could be those phytoplasmas ingested by the bugs (in the food bolus) that we detected.

However, our experience with a very large number of PCR analyses carried out on insects in Ghana would suggest that the phytoplasmas were in quite a high concentration in the insect and could therefore have multiplied there systemically (not just the phytoplasmas in the food bolus). This bug is therefore a potential candidate for LY transmission in Mozambique, at least in Cabo Delgado province. This would be the first time that a bug has been acknowledged as a vector of phytoplasmas. And it is the first insect suspected of transmitting LY in Mozambique. Remember that, in Africa, the vectors of the various LY or LDT diseases remain unknown.

4.3.2. Palma

Five samples from the same palm were tested. They all gave amplification with P1/P7, and nothing with the LDT primers. The sequences obtained revealed that LY phytoplasmas were indeed involved (100% homology with West African LY).

4.3.3. Nanjaba

Five samples from the same palm in Nanjaba gave amplification products with both P1/P7 and the primers of LDT (Lethal Decline Tanzania), a disease considered to date to be confined to Tanzania. This result shows for the first time that "LDT" phytoplasmas are not restricted to Tanzania, but also exist in Mozambique. Consequently, two different phytoplasmas – but from the same group, 16S rRNA IV – can be associated in the same province with the same coconut Lethal Yellowing syndrome. This is not really surprising because political borders have never been barriers to epidemics and the passage of vector insects. However, it is a fact that to date all articles refer to LDT exclusively in Tanzania, and LY in Mozambique (Tymon *et al.* 1998; Mpunami *et al.* 1999).
RFLP analysis of all the amplificates obtained (Fig. 37) clearly showed that the restriction profile of the phytoplasmas from Nanjaba (LDT) differed from that of the phytoplasmas from Mecufi, Palma and Quelimane.

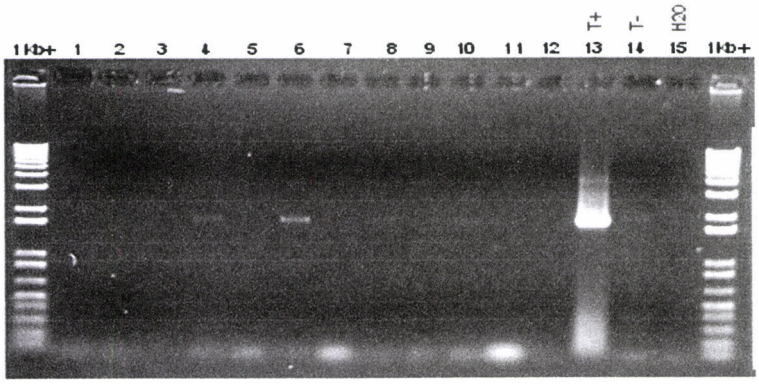


Fig.35. Direct PCR P1/P7
Lane 1 = Molecular weight marker 1Kb+
2-12 = Individual pentatomids DNA
13 = positive control
14 = Negative control
15 = Absolute control (distilled water)

