OVERVIEW OF THE EPIDEMIOLOGY OF COCONUT LETHAL YELLOWING DISEASE (LYD) IN MALAYAN YELLOW DWARF x VANUATU TALL (MYD x VTT) HYBRIDS IN THE WESTERN AND CENTRAL REGIONS OF GHANA

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ABSTRACT

The Ghanaian form of lethal yellowing disease (LYD) of coconut is known as the Cape Saint Paul Wilt Disease (CSPWD). The disease is caused by phytoplasmas, and has been active in the country since 1932. The study was conducted to update the disease situation among Malayan Yellow Dwarf x Vanuatu Tall (MYD x VTT) hybrids in the Western and Central Regions of Ghana. It was also to compare the level of LYD susceptibility in the local West African Tall (WAT) variety and the MYD x VTT hybrid, recommended for replanting under the Coconut Sector Development Project (CSDP). Structured survey questionnaires were administered for 15 months to obtain information on every CSPD coconut farm. Categories of data included: farm identity, hydrography, topography, soil properties, agronomic and ecological condition, and disease situation around each coconut farm. Losses to LYD were higher in the Central Region than in the Western Region. Overall, only 4.8 % of total hectarage of MYD x VTT coconut plantings have been affected by the disease as at December, 2009. LYD incidence was significantly higher in the WAT variety than the MYD x VTT hybrids. Mathematical calculations also showed that, the epidemic rate (r) of LYD was higher in the WAT variety than the MYD x VTT hybrids. There was variation in disease incidence and severity in MYD x VTT hybrids between the different coconut growing areas. The susceptibility of the MYD x VTT hybrids under intense disease pressure calls for the screening of more coconut varieties, to identify truly resistant types to the lethal yellowing disease.

INTRODUCTION

Lethal yellowing (LY) is a disease that affects about 40 species of palm world-wide. Many of the disease symptoms are caused by plant pathogens called phytoplasmas (Dollet *et al.,* 2008). Phytoplasmas are transmitted by insect vectors. However, the vector(s) of the phytoplasma responsible for coconut lethal yellowing disease in West Africa is unknown (Philippe *et al.,* 2007).

In Ghana, the first incidence of lethal yellowing disease was in 1932, at Cape St. Paul, Woe, in the Volta Region. It was detected later in 1964 at Cape Three Point in the Western Region, and by 1983, the disease had spread to Ayensudo in the Central Region (Ofori and Nkansah-Poku, 1995).

Several thousands of hectares of coconut plantings in the main coconut producing regions

namely, Western, Central, and Volta Regions have been devastated by the disease, and it is still spreading (Dery and Arthur,1996).

The disease starts by infecting a few coconut palms (localized infections) and spreads by leaps over variable distances in any direction (jump infection). Disease foci occur in patches that sometimes merge.

The symptoms of LYD in Ghana include premature nut drop with or without yellowing of fronds. This is followed by progressive yellowing or, sometimes browning of the crown starting with leaves. The whole crown then turns yellow, dries up and falls off, leaving only the stipe standing (Dery *et al.,* 2008). Symptoms may vary with variety, for example, in Malayan yellow dwarf (MYD) x Vanuatu tall (VTT) hybrids, leaves turn bronze-like in colour instead of yellow (Dery *et al.,* 2008).

The history and epidemiology of this disease in Ghana have widely been reported by Ofori and Nkansah-Poku (1995). Attempts by Researchers to control the disease have been going on for decades. In 1942, investigations into the nature, causes and control of the disease were initiated by the Crops Research Institute (CRI) of Ghana but there was no breakthrough (Chona and Andoh, 1970).

The Ministry of Food and Agriculture (MoFA) conducted a LYD resistance screening trial involving 27 coconut varieties at seven locations from 1981-1983. Three coconut varieties namely, Sri-Lanka Green Dwarf (SGD), Malayan Yellow Dwarf (MYD) and Vanuatu Tall (VTT) from the trial showed great promise and were therefore, crossed to produce hybrids (MYD x VTT and SGD x VTT).

