Cover Page

Project Title

On site detection of three viruses infecting hop by Replicase Polymerase Amplification (RPA) combined with portable lateral flow device.

Project Summary

The West Coast of the United States has soils and climates that are conducive to hop production, accounting for 98% of the domestic output. However, many challenges exist that can influence the cost of production, yield, and quality of the cone. Diseases caused by viruses are a critical factor affecting the potential profitability of hop producers. This research proposes the development of three Reverse Transcription Replicase Polymerase Amplification (RT-RPA) assays for the rapid, on-site, sensitive detection of three carlaviruses most frequently infecting hop: Hop Mosaic Virus (HpMV), Hop Latent Virus (HpLV), and American Hop Latent Virus (AHLV). The advantage of RT-RPA over RT-PCR is that it is simple and can be easily transferred to hop nurseries or farms as a technique for effective integrated disease management. Isothermal reactions are performed at 37°C, with no Thermocyclers (PCR machines) needed, but economical mini dry baths. Moreover, results are readable in lateral flow devices, like at-home COVID-19 tests. Although the relevance of other pathogens infecting Hop, such as the ilarvirus Apple mosaic virus (ApMV), the nepovirus Arabis mosaic virus (ArMV), and the important viroids Hop latent viroid (HpLVd) and Hop stunt viroid (HpSVd) is well known and documented, the carlavirus are considered being prevalent and priority. Implementation of RPA in nurseries or farms will update hop disease management to 21st-century technology. The team addressing this research proposal has experience working with hop pathogens and developing RPAs.

Proposed Duration: 1 year

Project Leader: Francisco, Ochoa-Corona. Oklahoma State University (OSU)

Co-PL or Technical Assistance:

- Charles Fontanier (OSU),
- Scott Harper (Washington State University), and
- Andres Espindola (OSU)
- Daniele Do Nascimento (Postdoctoral Scientist)

Amount Requested: $75,000.00

Send Funding To:

OSU Grants
P.O. Box 248957. Oklahoma City, OK 73124-8957
Email: gcfa@okstate.edu Phone :405-744-6097

Proposal Narrative

Project Title

On site detection of three viruses infecting hop by Replicase Polymerase Amplification (RPA) combined with portable lateral flow device.

Statement of Problem

Diseases caused by viruses and viroids negatively affect the potential profitability of hop producers. There are six common viruses or virus-like organisms infecting hops in the U.S., of which *Carlavirus latenshumuli* formerly Hop latent virus (HLV), *Carlavirus americanense,* formerly American hop latent virus (AHLV), and *Carlavirus humuli* formerly Hop mosaic virus (HMV) are amongst the most common (Pethybridge and Turechek 2003), due in part to their aphid transmissibility and ubiquity of their vector species. However, it is essential to consider the hop diseases reported in the U.S. about the global hop virome. To date, 16 viruses and four viroids have been reported to affect hops (Pethybridge et al. 2008). Since some of these viruses and viroids are distributed worldwide, whereas others are reported locally (Gargani et al. 2017), the risk of new unwanted virus introductions may occur due to international trade and the lack of rapid detection methods for monitoring germplasm movement and selection. Sanitary measures to prevent the spread of viral infections require rapid diagnostic of the presence of key pathogens. Current methods of virus detection are limited to observation of virus symptoms, ELISA (for ApMV), and PCR-based assays. Unfortunately, visual symptoms are not always reliable because viruses may remain undetected during the latent stage of infection, ELISA methods are largely unavailable for most viruses and take time to develop and validate, and PCR-based assays require significant laboratory investment. Efforts to develop less expensive virus detection methods that can be used on-farm are needed for effective detection of virus infections in nurseries and hop yards.

Justification and Importance of Proposed Research

The implementation of combined RPA and Lateral Flow Immunoassay in virus detection has recently become widespread; for viruses whose genome is composed of RNA, Reverse Transcription (RT) is required to convert RNA into complementary DNA (cDNA), which serves as the template for RPA and also for PCR-amplified copies. Therefore, detection by Reverse Transcription Replicase Polymerase Amplification (RT-RPA) is the method to follow for carlaviruses because their genomes are composed of RNA. A second piece of technology we will use, combined with RT-RPA, is the portable lateral flow device (PCRD-2) assay, which allows for the rapid, on-site, and sensitive detection of the three targeted carlaviruses most frequently infecting hop: HpMV, HpLV, and AHLV.

RT-RPA can be easily transferred to hop nurseries or farms as a technique for effective integrated disease management. RPA implementation requires minimal capital investment if compared with PCR and ELISA. Moreover, RT-RPA is easy to teach and train because it involves fewer steps and utilizes simpler equipment.

