NATIONAL INSTITUTE FOR MICROBIAL FORENSICS & FOOD AND AGRICULTURAL BIOSECURITY

NIMFFAB

OKLAHOMA STATE UNIVERSITY

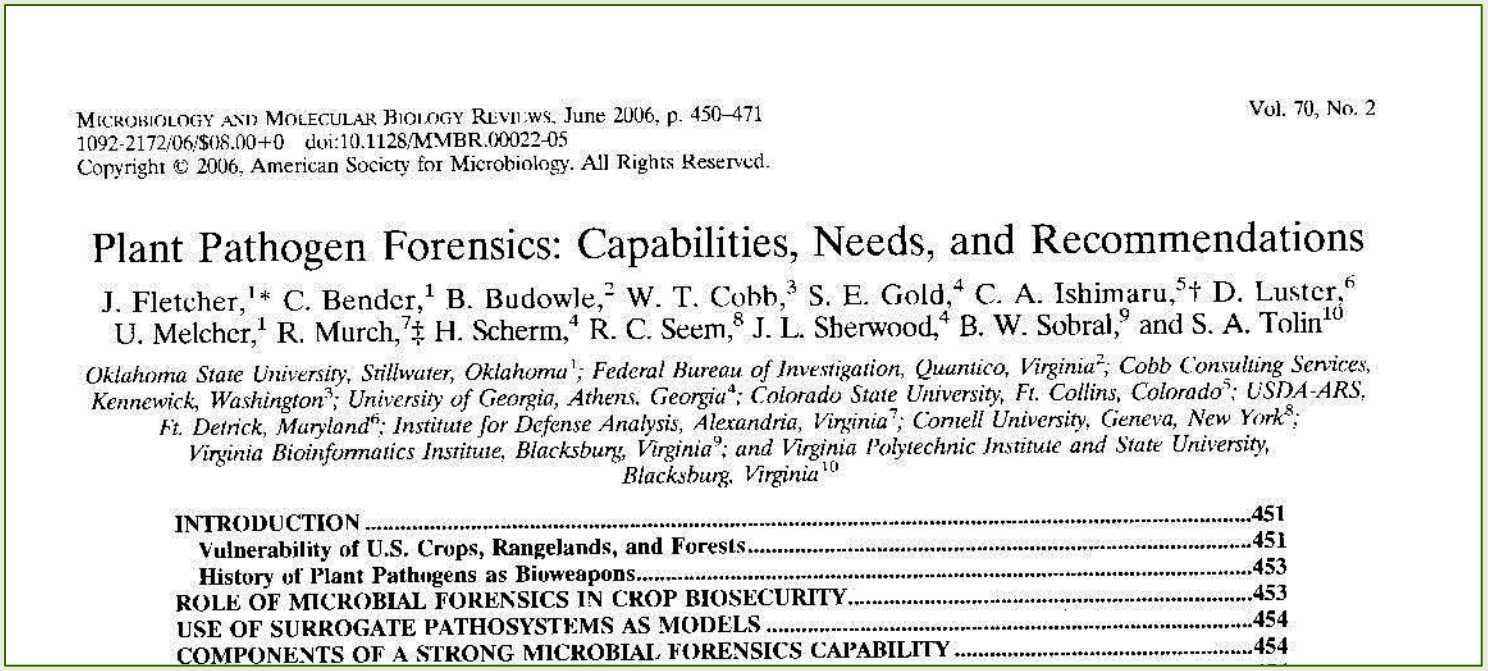
Jacque Fletcher - NPDRS Workshop – March 2011

National Academy of Sciences 2002
A strong national security plan should include:

- Early detection and diagnostic systems
- Epidemiological models for predicting pathogen spread
- Reasonable but effective strategies and policies for crop biosecurity
- Distributed physical and administrative infrastructure
- National response coordination plan and infrastructure
- ***Strategies for forensic investigation and attribution in cases of intentional or criminal activity***

U.S. capability in microbial forensics

- 2002 – Study commissioned by US defense community found need for greater capability in microbial forensics
  - *Included specific language with respect to plant pathogen forensics*



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Plant Pathogen Forensics: Capabilities, Needs, and Recommendations

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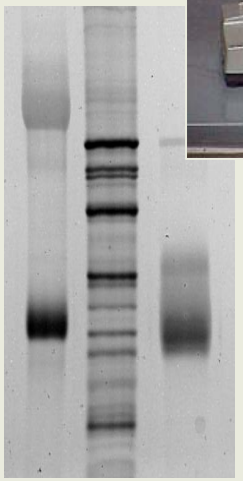
Goals of microbial forensics

