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1st - 3rd MAY 2019 Centro de Investigación Científica de Yucatán (CICY) Mérida, Yucatán (México)

Lethal Yellowing (LY) is causing significant losses in coconut production that are affecting the trade and import of coconut agricultural products and materials worldwide. The disease has killed millions of coconut palms in different tropical and subtropical countries, therefore, a wide understanding of its symptoms, transmissions routes, control and management measures, is needed to manage it in the most appropriate manner. The TROPICSAFE training session will allow you to recognize the disease in the field and which techniques and practices are being used and/or developed within the project to manage the LY.

OBJECTIVES

- Provide information for the identification of LY symptoms in coconut palms, alternative host plants, and how to collect insect vectors.
- Provide an opportunity to visit a site where LY resistant coconut palms are being cultivated under organic conditions.
- Acquire the capacities: (a) for collecting samples of insects and plants and (b) for the fast molecular detection of phytoplasmas associated with the LY disease.
- Improve the knowledge on management strategies to reduce LY economic impact.

TARGET

- >> Staff of companies or organizations involved in coconut palm propagation, cultivation and utilization.
- >> Technical advisers and consultants.
- >> Local plant protection organizations.





DAY 1: THE TROPICSAFE PROJECT AND THE LETHAL YELLOWING DISEASE TECHNIQUES FOR DETECTION OF LETHAL YELLOWING ASSOCIATED PHYTOPLASMAS

08:30-08:45 Welcome. Carlos Oropeza

08:45-09:15 Presentation of the TROPICSAFE project. Assunta Bertaccini

09:15-10:30 An overview of the Lethal **Yellowing (LY) disease.** Carlos F. Ortiz and Carlos Oropeza

- Symptomatology of LY in coconut palms and other host plants
- » Time-space dispersal of LY
- » Phytoplasmas associated with LY
- Screening for LY resistance and management

10:30-10:45 Coffee break

10:45-11:45 LY in TROPICSAFE countries in the Americas (Jamaica, Cuba, Mexico) and Africa (Ghana). Wayne Myrie, Carlos Oropeza and Matthew Dickinson

11:45-13:00 Techniques for the detection of LY phytoplasmas:

- » Polymerase Chain Reaction (PCR) analysis and its applications. Luis Sáenz
- » Loop-mediated isothermal amplification (LAMP) analysis and its applications. Matthew Dickinson

13:00-15:00 Lunch

15:00-16:30 Explanation of the field visit. Carlos Oropeza

- Sites to visit and what to find and do in each site
- Sampling of plant tissues, description of methodology
- Collection of insects, description of methodology

16:30:17:30 Visit to laboratories in CICY: facilities and equipment. Luis Sáenz

DAY 2. VISIT TO SITES WITH COCONUT PALMS OR OTHER PALMS AFFECTED BY LY FOR SYMPTOM OBSERVATION, SAMPLING AND CAPTURE OF INSECTS

Wayne Myrie, Carlos Oropeza and Matthew Dickinson

07:45-08:15 Transfer from hotel to MAPSA site

08:15-10:00 Visit to MAPSA near Merida:

Visit to LY resistant coconut palms cultivated under organic conditions

10:00-11:30 Transfer from MAPSA site to San Crisanto

11:30-12:30 Visit of two sites in San Crisanto:

- Suadalupe site. Visit to the collection of germplasm that was a previous resistance screening trial
- Caridad del Cobre site*. Watching and collecting of insects in a site with a recent LY outbreak

12:30-13:00 Transfer from San Crisanto to Telchac

13:00-14:00 Visit to Telchac Town:

Observation of LY symptoms in palms, sampling of plant tissue and demonstration of rapid DNA extraction and setting up of Loop-mediated isothermal amplification (LAMP) diagnostics in the field; observation and collection of insects

14:00-15:30 Lunch in Telchac

15:30-17:00 Transfer from Telchac to the hotel

DAY 3. LABORATORY SESSIONS

08:30-13:30 Analyses of samples using qPCR and LAMP. Luis Sáenz and Matthew Dickinson

- Distribution to trainees of documents detailing the qPCR and LAMP protocols
- Demonstration of the process for the extraction of DNA from samples
- » qPCR assay of DNA extracts from palm and insect tissue samples
- LAMP assay of DNA extracts from palm tissue and insect tissue samples

13:30-15:00 Lunch

15:00-16:00 Results session. Carlos Oropeza, Matthew Dickinson and Luis Sáenz

 Observation of the results obtained with both techniques, discussion and conclusions

*Trainees will be provided with kits for insect collection.





