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Département du Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)

GHANA - FRANCE - COTE D'IVOIRE

RESEARCH PROJECT ON THE WESTERN REGION COCONUT DISEASE

REPORT ON THE MISSION BY MR. J.F. JULIA, IRHO ENTOMOLOGIST (24th June - 7th July 1989)

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I - INTRODUCTION - PURPOSE OF MISSION

This mission was essentially devoted to the entomological aspects of Cape Saint Paul Wilt disease, also known as "Western Region Coconut disease", which is a type of coconut lethal yellowing associated with a mycoplasma. Hereafter, we shall refer to this disease as LY.

During the visit by Messrs. G. de Taffin and H. de Franqueville to Ghana (12 - 18/3/89) a Homoptera Cixiidae type insect was seen in the new LY focus at Ayensudu, in the Central Region (see IRHO Document No. 2186, June 1989). This insect has now been identified as *Myndus adiopodoumeensis* described as being from Côte d'Ivoire, a country where LY does not occur, and also known in Nigeria.

Cixiidae have nymph sites at ground level or underground on the collar or roots of plants, which, for numerous species, differ from those frequented by adults. This family contains several species which are virus and mycoplasma vectors, including *Myndus taffini*, the vector of FDMT (viral disease of coconut in Vanuatu, South Pacific) and *M. crudus*, the vector of Lethal Yellowing in Florida and Jamaica (mycoplasma disease very similar to LY in Ghana).

In addition to the fact that it belongs to a group of insects that transmit mycoplasma diseases, the outbreak of *M. adiopodoumeensis* within a one-off, newly occurring focus perfectly isolated from the other LY foci, suggests *a priori* that this insect is possibly and even quite probably a vector of this disease.

However, it is essential to prove this experimentally to be absolutely certain.

The detection of an insect as the vector of a disease is essential for acquiring an understanding of the disease's epidemiology, for studying any control possibilities and attempting to develop cage tests to rapidly establish the susceptibility of numerous cultivars and crosses.

The purpose of this mission could thus be defined as follows:

1. Check the soundness of the hypothesis that *M. adiopodoumeensis* is an LY vector in Ghana.
2. Check whether other insects could be considered as potential vectors.
3. Acquire a minimum of basic knowledge about *M. adiopodoumeensis* in particular and, if possible, discover the nymph sites.
4. Draw up a research project, mainly based on obtaining LY symptoms by the controlled introduction of insects in cages containing healthy coconut seedlings at the susceptible stage at the beginning of the experiment.

II - MISSION SCHEDULE

24/6 Paris-Accra. Met by Messrs. E.D. Arkhurst and R. Huguenot.

25/6 Free in Accra.

26/6 Morning

Initial meetings, accompanied by Mr. E.D. Arkhurst, Project Manager, Ghana-France-Côte d'Ivoire Project, the with following people in order:

- Mr. J. de Mones, Cultural Attaché, French Embassy,
- Mr. E.S. Otinkorang, Ag. Director, Crops Services Department
- Mr. K. Kyei Antwi, Plant Protection and Regulatory Service Department Officer.
- Commodore S.G. Obimpeh, PNDC Secretary for Agriculture.

Afternoon

Trip to Takoradi with Mr. E.D. Arkhurst and meetings with the following people in chronological order:

- Dr. D.O.C. Atuora, Director of Agriculture, Central Region, Cape Coast,
- Dr. F.J. Abu, PNDC Deputy Regional Secretary for Agriculture, Western Region,
- Miss Hilda Tamakloe, Director of Agriculture, Western Region.

Late afternoon: meeting with the Franco-Ivorian mission, including Messrs. M.A. Fataye, entomologist at the Marc Delorme Station, H. de Franqueville, phytopathologist at the Dabou Station and their guide Mr. Madi Ouedraogo from the Marc Delorme Station.

27/6 Visits to disease foci at Funko, Busia Town, Dixcove (including the performance trial) and on the road from Princess Town to Ben Akosi.