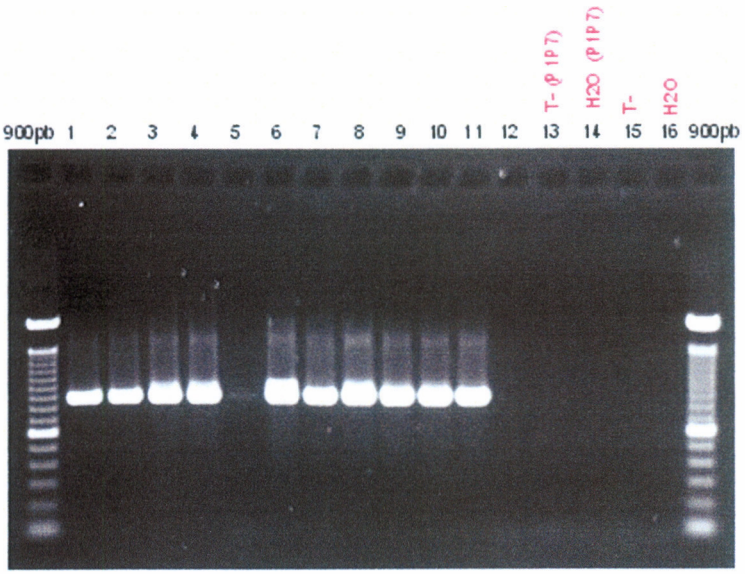


Fig.36. Nested PCR P1/P7 then G813/GAKSR
Same lanes as for Fig.35

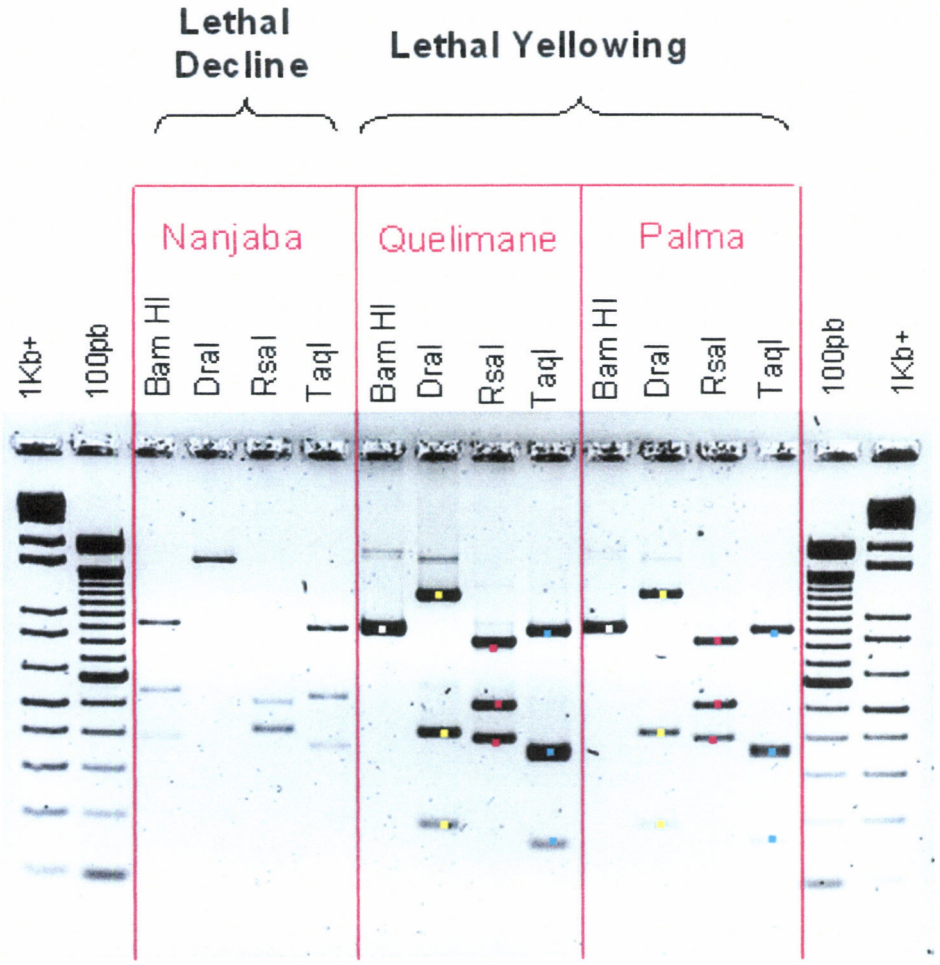


Fig.37. RFLP on the amplificates obtained from diseased coconut samples from Nanjaba.

Conclusions

Coconut Lethal Yellowing is continuing its devastation in Zambezia in the coconut plantings around Quelimane. Due to the crownless stems that it leaves, this disease encourages outbreaks of *Oryctes* beetles, which also cause some considerable damage. In order to solve the *Oryctes* problem, the Madal Group has embarked upon rapid eradication of coconut palms affected by LY and of the neighbouring palms that could be in the incubation period. This is undoubtedly a very good initiative. However, the results of PCR analyses carried out in Montpellier show that, while it is important to fell palms affected by LY, it would be a pity to fall into the "preventive excess" of felling palms that display somewhat dubious symptoms but which are not affected by LY. Consequently, yes to eradication but only when the palms display clear, unarguable LY symptoms.

The phytoplasmas diagnosed in 2007 are identical to those studied in 2003 at the same plantations. But as only one sample was sequenced this year it is impossible to draw any final conclusions regarding current variability. Remember, however, that in 2003-2004 (samples taken in November 2003 and October 2004), if very little variability was found, the same "strain" was always involved.

In Cabo Delgado province, a visit was made to some estates equivalent to the Madal estates in Zambezia. It is therefore possible to make a real comparison between LY syndromes and the damage they cause in these 2 provinces.
In coconut smallholdings, no devastation was seen due to LY. The only estate seen at Cabo de Ulo near Macimboa da Praia was an old abandoned plantation. All the coconut palms were suffering from senility, severe deficiencies and *Oryctes* damage. It cannot be confirmed that there were no cases of LY in that plantation, but there were many other reasons why the coconut palms were dying.

