

CROP PROTECTION PROGRAMME

Coconut lethal yellowing disease: development of new diagnostic tools and laboratory support to promote their application

R 8309 (ZA 0581)

FINAL TECHNICAL REPORT

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Executive Summary

Coconut lethal yellowing and related diseases associated with phytoplasmas continue to threaten the livelihoods of resource-poor farmers and consumers in developing countries. Management has been based on the identification and rapid deployment of less susceptible varieties but a new epidemic of disease in Jamaica, in varieties that were previously considered to be resistant, has undermined this strategy. This project should have provided scientific support to coconut rehabilitation programmes by refining, validating and supporting the trial commercial development of new diagnostic techniques to detect and identify phytoplasmas in the early stages of disease using monoclonal antibodies developed at Rothamsted Research. Had the project been successful these would have facilitated reliable screening of new varieties for resistance to the disease and enable other control options, such as timely phytosanitary measures, to be evaluated.

The following outputs were achieved:

1. Standard operating procedures for non-destructive sampling of palms for serological and PCR tests developed, validated and documented.
2. Standard operating procedures transferred to CRP laboratory at Takoradi and skills of staff updated.
3. Laboratory experimental reporting procedures established.
4. A diagnostic capability to fully support the needs of the coconut rehabilitation project establishes at Takoradi provided that donor funding to sustain activities and back-stopping from a European laboratory are available.

These outputs planned in the PMF were not achieved in full

5. Validation of monoclonal antibodies by parallel ELISA and PCR assays.
6. Evaluation of the needs and opportunities for linkage with the proposed CFC LY project.
7. Develop and evaluate a trial batch of diagnostic kits, and draft agreements for subsequent licensing, production and commercial sales.

The outputs were not achieved because of problems with resurrection of the monoclonal cell lines. 19 cell lines had been selected for secretion of monoclonal antibodies with specificity to the LY phytoplasma. As we geared up for production unscheduled building works at Rothamsted, forced us to freeze all the cell lines in liquid nitrogen until completion. Following completion of the work we started to resurrect the cell lines, but no lines proved to be viable. Our investigations suggested that interruptions with the supply of liquid nitrogen to our freezer had resulted in death of the cell lines.

Background

Lethal yellowing-type diseases (LYD) caused by phytoplasmas are one of the major constraints to coconut production 'world-wide (Harries, 1978). These diseases have destroyed millions of coconut palms in the Caribbean, East and West Africa. In Jamaica, cyclical epidemics were confined to the eastern end of Jamaica until the 1960's, where they restricted coconut cultivation to isolated plantings, and over 4 million palms were destroyed when the disease subsequently spread to extensive cultivations in central and western regions (Steer, 1997). Recent spread of the disease to Mexico, Belize and Honduras has decimated the mainly smallholder plantings along the western coast. In Tanzania, LYD has destroyed about 40% of coconut palms on the mainland within the past 30 years (Schuiling et al. (1992). Similarly, in Ghana, entire coconut groves (over 10,000 ha) in the Volta region were totally destroyed during the 1950s. In recent years the disease has destroyed over 5,000 ha of coconut plantations in the Western and Central regions of Ghana (Ofori & Nkansah-Poku, 1997). One of the consequences is that many rural communities in the coastal areas of these countries have lost their main source of livelihood since the palms provide food, shelter and cash income. In Ghana, about 20% of the rural communities in the Western region have depended on the coconut for their sustenance, but are now deprived (Adams, 1996). The economic and social importance of coconut in the livelihoods of poor communities is frequently understated by national or global statistics that tend to focus on a small number of traded commodities. In consequence, insufficient attention has been given to the truly global potential for LYD to have a negative impact on

income stability, nutrition and food security and ecosystem sustainability for some of the most vulnerable producers and consumers (Doyle, 1999).

Research in Jamaica and the UK supported by DFID (then ODA / ODM) in the 1970's showed that phytoplasmas are the likely cause of LYD in both Africa and the Caribbean regions, and the diseases are spread by leafhopper or planthopper vectors (Dabek et al.; 1982). Extensive transmission tests have been carried out in several regions but positive results have only been obtained in Florida, with the planthopper *Myndus crudus* (Howard et al., 1983). Using DNA- based diagnostic techniques developed under an EC research project and related work funded by the CPP between 1992 - 1997, investigations on the presence of strains of the pathogen and its putative insect vectors showed that at least two strains and two suspected insect vector species could be found in Tanzania (Mpunami et al., 1999, 2000). However, the vector status of these insects has yet to be confirmed and no suspected vectors have been identified in Ghana, where large numbers of insects have been screened (Offei et al., 1997). Further development and refinement of these techniques has revealed the presence of considerable diversity both between and within LYD phytoplasma from different regions (Harrison and Jones, 2002), raising the possibility that different strains of the pathogen could account for observed differences in the varietal susceptibility of coconut palms in these regions.