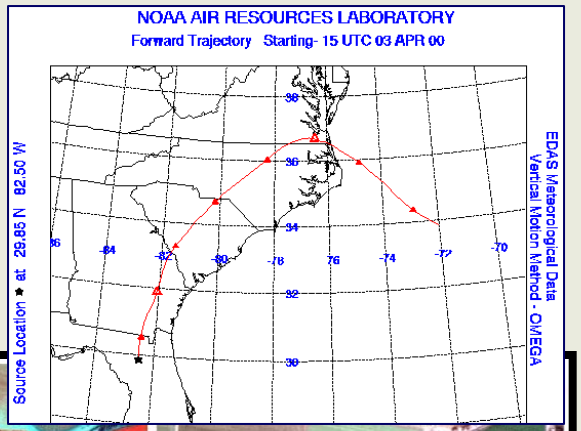
- Collect very specific forensic (microbial and physical) evidence via tests that are
  - Specific, standardized and validated
  - Very high confidence levels
  - Sufficiently robust to withstand rigorous adversarial peer review for use in a court of law
- Attribution
  - Determine source of biothreat agent
  - Identification of the perpetrators
  - Criminal prosecution
- Deterrence of future attempts

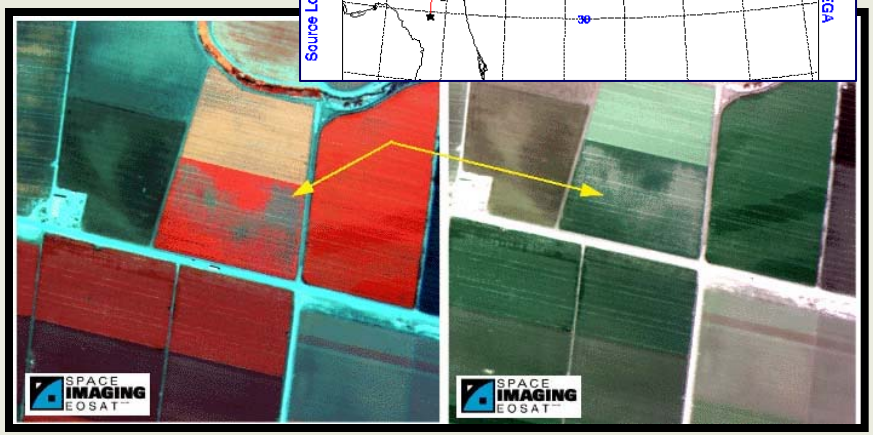


What relevant knowledge and technologies do we already have?

- Bring together elements from established and emerging fields of science:
  - Classical microbiology
  - Microbial genomics
  - Phylogenetics
  - Epidemiology
  - Microbial ecology
  - Bioinformatics
  - Human forensics
  - Plant pathology
  - Entomology
  - Food microbiology
- Identify gaps and needs

Trajectory analysis





Remote sensing

-R. Seem

Special issues for forensic plant pathology:

- 100s of plant spp, each w/many pathogens
- Lack of information on pathogen biology
- Lack of effective molecular detection tags: primers, probes and antibodies
- Some diagnostics based on time-consuming tests (reactions on plant "differentials", mating types)
- Plant pathogen entries in key databases (NCBI, GeneBank, BIOLOG, FAME, etc) limited
- Funding for plant disease research has been very limited, and even post-2011 targeted funds have been discontinued

How can we fill the gaps & meet the needs?

NIMFFAB's new home

Henry Bellmon Research Center, OSU

NIMFFAB mission

To **identify, assess, prioritize, facilitate** and **conduct research, education** and **outreach** related to national needs in microbial forensic science with respect to pathogens of crops, forests, rangelands and fresh produce.

NIMFFAB *will build on, connect and enhance existing programs that support and address issues of crop and food security.*

NIMFFAB strategies & approaches

- **Foster a forensic plant pathology community**
  - **APS Microbial Forensics Interest Group** – APS annual meeting
- **<u>Liaisons with end users</u>: Law enforcement and security communities, policy makers, funding agencies**
  - **FBI** – Scientific Working Group (currently inactive)
  - **DHS/BNBI** – National Bioforensic Analysis Center (NBFAC)
  - **DOD –** Department of Defense
  - **USDA –** Office of Homeland Security
  - **USDA –** APHIS, ARS, NIFA
  - **USDA –**NPDN, NPDRS
  - **Others** – Other government agencies, scientific societies, etc.
- **New education, research, outreach initiatives**
- **Advocacy for targeted funding programs**

NIMFFAB faculty

Jacqueline Fletcher

Director

Plant pathology, microbiology, forensics

Astri Wayadande

Asst. Director

Vector-pathogen interactions

Francisco Ochoa Corona

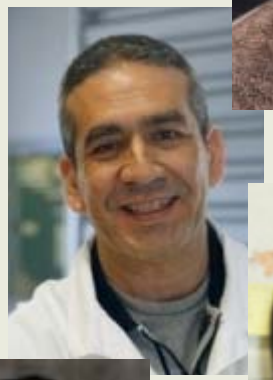
Diagnostics & detection, molecular biology