The Oil Palm Research Institute (OPRI) of Ghana, in 1996, carried out an adaptive trial to assess the on-field performance of the MYD x VTT hybrid. The MYD x VTT hybrid proved resistant or tolerant to the coconut lethal yellowing disease, and was produced commercially.

Following the above, the Ministry of Food and Agriculture (MoFA) with funding from Agence Francaise de Developpement (AFD) launched a replanting programme under the Coconut Sector Development Project (CSDP) in 1999. The project planted 1,300 ha of land to the MYD x VTT hybrids. However, the MYD x VTT hybrids succumbed under intense disease pressure.

Control of the disease has proven difficult since. The use of resistant coconut varieties has been identified as the best way of controlling coconut lethal yellowing disease and lethal yellowing diseases in general (Dery, and Arthur, 1996); Harries(1995); Mariau *et al.* (1996). It has been observed that most secondary spread of the disease occurs within 100 m of a new focus. Elimination of disease focus by felling of the first infected palm(s) is effective in slowing the spread of disease, especially when practiced rigorously in the early stages of an outbreak (McCoy *et al*., 1976).

This paper looked at the incidence, severity, and the epidemiology of coconut lethal yellowing disease in Ghana, compared incidence levels and epidemic rates of LYD in the local West African Tall (WAT) coconut variety and the Malayan Yellow Dwarf (MYD) x Vanuatu Tall (VTT) hybrid. Also, recommendation on the way forward for coconut research and the industry in the country has been suggested.

MATERIALS AND METHODS

CSDP Farms

Between April, 1999 and December 2004, a total of 1 300 ha of Malayan Yellow Dwarf x Vanuatu Tall (MYD x VTT) coconut hybrid was planted in the Central Region (360 ha) and Western Region (940 ha) of Ghana, by the Coconut Sector Development Project (CSDP) with funding from Agence Française pour le Développement (AFD). The MYD x VTT hybrid was recommended for the replanting programme because of its good agronomic performance and presumed resistance to CSPWD. It subsequently proved susceptible to the disease. These farms were used for the study.

Data collection

A survey of the CSDP farms was conducted using a structured questionnaire (Appendix 1) to obtain information on every farm. The survey covered six districts: two in the Central Region (Abura-Asebu-Kwamankese, AAK and Komenda-Edina-Ebrim-Aguafo, KEEA), and four in the Western Region (Nzema East, Wassa West, Ahanta West, and Shama Ahanta East Metropolis, SAEMA). Six Technical Officers (T.O.) were employed to visit the farms and gather information. Data collected included the precise geographical location of the farms (determined with a Global Position System, GPS) and the phytosanitary status of the farms. The original GPS files were in Garmin Mapsource format and the phytosanitary data was inputted into a computer file in a Sphinx software format.

Data management

A software developed by the International Centre for Co-operation in Agricultural Research for Development (CIRAD-France) with Microsoft Access, a relational database management system was used to manage the data collected from the epidemiological survey: list of farmers established by the Coconut Sector Development Project (CSDP), GPS files on precise geographical location of farms as well as phytosanitary status (diseased or healthy) of the farms.

Data were organized in 10 main tables linked by logical relations (Figure 1). Dedicated queries were written in order to extract the data from the tables and to export them to files readable by statistical softwares.

