Study on the transmission of coconut Lethal Yellowing in Ghana

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Abstract: *Research on the Cape Saint Paul Wilt Disease (CSPWD) vector in Ghana began from 1990 (1990-1997; 2002-2004) and did not give convincing results. From July 2005, new test standards were applied: shading, daily collections and releasing of insects at the less hot hours and use of various sizes of cages and test plants. More than 70,000 Myndus. adiopodoumeensis were introduced in cage for 28 months (520 adults/seedling/month). Controls in polymerase chain reaction (PCR) on the five coconut of this Myndus cage and on 935 adults were always negative. The tests of transmission with M. adiopodoumeensis apparently not a vector of the disease were thus stopped. The phytoplasma of the CSPWD was identified by PCR in a coconut having received 4,380 Diostrombus (four species of Derbidae) 4 months after the beginning of the test. This coconut never presented symptom of the disease 28 months later and all the successive PCR were negative. Auchenorrhyncha collected by sweeping on the adventitious plants in and around the plot during the day were also tested without success. The hypothesis of a ground transmission was also taken into account because of the presence of scale insects and nematodes.*

Key words: *lethal yellowing, vector, Homoptera, Auchenorrhyncha, scale insects, coconut, Ghana*

Phytoplasmas are known to be associated with coconut Lethal Yellowing (LY). It is also known that all phytoplasma diseases are transmitted by leafhoppers (Membracidae, Cicadellidae), planthoppers (Delphacidae, Cixiidae, Derbidae, etc.) or Psyllidae [1]. These Homoptera Auchenorrhyncha [12-15] also play a role in transmitting viral diseases of palms [2-6].

To date, only the Cixiidae *Myndus crudus* has been experimentally identified as an effective vector of LY, and only in Florida [7]. Thus, it is only in Florida that it has been possible to detect the existence of the LY phytoplasma in the body of *M. crudus* through an electronic microscope. At that time, there was no check by polymerase chain reaction (PCR). Cloning and sequencing were not in practice. So, it is difficult to say that the phytoplasma observed was really that of coconut LY in Florida.

However, it has so far been impossible to confirm the role of *Myndus* as a vector in Jamaica or in Mexico.

In Tanzania, the Derbidae *Diostrombus mkurangai* Wilson and *Meenoplus* sp (*Meenoplidae*) are suspected by the sole fact that LY phytoplasma has been detected by the PCR technique [8].

Identification of vector insects by experimentally reproducing disease symptoms under controlled conditions is essential [9]. It will enable optimum application of all the researches to be undertaken for a better understanding of the epidemiology and for designing control methods.

We describe here the work being conducted in Ghana to search for the Cape Saint Paul Wilt (CSPW) vector.

History of disease transmission studies in Ghana

The first disease transmission studies were undertaken with World Bank funding from 1990 to 1992. A European Union project (STD 3) then took over from 1993 to 1997.

Starting in 1990, 18,000-20,000 *Myndus adiopodoumeensis* Synave (Cixiidae) and around 20,000 Derbidae and other Homoptera were tested in large cages (6.7 × 6.7 × 7.5 m) with one 6- to 7-year-old West Africa Tall (WAT) per cage. Some enigmatic results were obtained, which were not backed up by PCR tests at the time [10, 11].

Starting in January 1996, attempts were made in medium-sized cages to transmit the disease with the same species after spending 3 days inside a large sleeve on a diseased coconut palm. The aim was to check for the existence of the phytoplasma in the insects and in the nursery plants using the PCR analysis method. A PCR analysis laboratory was set up by DFID (ex-NRI) at the University of Legon (Accra). The nursery plants did not display any yellowing symptoms and the PCR analyses were negative.

From the end of 2002 onwards, an Agricultural Services Sub-Sectors Investments Programme (AGSSIP) budget made it possible to relaunch two series of disease transmission trials. The insects also underwent an acquisition phase. No positive PCR case was obtained. In the second series, a plant onto which all the Derbidae species had been released gave a positive PCR analysis. DNA sequencing showed that it was not that of the phytoplasma responsible for CSPW.