Of the three viruses, HpMV is the most likely to cause observable symptoms and crop damage because on sensitive cultivars, chlorotic mosaic can develop between major leaf veins. Severely affected plants may establish poorly when planted, have weak bine growth, and often fail to attach to the string, followed by premature plant decline and death. Cultivars with Golding-type parentage are most susceptible to this virus, although this is suspected to be virus-strain specific. Cone yields and weights can be reduced by up to ~50% in cultivars sensitive to HpMV, as well as reductions in alpha- and beta-acids of up to ~20% (Probasco and Murphey 1996; Pethybridge et al. 2000; Pethybridge et al. 2002a; Pethybridge et al. 2008). HpLV is largely latent on most cultivars, though has been reported to cause chlorotic flecking on cultivars Cluster and Hersbrucker Spat, but not on cultivars from the Golding or Wye lineages, which remain asymptomatic (Probasco and Skotland 1978; Adams and Barbara 1982; Pethybridge et al. 2008). HpLV has also been associated with reduced growth, shorter internodes and laterals, and fewer leaves on some cultivars, and yield reductions of between 10-70% (Probasco and Murphey 1996; Pethybridge et al. 2002a), with corresponding reductions in alpha-acids. No diagnosable symptoms have been reported for AHLV on any cultivars, though it, along with HpLV may synergistically increase loss when in combination with more pathogenic viruses (Pethybridge et al. 2002a). The interaction of multiple viruses or viroids is often enhanced when combined with extreme weather conditions like heat and drought or other biotic pests such as mites. The primary means of spread of these carlaviruses, and likely why they are so common across the Pacific Northwest is because they are transmitted by aphid species. All three viruses are transmitted by the hop aphid (*Phorodon humuli*) (Adams and Barbara 1982), while HpMV and HpLV are also transmitted in the field by *Macrosiphum euphorbiae,* the potato aphid, and *Myzus persicae*, the green peach aphid, a species that is prolific and highly mobile (Crowle et al. 2006).

Thus, the impacts of viruses in perennial plant systems are likely to worsen over time, as well-documented climate changes, which include global warming, are expected to favor the expression and effect of diseases caused by viruses (Broecker, 1975). From here, it becomes apparent that the significance of developing rapid, on-site, and applicable detection methods, such as RT-RPA, lies in implementing control measures in seed stock foundational blocks and breeding programs.

The use of virus-tested planting stock is the most practical method for limiting the entry of these viruses into new plantings. However, preventing aphid transmission in areas of high incidence is a challenging task. The application of insecticides to control aphids is inefficient for limiting the introduction of viruses, as they are transmitted during very short host acquisition times during early feeding on plants before the viruliferous aphids are killed. Additionally, these aphids have a broad host range, making targeted sprays ineffective. However, reducing local aphid populations using contact or systemic insecticides can reduce the rate of secondary transmission within a hop yard. Therefore, early detection and removal of infected bines are the most effective means of control; however, to achieve this, rapid and less expensive detection assays are required.

Understanding RPA

The RPA is a technique with applications in pathogen diagnostics. RPA is a molecular and sensitive, low-temperature, isothermal (constant temperature) DNA amplification method that does not require thermal cycling (alternation of a set of temperatures). RPA requires two oligonucleotide primers (like PCR), which are short DNA sequences that initiate the synthesis and massive amplification of the targeted diagnostic virus genomic sequence. A mixture of a) recombinase enzymes, b) a single-stranded binding protein, and c) a strand-displacing DNA polymerase carries out the RPA reaction. The reaction is performed at a constant temperature, which may vary between 37 °C and 42°C. These low temperatures (compared to PCR) facilitate translating the technique to field conditions. RPA can also be used to detect RNA targets by incorporating reverse transcriptase into the reaction and so being named Reverse Transcription RPA (RT-RPA). The enzyme reverse transcriptase synthesizes a complementary sequence of the targeted viral sequence to generate a complementary DNA (cDNA) template from RNA, thereby initiating the RPA DNA amplification reaction. Subsequently, the Amplified DNA can be visualized using various detection methods, including commercially available lateral flow assays, such as the PCRD-2 devices.

What is the Lateral Flow Immunoassay PCRD-2

PCRD-2 is a nucleic acid lateral flow test used for rapid readout of post-isothermal amplification reaction products. The name PCRD-2 is a trademark assigned to the rapid lateral flow immunoassay, designed to detect and visualize the successful amplification of targeted RPA DNA sequences (Fig. 1).