Practical control was achieved in Jamaica and elsewhere in the Caribbean following discovery of resistance in fortuitous introductions of 'Malayan Dwarf', Panama Tall and subsequently other varieties and hybrids introduced or produced locally with DFID and FAO assistance (Been, 1981). However, the MD proved more susceptible and poorly adapted under African conditions. An extensive varietal introduction and screening programme in Tanzania has failed to identify a useful degree of resistance in any exotic variety or hybrid (Kullaya et al., 1997). It was thought that resistance might reside in populations of the indigenous East African Talls that have survived earlier epidemics and different EAT sub-populations are being used in crosses with survivors from selected introduced varieties and as a source of seed nuts for establishment of an EAT seed farm. In Ghana, preliminary results indicate that two introduced varieties are less susceptible to the disease (Dery et al., 1997). Such is the demand for action that, despite this as yet slender evidence for resistance to the disease, the government of Ghana is actively involved in producing hybrid seed to replant devastated areas, through bilateral agreements with local NGOs and French technical assistance (renewed in June 2005).

The need to intensify and co-ordinate research on these diseases in affected countries in East and West Africa with links to research teams in Europe, USA, Mexico and the Caribbean, was recognised by the EC project and this has been consistently accorded the highest priority for coconut research by a wide range of stakeholders at regional meetings coordinated by Burotrop. It will be one of the lead themes for the new Global Coconut Research programme (PROCORD, 2002). The problem has achieved greater urgency since 1998, when reports began to emerge of a new epidemic in Jamaica amongst coconut varieties and hybrids previously considered resistant to the disease. The situation was reviewed at an expert meeting in January 2002, convened under CFC Fast Track facilities (see Proceedings, 2002) and is the subject of a research proposal focussed on the Caribbean and Central America that finally started, after much delay, in August 2005.

The development of sensitive and specific DNA-based techniques for detecting and differentiating these non-cultivable organisms, for identifying putative vectors, for early detection of infected palms before these contribute to the epidemic, and for diagnosing and monitoring infection in resistance trials. However, these techniques require careful supervision, expensive and fragile lab equipment and consumables, and are not well adapted for use under tropical conditions subject to unstable or unreliable infrastructure and services. Very recently, Rothamsted has made progress in developing monoclonal antibodies (mabs) that show high activity and specificity to LY phytoplasmas. Once selected, mabs can be produced relatively cheaply and applied in immunological techniques that tend to be more robust and more easily adapted than DNA-based technologies for use under less sophisticated conditions. Simple diagnostic test kits using mabs are already in commercial production for other plant diseases and the project was to subcontract production of a prototype kit for coconut LY in accordance with CPP guidelines. Novel commercial arrangements were to be explored that not only guarantee access to IPR by DFID for use in developing countries but also seek to use income from commercial royalties or product sales in developed countries (such as the USA) to support production or sales in developing countries. The proposed project would thus help to:

- Ensure that laboratory practices and techniques are standardised as far as possible between the laboratories in Africa and the Caribbean so that results are fully comparable.
- Establish monitoring of seed gardens to ensure disease-free status of seedlings for replanting programmes.
- Establish monitoring of the performance of BTM so as to detect early stages of disease and possible atypical symptoms, thus reducing the time required by breeders to select resistant materials.
- Establish monitoring of farmers' gardens chosen for intensification trials.
- Validate methods for early (pre-symptomatic) detection of disease and assess the potential application to disease epidemiology and reducing rates of spread, including possible relationships between LYD epidemiology and different phytoplasma strains.
- Trial commercial production of a diagnostics kit and explore novel licensing arrangements through which royalties from sales in developed countries (notably the USA) could be used to support provision of kits to developing countries.

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Project Purpose

During the life of the project it provided scientific support to the Ghana coconut rehabilitation programme by drawing up standard operating procedures for sampling and testing coconut palms it did not progress to the trial and commercial development of new diagnostic techniques to detect and identify phytoplasma pathogens in the early stages of disease. Nor was it able to liaise with the CFC lethal yellowing project because of delays in implementing that project.

Research Activities

1. (a) Draw up and validate standard operating procedures for the non-destructive sampling of palms. 3 seed gardens and over 100 CSPWD-affected and 50 healthy palms were used. The methods included trunk boring, inflorescence, leaf and root sampling. Quality of sampling was assessed by PCR assays.
1. (b) By experimentation we devised standard operating procedures for PCR and ELISA testing of palm samples. Various combinations of primer and PCR cycling parameters were tested on standardised samples of inflorescence, trunk and root material.

Two visits were made to the Coconut Research Programme at Sekondi in February and July 2004. the first visit was delayed because of problems with the French counterparts who did not want a UK scientist to be the first into the molecular laboratory that had been funded by their government. A good working relationship was established with the French scientist and SOPs were drawn up in collaboration with the Ghanaian counterpart Robert Quaiocoe. During both visits consumables were purchased in the UK and hand carried to Takoradi to supply the laboratory.