New transmission studies under project FSP 2004-34

The CSPW transmission trials began in July 2005 with new experimental conditions:

– Construction of a shade over each cage;

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– Insects were collected in the morning from 6 a.m. to 8 a.m. and in the evening from 4 p.m. to 5.30 p.m.;
– Those insects were released immediately into the respective cages after each of the two daily collections.

Material and method

Medium-sized cages

An initial set of five shaded cages (2.2 × 2.2 × 2.2 m) housed Myndus and Derbidae; All Homoptera that are collected with sweep net from the plant cover under the coconut palms and around the coconut plot; a control with shade; a control without shade – six 12 to 18-month-old nursery plants (3 Malayan Red Dwarf palms [MRD] and 3 Malayan Yellow Dwarf palms [MYD]) per cage; each plant was fitted with a sleeve.

A medium-sized cage was reserved for the small and large sucking insects collected at the two periods of the day using a sweep net in the herbaceous cover inside and around the plot.

Those insects belonged to two major groups of Homoptera: leafhoppers (Cicadellidae and Cercopidae) and planthoppers (Delphacidae, Achilidae, Meenoplidae), along with a few Heteroptera (Pentatomidae).

First series: Six nursery plants (three MRD and three MYD) aged 12 to 18 months per cage; each plant was fitted with a sleeve. From July to December 2005, it proved possible to release a total of 9910 insects, that is, an average of around 275 insects per plant per month.

Second series: Two plants were kept (one MYD and one MRD) from the previous six. One plant received only small leafhoppers, the other only large leafhoppers + Pentatomidae + Flatidae + Cercopidae *(table 1)*.

Third series: Two new plants were introduced (MYD × VTT = Vanuatu Tall) *(table 1)*.

Large cages

***Myndus adiopodoumeensis*[1]**

A large cage (3 × 3 × 3.5 m) was constructed to house five hybrid coconut seedlings (MYD × VTT) not covered with a sleeve.

***Derbidae*[1]**

Three large cages (4 × 4 × 4 m) housed, respectively, four species of *Diostrombus*: *D. mayumbensis*, *D. nitidus*, *D. luteus*, *D. dilatatus*; *Metaphenice stellulata* + *Patara armata*; one control cage. These insects were released onto three plants (MYD × VTT) over 2 years old, placed in each cage without sleeve.

Note: The cages have a red wooden or metal (angle iron) frame and they are covered with a polyester sleeve (Fyltis) with 600 or 800 µ meshes.

[1] Homoptera Auchenorrhyncha determinations were carried out by Jacques Bonfils, a retired entomologist, Pentatomidae and Cercopidae by the CIRAD faunistics laboratory at Baillarguet (Henri-Pierre Aberlenc & Jean-Michel Maldès)

Table 1. *Tests of Homoptera collected in sweep nets for lethal yellowing transmission on coconut palms aged 12–18 months.*

Species	**Jan–Oct 2006 (Total insects/ three plants/10 months)**	**Average insects/ month/plant**
Small Cicadellidae	4,801	160
Large Cicadellidae + Cercopidae + Pentatomidae + Flatidae	12,822	427
Total: Jan-Oct 2006	17,623	587
Species	**Nov 2006–Mar 2008 (Total insects/two plants/17 months)[a]**	**Average insects/ month/plant**
Small Cicadellidae	9,498	279
Large Cicadellidae	22,343	657
Pentatomidae	421	12
Cercopidae	3,435	101
Flatidae	275	8
Total: Nov 2006-Mar 2008	35,972	1,058

[a]The plants of the second series from November 2006 to March 2008 were different from those in the first series from Jan to October 2006.

Results

With medium-sized cages

Myndus adiopodoumeensis

This test was halted after 4 months and the plants were kept under a veranda that was sunny but protected from any visiting Homoptera. None of the coconut plants receiving 1,400 *M. adiopodoumeensis* adults for 4 months displayed any disease symptoms 27 months after the trial was halted.

Derbidae

Three groups of two plants each fitted with a sleeve were placed in the medium-sized Derbidae cage: first group with *Metaphenice stellulata* (Boheman), a species in which a phytoplasma different from that of CSPW has been found; second group with *Patara armata* (Van Stalle); third group with four species of *Diostrombus* in a mixture: *D. mayumbensis* (Synave), *D. dilatatus* (Westwood), *D. luteus* (Muir), *D. nitidus* (Muir).