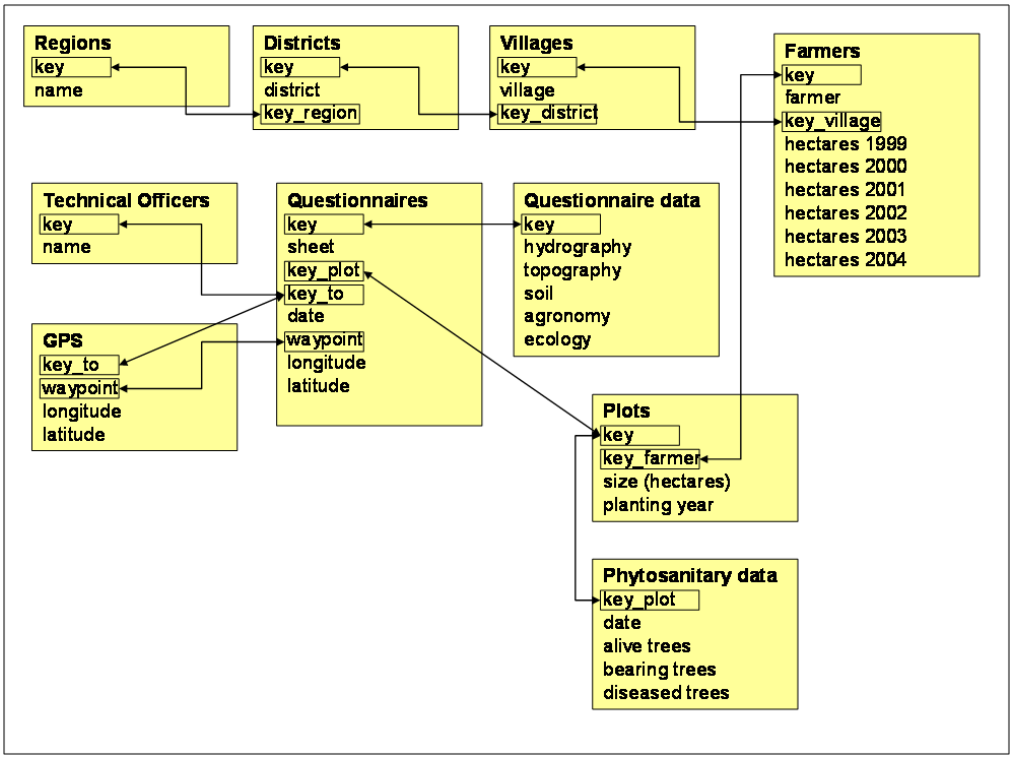

Fig: 1: Organization of the database on surveys in CSDP farms. Large rectangles represent tables, small rectangles represent keys and arrows represent relations between tables

At the end of the phytosanitary audit, eight hectares of the CSDP farms were selected and monitored as a study in each region. Because the CSPD farms are typically 1-2 ha in size, contiguous farms were chosen to simulate a single large farm. This is necessary, to obtain large population (or sample) size for statistical analysis, and also minimize variations in disease incidence due solely to differences in micro-climate. The farms in the Central Region (four farms, 1 083 palms) were located in Empiro and Batanyaa. Those in the Western Region (five farms, 1 166 palms) were located at Aluku and Fawomanye. In addition, a 2-km stretch of coastal coconut strip (774 palms) located at Asanta-Kikam in the Nzema East district of the Western Region, with active disease focus was used for the analogous studies on the WAT variety. The palms in each farm were spot mapped using Global Position System (Garmin GPS 70) and tagged with numbers on the trunks.

On-field assessment of diseased palms was by visual observation of symptoms according to the scale described below and shown in Plate 1.

Stage 1: Pre-mature nut fall/button drop (≥ 8 premature nut/buttons);

Stage 2: Generalized yellowing of coconut fronds/leaves starting from the bottom of the crown;

Stage 3: Wilting and death of entire coconut crown;

Stage 4: Drooping and falling-off, of entire crown leaving only the coconut stipe/trunk standing.

Visual diagnosis was confirmed by subjecting suspect diseased palms to Polymerase Chain Reaction (PCR) assay, using both universal phytoplasma primers and LYD-specific primers for DNA amplifications.

Determination of LYD cycle of coconut (WAT variety)

At the start of the study, 20 WAT palms showing stage one disease symptoms (premature nut drop) at Asanta-Kikam in the Nzema East district of the Western Region were monitored for disease progress to determine the average cycle of the disease. These palms were the only ones showing stage one symptom according the disease scale shown in section (2.2) under Materials and Methods. The latent/incubation phase of the disease cannot be determined by visual observation; therefore, the study was carried out solely on the symptomatic phase of the disease. Data on disease progress was collected every two weeks for six months.