28/6 Joined by Messrs. K. Kyei Antwi and P. Fiscian, entomologist from Cape Coast University, visits to the Aiyanasii seed garden and the focus zone at Agyembra.

29/6 Visit to Ayensudu focus, near Elmina in the Central Region.

30/6 Visit to Pumpuni focus. Departure of the Franco-Ivorian team late morning. Additional visit to the Funko focus.

1/7 Visit to Princess Town focus (including performance trial) and visits to performance trials, outside the foci, at Dadwen and Agona Junction.

2/7 Free in Takoradi

3/7 Classifying notes. Sorting of the insects collected. Survey of coconuts and oil palms near Animens Hotel.

4/7 Visit to Ayensudu focus, occasional surveys between Ayensudu and Sekondi. Work discussions with Dr. D.O.C. Atuora at Cape Coast, then with Dr. F.J. Abu at Sekondi.

5/7 Visit to Bakado focus near Sekondi, then return to Accra.

6/7 Rapid return trip Accra-Volta Region, tour round Anloga performance trial near Cape Saint Paul.

7/7 Discussions in Accra with His Excellency, Mr. Auchère, French Ambassador, His Excellency Mr. Konan N'Da, Ivorian Ambassador, Mr. B.K. Hama, Deputy Director, Crops Services Department, Mr. E.S. Otinkorang, Ag. Director, Crops Services Department, Mr. R.S. Owusu Appiah, entomologist Oil Palm Research Institute, Mr. François Tobokoué Koyeman, Cultural Attaché, Côte d'Ivoire Embassy and Mr. J. de Mones, Cultural Attaché, French Embassy.

III - VERIFYING THE SOUNDNESS OF THE HYPOTHESIS THAT *MYNDUS ADIOPODOUMEENSIS* IS A VECTOR OF LY IN GHANA

In order for a disease transmitted by an insect to be expressed in a susceptible plant, the pathogen and the vector have to be present and active at the same time. The example of *Myndus crudus* is particularly indicative in this respect. The insect is present in all the LY foci in Jamaica, Florida and Mexico, but it is also found in healthy zones, as in Mexico and in very distant countries which have been completely spared, such as Lower Amazonia in Brazil. In these healthy zones where, *Myndus crudus* outbreaks can occur on varieties susceptible to LY, it is obvious that the pathogen is either absent or latent.

If *Myndus adiopodoumeensis* is the sole LY vector in Ghana (if not, it is highly probable that the other vector is also a cixiid), it can be thought, on the one hand, that it may be present in healthy zones (as in Côte d'Ivoire) and, on the other hand, that it is necessarily present in all the LY foci.

However, detection of this type of insect is not always easy, since it can be quite rare or there may only be occasional outbreaks and it may prefer to remain principally on the higher leaves of adult coconuts. In order to check that this *M. apododoumiensis* hypothesis is sound, the insect has to be found without too much difficulty in easily observable sites (with small trees) within active LY foci.

The observation and capture of a single adult is enough to prove the insect's presence, but a negative result only indicates that it was not possible to see it under the precise observation conditions laid down.

We obtained the following results in focus zones:

1) a) Funko - 1.6 km away coming from the junction and 200 m to the left-hand side - survey negative.

   b) Funko - 2.7 km away coming from the junction and a few metres to the right-hand side - survey positive with insects in several trees.

   c) Funko - along the road between the previous two sites, survey positive on 20% of the trees.

   It can therefore be considered that the insect is present in the surveyed zone of the Funko focus.

2) Busia Town - At the edge of the road, on the right-hand side, 1.8 km before the village. 4 insects seen on a dozen trees - survey positive.

3) Dixcove performance trial - survey positive - 5 insects captured on around 50 surveyed trees.

4) Agyembra road, place known as Aduano. Positive result, but only one insect seen and captured after a 2-hour search.

5) Pumpuni - on the edge of the LY front. Negative result after searching for several hours.

6) Princess Town performance trial - Positive result, 5 insects seen and captured on the fifty or so trees surveyed. This site is an old focus, with no living coconuts in the immediate vicinity of the performance trial where the young coconuts have no symptoms, but there is an active focus very close by.