– One plant that received an average of 4380 *Diostrombus (table 2)*, tested positive in PCR. In addition, the *DNA was identified after cloning and sequencing as being that of the LY phytoplasma*.

– Unfortunately, that plant did not display disease symptoms. Subsequent PCR analyses (2 and 4 months later) did not reveal the presence of the phytoplasma.That may have been due to the fact that:

• the plants were too young and the sap did not offer good conditions for phytoplasma development and,

• the plants under shade were not traumatized and were able to more effectively resist any phytoplasma aggression.

This plant was also kept under the sunny veranda protected from any visiting Homoptera. It did not express any disease symptoms 27 months after insect releases were halted.

Table 2. *Tests of Homoptera Auchenorrhyncha for lethal yellowing transmission on coconut palms aged 12-18 months.*

Species	**July-Nov 2005 (Average insects/plant)**	**Dec 2005-Oct 2006 (Total insects/one single plant)**
Four species of *Diostrombus*	4,380	24,050
Metaphenice stellulata	1,320	10,030
Patara armata	1,800	5,500

It should be noted that these species remained on the underside of leaflets throughout the day.

Other Homoptera collected in sweep nets

This test was organized to try and see if the vector might be sheltering in the plant cover of the coconut plot or the surrounding area during the day.

So far, two-monthly PCR analyses have yet to reveal the existence of phytoplasma in coconut plants subjected to the pressure of more than 40 species of Cicadellidae (small and large), Pentatomidae, Cercopidae and Flatidae.

Other so-called "rare" Homoptera collected from fronds

Since November 2006, other Homoptera found at a very low frequency on fronds have been released into a medium-sized cage; these are in decreasing order of abundance: *Proutista fritillaris* (Boheman) (Derbidae), *Nisia nervosa* (Motschulsky) (Meenoplidae), *Diostrombus annetti* (Muir), *Diostrombus nitidus* Muir (Derbidae) *(table 3)* and others in very small numbers but not counted such as the Derbidae, *Zorabana maculata* (Van Stalle), *Pamendanga nealei* (Distant), *Diostrombus (Lyddastrombus) lineaticeps* (Muir), the Meenoplidae *Anigrus lugens* (Muir) and *Meenoplus stramineus* (Stal), and a species of Lophopidae, *Elasmoscelis trimaculata* (Walker).

So far, none of the coconut seedlings that have received the pressure of some tens to some thousand insects has yet expressed the symptom of the disease.

Large cages

Myndus adiopodoumeensis

The main problem encountered is the high mortality of Myndus inside the cage: only between 0 and 5% of insects introduced were alive the next morning. *M. adiopodoumeensis* were excessively attracted to light. They therefore flew to the Fyltis netting and only rarely visited the coconut fronds. On the Fyltis netting, they soon died from exposure to the sun; they were caught in spider webs in the upper corners of the cages or they were attacked by ants. *Myndus* flights to the Fyltis netting were greatly reduced by installing mosquito netting over the plants. The adults thus remained longer on the fronds. Consequently, 46% of released insects were still alive the next morning. However, despite this substantial improvement of their conditions in captivity, the adults do not survive more than 4 days *(figure 1)*. The adults, if they were under the leaflets, were not moving much, but it is difficult to determine exactly if they were in position to stick in the leaflet and withdraw the sap or not.

So far, the five coconut seedlings have not yet expressed the symptom of the disease after 28 months despite the number of *M. adiopodoumeensis* released per plant and per month *(table 4)*. It is therefore unlikely that this species is the vector of the CSPW phytoplasma.

Derbidae

Table 5 indicates the *Derbidae* spp. tested and their numbers released in large cages.

Derbidae mostly remained on the coconut palms. They were not much attracted by the Fyltis netting and sunlight. Nevertheless, mortality was not insubstantial in most of the species tested. The most resistant species to heat was *D. dilatatus*.