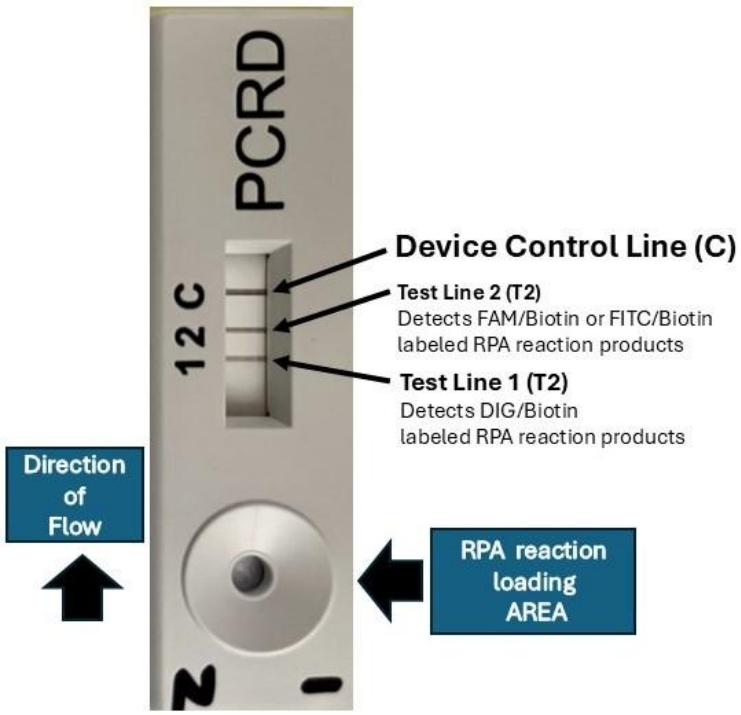

Fig.1. A Lateral Flow Immunoassay PCRD-2 device showing the area RPA reaction loading, flowing direction of the reaction, and meaning of the resulting lines 1 and 2, which mean positive detection of the targeted virus and the corresponding internal control for the host, and the device control line.

Objective(s)

1. To develop three Reverse Transcription Replicase Polymerase Amplification (RT-RPA) assays for the rapid, on-site, sensitive detection of three carlaviruses frequently infecting hop: Hop Mosaic Virus (HpMV), Hop Latent Virus (HpLV), and American Hop Latent Virus (AHLV).
2. To validate the method with field samples collected in the Pacific Northwest.
3. To transfer the technology to growers through an outreach webinar demonstration, one factsheet including the cost of the method per sample, equipment, and reagents required. One scientific article.
4. Attending

Procedures/Methods to accomplish objectives

Nucleic acids extractions

Total nucleic acids and total RNA will be extracted from hop tissue. Approximately 100 mg will be loaded into 2 mL microcentrifuge tubes or plastic mesh bags and homogenized using liquid nitrogen and/or pestles. Different ratios of tissue:buffer will be tested. Total RNA will be extracted and purified using the RNeasy plant mini kit (Qiagen Inc., Valencia, CA) as the reference kit. For virus-infected lyophilized reference positive controls, 450 µL of RLT buffer (from the Qiagen RNeasy Plant Mini Kit) will be directly aliquoted into the positive control vial, vortexed for 30 seconds, and the total RNA extracted following Qiagen's instructions. Alternative methods, such as direct trapping into PCR tubes, will also be tested (Babu et al. 2017).

The quantity and quality of the extracted RNA will be quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). Standards will be prepared by serial dilution from nanogram to femtogram/µl.

Primer design

RPA primers are 30-37 nucleotides (nt) long and will be designed using the Primer3 application. The thermodynamics and tendency to form self-dimers will be further analyzed using mFold and PrimerChecker. The selected parameters for optimal RPA primers are described in the TwistAmp Design Manual. The specificity of the primer sets will be tested *in silico* using BLASTn and Primer-BLAST. A specificity panel will be used to assess the *in vitro* specificity of the RPA primers, which will be synthesized by Integrated DNA Technologies (IDT). To combine the RPA reaction with a subsequent lateral flow technology, modifications are to be added to the primers. These modifications consist of labeling with Biotin at the 5' termini of the forward primer (sense) and FAM or DIG at the 5'termini of the reverse primer (antisense).

RPA and Lateral flow PCRD-2 optimization

The RPA reaction will be performed using the RPA TwistAmp® basic (TwistDx, UK) according to the manufacturer's protocol with modifications. Betaine (10 µL, 5 M; Thermo Fisher, USA) may be added to the RPA reactions if high levels of oxidation are observed. The RPA reactions will be incubated in a mini dry bath incubator (GeneMate, USA) at a constant temperature of 35 °C to 40 °C for 20 min to 40 min. Reactions will then be finished at 80 °C for 5 min to deactivate the enzyme complex after DNA amplification. The amplified RPA product will be loaded into lateral flow PCRD-2 as per the manufacturer's instructions. Additionally, the product will be purified using the QIAquick PCR Purification Kit (Qiagen, USA) to enhance the visual discrimination of

products in 1% agarose gel electrophoresis in 1X TAE buffer, stained with SYBR Safe (Invitrogen, USA) added to the gel (Fig. 2).

