# RAPID IN-FIELD DETECTION OF COCONUT PHYTOPLASMAS IN AFRICA

■ THE PROBLEM ADDRESSED

## The importance of slowing down the death of coconut palms from infection by phytoplasmas in Africa

Phytoplasmas of coconut in Africa are associated with lethal-yellowing (LY) type diseases, and have been responsible for the death of many palms in coastal regions of west and east Africa. These diseases are also responsible for destroying the livelihoods of many small-scale subsistence farmers and for the collapse of the coconut industry in countries such as Ghana and Mozambique. The original epidemic in Ghana caused the collapse of the coconut industry in the Volta region by the 1950s, and the more recent epidemic in the Central and Western regions has killed well over one million coconut palms to date (Eziashi and Omamor, 2010). These phytoplasmas have been classified into the 16SrXXII-A '*Candidatus* Phytoplasma palmicola' in the Cameroon, Nigeria and Mozambique, 16SrXXII-B '*Ca.* P. palmicola'-related strains in Ghana and Cote d'Ivoire, and the Tanzanian lethal disease (TLD) group in Tanzania and Kenya. These latter phytoplasmas are different from those associated with LY disease of coconut in Mexico and the Caribbean, which all belong to the 16SrIV groups (Harrison *et al.,* 2014).

Promising resistant hybrid palm genotypes have been screened in Ghana to the 16SrXXII-B phytoplasmas, and the only current efficient management options for the disease are the rapid and systematic removal and burning of infected palms, to remove the reservoir of phytoplasmas, followed by replanting with healthy palms. One factor that can significantly improve the success of such a strategy is the rapid detection of infections in palms, so that they can be removed before they have had the chance to spread the disease to the neighbouring palms. This factsheet details the development and deployment of a rapid 20 minutes in-field system for the 16SrXXII *'Ca.* P. palmicola' detection in coconut in Africa.

• Dead coconut palms resulting from infection with the 16SrXXII-B '*Candidatus* Phytoplasma palmicola' in Ghana. Photo by Fabian Pilet, Cirad.


This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727459

■ LATEST RESEARCH RESULTS

# Rapid diagnostic method to fight the spreading of coconut phytoplasmas

Diagnostic methods for coconut phytoplasma detection worldwide generally require samples of trunk borings collected from palms in the field to be transported to the laboratory for DNA extraction, then testing by conventional polymerase chain reaction (PCR) with the results analysed through gel electrophoresis. Because of the remoteness of diseased areas in many countries, particularly in sub-Saharan Africa, this can often take two or more days from sampling to final results. In-field loop-mediated isothermal amplification (LAMP) detection systems have been shown to be much quicker than PCR with the advantage that they can work on relatively impure DNA samples. Portable, battery operated LAMP machines that can be deployed in the field in remote locations have been developed, which display the detection of LAMP reaction products within 15-20 minutes. In addition, LAMP reagent master mixes have been developed that are stable at ambient temperatures for at least a month, so can be easily transported to these remote locations.

• Collecting trunk borings from coconut palms for DNA extraction in the field in Ghana.



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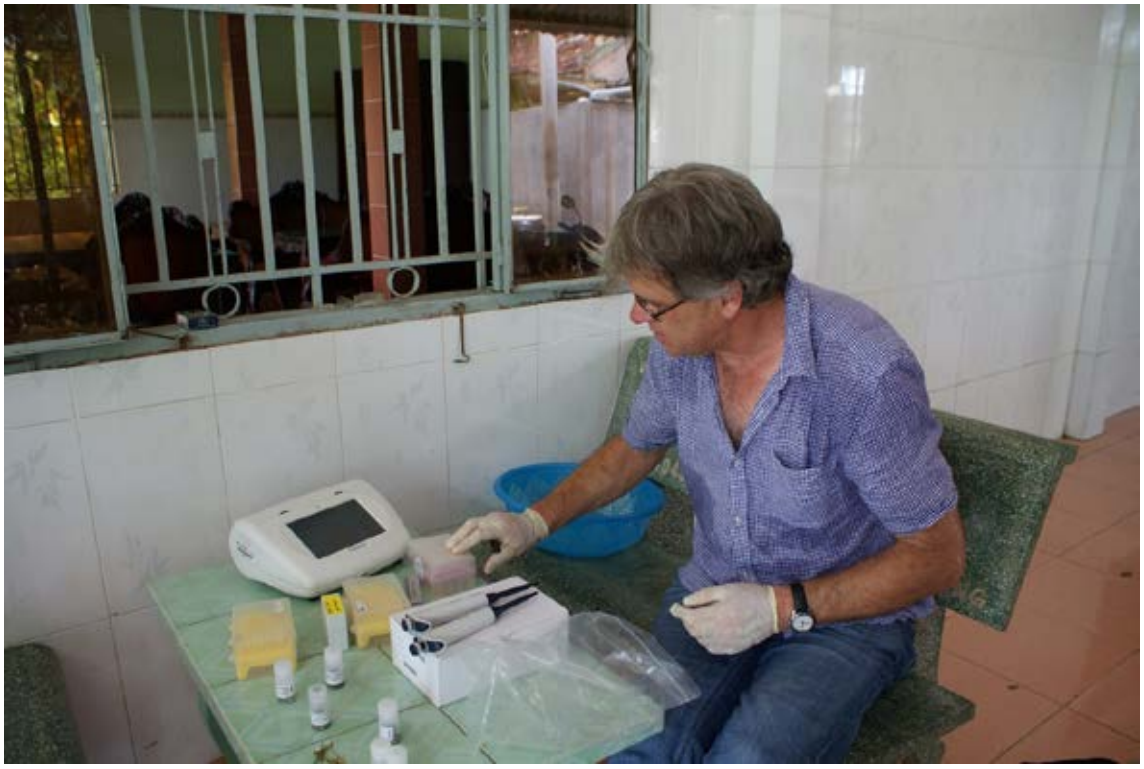
■ THE TROPICSAFE RESEARCH AND DEVELOPMENT ACTIVITY

