PLANT HEALTH TASK FORCE & FOCUS

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• Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP)

Dr. Rose Hammond  
• United States Department of Agriculture – Agricultural Research Service (USDA-ARS)

Dr. Della Johnston  
• Agriculture & Agri-Food Canada (AAFC)

PLANT HEALTH

▪ Promote joint research projects
▪ Capacity building and linking specialists and projects for proactive research on invasive pests and diseases
▪ Promote knowledge sharing on pests/diseases of tri-lateral interest through several means
▪ Carry out outreach activities with other countries and regions in LAC
PLANT HEALTH TASK FORCE

• Plant Health Task Force (PHTF) was formed in 2011
  • Harmonize protocols and share knowledge for insect identification
• 1st meeting and workshop in Vineland, NJ., 2013
  • focused on Brown Marmorated Stink Bug (BMSB) and parasitoids
• 2014 Workshop in Washington, DC
  • DNA barcoding of insects; tour of USDA-ARS insect collections at the Smithsonian
• 2015 Workshop in Monticello, Mexico
  • Molecular Insect Taxonomy
• 2016 Workshop in Ottawa, ON, Canada
  • Pests & Diseases of Solanaceans in North America: Trilateral approaches for their management
PLANT HEALTH TASK FORCE
WORKSHOP 2017
Beltsville, Maryland, U.S.A.
Initial Observations on the invasive Bagrada bug (Bagrada hilaris) in Mexico (Saltillo)

Reyna Ivonne Torres-Acosta*, Moisés Felipe Victoriano, Veronica Hernandez-Hernandez*, Richard A. Humber ** And Sergio Sánchez-Peña*

*Departamento de Parasitología, Universidad Autonoma Agraria Antonio Narro, Saltillo, Coahuila, Mexico
**USDA-ARS, Ithaca NY
*Bagrada hilaris* (left) and *Murgantia histrionica*, (Harlequin bug)

Harlequin bug is usually a very minor pest

**Bagrada: PRIMARY PEST**

Bagrada bug: Early season
Harlequin Bug: Late season

Harlequin: Usually low populations

In total, 213 naturally infected insects by five genera of entomopathogenic fungi

Collected Insects: 600

Mycosed (infected) Insects: 213

Overall % infection: 35.5 %
Parasitoid Exploitation of Native and Invasive Stink Bug Species in Canada: A Molecular Approach

Tara D. Gariepy
Agriculture and Agri-Food Canada
London Research and Development Centre
Ontario, Canada
Egg Masses are Hard to Find!

MORPHOLOGICAL IDENTIFICATION

MORPHOLOGICAL IDENTIFICATION

Molecular Tools?

Molecular Tools: Modified Barcoding

- DNA barcoding = great for identifying unknown species – large public database of sequences. BUT: Universal barcode primers = amplify everything
  - PCR primers specific to Pentatomidae and Scelionidae, nested within DNA barcode region
  - Identification of unknown or unexpected species for both host and parasitoid

Gariepy et al. 2013, Molecular Ecology
‘Candidatus Phytoplasma’: Tools for Detection and Identification

PROCINORTE – Plant Health Task Force Workshop
Working Beyond Boundaries to Secure Plant Health and Productivity

Beltsville, MD
October 11-13, 2017

Robert E. Davis
Molecular Plant Pathology Laboratory,
USDA-Agricultural Research Service, Beltsville, MD USA
Phytoplasmal Infection Derails Meristem Fate and Alters Plant Architecture


The shoot meristem gradually transitions from vegetative to reproductive.

Each symptom corresponds to a distinct phase in meristem fate derailment.

Gene expression: altered in relation to degree of stem cell commitment to floral destiny

iPhyClassifier output, example

16S rDNA sequence is identical to that of "Candidatus Phytoplasma pruni" rrnB reference strain (GenBank accession: JQ044392). The phytoplasma under study is either the "Candidatus Phytoplasma pruni" rrnB reference strain or a closely related strain that cannot be distinguished by 16S rDNA sequence alone.