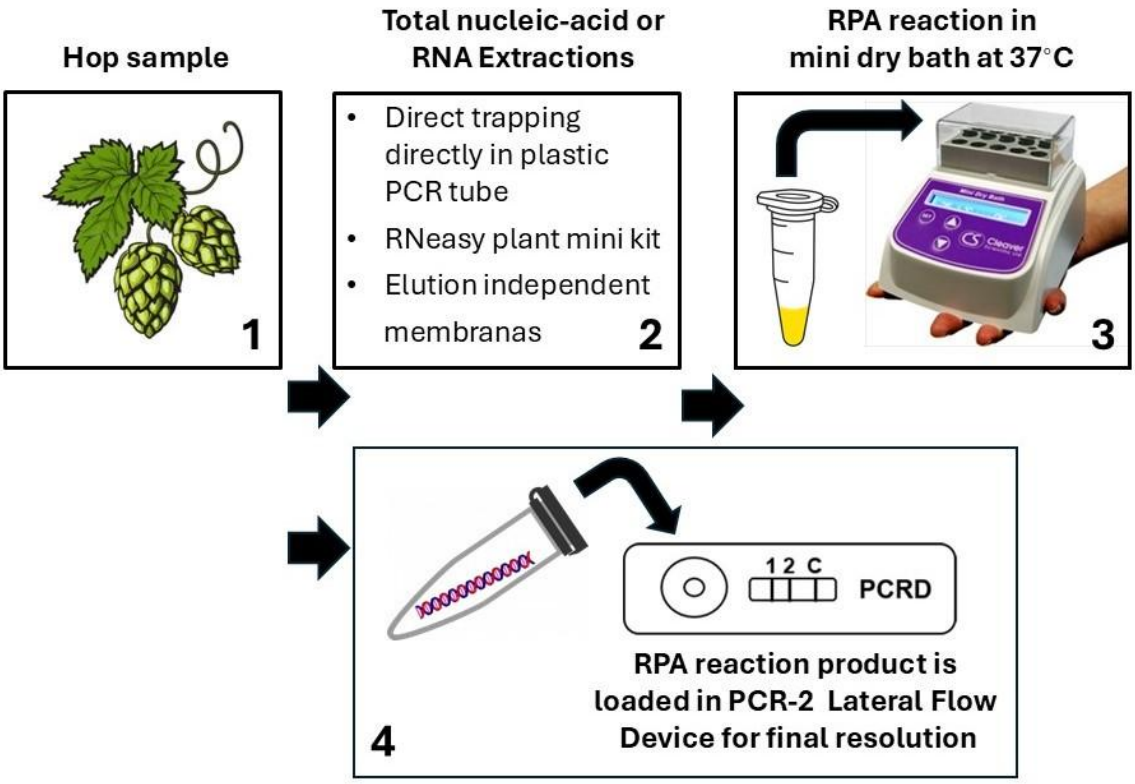

Fig. 2. Schematic representation of the four main steps of the RPA assay from sample tissue to lateral flow resolution and visualization.

Outcomes

1). One Laboratory- field protocol containing detailed guidelines to perform the method at farms or nurseries. 2). One outreach webinar organized in conjunction with the Hop Research Council/Washington Hop Commission. 3). One Extension factsheet. 4). One scientific article published in a refereed journal.

Steering supervision

Co-Pi Andres Espindola will oversee the project development and practices and also will provide sufficient technical knowledge guiding and supervising the grant activities to ensure proper and efficient administration of funds and time. He will participate in scheduled monthly meetings during the duration of the grant.

Extension and Outreach Activities

The research team proposes creating a series of outreach products to be shared in person and through online platforms, targeting hop industry stakeholders. The primary deliverables are the protocols for the three RPA techniques, which will be described in terms that can be understood and reproduced by both technical and non-technical personnel. The protocols will

include a detailed list of equipment and reagents needed with the estimated cost per unit. These protocols will be delivered as both a printable Extension document (pdf factsheet) and as an instructional video that can be shared via YouTube or a similar platform.
Scientists will participate in the American Hop Convention to meet in person with hop growers and associated stakeholders, offering a presentation about the research funded by the grant, as well as progress updates. This will include an in-person RPA demonstration at the annual HRC summer meeting. Moreover, the team will publish an online extension factsheet note to address the Hop audience nationally and internationally, promoting the support provided by the Hop Research Council and the Washington Hop Commission for the described problem-solving research. Furthermore, Co-PI Scott Harper will use his extensive network of growers and nursery propagators to ensure project outcomes are delivered to key stakeholders.

Time Frame for Objectives:
Include a time frame for accomplishment of specific objective goals, including associated anticipated costs per objective in relation to the total amount being requested. Use of a Gantt style worksheet is encouraged.

OBJECTIVE	FEB-APR 2026	MAY-JUL 2026	AUG-OCT 2026	NOV/26-ENE-/27	APROX BUDGET $75,000
1.- To develop three RPAs for three viruses infecting hop	X	X	X	X	$30,000
2.- To validate the three methods			X	X	$25,153
3.- To transfer the technology				X	$2,450
4.-To attend the Sumer Hop convention.		X			$1,882

Project Budget:

Expenditure	Hop Research Council Request	Commission/Other Request (specify)		Total Amount Requested
		State: N/A	Other: N/A	
		Amount (cash or in-kind)	Amount (cash or in-kind)	
Salaries	$49,500	-	-	$49,500
Employee Benefits	$5,653	-	-	$5,653
Temporary or hourly workers	-	-	-	$0
Travel	$1,882	-	-	$1,882
USA Hop Convention Registration	$800	-	-	$800
Equipment	-	-	-	$0
Other (specify)	$17,165	-	-	$17,165
Total	$75,000	$0	$0	$75,000

The salary request is to fund a full-time postdoctoral scientist. Responsibilities to include designing and developing an Artificial Positive Control; performing their RPA experimentation which include data analysis, optimization, validation, and cost analysis; and writing a protocol and a draft of a scientific article. Fringe benefit rates are negotiated annually with the Office of Naval Research and will be adjusted accordingly. The calculated rate for OSU – Agricultural Sciences for FY26: 11.42% (post-doc).

