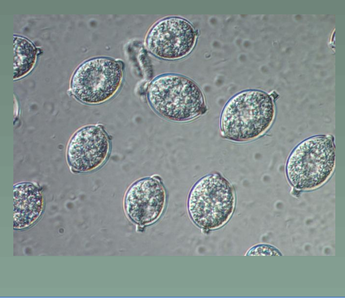
Deployment of Rapid Diagnostic Tools for *Phytophthora* on Horticultural Crops in Central America

Jean Beagle Ristaino[1], Kelly Ivors[1], Monica Blanco Meneses[2] , Dinie Espinal Rueda[3], Peter Bonants[4], and Jose Melgar[5]. [1]NC State University, Raleigh, NC; [2]Universidad de Costa Rica, San Jose, Costa Rica; [3]Zamarano University, Honduras, [4]Plant Research International, NL and [5]Earth University, Costa Rica.

ABSTRACT

Plant disease is a limiting factor in agricultural production in Latin America. Diseases are difficult to control due to high rainfall conditions and the presence of a diversity of plant pathogenic microorganisms and cause losses estimated to be as high as $30 billion per year. The accurate identification of *Phytophthora* has important implications for growers in Latin America and the US and can improve our knowledge of pathogen biology and ultimately treatment and control of tropical plant diseases. The risk of accidental introduction of *Phytophthora* species with trade requires continued monitoring and improved diagnostic capabilities. Our overall objective is to produce a platform of tools needed to detect, identify, and ultimately prevent entry of novel species of *Phytophthora* into the US with a major focus on development of surveillance tools for common and high threat species of *Phytophthora* on horticultural crops including potato, cacao and floriculture crops from Central America. We deployed a series of "shovel ready" technologies including: a Phytophthora diagnostics workshop held in San Jose Costa Rica in 2010, a Lucid key for species identification, PCR-RFLP and Padlock probes and digital diagnostic identification systems to identify *Phytophthora* species and improve the diagnostic capabilities for important plant disease clinics in the region. Most recently, the workshop was repeated in 2013 at Zamorano University in Honduras. We are working with collaborators including, FHIA in Honduras, Universidad de Costa Rica, CATIE, The World Cacao Foundation, DOLE Foods, and the Organization of Tropical Studies to conduct surveys of *Phytophthora* species on horticultural crops in the region. The accurate identification of *Phytophthora* has important implications for growers in Latin America and the US and resulted in the development of a Latin American Phytophthora Diagnostic Network (LAPDN) in 2010.

Fig. 1. Plant disease diagnosticians from six Latin American countries convened at Zamorano University in Honduras for the workshop. The university is also the site of the Horticulture Innovation Lab Regional Center.



PHYTOPHTHORA DIAGNOSTICS WORKSHOP

A course on the "Deployment of Rapid Diagnostic Tools for *Phytophthora* on Agricultural Crops in Central America " was held at Zamorano University, Honduras, September 29 – October 4, 2013. During the week 21 participants from six countries (Guatemala, Honduras, Nicaragua, Costa Rica, Mexico, and Chile) learned a number of "rapid" technologies to analyze and identify species of *Phytophthora* using morphological and molecular methods. The workshop provided opportunities for participants to network and to become involved with the Latin American *Phytophthora* Diagnostics Network (Fig. 1 – 5).

SPECIFIC PROJECT OBJECTIVES

- Deploy a series of "shovel ready" technologies for improving diagnosis of Phytophthora diseases by:
  - -Conducting a Phytophthora diagnostics workshop
  - -Deploying a Lucid key, a protocols book, molecular and digital diagnostic tools that can be used in plant disease clinics and labs in Latin America
  - -Conducting a survey to identify *Phytophthora* species on cacao, potato, wild Solanaceous sp. and floriculture crops in Latin America
  - -Improving the diagnostic capabilities for important plant disease clinics in the region
- Focus on Hort Crsp Priorities: sustainability of production systems, gender and horticultural development and capacity building