Assunta Bertaccini is a plant pathology professor at the Alma Mater Studiorum - University of Bologna (UNIBO), Italy. In more than 40 years of research her major studies have been devoted to plant diseases associated with phytoplasmas and bacteria, focusing on their biology and epidemiology. She has received numerous awards, including the Emmy Klienenberger-Nobel Award for distinguished research in mycoplasmology. She is Editor-in-Chief of Phytopathogenic Mollicutes, Senior Editor of Phytopathologia Mediterranea, reviewer of international scientific journals and founder and head of the International Phytoplasmologist Working Group (IPWG). Chair of COST action FA0807 "Integrated management of phytoplasma epidemics in different crop systems", and she is the coordinator of the TROPICSAFE project.

Carlos Oropeza is a researcher at Centro de Investigación Científica de Yucatán (CICY) in México. He has been part of the coconut group for nearly 30 years, working on studies of the lethal yellowing disease affecting coconut and other palms, and developing protocols for the micropropagation of coconut based on somatic embryogenesis. He has extended this collaboration to the coconut value chain in Mexico, with producers in particular. From a practical point of view, these results are allowing improvements to LY management, mainly through propagation of LY resistant coconut palms for the replanting programs and to strengthen coconut cultivation and the coconut value chain as a whole. The CICY's coconut group is currently participating in the TROPICSAFE project.

Luis Sáenz Carbonell is a professor-researcher at CICY, México, in which he is part of the coconut group. He has devoted more than 15 years to the study of in vitro somatic embryogenesis in coconut, contributing significantly to the establishment of a micropropagation protocol for this species that now is at the semi-commercial scale. Besides, he has dedicated the last eight years to the development of new methods for the detection of phytoplasmas associated with the Lethal Yellowing (LY) disease in palms. He has also worked on the isolation and characterization of resistance gene candidates and the development of molecular markers for resistance or susceptibility to LY that could help to find coconut palms resistant to LY. The CICY's coconut group is currently participating in the TROPICSAFE project.





Carlos Fredy Ortiz García is a professor of plant pathology at the Tabasco Campus of the College of Postgraduate Studies in Agricultural Sciences, (COLPO) Mexico. He has over 30 years of research experience focused on the diseases of tropical plants such ascoconut, cocoa, citrus and banana, among others, associated with fungi, bacteria (phytoplasma), etc. He has received national and international distinctions and awards. He is responsible for the COLPO's phytopathology laboratory, mainly studying diseases of tropical crops to develop strategies for their sustainable management. He is a member of the TROPICSAFE consortium carrying out research in Tabasco, Mexico on the diversity of 16SrIV phytoplasmas in plant hosts and vectors of LY.

Dr. Wayne A. Myre is a Plant Pathologist/Molecular Biologist at the Coconut Industry Board (CIB), Jamaica. PhD in Biochemistry from the University of the West Indies, Mona, Jamaica, MSc in Agriculture, Plant Protection from Gödöllö Agricultural University (Szent István University) Gödöllö, Hungary, ASc in Agriculture from College of Agriculture (College of Agriculture Science and Education) Portland, Jamaica.

Matthew Dickinson is a Professor of Plant of Plant Pathology at the University of Nottingham (UNOTT), United Kingdom. He has been working on phytoplasma diseases of coconut for over 25 years, with previous PhD students and projects with Tanzania, Ghana, Sri Lanka and Malaysia. Much of this work has involved development of molecular diagnostic techniques for detection of phytoplasmas and also development of methods to improve the taxonomic classifications of these organisms. He has been involved in developing the Loop Mediated Isothermal Amplification (LAMP) technique for rapid in-field detection of phytoplasmas that can be used in remote locations with minimal equipment. He has undertaken previous training workshops in the LAMP technology in Ghana, Malaysia and Vietnam. He is part of the TROPICSAFE consortium.

Dates:

May 1st,2nd,3rd (Wednesday to Friday)

Contact:

Tel:(52) 999-942-8330 Ext. 191 and 194

Venue:

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