Polymerase Chain Reaction Assay

Samples for PCR assay were collect by harvesting inflorescences from 12 palms in front of the advancing disease focus in each diseased farm.

(a) **yellowing of fronds**

(b) **wilting and death of crown**

(c) **crown fall, leaving stipe/ trunk**

Plate 1a, b, &c. Progressive stages of lethal yellowing disease of coconut.

Where palms lacked unopened spathe, spear leaf samples were collected for PCR assay. The de-oxyribose nucleic acid (DNA) from samples was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) DNA extraction protocol by Daire, described below:

1. Crush 1 g of sample tissue in 5 ml of CTAB buffer (Cetyl Trimethyl Ammonium Bromide);
2. Transfer 1 ml of the crushed tissue into 2 ml eppendorf microfuge tube;
3. Incubate at 65°C for 20 minutes;
4. Add 1 ml of chloroform: isoamyl-alcohol;
5. Centrifuge for 10 minutes at 10 000 rpm;
6. Transfer the aqueous phase into another 2 ml eppendorf microfuge tube;
7. Add 1 ml (or equal volume) of chilled isopropanol;
8. Centrifuge for 10 minutes at 10 000 rpm;
9. Discard the supernatant;
10. Further add 1 ml of 70 % ethanol;
11. Centrifuge for 5 minutes at 10 000 rpm;
12. Discard the supernatant;
13. Dry the pellet of DNA;
14. Suspend the DNA in 150 ml of ET.

A pair of primers, a forward primer and a reverse primer, which are specific to a particular segment of the 16S rDNA, was used together with a reaction buffer, $MgCl_2$ solution, deoxyribonucleotide triphosphates (dNTPs), and a thermostable DNA polymerase, to set up a reaction mixture. The test DNA was then added, and subjected to a cycle of three temperature changes called: denaturation, primer annealing, and primer extention/elongation as illustrated below:

The cycle of DNA denaturation, annealing, and extension is then repeated many times.

Epidemic modeling of LYD of coconut in Ghana

Mathematically, a disease in which the pathogen produces several reproductive cycles (polycyclic pathogen) in the life of the host is governed by the Van der Plank's (1963) equation below:

$$\mathbf{X} = x_o e^{rt} \qquad (1)$$

Where

X = the number of diseased palms at any one time;
$\mathbf{x_o}$ = the initial number of diseased palms;
r = average rate of infection;
t = time during which infection occurred;
e = base of natural logarithm ($e= 1+{}^{1}/n)^{n}$,

where n is a very large number, =2.718…)

The factor (**r)** provides an overall measure of the rate at which the epidemic is progressing and can be used to compare epidemics of the same disease in different localities and within different cultivars. It is derived by taking logarithms through the Van der Plank's equation and transposing. Thus:

$$\mathbf{r} = {}^{1}/_{t} \log_e ({}^{x}/x_o) \qquad (2)$$

Provided, therefore, we have an initial amount of disease and a second amount some time afterwards we can calculate the average rate of increase over the period of time. By convention, the first measurement is $\mathbf{x_1}$ (assessed at

Cycle	Denaturation	Annealing	Polymerization
First cycle	5 mins at 94°C	2 mins at 50°C	3 mins at 72°C
Subsequent cycles	1 min at 94°C	2 mins at 50°C	3 mins at 72°C
Last cycle	1 min at 94°C	2 mins at 50°C	10 mins at 72°C

time t_1) and the second x_2 (assessed at t_2). **Average infection rate is given by:**

$$r = {}^{1}/_{t2 - t1} \log_e (x_2/x_1) \quad (3)$$

All the statistical analyses were done using Genstat software package. Comparison between means was done using two-sample t-test. Significance was determined at the 5 % significance level.