Li Ma

Produce safety, human pathogens on plants, traceback

Rakesh Kaundal

Metagenomics, next-generation sequencing, bioinformatics



Training & education

USDA National Needs
NIMFFAB Graduate Fellowship Program
2006-2015

First graduate program to
blend coursework & research experience
in plant pathology & forensic sciences

NNF Fellow research

M.S. (OSU Forensic Sciences Department)

- Adapting human DNA detection technology to plant pathogen detection

Dr. Rob Allen, Chair, Forensic Sciences

Jesse Carver

Charlene Beaumann (on left)

Andrew Taylor

NNF Fellow research

Stephanie Rogers

TeeCie West Brown

Ph.D.

(Plant Path; Biochem & Molec Biol)

- **Microarrays and SNP typing** for plant pathogen detection
- A "**decision tool**" for use by **law enforcement** personnel at a potential field crime scene

NNF Fellow internships

TeeCie **Stephanie**

FBI Laboratory

- 3 months
- FBI forensic research projects
- Visits to other related agencies
  - USDA, Ft Detrick, MD
  - DHS NBFAC, Ft. Detrick, MD

Was a plant disease outbreak:
intentional or natural?

Natural or intentionally caused?

Issues

1.99.99999….% of plant diseases are incited under natural conditions

2.This disease occurs in this area and looks familiar

3.“Suspicion inertia”

Questions

1.What features of an outbreak would trigger concern on the part of the first detector?

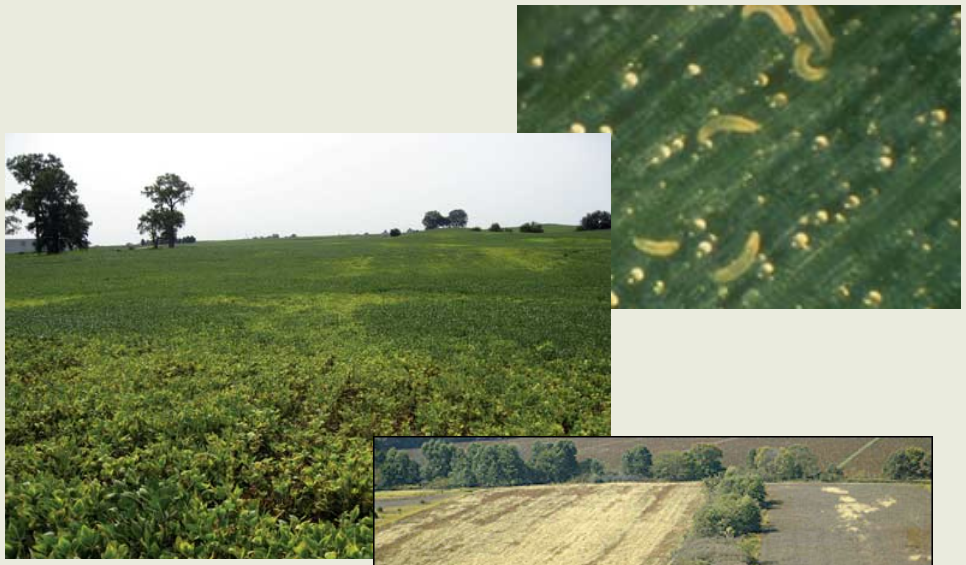
1. Unusual symptoms
2. Unusual circumstances
3. Odd coincidences

2. What would prompt a call to law enforcement?

3. When would law enforcement be called in?

Crop Bioagent Introduction Intent Assessment Tool (CBIIAT)

1. Geographical issues
2. Vector issues
3. Pattern of disease
4. Weather factors
5. Timing
6. Field history of disease
7. Adjacent areas
8. Crop rotation
9. Human activity
10. Physical evidence
11. Motive
12. Pathogen features



NIMFFAB research

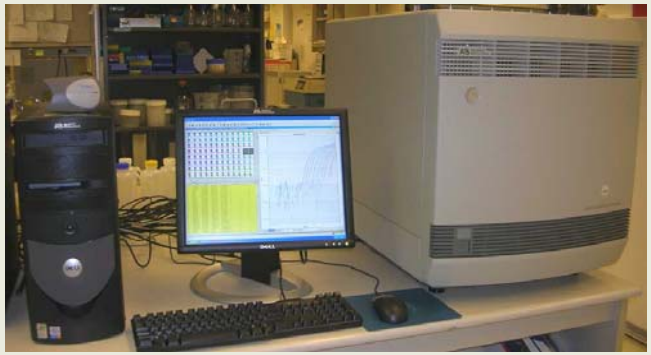
Microbial Rosetta Stone Database

- Added 100 high consequence plant pathogens
- Agents were selected with community (**APS MFIG**) input