Field visits were made during both trips and sampling procedures devised to suit the conditions in Ghana. For example local hand drills were of poor quality and so a cordless electric drill and quality steel drill bits were purchased and taken out. This speeded up the collection of samples considerably. SOPs for good laboratory practice, non-destructive sampling, DNA extraction and PCR were prepared and tested at Takoradi and Rothamsted.

Quality of sampling and DNA extraction allowed single hole sampling of palms as early as infection stage 2 to be used routinely.

2. The level of competence of the laboratory personnel at CRP will be assessed, according to the results of this assessment we will devise and conduct appropriate training to bring their skills to at least those of good laboratory practice (molecular biology) level.

Training was given to Robert Quaiocoe to update his molecular biology skills, however his level of competence will necessitate constant use in the laboratory and this in turn will require a good supply of laboratory consumables. At the time of my visits Robert was the main Ghana scientist at the Coconut Program to do molecular work and the demands on his time were such that there was competition between laboratory and field work. The ideal solution would be to have a dedicated molecular biologist running the laboratory analyses.

3. Field samples will be used to conduct both ELISA and PCR tests. Results from both will be compared to assess the usefulness of the monoclonal antibodies for CSPWD diagnosis. The panel of monoclonal antibodies will be tested in ELISA against a range of healthy and infected palm tissues. Results from ELISA will be validated against results from parallel samples obtained by PCR.

Collections of field samples were made, dried and brought to Rothamsted. Initial tests were made using the monoclonal antibodies with promising results. We were unable to complete the large scale testing and validation of the monoclonal antibodies due to the death of the cell lines

4. Laboratory reporting procedures will be implemented to achieve at least a minimum level of QTL to ensure that all results are logged and reported with clarity and in a timely manner.

Instructions into laboratory reporting procedures were given and laboratory notebooks established. The importance of regular entries to record laboratory activities and results was stressed and examples given as to how these can be incorporated into publications and reports.

5. (a) The UK partner will provide logistics for the purchase and delivery of laboratory consumables, the monitoring of procedures, and repairs to equipment where necessary.
5. (b) The Ghanaian partner will provide all local transport and field staff for sample collection, together with the laboratory staff.

Laboratory consumables and chemicals were ordered and provided by the UK partner. In country transport was arranged by the Ghana Partner.

6. (a) A visit will be made to proposed CFC project regional partners in the Caribbean to assess the scope, needs and opportunities for collaborative and shared research on diagnostic techniques and their application, including with African groups. This would include assessment of the facilities and level of competence of local laboratory personnel, as well as the interest, availability for participation in "a global programme and possible access to training provisions, exchange programmes or other sources of funds. (Joint funding might be available from the CFC, but the CFC have taken a policy decision to focus on the Caribbean/C America and is not expected to fund work of a global dimension).
6. (b) Main points of discussions will be summarised and, depending on stage of development of the CFC proposal, used to inform that proposal and/or development of a new CPP proposal to complement the CFC-funded work.

These activities were not undertaken because of the delay in implementing the CFC Project. At the time of completing this FTR I understand that the Project documents and at last been received by the participants, and the project commenced in August 2005. No official discussions have been held with CFC participant countries.

7. (a) Pocket Diagnostics (PD) will be sub contracted for an agreed sum of £5000, to develop a lateral flow test for the lethal yellowing phytoplasma based on the Rothamsted mabs. Rothamsted Research will provide either cell lines or partially purified mabs together with suitable test materials, which PD will incorporate into a prototype test kit.
7. (b) Following initial trials, troubleshooting and if necessary further refinement by Rothamsted Research and PD, PD will provide at least 1000 prototype lateral flow tests for evaluation and validation. Initial field trials will be carried out in Ghana and Jamaica. Results will be collated and reported, together with feedback on ease of use, initial assessment of likely demand from public and commercial sectors, and proposals for wider distribution and test marketing. If time and resources allow more extensive testing will include Tanzania, Honduras, Mexico and the USA and a more detailed market assessment will be carried out; otherwise it will be completed under a possible CPP follow-up proposal in association with the CFC project.

Outputs

1. Standard operating procedures for non-destructive sampling of palms for serological and PCR tests developed, validated and documented in a form suitable for adoption by users in developing countries.
2. Standard operating procedures transferred to and established at CRP laboratory in Takoradi and skills of CRP laboratory personnel updated
3. Validation of monoclonal antibody ELISA tests by parallel PCR assays.
4. Laboratory experimental reporting procedures established.
5. A diagnostic laboratory to fully support the needs of the Coconut rehabilitation project.
6. Evaluation of the needs and opportunities for linkage with the forthcoming CFC project and terms of reference for a possible new CPP proposal.
7. Under a suitable materials transfer agreement with the commercial subcontractor (pocket Diagnostics), develop and produce a trial batch of diagnostic kits, and draft agreements for subsequent production and commercial sales.

Contribution of Outputs to developmental impact

The intended outputs were not achieved.