The height of the cages (4 m) made it difficult to construct a bamboo or a thatch shade which would quickly be destroyed by a strong gust of wind (occurring approximately annually). Percale fabric (flour bags) was tested and gave sufficient shade to reduce the temperature by 2 °C. Apparently, that drop in temper-

Table 3. *Tests of so-called "rare" Homoptera Auchenorrhyncha for lethal yellowing transmission on coconut palms aged 12–18 months.*

Species (Families)	Nov 2006 to March 2008 (Total insects/two plants/17 months)	Average insects/ month/plant
Proutista fritilairis (Derbidae)	3,421	101
Nibia nervosa (Meenoplidae)	1,286	38
Diostrombus annetti (Derbidae)	201	6
Diostrombus nitidus (Derbidae)	728 (since Nov 2007)	21
Lophopidae sp	418	12
Other species	50	1

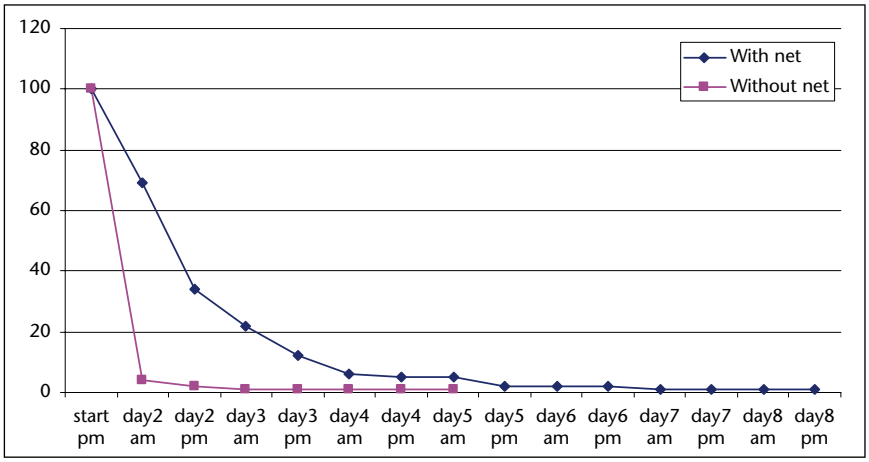

Figure 1. *Survival of M. adiopodoumeensis with or without mosquito net.*

Table 4. *Tests of M. adiopodoumeensis for lethal yellowing transmission on coconut palms around 2 years old.*

Species	Dec 2005–Mar 2008 (Total insects/five plants/28 months)	Average insects/ plant/month
M. adiopodoumeensis	72,943	521

Table 5. *Tests of Derbidae for lethal yellowing transmission on coconut palms around 2 years old.*

Species	Nov 2006–Mar 2008 (Total insects/three plants/17 months)	Average insects/ month/plant
D. mayumbensis	21,348	418
D. nitida	661	13
D. luteus	8,673	170
D. dilattatus	8,395	164
M. stellulata	24,496	480
P. armata	11,811	231

ature inside the cage did not lead to a significant drop in insect mortality. This suggests that the confinement aspect (absence of air circulation) might play a definite role in insect survival in the cage. Remember that outside the maximum temperature was around 35 C-36 °C but air circulation makes the heat more bearable.

So far, the two batches of three coconut seedlings more than 2 years old have not yet expressed the symptom of the disease after 17 months *(table 5)*.

Other observations

Nematodes[2]

A sample of soil + pieces of roots, taken from around two diseased coconut palms, was analysed in the Institute of Research for Development (IRD) at Montpellier (France). The roots were virtually uninfested, as the *Pratylenchus* population levels were very low. On the other hand, the *Helicotylenchus*, especially *Heterodera* (cyst nematode) and *Meloidogyne* (root-knot nematodes) were pretty high. No cysts or root-knots were found on the roots.

The nematodes, vectors of viruses, practised only mechanical transmissions through their stylet, which makes it very unlikely the transmission of phytoplasma. Nevertheless, this possible soil effect is being taken into account in our transmission trials with five cages (2.5 × 2.5 × 2.5 m) in which the coconuts were planted directly in the soil without any insects releasing in the cages.

Soil scale insects[3]

Scale insect searches have been resumed in the soil specifically around diseased coconut palms. It has been possible to detect five species of Pseudococcidae: *Geococcus coffeae* Green, *Planococcus minor* (Maskell), *Rhizoecus* sp, *Ferrisia virgata* (Cockerell), *Paracoccus* sp and one species of Aphididae: *Geoica* sp. So far, no Margarodidae have been found (ground pearls).