16S group/subgroup classification -- The virtual RFLP pattern derived from the query 16S rDNA F2nR2 fragment is identical (similarity coefficient 1.00) to the reference pattern of 16Sr group III, subgroup A rrnB (GenBank accession: JQ044392). The phytoplasma under study is a member of 16SrIII-A.
Invasive Pests:
ARS Biological Control Update

Kim Hoelmer
USDA Agricultural Research Service
Beneficial Insects Introduction Research Unit
Newark, DE, USA
Landscape pests move between wild hosts and susceptible crops

**Trissolcus japonicus**

“samurai wasp”

- solitary egg parasitoid
- high % of eggs in mass attacked
- 2 - 3 weeks / generation
- Sib-mating with female-biased sex ratio
- Parent females aggressively guard egg masses
- 65 to 90% BMSB parasitism in Asia

C. Dieckhoff/ARS
Populations of *Psyllidae* and *Circulifer tenellus* as vectors of plant pathogens in *Capsicum annuum* L. at Central Mexico

Dr. Jaime MENA (INIFAP Zacatecas)
Dr. Rodolfo VELÁSQUEZ (INIFAP Zacatecas)
Dr. Joseph MUNYANEZA (USDA NPL, Washington, DC)
Dr. Kylie SWISHER (USDA ARS, Wapato, WA)
Many different Symptoms

DEFOLIATION

STUNTED GROWTH, NO FRUITS

STUNTED GROWTH

FRUIT DROPPING

A FEW FRUITS

SOME BIG BUD SYMPTOMS ON PEPPERS IN NEW MEXICO

Yellow foliage
Stunt growth
Witch broom
AND BCTV

Hyper leaf development, witch broom


Mosquita Blanca / Begomovirus (Bemisia spp)

Bemisia vs Trialeurodes
Vector vs No vector
Biology and natural enemies of the pepper weevil


Harrow Research and Development Centre
What parasitoids are present?

Three distinct *Nealiolus* spp.

Braconidae, Heliconinae

Dr. J. Fernandez-Triana

Pepper field of cascabelillo cultivar in Querétaro

2017 IICA Research Internship Assistance Program
Catalina Fernandez, PhD Candidate
Dr. Rodriguez-Leyva

*Jaliscoa hunteri* female ovipositing on alternate host larva
Biology, Impacts, & Monitoring of the Tomato Leaf Mining Moth *Tuta absoluta* (Meyrick)

Julia Mlynarek, Ph.D.
(Julia.mlynarek@canada.ca)
Harrow Research & Development Centre

`tutaabsoluta.com`
Ecological equivalent in North America

- *Tuta Absoluta*
- *Keiferia lycopersicella* (tomato pinworm)
- Other Species
  - *Sinoe capsana*
  - Native *Tuta* sp.

Parasitoids

- *Trichogramma* spp. – Aphelinidae (Argentina)
- *Encarsia poteri* – Aphelinidae (Europe & South America)
- *Apanteles* spp. – Braconidae (South America)
- *Bracon* spp. – Braconidae (endo) (Argentina)
- *Copidosoma* spp. – Encyrtidae (Argentina)
- *Campoplex haywardii* – Ichneumonidae (endo) (Argentina)
- *Diadegma ledicola* – Braconidae (endo) (Europe)
- *Habrobracon hebetor* – Braconidae (Mediterranean)
- *Temelucha* spp. – Ichneumonidae (endo) (Argentina)
- *Necremnus* spp. – Eulophidae (Europe)
- *Neochrysocharis formosus* – Eulophidae (Argentina)
- *Stenomesius* spp. – Eulophidae (Europe)
- *Pseudapanteles* spp. – Braconidae (endo) (Argentina)
- *Dineulophus photorimae* – Eulophidae (Europe & Argentina)
- *Spilochalcis* spp. – Chalcididae (Argentina)
The USDA National Plant Diagnostic Network: Protecting US Agriculture

Rubella S. Goswami
National Program Leader
Institute of Food Production and Sustainability
PYTHIUM APHANIDIERTUM CROWN ROT OF INDUSTRIAL HEMP

Jenifer Swiney, Jack Wilhelm and Shuhua Wang, Nevada Department of Agriculture Plant Pathology Laboratory

Cultivation of industrial hemp (Cannabis sativa) was first approved in 2014 for the purpose of research and development. The federal Farm Bill Section 7606 authorizes state agencies to conduct pilot trials on the crop to assess crop viability for the creation of an industry in prospective states. In Nevada, the Department of Agriculture authorizes the production of hemp crops for research purposes. The acreage of hemp production in Nevada is relatively small in comparison to the acreage in other states. However, plant diseases associated with hemp crops have been occurring in Nevada in recent years. In 2016, the Nevada Department of Agriculture Plant Pathology Lab detected Fusarium root rot and sudden death disease from an industrial hemp crop, and Fusarium wilt from medical marijuana plants. Here we describe a newly detected hemp disease, Pythium aphanidermatum crown rot.