# The TROPICSAFE contribution to improve the in-field LAMP detection systems

The aim of the work within TROPICSAFE is to design and validate primers that are specific for the coconut phytoplasmas. These can then be incorporated with the master mixes and detection systems for in-field LAMP detection of the coconut diseases, and also to develop and validate a rapid DNA extraction system from trunk borings that can be undertaken with minimal equipment. The overall goal is to develop and validate a detection system that can detect the presence of the specific phytoplasmas in coconut palms within 20 minutes from start to finish in remote locations using a minimal equipment.

LAMP primers have been designed based on the sequence of the '*Ca.* P. palmicola' *leuS* gene that are able to detect the 16SrXXII-A and 16SrXXII-B phytoplasmas within 15-20 minutes in the LAMP diagnostic assay system. These primers have been validated against samples from Ghana, Nigeria and Mozambique, and have also been shown to have no cross reaction to the TLD phytoplasmas from coconut in Tanzania, DNA from 16SrIV-A (coconut lethal yellows) phytoplasmas from the USA and Mexico, 16SrIV-D phytoplasmas from coconut in Mexico, or phytoplasmas from all other taxonomic groups tested (16SrI, 16SrII, 16SrIII, 16SrV, 16SrVI, 16SrIX, 16SrX, 16SrXI and 16SrXIV). In addition, a DNA extraction procedure has been validated based on the alkaline PEG method of Chomczynski and Rymaszewski (2006), in which 10-20 mg of coconut trunk borings is placed directly from the drill tip into 500 µl alkaline PEG buffer and it is grounded for 30 seconds with a disposable plastic micropestle. One microlitre of the supernatant is then used directly in the LAMP reaction. To confirm that the quality of the extracted DNA is suitable for LAMP, a second set of primers has also been designed that detect the coconut cytochrome oxidase gene.



• Setting up LAMP diagnostics with a portable equipment for DNA extraction and phytoplasma detection in the field.



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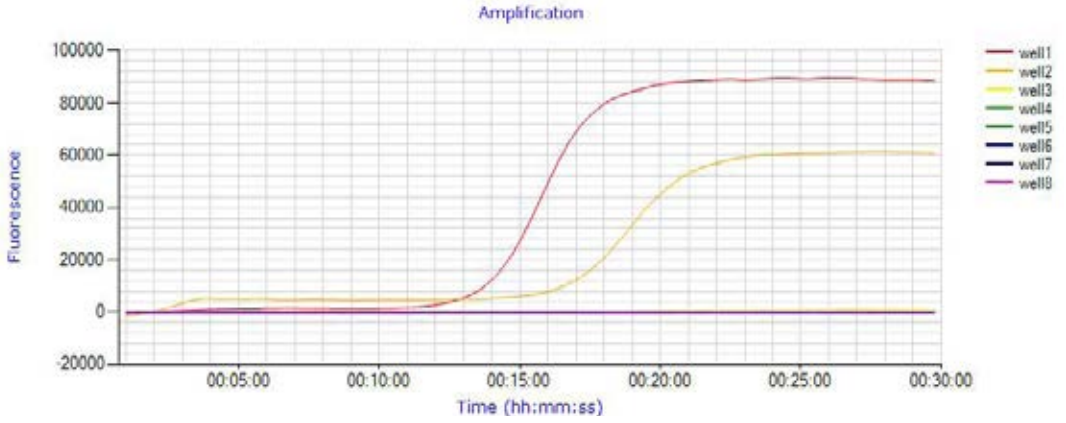
■ SCIENTIFIC DATA AND FIRST RESULTS

# First tests in the field in Ghana and potential for the testing in Caribbean and America

A LAMP diagnostic assay has been developed for the use in the field for rapid and specific detection of the 16SrXXII coconut lethal yellowing phytoplasmas in Africa. This assay has been combined with a rapid DNA extraction system that uses minimal equipment such that trunk boring samples can be tested from individual palms in the field and results confirmed within 20-30 minutes from the extraction. In effect, each trunk boring sample is tested with two sets of primers simultaneously in the portable LAMP machine, one set for the 16SrXXII phytoplasma DNA, and the second set for the coconut DNA. Any sample that tests positive with the phytoplasma primers can be deemed positive for the presence of the phytoplasma, whilst any sample that tests negative with the phytoplasma primers but positive with the coconut primers can be deemed to be negative for the presence of detectable levels of phytoplasmas and therefore most likely uninfected. Any sample that tests negative with both sets of primers is deemed to contain inhibitors of the LAMP reaction enzymes (or not containing DNA) and would need to be retested from a new DNA extraction. The method has been tested in the field in Ghana and with stored trunk boring samples sent to the University of Nottingham, UK. In addition, a separate set of primers have been developed for specific detection of the 16SrIV-A and the 16SrIV-D phytoplasmas of coconut, which have the potential for rapid in-field detection in the coconut palms in the Caribbean and the Americas, where these phytoplasmas are the ones associated with the coconut lethal yellowing.




• A LAMP detection profile for the 16SrXXII phytoplasmas. Wells 1 and 2 show positive reactions from phytoplasma infected palms whilst wells 3-7 show negative reactions and well 8 is a water control.

## KEY WORDS

LAMP, lethal yellowing diseases, in-field detection, phytoplasmas

## FURTHER INFORMATION

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February, 2019



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727459