Fig 2. Workshop organizers from left to right: Dr. Kelly Ivors, Dinie Espinal Rueda, Dr. Jose Melgar, Dr. Jean Ristaino, and Dr. Monica Blanco

PHYTOPHTHORA SPECIES IDENTIFICATION USING MORPHOLOGICAL TECHNIQUES

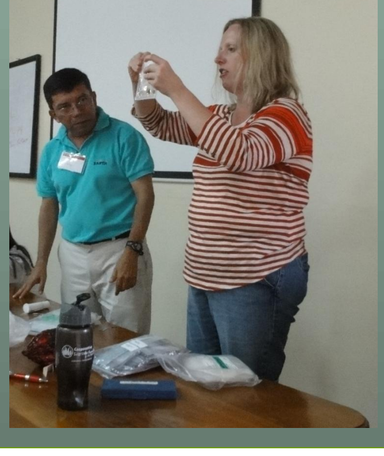
Fig 3. Students were taught rapid isolation techniques for isolating from infected tissue. In addition, students were given an unknown *Phytophthora* culture to identify using morphological characters and the Lucid key developed in conjunction with this course.

JUSTIFICATION

Plant disease is a limiting factor in agricultural production in many areas of the world. In the humid tropics, diseases are difficult to control due to high rainfall conditions and the presence of a diversity of plant pathogenic microorganisms. We live in a global community and plant diseases do not recognize country borders. During the ten-year period from 1989 through 1998, imports from Central America to the US have grown from $11.4 million to $28.1 million (a 147% increase). Small landowners and women farmers are actively involved in horticultural crop production and need access to well-trained plant pathologists that use state-of-the-art plant pathogen diagnostic capabilities in their laboratories. An ultimate goal of this project is to develop a coordinated network of trained extension, local government, university scientists and individuals in plant diagnostic clinics within Latin America that will have the tools needed for implementing more sustainable food production for small subsistence farmers in the region.

MOLECULAR DIAGNOSTICS FOR *PHYTOPHTHORA* SPECIES IDENTIFICATION

Fig 4. In addition to physical characters, students utilized molecular techniques, including PCR, PCR-RFLP, and real time Taqman PCR in order to identify their unknown culture to species.



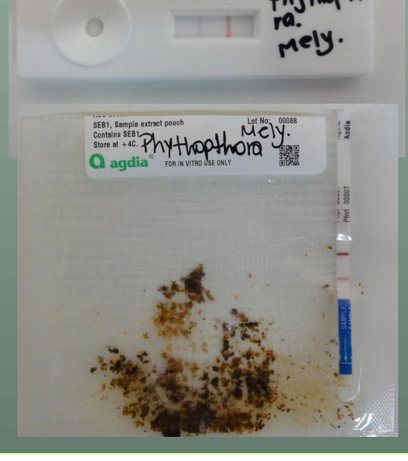


Fig 5. Instructors Jose Melgar and Kelly Ivors demonstrate the use of a rapid immunoassay kit for detecting *Phytophthora*.

Many Phytophthora diseases have reached epidemic proportions due to the development of resistance to fungicides such as metalaxyl. However, *Phytophthora* species represent a significant and emerging biosecurity threat, in large part due to increases in plant movement via international trade. Several species including *P. quercina*, *P. kernoviae*, *P. pinifolia*, *P. meadii*, and *P. tentaculata* are currently identified as highly important potential pathogens in plants imported into the United States and have been identified by an important stakeholder group, the regulators of the USDA-APHIS, who develop regulations that impact the nursery trade and industry.

ACKNOWLEDGMENTS

Thanks to the many Phytophthora researchers worldwide who have contributed their images for fact sheets and are credited in the figure legends of the Lucid key. Thanks to Julia Hu and Marion Haege for work on the Lucid key. This poster is made possible by the generous support of the American people through the United States Agency for International Development (USAID). The contents are the responsibility of the Horticulture Innovation Lab *Phytophthora* Diagnostics project and do not necessarily reflect the views of USAID or the United States Government.