7) Ayensudu - Positive result after a *M. adiopodoumeensis* outbreak on adult and young coconuts, and on oil palm (around a hundred individuals per tree).

8) Bakado focus, near Sekondi - Positive result, 1 insect found after a 20-minute search.

9) Anloga performance trial, near Cape Saint Paul - Negative result, but this site can no longer be considered as a focus.

These survey results are given in Annex I.

Hence, only one LY focus site was found to be without any detectable *Myndus adiopodoumeensis* under our observation conditions. The insect was therefore seen in 7/8 or 86% of the sites observed inside or in the immediate vicinity of active foci. Its presence in all the LY foci in the region is therefore considered to be highly probable. It was only at the Ayensudu site that an actual outbreak was seen, for the obvious reason, as we shall see later, that this site is also a substantial nymph site from which the adults emerging are no doubt capable of flying great distances.

The results of this survey in LY foci therefore confirm the soundness of the hypothesis put forward as to the role of *M. adiopodoumeensis* as a possible disease vector and justify the setting up of transmission experiments in cages as soon as possible.

IV - BRIEF OBSERVATION OF OTHER INSECTS THAT MIGHT BE SUSPECTED OF TRANSMITTING LY IN GHANA

It is obvious that a brief survey lasting just a few days and mainly concentrating on a single species will not provide a valid inventory of the homopteran entomofauna frequenting coconut in a coastal region over 100 km long.

Nonetheless, whilst searching for *M. adiopodoumeensis* we also observed the other homopterans and attempted to capture the most interesting ones. Although the search was made in a period favourable to these insects (end of the rainy season), they seemed to us to be neither very varied, nor very numerous. The Derbidae, a family which, to date, contains no known disease vector, make up the great majority of the insects present. Apart from a small yellow species, also found in Côte d'Ivoire, we saw very few Cicadellidae. There are, however, one or more *Recilia* species, which should not be overlooked, despite their apparent rarity, because Blast, a mycoplasma disease in young oil palms and coconuts in Africa, is transmitted by *R. mica*.

In addition, we quite often encountered a species of Dictyopharidae, but the other interesting families (Delphacidae, Cercopidae) did not seem to be present on the trees observed.

If *Myndus adiopodoumeensis* had been absent, we would probably not have been led to seriously suspect any other species. Nonetheless, if an experimental programme is conducted with *Myndus*, it will also be necessary to test the other homopterans seen on the coconuts in foci, to ensure scientific consistency, even if this only involves one treatment with a mixture of these species.

V DISCOVERY OF A SUBSTANTIAL *M. ADIOPODOUMEENSIS* LARVA SITE AND THE SEARCH FOR ADULT INSECTS OUTSIDE LY FOCI - INITIAL GLIMPSES OF THE INSECT'S BIOLOGY

At Ayensudu, at the very centre of the focus, the natural vegetation is basically made up of Guinea grass, *Panicum maximum*. There is also sugarcane and maize growing, along with other grasses. We took root samples from these plants and from a coconut palm. It was exclusively on *Panicum maximum* in a marshy area that we found egg-laying, larva, and some adult sites typical of Cixiidae, in abundance, on virtually all the plants in the zone observed. All indications point to *M. adiopodoumeensis*. We asked our colleague, P. Fiscian, to isolate a few plants with larvae in a cage, so as to observe the emergence of adults and confirm their identification.

Thereafter, we directed our *M. adiopodoumeensis* surveys to healthy sites, preferably with coconuts near to *Panicum maximum* patches in marshy areas.

The results are given in annex I. It is obvious that the presence of the insect is linked to that of *Panicum maximum* in marshy areas. This is especially the case at Ayensudu, Dompuasi and Sefwi, where there are *M. adiopodoumeensis* outbreaks.

We were unable to return to each of the focus sites surveyed before the discovery of nymphs at Ayensudu to look for larva sites. The insect is most probably capable of flying several hundred metres, especially with the wind behind it. *Panicum maximum* far away from marshy areas must be less propitious for the establishment of nymph sites and there must also be other factors, maybe biotic (larva predators?), that can limit nymph populations in certain marshy areas.