NIMFFAB research
National Bioforensic Analysis Center

US Department of Homeland Security/Battelle

1. **NIMFFAB: Spoke Lab** for forensic plant pathology capability
2. **Communication bridge -** to the plant pathology community
3. **Assay development -** Real time PCR
   - Plant pathogens of high consequence
   - PCR primers, optimization
4. **Assay validation**
   - Error rates, confidence
   - Multiple operators
   - Screening panels
   - Limits of detection



DOD

Technical Applications for Security Enhancement

University Multispectral Laboratory

NIMFFAB – Plant pathogens
School of Veterinary Medicine – Animal pathogens

- **Field Portable PCR Assay** - Real time PCR
  - Plant pathogens of high consequence
  - PCR primers, optimization
- **Assay validation**
  - Error rates, confidence
  - Multiple operators
  - Screening panels
    - Near neighbors – taxonomic
    - Near neighbors – niche community
    - Pathogens of interest to NBFAC
  - Limits of detection

USDA Plant Biosecurity Grant

**This grant program has been discontinued*

Massively Parallel Sequencing as a Diagnostic & Forensic Analysis Tool for Plant Pathogens

NIMFFAB - USDA ARS - NBFAC Collaboration

Research:

- Rapid ID of causative microbes in unexpected disease outbreaks
- Screen for multiple pathogens in a complex sample
- Identify unexpected, unknown, and uncultivable pathogens
- Identify signatures of genetic modification

Education & Outreach:

- Graduate student fellowships
- Summer internships: USDA ARS, Ft. Detrick
- Summer 4-H mini-camp
- Summer workshop for high school teachers

Enhanced sampling, detection & discrimination technologies – F. Ochoa Corona

DNA extraction

PCR amplification and cloning

Plasmid DNA purification

End point PCR

1. Primer sets
2. High sensitivity and specificity
3. Rapid detection (~2h) and high sensitivity

Real time PCR

1. Primer sets
2. High sensitivity and specificity
3. Rapid detection (~1h)

HDA at 60°C

1. Primer sets
2. Good sensitivity and specificity
3. Rapid detection (~1.5h)
4. No need of thermocycler

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PLANT AND FOOD BIOSECURITY

EU Network of Excellence: Plant & Food Biosecurity

SEVENTH FRAMEWORK PROGRAMME

European Commission's Security Programme

- 5 years,13 partners, 9 nations, 3 continents
  - NIMFFAB – OSU
  - Kansas State University
- 8 Work Packages
- To increase the quality and impact of plant and food biosecurity training and research in Europe
  - Enhancing preparedness and response capabilities
  - To prevent, to respond and to recover from a possible use of plant pathogens against crops in the European agrifood system.

NIMFFAB

OKLAHOMA STATE UNIVERSITY AGRICULTURE

USDA NIFA; CPS
Human pathogens on plants

- **Astri Wayadande**
- **Li Ma**

AP Photo

Sep 17, 2006:
Spinach Pulled From Stores Across US

Getty Images

July, 2008:
Salmonella on tomatoes – or was it peppers??

NIMFFAB

Oklahoma State University Agriculture

NIMFFAB outreach – Workshop 2007

January 2007, Oklahoma City, Oklahoma

Workshop: Plant Pathogen Forensics: Filling the Gaps

Goal: *Bring together the plant pathology and the law enforcement and security communities to prepare for a timely and effective response to a plant disease emergency.*

Participants:

- USDA: APHIS, ARS, CSREES
- FBI Laboratory
- National Bioforensic Analysis Center
- National Laboratories
- Academic community
- International members

NIMFFAB outreach - APS

APS Microbial Forensics Interest Group (MFIG)

- To facilitate interaction and communication among APS members involved in aspects of forensic plant pathology.

- A forum for prioritizing needs, providing community input, and developing collaborative initiatives in forensic plant pathology.

Attendees:

USDA: APHIS, ARS, CSREES

FBI Laboratory

Academic community – OSU & nationwide

Oklahoma agricultural security community

NIMFFAB outreach – FX & TTX 2009, 2010

Partnering for Success During a Plant Health Response

USDA APHIS – Preparation of Situation Manual

NPDN – Carla Thomas – Facilitation

OHS, DHS, FBI, Security Community: Collaboration & planning

- **Intentional pathogen introduction in a field setting**
- **Issues**
  - Roles of each agency
  - Identifying intent
  - Determining what is evidence
  - How to collect, store, transfer evidence
  - What tests to use
  - How to interpret them


There is a need for:
New knowledge
New technologies
New forensic capabilities
Trained personnel

NATIONAL SECURITY
DEPENDS UPON FOOD SECURITY

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