In addition to the full registration packet of $800 to attend the annual American Hop Convention, which will be in Tucson, AZ, travel expenses include 3 nights at the Hilton El Conquistador Hotel $250 each, Subtotal $750 + Roundtrip airfare Stillwater-Tucson by American Airlines $832 + Per diem, first and last day $60 each plus a regular working day $80, subtotal $200 + Taxi transportation from Airport to Hotel and back $100.

Other direct costs include:

- Materials and Supplies - $14,595
    - Disposables such as pipette tips, gloves, and microcentrifuge tube, PCR tubes.
    - Reagents such as kits for RNA extraction (Reverse Transcription PCR (RT-PCR), Replicase Polymerase Amplification (RPA), Artificial Positive Control plasmid (APC), oligonucleotide primers for PCR and RPA, Nuclei Acid Lateral Flow Immunoassay cassettes for combined Molecular & Lateral Flow assays Technology. Reference positive controls.
- Publication - $2,450
    - One scientific article in a highly ranked journal, Phytofrontiers.
- Shipping - $120
    - FEDEX shipping of samples.

Literature Review

- Adams, A. N., & Barbara, D. J. (1982). Host range, purification and some properties of two carlaviruses from hop (*Humulus lupulus*): hop latent and American hop latent. *Annals of Applied Biology*, 101(3), 483-494.

- Broecker, W.S. (8 August 1975). "Climatic Change: Are We on the Brink of a Pronounced Global Warming?". *Science.* 189 (4201): 460–463

- Crowle, D. R., Pethybridge, S. J., & Wilson, C. R. (2006). Transmission of hop latent and hop mosaic carlaviruses by Macrosiphum euphorbiae and Myzus persicae. Journal of Phytopathology, 154(11-12), 745-747.
- Gargani, E., Ferretti, L., Faggioli, F., Haegi, A., Luigi, M., Landi, S., Simoni,S., Benvenuti, C., Guidi, S., Simoncini, S., D'Errico, G., Amoriello, T.,Ciccoritti, R., Roversi, P. F., and Carbone, K. 2017. A survey on pests and diseases of Italian hop crops. Italus Hortus 24:1-17.

- Pethybridge, S. J., & Turechek, W. W. (2003). Analysis of the association among three viruses infecting hop in Australia. *Plant Pathology*, *52*(2), 158-167.

- Pethybridge, S. J., Wilson, C. R., Sherriff, L. J., Leggett, G. W., & Munro, D. (2000). Virus incidence in Australian hop (*Humulus lupulus* L.) gardens and cultivar differences in susceptibility to infection. Australian journal of agricultural research, 51(6), 685-689.

- Pethybridge, S. J., Wilson, C. R., Hay, F. S., Leggett, G. W., & Sherriff, L. J. (2002). Effect of viruses on agronomic and brewing characteristics of four hop (Humulus lupulus) cultivars in Australia. *Annals of Applied Biology*, *140*(1), 97-105.

- Pethybridge, S. J., & Madden, L. V. (2003). Analysis of spatiotemporal dynamics of virus spread in an Australian hop garden by stochastic modeling. *Plant Disease*, 87(1), 56-62.

- Pethybridge, S. J., Hay, F. S., Barbara, D. J., Eastwell, K. C., & Wilson, C. R. (2008). Viruses and viroids infecting hop: Significance, epidemiology, and management. *Plant Disease*, *92*(3), 324-338.

- Probasco, E. G., & Skotland, C. B. (1978). Host range, general properties, purification and electron microscopy of hop latent virus. *Phytopathology*, 68, 277-281.

- Probasco, G., & Murphey, J. M. (1996) The effects of hop viruses on brewing and agronomic characteristics in the hop variety chinook. *MBAA technical quarterly* 33(3), 160-165.

Francisco M. Ochoa-Corona
Professor. Institute for Biosecurity & Microbial Forensics and Department of Entomology & Plant Pathology
Oklahoma State University. 127 NRC, Stillwater Ok 74078.
https://experts.okstate.edu/ochoaco / https://orcid.org/0000-0002-4112-8209

Education and Training

- 1982. Universidad del Zulia, Venezuela, Agronomy. Agricultural Engineering.
- 1990. Universidad Central de Venezuela, Plant Protection. M.Sc.
- 2001. University of Florida, Gainesville, Plant Pathology-Virology. Ph.D.
- 2001-2002. Postdoctoral at University of Florida, GCREC. Plant Virology.

Research and Professional Experience

Positions:

2019-present **Professor.** Oklahoma State University, Natl. Institute for Microbial Forensics & Food and Agricultural Biosecurity (NIMFFAB), and Department of Entomology & Plant Pathology, 90% research and 10 % teaching respectively, Stillwater, OK. USA.

2014-2019 **Associate professor.** Oklahoma State University, Natl. Institute for Microbial Forensics & Food and Agricultural Biosecurity (NIMFFAB), Department of Entomology & Plant Pathology, Stillwater, OK. USA.

2008-2014 **Assistant Professor**. Oklahoma State University, Natl. Institute for Microbial Forensics & Food and Agricultural Biosecurity (NIMFFAB), Department of Entomology & Plant Pathology, Stillwater, OK. USA.