It should be noted that in two foci which are still very limited - Sekondi and Ayensudu - the centre of the focus is marshland with *Panicum maximum*.

An investigation we made in the Cape Saint Paul region revealed that the shore of the lagoon, which is totally cultivated and free of *Panicum maximum* formerly consisted of numerous marshy areas propitious to this plant.

It is not impossible that other grasses could constitute larva sites. This point, along with the biotic and abiotic factors likely to limit larva outbreaks should be carefully examined.

VI - RESEARCH PROJECT ON TRANSMISSION OF THE MYCOPLASMA CAUSING LY BY AN INSECT VECTOR, PAYING PARTICULAR ATTENTION TO *M. ADIOPODOUMEENSIS*

This is a simple project aimed at proving disease transmission, initiating the study of *M. adiopodoumeensis*, which is currently greatly suspected of being the vector, and verifying the presence of the mycoplasma, particularly in the plants that undergo transmission trials. This project is due to take two years and could then be replaced by a more in-depth national or international project, particularly with respect to mycoplasmology and experimental research into LY-tolerant coconut varieties.

VI.1 Transmission Experiment using Insects introduced into Cages

a) General Conditions and Choice of Site

The purpose of this experiment is to obtain disease symptoms, then isolate the pathogen under artificial and controlled conditions.

In order to achieve this goal, healthy test plants are required at the outset, of a sensitive variety at the disease susceptibility stage, along with a large number of insects that could have acquired the pathogen and capable of transmitting it.

There is a very common susceptible variety in Ghana, the WAT. However, trees under 6 years, whilst frequented by similar fauna to that on other trees, do not express LY symptoms in Ghana. It is not known whether these immature trees are immune or whether the mycoplasma remains in its latent state, in incubation.

It is therefore highly probable that using young plants only, as was the case in the trials conducted on numerous diseases (Blast, Dry Bud Rot, LY in America, FDMT and Hartrot), is not absolutely suitable for obtaining LY symptoms in Ghana.

Consequently, although this type of trial is much more uncertain, difficult and costly to undertake on yielding adult trees, it is trees at this stage that will have to be worked on in the main.

As it is also necessary to have maximum guarantees as to the non-infected state of the test plants at the beginning of the experiment and in view of the fact that LY can spread rapidly (up to 3 km per year in the Western Region), the trial should be located 3 km from the nearest advancing LY front, at a site which is absolutely disease-free. In order to ensure a good survival rate of the insects being handled, a site over 10 km from the advancing disease front should not be chosen. Precautions will be taken, but the risks of creating a small disease focus cannot be totally excluded.

On the right-hand side, 6.2 km from Ayensudu and around 600 m before Akawi Town, we located a suitable site for this type of experiment. It is a small orchard with numerous trees starting to bear. There is little or no *Panicum maximum* in the immediate vicinity. The very poor weather when we were there prevented us from carrying out a proper survey to detect the presence of *M. adiopodoumeensis*.

If the current focus does not advance too quickly in this direction, this site could be used.

b) Experiment Protocol

Transmission trials will be conducted in different types of cages.

Details of the experiments to be conducted will be proposed at a later date.

VI.2. Study of *M. adiopodoumeensis* biology

This part of the programme will only become essential once it has been proved that this insect is the vector and it should be abandoned if trials show that a different insect is to blame. Given the probability of LY transmission by *Myndus*, and in order to save time, this study should be started at the same time as the transmission trials.

VI.2.1. Field Observations

- Search for nymph sites around the Ayensudu focus, in other focus zones and in very heavily infested healthy sites, such as Dompuasi and Sawfi.

This research will include the examination of other grasses, especially *Panicum*, likely to serve as a larva site. Attempts will be made to characterize site ecology and to draw up an inventory of any parasites or predators which would limit nymph populations or prevent their establishment.