Pythium aphanidermatum crown rot occurred in a commercial hemp field, with approximately 5-10 percent of plants affected. Infected plants were noticed by leaf yellowing, curving, necrosis, and the eventual death of entire plants (Fig A). White-colored mold (Pythium mycoides) growth on the surface of the crown area was frequently observed when the plant was pulled from the ground (Fig D). Close examination of the stalk revealed extensive water-soaked lesions and cankers around the crown and basal stalk regions (Fig C). With disease progression, the majority of stalks became completely necrotic or rotted (Fig F). Some affected plants had mild root rot. In the early stage of the disease, only mild internal discoloration of the basal stalk tissue was observed (Fig B). In later stages, cankers spread from the crown area to lower branched stems (Fig E). Affected tissue plated on potato dextrose agar (PDA) medium amended with streptomycin did not yield growth of any pathogens. On selective PPAR medium, a fast-growing Pythium was obtained from all pieces of stem tissue plated. This isolate grew into a full plate (10mm diameter) on PDA medium within 24 hours at 22°C in the dark (Fig G), and produced copious, anthracitic and sporemass on corn meal agar (CMA) medium. Based on both morphology and the DNA sequence of the ITS region of rDNA, the isolate was identified as P. aphanidermatum. This disease can be detected using Agdia’s Phytophthora immunostrip as it cross reacts with Pythium aphanidermatum.

Hemp crown and root rot caused by Pythium aphanidermatum was recently reported in Indiana in June, 2017. It was found in a small research plot where hemp seeds were planted. The disease described here...
Changing genetics of the mycotoxin-producing pathogen *F. graminearum*: implications for mycotoxin surveillance

Mark Sumarah

October 13th 2017
**F. graminearum – 4 or more chemotypes?**  
(implications for monitoring)

Detection of mycotoxins from maize (spectral library)

- Deoxynivalenol (DON)
- 3-acetylDON
- Nivalenol (NIV)
- 15-acetylDON
- NX

![Image of mycotoxin detection from maize](image_url)
Fungal identification in a regulatory environment

Megan Romberg
National Taxonomic Specialist in Mycology
USDA, APHIS, PPQ National Identification Services
Beltsville MD
Estimated number of species vs. described number of species


Port of Entry identifier locations

B Botany identifiers
E Entomology identifiers
P Pathology identifiers

Plant Inspection Stations

Puerto Rico

Hawaii
INIFAP’s Research on Mycotoxins in Crops of Southern Mexico

Eduardo R. Garrido-Ramírez
Francisco J. Cruz-Chávez
Néstor Espinosa-Paz
Elizabeth Hernández-Gómez
Carolina Orantes-Garcia

October 11 - 13, 2017 Beltsville, Maryland
Isolation, identification and monosporic culture selection.

Samples PV2006

Single spore colonies → Isolation on PDA-A → Morphological identification

Conservation

Mycotoxins determination

221 samples analyzed

- Absorbance lecture
- RIDASOFT software

ELISA Kit
RIDASCREEN FAST (R-Biopharm)

immunoaffinity columns on coffee
E-probe Diagnostic Nucleic acid Analysis (EDNA) for plant pathogen detection

Jacque Fletcher
Ulrich Melcher
Francisco Ochoa Corona
Carla Garzon
Tony Stobbe
Jon Daniels
Andres Espindola
Ruchi Verma
Trenna Blagden
Sharon Andreason
Astri Wayadande
Oklahoma State University
Stillwater, OK

William L. Schneider
Diana Sherman
Andrew Stone
Aaron Sechler
USDA-ARS
FDWSRU
Fort Detrick, MD

USDA

NIFA

United States Department of Agriculture

National Institute of Food and Agriculture
Nextgen Sequencing

Thousands and thousands of short sequences generated for a given DNA sample (e.g. Roche 454, AB SOLiD, Solexa)

Comprehensive picture of the entire microbial profile

Can sequence 400 megabases of DNA per 4.5-hour run. Enough to fully sequence 2 bacterial genomes

EDNA:
E-probe Diagnostic Nucleic acid Analysis
Bioinformatics tool designed to ignore irrelevant sequences and limit processing

Control the size of the reference database: Dump raw non-assembled sequence data into its own database (create a mini-genbank).

Control the size of the query set: Query the raw sequence data base with a series of signature diagnostic sequences (“e-probes”).