2005-2008 **Principal Adviser Virology**. Ministry of Agriculture and Forestry (MAF). Biosecurity New Zealand (BNZ). Investigation and Diagnostic Centre (IDC), Plant Health & Environment Laboratory (PHEL), Auckland, New Zealand.

2002-2005 **Scientist II-Molecular Plant Virologist.** Biosecurity New Zealand, Ministry of Agriculture and Forestry. Auckland, New Zealand.

2001-2002 **Postdoctoral Associate**. University of Florida. Gulf Coast Research & Education Center. Plant Virology Laboratory, Dr. Jane E. Polston.

1996-2000 **Graduate Research Assistant**. University of Florida. Plant Pathology Department. Citrus Research & Education Center. Plant Virology Laboratory, Dr. Richard F. Lee, and Plant Virology Laboratory, Dr. C.L. Niblett. Gainesville.

1994-1995 **Instructor and Lecturer of Plant Pathology**. Department of Botany, Section of Plant Pathology, Universidad Central de Venezuela (University of Central Venezuela), Venezuela.

1991-1993 **Professor and F.V.P.I. Scientist**, Department of Botany, Section of Plant Pathology, Universidad Central de Venezuela (University of Central Venezuela). (The F.V.P.I. Program, National Council for Technology and Research of Venezuela, supports scientists selected for novel work.)

1990-1991 **National Citrus Program Leader**. Farmer Service Foundation, Venezuela (FUSAGRI).

1984-1987 Researcher and Extension Educator. Citrus Program. FUSAGRI.

1982-1984 Researcher and Extension Educator. Low Valley Tropical Fruit Program. FUSAGRI

Teaching Experience:

Instructor of record for PLP 5014 'Plant Virology', graduate course, 2014-present. PLP 2143 'Global Agricultural Biosecurity and Microbial Forensics', undergraduate course, 2009-to present.

Honors and Awards:

- 2021-2028 Sarkeys Distinguish Endowed Professorship.
- 2015 Berry Faculty Fellow Award. The OSU Oklahoma Water Resources Center (Water Center) recognize the Division of Agricultural Sciences and Natural Resources (DASNR) faculty, Extension

educators, and district specialists who are making outstanding contributions in research, Extension, and/or education. I was selected the 2015-2017 Berry Faculty Fellow.

- 2012 Oklahoma State University, President's Cup For Creative Interdisciplinary Award. 3rd Place. EDNA: Powerful New Technology for Electronic Diagnostic Nucleic Acid Analysis.
- 1994 Outstanding Lecturer Award. Awarded by the XLVI Class (1994) of Engineers in Agriculture. Universidad Central de Venezuela. 1994.

Grants Received:

Total 40 projects granted at Oklahoma State University. Total Funds granted: US$13,675,206.9

Publications

Peer Reviewed Publications in the Last Five Years:

2025

1. Lizbeth Peña-Zúñiga, Andres Espindola, Francisco Ochoa-Corona. 2025. Assessment of viral limit of detection in spiked, unassembled High Throughput Sequence datasets. Phytofrontiers. 2025. Accepted. Available at 'First Look' https://doi.org/10.1094/PHYTOFR-11-24-0121-FI
2. Marcos Roberto Ribeiro-Junior, Andres Espindola, Daniele Maria Nascimento, Felipe Barreto Da Silva, Renate Krause-Sakate, Francisco Manuel Ochoa-Corona. An attempt toward the global screening of soybean viruses using EDNA-MiFi based electronic probes " Phytofrontiers. 2025. Accepted. Available at 'First Look' https://doi.org/10.1094/PHYTOFR-12-24-0141-FI
3. Kuhn et al. Annual (2024) taxonomic update of RNA-directed RNA polymerase-encoding negative-sense RNA viruses (realm *Riboviria*: kingdom *Orthornavirae*: phylum *Negarnaviricota*). Journal of General Virology. 2025. Journal of General Virology 2025;106:002077 DOI 10.1099/jgv.0.002077 https://doi.org/10.1099/jgv.0.002077
4. First report of *Potyvirus phytolaccae*, formerly Pokeweed mosaic virus (PkMV), in Oklahoma Rafaela Gomes Ruschel, Camilla Austin, Francisco M. Ochoa-Corona. Plant Disease. 2025. *Submitted and under review* (manuscript PDIS-1224-2555-PDN).
5. Renate Krause-Sakate, Rafaela Gomes Ruschel, Francisco Ochoa-Corona, Sharon A. Andreason, Reese Trujillo, Marcos Roberto Ribeiro-Junior, Daniele Maria Nascimento do, Bruno Rossito de Marchi, Hugh A. Smith, Samuel F. Hutton, Sara Wallace. 2025. First detection of *Bemisia tabaci* (Hemiptera: Aleyrodidae) MED in Oklahoma and development of a high-resolution melting assay for MEAM1 and MED discrimination. Journal of Economic Entomology: 118 (1):45-56. doi: 10.1093/jee/toae228.2023