VI.2.2. Breeding Trial on *Panicum maximum* on Trays in Cages

Initially, orientation tests could be carried out in the actual focus at Ayensudu in small cages placed inside a large cage 5 m x 6 m x 2m constructed on site with 600 µ mesh material in the immediate vicinity of a permanent water supply. The purpose of these tests will be to acquire general knowledge about the insect's development cycle (pre-oviposition, egg-laying potential, incubation time, duration of larva instars and immaturity periods, then adult fertility).

The second purpose of the trial, in a subsequent project, will be to develop a mass breeding technique which could be very valuable, both for LY susceptibility tests on coconut varieties and crosses and for mycoplasma studies (acquisition, transmission, persistence, etc.).

VI.3. Mission by a virology-mycoplasmology specialist

This visit should make it possible to increase our very limited knowledge on the mycoplasma responsible for LY. It would be wise for it to take place as soon as disease transmission has been confirmed in cages.

The aims of this visit would be as follows:

a) Confirm the existence of the mycoplasma in diseased plants (in the field and in cage trials).

b) Search for the mycoplasma on healthy plants exposed to LY (young coconuts and oil palms in focus sites, young plants exposed to the vector in cages where transmission took place).

   If it is proved that the young coconut plants are healthy carriers, this could be taken into account in tolerance tests.

c) Draw up a future research programme to study mycoplasma acquisition, transmission and characterization.

VI.4. Resources required

a) Human resources

We propose that a Ghanaian project manager be placed in charge of the general administration of the project and liaison with partners from the Ministries of Agriculture and Research. We suggest that Mr. ARKHURST, who knows the terrain very well, be in charge of the project, given that this new responsibility should not be too onerous. A French entomologist will be responsible, with his Ghanaian counterpart, for programme implementation in the field and staff supervision. The French entomologist will be a qualified National Service Volunteer (VSN).

The Ghanaian counterpart and the VSN will take a fortnight's training course in Côte d'Ivoire, where studies are being carried out on vectors and the transmission of juvenile diseases (Blast and Dry Bud Rot). The French entomologist will devote all his time to the project. The Ghanaian entomologist's programme will depend on the person chosen. We propose either Mr. Fiscian, from the University of Cape Coast, or Mr. C.J.B. Babe (VORADEP), who has already followed a training course in Côte d'Ivoire on vector insects (1984).

Support missions, lasting around ten days each, will be undertaken every two months by the Head of the Entomology Service at the La Mé Station in Côte d'Ivoire.

A mission by an IRHO virologist is planned under this project (fortnight). Allowance should also be made each year for a two-week visit by the Director of the IRHO Entomology Division.

The supervisor, with experience in the field and able to write up a report, preferably resident in Ayensudu. The labourers must live in Ayensudu, so as to simplify transport problems. Their time will be devoted exclusively to the project.

b) Equipment and facilities (suggestions)

Housing

The IRHO specialist will be housed in Cape Town in a fully furnished and equipped apartment or villa with living room, kitchen, laundry room-storage room, bathroom and toilet and air-conditioned bedroom.

The counterpart will necessarily be resident in Cape Town.

Vehicles

The IRHO specialist and his counterpart will have a service vehicle, enabling the transportation of a dozen or so people (Four-wheel drive pick-up). The supervisor will have a motorbike. The project will cover the transport costs incurred by the project manager whilst working on the project (10,000 km/year)

Office-Laboratory

The team will be provided with a work room in Cape Town. It should be 30 m^2, to take 3 desks, 6 chairs and office armchairs and, if possible, there should be 1 m working surface in one corner, with a sink.

Binocular Magnifyer

For insect recognition and dissection, the team will have a binocular magnifyer (magnification: 5 to 50), with a 10x lens attachment and a 20x lens attachment, along with a device for slide and lateral lighting, and the transformer. 4 sets of spare lightbulbs will be required.

Small items of laboratory and field equipment

Miscellaneous constructions and small items of equipment in the field

We have mentioned the need to construct a shelter from planks, with a corrugated tin roof in the field, along with a large breeding cage. A cage drawing is given in annex III. Small items of agricultural equipment are listed in annex IV.