Control experiments with nematodes and scale insects prove very difficult. In fact, they are conducted blind, that is, every month the soil in the large tubs containing the tested coconut palms is changed in two cages. Soil is taken from around diseased coconut palms; it also contains pieces of diseased coconut palm roots.

Insects in frond axils

Searches with a brush in frond axils have revealed some larvae of a sucking species. The eggs were placed in a hatchery and gave a Derbidae known as *Metaphenice stellulata*, a species in which a phytoplasma has been found, but not that of CSPW. A Derbidae species is known thereby to complete its entire cycle on coconut. In addition, the CSPW phytoplasma was not found by PCR analysis.

Another species of white larvae (probably Cicadellidae) was spotted once in the plant debris in frond axils, without obtaining any adults, which makes impossible the determination of the species.

Insects on inflorescences

Thrips are known to exist on coconut inflorescences. Observations of a series of inflorescences dissected on flowering revealed the effective existence of a black Thrips species. Some black Thrips samples have undergone PCR analysis without any positive responses.

Discussion and prospects

The search for the coconut LY vector in Ghana was launched following observation of the existence of *M. adiopodoumeensis* species in the first focus at "Ayensudu" which started in 1983. In fact, that species of Auchenorrhyncha which is well-known as an intra-phloemic sucking species is already responsible for the transmission of a phytoplasma in Florida, with the species *M. crudus*, and of a viroid in Vanuatu with the species *M. taffini*.

At the same time like *M. adiopodoumeensis*, all the other diurnal species found on the underside of leaflets have been tested. They belong to the families of the Derbidae: some are abundant like certain species of *Diostrombus* and others less frequent like *Proutista*. The Meenoplidae are not very frequent, likewise the Lophopidae.

More than 40 species of Cicadellidae, Pentatomidae, Cercopidae and Aphrophoridae harvested with sweeping net, twice daily, among the weeds or cover crops inside and around the experimental plot, have also been tested in a medium cage.

Investigations were then extended to insects (scale insects) and other organisms (nematodes) in the soil. This soil effect needs to be taken into account without being able to set up precise trials as it is difficult to collect a large number of scale insects that are very delicate to handle. Tests with nematodes also present the same problems in practice (collection, counting of individuals, releases, mortality monitoring, etc.).

The diurnal investigations therefore seem complete and no disease symptoms have been expressed in cages after two and a half years. Nocturnal observations have therefore begun, collecting insects directly from leaflets. Dozens of species of Cicadellidae not found in the daytime with sweeping net have been collected in that way.

Two groups seem to be of interest: Cicadellidae of the subfamily Deltocephalinae, which contains numerous species that are potential disease vectors, along with the family of the Delphacidae.

The next tests in cages will be conducted with species from these two groups of insects.

Conclusion

Results of PCR analyses on insects showed that a species of Derbidae might potentially be the disease vector. First of all, a coconut plant around 1 year old was infested by a group of four *Diostrombus* (Derbidae). However, disease symptoms had yet to occur on the plant 18 months after the phytoplasma was detected. It may be that the age of the plant was not favourable for pathogen development. It tested negative on subsequent occasions.

Next, a pathogen test was carried out with the four Derbidae species involved to see whether phytoplasmas could be found in the food bolus. An individual of the species *D. mayumbensis* carried the CSPW phytoplasma: one positive nested-PCR was obtained in the CIRAD/UPR29 laboratory at Montpellier.

After 27 months of trials meticulously conducted in Ghana with the species *M. adiopodoumeensis*, more than 70,000 adults have been released on five seedlings in insect-proof cage. So, in the absence of any positive PCR analysis responses, be it in coconut palms in cage or in *M. adiopodoumeensis* adults, it turns out that this species seemed not be the disease vector in our experimental conditions.

In addition, none of the coconut plants exposed in cages to whatever species of Homoptera Auchenorrhyncha has expressed any LY symptoms to date.

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[2] Determination by Thierry Mateille, IRD Nematologist, UMR 1062 INRA/IRD/SUPAGRO/CIRAD/CBGP, Campus de Baillarguet, France.

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