2024

1. Ali Pasha, Andres S. Espindola, Heiko Ziebell, and Francisco M. Ochoa-Corona. Highly Curated and Reliable E-Probes for Detection of Viral Pathogens in Unassembled HTS Datasets from Hops. Phytofrontiers. Accepted 24 December 2024, in press. First look. https://doi.org/10.1094/PHYTOFR-09-24-0106-FI
2. Rosa Lilia Ferrucho, Gustavo Adolfo Marín-Ramírez, Francisco Ochoa-Corona and Carlos Ariel Ángel-Calle. 2024. PCR-based detection for the quarantine fungus *Colletotrichum kahawae*, a biosecurity threat to the coffee (*Coffea arabica*) industry worldwide. Plant Disease. Vol 108: 2615-2624. https://apsjournals.apsnet.org/doi/epdf/10.1094/PDIS-09-23-1788-SR
3. Ribeiro-Junior, M.R., Barreto da Silva, F., Marubayashi, J.M. et al. Molecular identification of thrips species in Brazilian agroecosystems. Phytoparasitica 52, 79 (2024). https://doi.org/10.1007/s12600-024-01198-8
4. Michele Digiaro, Toufic Elbeaino, Kenji Kubota, Francisco M. Ochoa-Corona, Susanne von Bargen. 2024. ICTV Virus Taxonomy Profile: Fimoviridae 2024. Journal of General Virology 105:001943. https://doi.org/10.1099/jgv.0.001943

2023

1. Rafaela Gomes Ruschel, Mason Taylor, Francisco M. Ochoa-Corona, Abdul Kader Jailani Amirudeen, Tobiasz Druciarek, Mathews Paret. 2023. An artificial positive control for routine detection of rose

rosette virus and *Phyllocoptes fructiphilus* that fit most primers for PCR, LAMP and RPA based assays. Ann Appl Biol. Vol. 183 (1) 67-79. https://doi.org/10.1111/aab.12834

2. Olmedo-Velarde A, Ochoa-Corona FM, Larrea-Sarmiento AE, Elbeaino T, Flores F (2023) *In-silico* prediction of RT-qPCR-high resolution melting for broad detection of emaraviruses. PLoS ONE 18(5): e0272980. https://doi.org/10.1371/journal.pone.0272980
3. Kuhn et al. Annual (2023) taxonomic update of RNA- directed RNA polymerase- encoding negative-sense RNA viruses (realm Riboviria: kingdom Orthornavirae: phylum Negarnaviricota). Journal of General Virology. 2023; 104:001864 https://doi.org/10.1099/jgv.0.001864

2022

1. Marcos Roberto Ribeiro-Junior, Felipe Barreto da Silva, Julio Massaharu Marubayashi, Juliana Uzan, Angelica Maria Nogueira, Cristiane Muller, Daniele Maria Nascimento, Valdir Atsushi Yuki, Nobuyoshi Narita, Marcelo Agenor Pavan, Francisco Manuel Ochoa-Corona, Renate Krause-Sakate. 2022. Molecular and biological characterization of an isolate of the potyvirus passiflora virus Y naturally infecting soybean (Glycine max) in Brazil. Archives of Virology. https://doi.org/10.1007/s00705-022-05605-5
2. 2022 taxonomic update of phylum *Negarnaviricota* (*Riboviria*:*Orthornavirae*), including the large orders *Bunyavirales* and *Mononegavirales.Kuhn.* Archives of Virology. (In press) https://doi.org/10.1007/s00705-022-05546-z
3. Nicolas Aparicio Claros, Madalyn Shires, Dimitre Mollov, John Hammond, Ramon Jordan, Francisco Ochoa-Corona, Jennifer Olson, Kevin Ong, and Lina Rodriguez Salamanca. Rose Rosette Disease: A Diagnostic Guide. Plant Health Progress 2022 23:4, 482-491. https://doi.org/10.1094/PHP-05-22-0047-DG
4. Felipe Cevallos, Lizbeth Peña-Zuñiga, Francisco Ochoa-Corona & John Damicone (2022): Frequency and genetic variability of the avirulence gene AvrLm4-7 among Leptosphaeriamaculans isolates collected in Oklahoma, USA, Canadian Journal of Plant Pathology, https://doi:10.1080/07060661.2022.2077449