Stobbe et al., Journal of Microbiological Methods

EDNA results
• Detects RNA and DNA viruses
• Detects bacteria
• Detects oomycetes and fungi
• Detects vectors
• Useful in pathogen discovery

SUCCESS
Because you too can own this face of pure accomplishment
Invasive Stink Bugs: Applied Semiochemistry (and a little bit about Biological Control)

Don Weber
USDA Agricultural Research Service
Insect Biocontrol & Behavior Lab
Beltsville, Maryland

PROCINORTE Plant Health Task Force
Workshop in Beltsville, 12 October 2017
**Pentatomidae:** 22 genera (±) for which pheromones are known (at least partially)

**Asopinae:** *Oplomus*
- *Perillus*
- *Podisus*
- *Stiretrus*
- *Tynacantha*

**Edessinae:** *Edessa*

**Pentatominae:**
- **Antestiini:** *Plautia*
- *Thyanta*

**Cappaeini:** *Halyomorpha*

**Pentatominae (cont’d):**
- **Carpocorini:** *Agroecus*
- *Euschistus*
- *Oebalus*
- *Tibraca*

- **Eysarcorini:** *Eysarcoris*

- **Nezarini:** *Chinavia*
- *Chlorochroa*
- *Nezara*

- **Pentatomini:** *Pallantia*
- *Pellaea*

- **Piezodorini:** *Piezodorus*

- **Rhynchocorini:** *Biprorulus*

- **Strachiini:** *Murgantia*

Captures using black pyramid traps baited with MDT (50mg) in VA & MD apple orchards, 2011

**Halyomorpha halys**
Brown marmorated stink bug

Asian native responsive to MDT

but usually only after harvest of apple crop, in late season

Leskey et al. 2012, Psyche 2012: S5S062, Figure 5b. Also shown by Khrimian et al. 2007, J.Ag.Food Chem.56: 197-203.
PLANT HEALTH TASK FORCE

• Relevance
  • Insect pests, plant pathogens and associated toxins with potential to become invasive are a primary concern for each of the 3 countries

• Effectiveness
  • Researchers in each of the 3 countries provide expertise in identification of native and invasive pests, plant pathogens and associated toxins

• Impact
  • Researchers collaborate and share information on native biological control agents (predators, parasitoids and entomopathogens) for control of invasive pests to other member countries
INIFAP National Project Lead for Plant Health will host a 2 ½ day workshop in Mexico (location TBD) in fall 2018

Workshop will focus on:

- Hemipteran insect pests and diseases that are vectored by them.
- Targeted pests include: Bagrada bug, Kudzu bug, Brown Marmorated Stink Bug, Pepper Weevil and Ambrosia Beetle. Tree fruits and small fruits will be the target host of the vectored viruses. In the case of Ambrosia beetle, *Fusarium* will be the vectored disease organism.
PLANT HEALTH TASK FORCE WORKPLAN AND BUDGET REQUEST 2018

• Outcomes
  • Knowledge transfer leading to harmonization of taxonomic methods in Canada, Mexico and U.S.A.
  • Tri-lateral collaboration and coordination of research in the area of insect pests and insect vectored diseases
  • Developing and enhancing networks of entomologists, pathologists and chemists in the three countries

• Budget requested - $19,500
  • To support travel of 10-15 scientists to workshop
  • Deliver Workshop
  • Sponsor participation at NAPPO 2018 (Tucson, Arizona, USA)
  • Sponsor participation at Entomology 2018 (Vancouver, BC, Canada)
Thanks!

- PROCINORTE and IICA
  - Audia Barnett & Gloria Ramirez

- INIFAP
  - Dr. José Isabel López-Arroyo; Dr. Sergio Sánchez-Peña, UAAAN; Dr. Jaime Mena-Covarrubias, INIFAP; Dr. Eduardo R. Garrido-Ramírez, INIFAP

- USDA/ARS
  - Dr. Rose Hammond; Dr. Joe Munyaneza; Dr. Donald Weber, USDA/ARS; Dr. Kim Hoelmer, USDA/ARS; Dr. Rubella Goswami, USDA/NIFA; Dr. Robert E. Davis USDA/ARS; Dr. Ronald Ochoa, USDA/ARS; Dr. Gary Bauchan

- AAFC
  - Dr. Della Johnston; Dr. Roselyne Labbé; Dr. Tara Gariepy; Dr. Mark Sumarah; Dr. Wen Chen; Dr. Julia Mlynarek
THANK YOU!!

Supporting Agriculture in North America
Guided by Science, Improved Technologies and Science-based Policies