RESULTS

Phytosanitary Survey

Percentage disease incidence in the MYD x VTT hybrids was higher in the Central Region than in the Western Region (Figure 1). The proportion of healthy farms was nearly the same in both regions. About 9.3 % of healthy farms in the Western Region were neglected whereas 7.0 % of the same were neglected in the Central Region. 45.0 ha representing 3.6 %

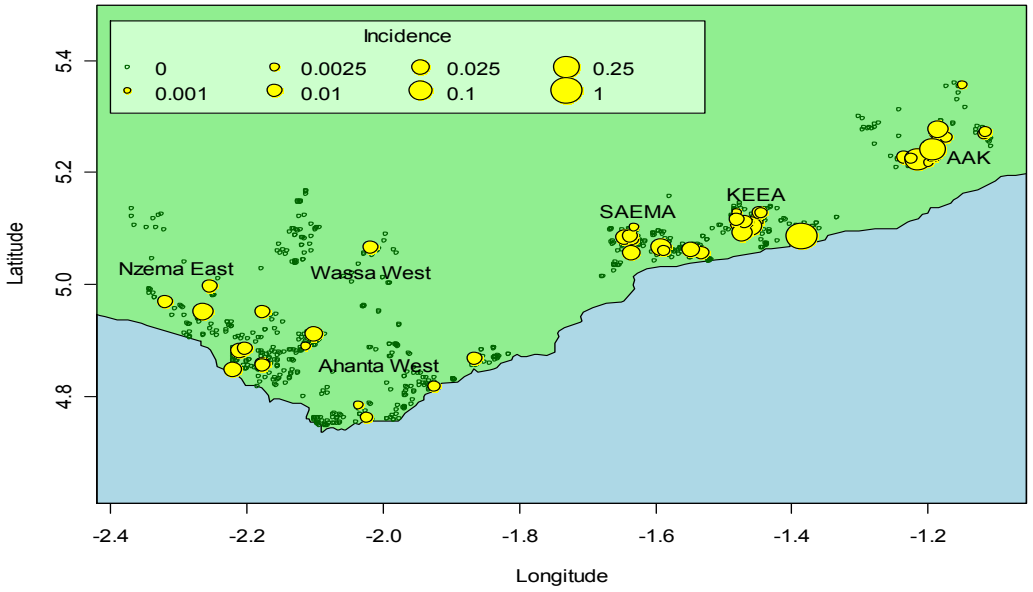

Fig. 1: Geographical distribution of CSDP farms infected by LYD in the Western and Central Region of Ghana. Each yellow circle represents an infected farm

Table 1a: Phytosanitary status of the 1 300 ha CSDP MYD x VTT hybrid farms in 2009

Region	Healthy farms (ha)	Diseased farms (ha)	Neglected farms (ha)	Unsurveyed farms (ha)
Western	868.0 (97.0 %)	27.0 (3.0 %)	81.0 (9.3 %)	45.0 (3.6 %)
Central	342.0 (95.0%)	18.0 (5.0 %)	24.0 (7.0 %)	0.0 (0.0 %)
Total	1 210.0 (97.0%)	45.0 (3.6 %)	105.0 (8.4 %)	45.0 (3.6 %)

N.B.: *A diseased farm is a farm in which there is at least, one diseased coconut palm*

of the farms in the Western Region could not be assessed during the survey. A total of 45.0 ha representing 3.6% of the 1 255 ha CSDP hybrid coconut farms surveyed were diseased (Table 1a).

LYD incidence in WAT and MYD x VTT hybrid coconut varieties

LYD incidence per annum was on the average four times higher in the local WAT variety than the MYD x VTT hybrids (Table 1b). Similarly, the LYD epidemic rate was in reality higher in the WAT variety than in the MYD x VTT hybrids (Table 1b). The difference in LYD incidence between the two coconut varieties was significant at $p < 0.05$.

LYD cycle in WAT coconut variety

All stages in the disease cycle preceding total death, namely nut drop; yellowing of lower fronds; and generalized yellowing, lasted approximately six weeks each. Time lapse between stage 1 (premature nut drop) and stage 4 (crownlessness) was approximately 22-24 weeks (Table 2).