2021

1. Andrea Salazar, Francisco M. Ochoa-Corona, Jennifer Olson, Binoy Babu, Mathews Paret. 2021. Probing Loop-Mediated Isothermal Amplification (LAMP) targeting two gene-fragments of rose rosette virus PLoS. 2021.08.in press; doi: https://doi.org/10.1101/2021.08.17.456656.
2. Espindola, Andres S., Daniela Sempertegui-Bayas, Danny F. Bravo-Padilla, Viviana Freire-Zapata, Francisco Ochoa-Corona, and Kitty F. Cardwell. 2021. "TASPERT: Target-Specific Reverse Transcript Pools to Improve HTS Plant Virus Diagnostics" *Viruses* 13, no. 7: 1223. https://doi.org/10.3390/v13071223
3. Salazar, A., Ochoa-Corona, F.M., Talley, J.L., Bruce H. Noden. 2021. Recombinase polymerase amplification (RPA) with lateral flow detection for three *Anaplasma* species of importance to livestock health. Sci Rep 11, 15962. https://doi.org/10.1038/s41598-021-95402-y
4. Andrea Salazar, Francisco M. Ochoa-Corona, Justin L.Talley, Bruce H. Noden. 2022. An elution independent collection device (EICD) for rapid collection of *Anaplasma marginale* DNA from blood samples. Acta Tropica Vol. 225, January 2022, 106201 https://doi.org/10.1016/j.actatropica.2021.106201
5. Ryan Domingo, Cristian Perez, Diksha Klair, HuongVu, Alika Candelario-Tochiki XupengWang, Amihan Camson, Jaclyn Nicole Uy, Mouauia Salameh, DarioArizala, Shefali Dobhal, Gamze Boluk, Jon-Paul Bingham, Francisco Ochoa-Corona, Md Emran Ali, James P. Stack, Jacqueline Fletcher, Jenee Odani, Daniel Jenkins, Anne M. Alvarez, Mohammad Arif. 2021. Genome-informed loop-mediated isothermal amplifcation assay for specifc detection of Pectobacterium parmentieri in infected potato tissues and soil. Scientifc Reports 11:21948. https://doi.org/10.1038/s41598-021-01196-4

Professional Service Activities:

- **2019-2025. Senior Editor.** Phytofrontiers, an American Phytoplathological Society (APS) peer-reviewed journal.
- **2014-2017**. **Senior Editor.** Plant Health Progress, an American Phytoplathological Society (APS) peer-reviewed journal of applied plant heath. ISSN 1535-1025
- **NCPNR – Rose. Attendance of Annual Tier II meetings for scientific input.**
- **2009-2010**. **National Academies of Sciences.** Washington. USA. Invited member of the Committee on Scientific Milestones for the Development of a Gene-Sequence-Based Classification System for Oversight of Select Agents.
- **2008-present Peer Reviewer for Referred Scientific Journals**
  Annals of Applied Biology, Applied and Environmental Microbiology,Crop Protection, European Journal of Plant Pathology, Journal of Virological Methods, Phytopathology, Phytopatologia Mediterranea, Plant Disease, Plant Disease notes, PLoS, Scientia Horticulturae, Scientific Reports_Nature, The International Journal of Molecular Sciences, Viruses.

RESEARCH TEAM BIOSKETCHES

Francisco M. Ochoa Corona is a Professor at the Institute for Biosecurity & Microbial Forensics (IBMF), and Department of Entomology & Plant Pathology, Oklahoma State University. Dr. Ochoa Corona is a forensic plant pathologist, specializes in developing and delivering reference diagnostics methods for exotic, naturalized, and indigenous plant viruses and other phytopathogens of relevance to agricultural biosecurity and microbial forensics. His work is applicable to plant pathogens, and more recently to waterborne plant viruses, insects, and also animal and public health viruses. Ochoa Corona's research in plant pathology contributes scientific input to regulatory officials regarding plant health emergencies and focuses on targeted aspects of forensic plant pathology that are relevant to agricultural biosecurity in Oklahoma, the southern plains, the United States, and other regions of the world such as the South Pacific and Central and South America.

Charles Fontanier is an associate professor in the Dept of Horticulture and Landscape Architecture. His research program primarily focuses on urban landscape management and ecology with particular emphasis in soil and water processes, applied stress physiology, and plant growth and development. In 2022, Fontanier began studying hops as a possible niche crop for diversified growers in the region. He built a 0.25-acre research hopyard to evaluate cultivar performance using conventional 18-ft trellising systems. The program also leads outreach education and conducts field days for interested growers and local craft brewers. Ongoing activities include evaluation of novel germplasm, development of low trellis systems, and use of plant growth regulators to enhance yield.

Andres Espindola Camacho is an Engineer in Biotechnology and Bioinformatician investigating the next generation sequencing (NGS) metagenome data analysis of plant-pathogen interaction systems to rapidly detect eukaryotic plant pathogens. Dr. Espindola contributed to innovating the bioinformatic tool EDNA (E-probe Diagnostic Nucleic acid Analysis) that effectively detects plant pathogens in sequenced metagenomes. He investigates the refinement of EDNA (termed EDNAtran) to detect actively infecting plant pathogens using transcriptomics and metatranscriptomic databases. Additional EDNAtran refinements included the detection of active metabolic pathways in plant-pathogen interaction systems, specifically the detection of metabolic transcripts involved in the production of aflatoxin in toxigenic Aspergillus flavus strains in corn. At present Dr. Espindola investigates at the Oklahoma State University Institute for Biosecurity and Microbial Forensics contributing new statistical and bioinformatic approaches to make EDNA effectively available to the research, regulatory and law enforcement

Scott Harper's research program is located at Washington State University, Department of Plant Pathology, and his research focuses on nonculturable systemic pathogens (i.e. viruses, viroids and phytoplasmas) of perennial crops in the Pacific Northwest, particularly those infecting fruit trees (*Malus* and *Prunus* spp.), grapevines (*Vitis* sp.), and Hops (*Humulus lupulus*), studying how particular interest in how these pathogens establish an infection, seasonal effects on distribution and pathogenicity, and in the epidemiology of vector transmitted pathogens in the interface between cultivated and natural environments.