However, cases of coconut "Lethal Yellowing" diseases do exist in Cabo Delgado province.
Cases of mortality were seen nearly everywhere in the villages – never more than two or three palms in the same "focus" after yellowing and, according to information from the villagers, after all the nuts fell two or three months earlier. Our PCR analyses, followed by sequencing, showed that LY (the disease already reported in Zambezia) does exist in Cabo Delgado, both in Pemba and in Palma (North and South of the province).
It would have required more time and visits to more, larger plantations to really judge the extent of LY in the province.
For example, we were told that there are some coconut estates in the Quirimba islands where cases of "Lethal Yellowing" diseases occur sporadically but the foci appear to be contained by rapid and regular eradications.

In the great majority of cases, in the villages, what was seen suggests the "Lethal Decline Tanzania" (LDT) type syndrome that is currently rife in Kenya south of Mombasa or in the North of Tanzania (which was visited in June 2007) rather than the Lethal Yellowing of Zambezia, which is much more devastating (rapid, step-by-step progression, creating large disease foci). At least in one case (Nanjaba), this impression was confirmed by PCR analyses followed by sequencing or RFLP. At Nanjaba, the samples taken harboured phytoplasmas that were considered to date to be solely associated with the LY syndrome of Tanzania and Kenya, known as Lethal Decline Tanzania (LDT). As our sampling was very limited, it can be imagined that LDT phytoplasmas exist in places other than Nanjaba, which is located around

200 km from the Tanzanian border as the crow flies. Between Nanjaba and the border, there are coconut plantings of various sizes. It is very interesting to see that the samples taken closest to the border, at Palma - around 30 km from the border – are, however, of the LY type as in Zambezia (map 2). It is therefore highly likely that the LY phytoplasmas (of the Zambezia type) also exist in Tanzania, at least south of Dar-es-Salaam. The existence of two different phytoplasmas on coconut in Tanzania might explain the differences found for a long time in the evolution (propagation rate among other things) of coconut Lethal Yellowing syndromes in that country (Schuiling et *al.* 1992). The difference could come from the phytoplasmas themselves or the difference between their vectors. Discovery of a pentatomid bug carrying LY phytoplasmas in one palm at Mecufi opens up new horizons in the epidemiology field. By analogy with what is known about coconut hartrot transmitted by a pentatomid bug (Louise *et al.* 1986), it can be imagined that a phytoplasma disease transmitted by those bugs would cause epidemics spreading more slowly than a phytoplasma disease transmitted by insects of the leafhopper or planthopper or psyllid (Auchenorrhyncha) type, as is generally the case.

Recommendations

Coconut is a crop that has retained all its agronomic, economic and cultural interest the length of the Mozambican coast, especially in Cabo Delgado province between Pemba and Palma, on the shoreline, but also in many villages inland. It is therefore necessary to halt the development cycle of *Oryctes* which causes considerable damage.

Oryctes and old coconut palms; eradication

- First of all, measures need to be taken for the systematic eradication/burning of palms that have recently lost their crowns for various reasons (LY or senility or lightning strike, etc.)
- The second priority is to eradicate all old coconut palms (60 years and older) which are at the irreversible tuft stage (very few small fronds, including two or three yellow-brown lower fronds and the stem culminating in a "pencil point" - see Figs. 14, 15 and 16). In these extreme cases, as at the Lusoma estate in Cabo de Ulo, a plantation that is over 50 years old at least (maybe sixty?), abandoned for more than 7 years, it is best to fell everything.

To be effective, such eradication calls for technical resources (vehicles, chainsaws, GPS), along with human resources for chopping and burning (it is very important to keep an eye on fires to prevent their getting out of control).
Eradication of old trees –not yet affected by *Oryctes* damages- could be self-funding, at least partially, if Cabo Delgado province (but this can also be done in the coastal provinces in the North - with Nampula, the nearest, or with Zambezia) took the initiative to develop workshops using coconut wood to make floorboards, timber, furniture, various handicrafts, etc. An estimation of the number of coconut palms in each district that are over 50-60 years old, spindly, in a pencil point, would make it possible to identify where such workshops would be set up. In fact, as the roads are not in very good condition, transport over long distances can hardly be envisaged. Such a policy was successfully launched recently in Kenya.

Lethal Yellowing and Lethal Decline Tanzania

- It seems important to determine rapidly the relative importance of the two phytoplasmas, LDT and LY, in the North of Mozambique and the true extent of their dissemination. It is all the more important because it is perfectly possible that the two diseases might have two different vectors, hence two different epidemiologies (e.g. different reservoir plants). It is also important for genetic control: a given variety of coconut palm might be resistant to LDT but not to LY.
For example, what is the situation in all the Quirimba islands, the entire region between Pemba and Mucojo, between Mucojo and Mocimboa da Praia?
But the study also needs to extend to the province of Nampula, since the positive LY sample from Mecufi was at the northern limit of Nampula. It is known that this type of pathology can flare up in a region for as yet unknown reasons, and it then becomes difficult if not impossible to confine its spread. What is today only a small disease focus at Palma or Mecufi may tomorrow become an intense propagation zone, as in Zambezia. The existence of these pathologies, once clearly identified (PCR possibly followed by RFLP or sequencing) would, in fact, deserve regular monitoring with the eradication of coconut palms as soon as they display the first symptoms.