Table 1b: Disease data on coconut farms used for LYD epidemiological studies

Location and coconut variety	Diseased trees per annum	Percentage disease per annum	Epidemic Rate per annum
Asanta-Kikam: WAT	130.0	13.0	0.1
Empiro-Batanyaa: MYD x VTT	21.0	2.1	0.1
Fawomanye-Aluku: MYD x VTT	3.6	3.6	0.1

N.B. ***Percentage LYD incidence was calculated per a 1,000 live coconut trees***

Table 2: Duration of lethal yellowing disease cycle in the WAT coconut variety

LYD Stage	Symptom	Duration (weeks)
1	Premature nut/button drop	4.0 – 6.0
2	Yellowing of lower fronds	4.0 – 6.0
3	Yellowing of entire crown	4.0 – 6.0
4a	Wilting and death of crown	6.0 – 8.0
4b	Crown fall/Pole stage	Not Determined

PCR assay of suspect diseased palms

Of the 179.0 samples from coconut palms showing lethal yellowing disease symptoms subjected to PCR analysis, only 42.0 samples representing 23.0 % tested positive for coconut

Table 3: PCR results of suspect diseased coconut palms subjected to molecular analysis

Location and coconut variety	Total number of palms	Total number of samples	Number of PCR positive samples
Asanta-Kikam: WAT	774.0	122.0	24.0 (20.0 %)
Empiro-Batanyaa: MYD x VTT	1 180.0	21.0	6.0 (29.0 %)
Fawomanye-Aluku: MYD x VTT	1 188.0	36.0	12.0 (33.0 %)
Total	3 142.0	179.0	4 2.0 (23.0 %)

phytoplasmal DNA (Table 3). Nearly all (175.0 palms) the suspected diseased palms eventually died.

DISCUSSION

Percentage disease incidence in the MYD x VTT hybrids was 2.0 % higher in the Central Region (5.0%) than in the Western Region (3.0 %). However, the number of neglected farms was 2.3 % higher in the Western Region (9.3 %) than in the Central Region (7.0 %). The proportion of healthy farms was nearly the same in both regions. A total of 45.0 ha representing 3.6 % of the farms in the Western Region could not be assessed during the survey (Table 1a). In all, 45.0 ha representing 3.6 % of the 1 255 ha CSDP hybrid coconut farms surveyed were diseased. Farm neglect or abandonment was due to death of farmer or disinterest in coconut farming resulting from LYD resurgence. Failure to survey all the farms was due to land tenure disputes. Studies on LYD cycle in the WAT coconut variety could not proceed beyond 24 weeks (six months) due to total death of diseased palms within the period.

Even though, by visual observation many of the palms displayed typical symptoms of coconut LYD during the dry season (November-January), only few tested positive for coconut phytoplasmal DNA upon PCR assay. This reinforces the unreliability of visual diagnosis and necessitates the need to evolve efficient on-field disease diagnostic methods.

Failure to detect all diseased palms by visual observation arose from the similarity of LYD symptoms to those arising from senescence, water stress or general mineral nutrient deficiency (Dery *et al.,* 2008*).*It may also be that, at the time of sampling, many of the suspected diseased palms were not infected at all, or at any rate, the amount of initial inoculum in infected tissues was too low to be detected by PCR assay (Pilet *et al*., 2008).

CONCLUSIONS AND RECOMMENDATIONS

Lethal yellowing disease of coconut within MYD x VTT hybrids coconut plantings was higher in the Central Region than in the Western Region.

The significantly ($p<0.05$) higher LYD epidemic rate in the WAT variety may have been due to the relatively ageing palms, rather than genetic inferiority. Like the WAT variety, the susceptibility of the MYD x VTT hybrids under intense disease pressure calls for the screening of more coconut varieties, to identify more resistant types to the lethal yellowing disease. Breeding for total resistance is difficult to accomplish.

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