VII - CONCLUSION

The mission undertaken by the IRHO Entomologist, Mr. J.F. Julia made it possible to confirm the soundness of the hypothesis that Coconut Lethal Yellowing in Ghana is transmitted by a cixiidae insect, *Myndus adiopodoumeensis*.

This insect belongs to a family and a genus well known as virus and mycoplasma disease vectors, especially on coconut.

Myndus adiopodoumeensis can be considered to exist in all the active focus zones. It can also be found in healthy coconut groves. Hence, the foci would appear to depend, as in the case of LY in America, on the presence and the activity of the mycoplasma responsible, which this insect transmits and propagates.

Myndus adiopodoumeensis is associated with Guinea grass, *Panicum maximum*, and, perhaps, with other grasses. The nymph sites are mostly, or even exclusively, found in certain marshy areas with *Panicum maximum*. However, indications are that adults are capable of flying quite large distances (up to 1 km?), especially with the wind behind them).

The two most isolated LY foci seen during the visit are in marshy zones with *Panicum maximum*: Bakado near Sekondi (little *Panicum maximum*, not very active focus) and especially Ayensudu (a great deal of *P. maximum* and a very active focus).

Much circumstantial evidence therefore points to *Myndus adiopodoumeenis* as being a potential LY vector. This justifies setting up a research project to prove vector transmission experimentally, whilst checking the possible role of the rest of the homopteran entomofauna found on coconut. This project should also study the biology of *Myndus adiopodoumeensis* in the field and under breeding conditions, so as to examine the possibilities of controlling the potential vector and, at a later stage, the possibilities of developing a rapid method for assessing the LY tolerance of coconut varieties and crosses.

This project, on a Ghanaian scale, will terminate at the end of 1991, but it could be resumed or extended in the form of a more general project at national or international level. Relations between the countries concerned by this disease could then be considered.

ACKNOWLEDGEMENTS

We should like to thank all those mentioned in this report, for kindly giving up their time and demonstrating such great interest in this serious problem of Lethal Yellowing in West Africa. We are convinced of their determination to help us in our task.

We were particularly honoured by the meetings granted to us by Commodore S.G. Obimpeh, PNDC Secretary for Agriculture and their Excellencies Mr. Auchère and Mr. Konan N'Da, French Ambassador and Ivorian Ambassador to Ghana, respectively.

We are also most grateful to Mr. J. de Mones, Cultural Attaché at the French Embassy, for his warm hospitality, for the information he supplied and for the assistance he afforded us.

Finally, our sincere thanks go to Mr. Eric D. Arkhurst, project manager of the Ghana-France-Côte d'Ivoire project on coconut. His kind attention, devotion and perfect knowledge of the people involved and of the situation in the field enabled us to complete our mission under the best possible conditions.

ANNEX I : SUMMARIZED RESULTS OF *M. ADIOPODOUMEENSIS* SURVEYS WITH RESPECT TO ACTIVE LY FOCI AND THE PRESENCE OF *PANICUM MAXIMUM* EITHER IN A MARSHY AREA OR NOT

	Sites Surveyed	*Paspalum Panicum* not in marshy area	*Paspalum Panicum* in marshy area	Search for *M. adiopodoumeensis*
I	FUNKO	+	o	++
N	BUSIA	+	o	+
	DIXCOVE	+	o	+
F	AGYEMBRA	+	?	+
O	AYENSUDU	+	+++	+++
C	PUMPUNI	+	o	o
U	SEKONDI	+	++	+
S	PRINCESS	+	+++	+
O	AGONA JUNCTION	+	+	o
U	TAKORADI	+	+++	++
T	DOMPUASI	+	+++	+++
S	DABOASE JUNCTION	+	?	+
I	GRAPO KROM	+	+	+
D	YAMORANSA	+	+	+
E	AIYANASII	o	o	o
	DADWEN	o	o	+
F	SEFWI	+	+++	+++
O	CAPE SAINT PAUL	o	o	o
C				
U				
S				

? = no data available
o = search unsuccessful
\+ = presence detected
++ = quite abundant
+++ = very abundent