Replantings

- If coconut palms are to be replanted after felling old or diseased palms, it is important to do so with seednuts of the most appropriate varieties for the local situation. Given the drought persisting in northern Mozambique, it is necessary to plant "rustic" varieties, at least outside villages. It is clear that the "East African Tall" (including the Mozambique Tall) coconut palms are undoubtedly the most appropriate. Variety performance trials in relation to LDT have been carried out in Tanzania. Several East African Tall populations in Tanzania and Kenya seem to perform well in relation to LDT. It remains to be seen how they react to LY. However, planting inside a zone, or very near a zone, affected by a developing Lethal Yellowing type syndrome must absolutely be avoided. Whatever the coconut variety or population, it can be imagined that everything will be destroyed sooner or later if there is strong inoculum pressure (Lebrun *et al.* 2007).

- For the moment, the simplest thing for Cabo Delgado province, and the least risky, would be to replant small areas of smallholdings regularly with seednuts from the province. In fact, there exist in the villages some very handsome palms (Local Tall type) that could be used as parents. There also exist in the villages some Red and Yellow Dwarf palms which, even though they show some signs of suffering from drought, seem to be overcoming it (provided large holes are not made in their stems!).

- As an empirical precaution, it seems preferable for the moment not to introduce any seednuts from Zambezia where the LY type syndrome is virulent.

- At the same time, the longer-term future needs to be prepared for. It would therefore seem important to continue analysing the genetic variability of the coconut palms in Cabo Delgado province (Baudouin *et al.* 2007) and see the correspondence with their reaction to the disease. A comparison with "LDT-tolerant" populations in Tanzania would be of great interest. It is highly likely that there are different types of "Local Tall" palms derived from introductions at different times, from different origins. CIRAD has developed molecular tools for establishing the genetic identity map of coconut varieties and could undertake this work in a minimum of time. A local selection plan would then be drawn up.

- For the long term, it is also important to test a wider range of genetic origins. For instance, in Ghana the Vanuatu Tall (VTT) and Sri Lanka Green Dwarf (SGD) varieties seem to be less susceptible to LY than all the other varieties to date. A seed garden of SGD x VTT hybrids has been planted in Ghana. As the LY phytoplasmas in Zambezia are identical to those of LY in Ghana, it can be imagined that this hybrid could be a component in the integrated control of LY wherever it occurs. However, its reaction to LDT is unknown, hence the utility of possessing a distribution map for the 2 phytoplasmas. There may be a difference in response either to the phytoplasmas or to their vector, if they are different.

- For Zambezia, in collaboration with CIRAD, under an AFD project, two variety performance trials have been set up on the Madal estates. The trials were planted in March-April 2007. It is now necessary to be patient and to wait for the inoculum to reach those plots to find out which varieties might be of interest in controlling LY. In the meantime, it is essential to ensure the upkeep of the plots in the early years, particularly with regard to drought – a more effective solution needs to be found for watering – and with regard to the different insect attacks and diseases affecting immature palms.

Based on the observations made at Quelimane, other trials to test the "colour" parameter (green, yellow, or brown nuts) in hybrid creation were proposed by Bourdeix in 2006.

- Search for the vector(s)

The only durable solution for controlling LY and/or LDT syndromes will be the integrated protection of coconut plantings. Planting less susceptible varieties will only give temporary results. Another way of preventing these diseases from causing their devastation would consist in preventing vector insects from acting. To do that, of course, it is necessary to identify them, then study their biology, their behaviour and their natural enemies. This is definitely not an easy task (cf. Philippe *et al.* 2007). However, knowledge of the vector would be a new key in controlling these diseases. In addition, identification of the vector(s) would make it possible to test coconut varieties or populations over a short period (by inoculation in cages) rather than waiting years for the inoculum to arrive in the field trial.

-Putting a stop to superstitious practices?

It was seen in the North of Cabo Delgado province that villagers, through superstition, made large holes in the base of coconut stems. As the coconut stem largely consists of phloem-ligneous vessels that serve to transport water and sap, those holes are highly prejudicial to the palm's water supply. Palms with such holes seem to suffer more than the others from drought. It seems important to embark on an information drive asking villagers to stop these harmful practices.

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