

A pair of hands is shown from a top-down perspective, cupping a small amount of dark soil. A small green plant with several leaves is growing out of the soil. The background is dark and textured.

Standardizing Diagnostics through SOPs

CPDN Diagnostic Training
June 2010

Carrie Lapaire Harmon, SPDN/UF

Standard Operating Procedure (SOP)



National Plant Diagnostic Network

**Standard Operating Procedure for
Plant Diagnostic Laboratories**

Southern Wilt/Brown Rot
Ralstonia solanacearum Race 3 Biovar 2



VERSION 2.3



Why?

- Most lab accreditation systems require SOPs
- Standardize the protocols so everyone uses the same test
- Training and continual resource for new personnel
- Communicate the best science available to multiple partners
- Foster trust and communication
- Same test, same result, every time – consistency and legitimacy!

Case Study: Southern Wilt

The geranium disease occurs on potatoe *solanacearum* (RS) solanaceous hosts especially-vexing temperate climate the southern U.S. of Select Agents. Southern wilt was greenhouse and in were destroyed to (tomato production) The pathogen was US.

**Why the plant c
up and talk abo
diagnostics...**



ot when it
f *Ralstonia*
various
var 2 is an
rives in
ains found in
-APHIS List
a production
0 geraniums
tion areas
at \$80,000.
the southern

et's back

Case Study: Southern Wilt

The [USDA Select Agent Program](http://selectagents.gov) concerns a limited list of highly-significant pathogens and toxins. This list was developed as part of the Agricultural Bioterrorism Protection Act of 2002. The list and other aspects of the program now resides at <http://selectagents.gov>.



The screenshot shows the APHIS Select Agent Program website. The main content area is titled "Agricultural Select Agent Program" and lists "USDA only agents and toxins" and "USDA/HHS overlap agents and toxins". A yellow circle highlights the "Plants" section of the USDA only agents and toxins list.

USDA only agents and toxins

Livestock

- African horse sickness virus
- African swine fever virus
- Akabane virus
- Avian influenza virus (highly pathogenic)
- Bluetongue virus (exotic)
- Bovine spongiform encephalopathy agent
- Camel pox virus
- Classical swine fever virus
- *Cowdria ruminantium* (Heartwater)
- Foot-and-mouth disease virus
- Goat pox virus
- Japanese encephalitis virus
- Lumpy skin disease virus
- Malignant catarrhal fever virus (exotic)
- Menangle virus
- *Mycoplasma capricolum* M.
- *F38M. mycoides capri* (contagious caprine o pleuropneumonia)
- *Mycoplasma mycoides mycoides* (contagious bovine pleuropneumonia)
- Newcastle disease virus (velogenic)
- Peste des petits ruminants virus
- Rinderpest virus
- Sheep pox virus
- Swine vesicular disease virus
- Vesicular stomatitis virus (exotic)

Plants

- *Candidatus Liberobacter africanus*
- *Candidatus Liberobacter asiaticus*
- *Peronosclerospora philippinensis*
- *Ralstonia solanacearum*, race 3, biovar 2
- *Sclerophthora rayssiae* var. *zeae*
- *Synchytrium endobioticum*
- *Xanthomonas oryzae* pv. *oryzicola*
- *Xylella fastidiosa* (citrus variegated chlorosis strain)

USDA/HHS overlap agents and toxins

- *Bacillus anthracis*
- Botulinum neurotoxins
- Botulinum neurotoxin producing species of *Clostridium*
- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- *Clostridium perfringens* epsilon toxin
- *Coccidioides immitis*
- *Coxiella burnetii*
- Eastern equine encephalitis virus
- *Francisella tularensis*
- Hendra virus
- Nipah virus
- Rift Valley fever virus
- Shigatoxin
- Staphylococcal enterotoxins
- T-2 toxin
- Venezuelan equine encephalitis virus



Case Study: Southern Wilt

RS 32 is on this list. Suspicion or identification of anything on the list involves the USDA-APHIS state and federal personnel and the FBI. Confirmation must be done at approved labs only, using an approved method. (A note about such lists – update them as need arises; the US is updating ours now.)

Plants

- *Candidatus Liberobacter africanus*
- *Candidatus Liberobacter asiaticus*
- *Peronosclerospora philippinensis*
- *Ralstonia solanacearum*, race 3, biovar 2
- *Sclerophthora rayssiae* var. *zeae*
- *Synchytrium endobioticum*
- *Xanthomonas oryzae* pv. *oryzicola*
- *Xylella fastidiosa* (citrus variegated chlorosis strain)



Case Study: Southern Wilt



SO, why the destruction of plants and financial loss if samples were processed by the federal lab with the federal protocol?

- PCR primers – one set of tests
 - Another set in the UK confirmed the samples as biovar 1. Another test in FL gave the same result – b1.
 - But by then, the news had been released and a quarantine and destruction order fulfilled.
- What did we learn?**
- Standardized protocols are great, as long as they are constantly updated with the best science.
 - Back-up labs and tests are a good idea.
 - Different types of tests (PCR and biochemical analysis, etc) make for a stronger diagnosis.
 - Confidential, in-house communication until we are sure is an absolute must.

Lessons learned from RS example

- Develop tests using all isolates available
- Tests must be properly validated and regularly updated
- Develop tests with multiple partners
- Multiple methods should be utilized
- Updates must be communicated throughout chain
- Partners must be trusted
- Protocols should be practiced during non-emergency times
- Communicate with all partners and users

Components of a Diagnostic SOP

- Background
 - Importance/impact (in-country and internationally)
 - Geographical information (include maps)
 - References
 - More basic information and refs for background
 - Other references (for protocols, etc) can go in an appendix
- Protocols
 - Sampling
 - Shipping
 - Triage screening
 - Communication
 - Confirmation
 - Response
 - Destruction of sample
- Laboratory forms
- Contact information

Components of a Process SOP

- Title
 - What is the purpose of the process?
- Author and date
- Ingredients
 - Where they are in the lab
 - Where you can obtain them
- Step-by-step walk-through of the process
- Include relevant photos, hints for success, etc.
- Laboratory forms if needed (PCR worksheet, ELISA map, etc.)
- Contact information and/or citations and references

NPDN SOPs

- Examples –RS32
- Walk-through
 - Content
 - Pathogens
 - Improvements to make
 - Designing a template for your SOPs
 - How do you find diagnostic data?
Other references?



National Plant Diagnostic Network

**Standard Operating Procedure for
Plant Diagnostic Laboratories**

Southern Wilt/Brown Rot
Ralstonia solanacearum Race 3 Biovar 2



VERSION 2.3



Discussion

- Do you use SOPs now?
- Do you write SOPs?
- Do you share your SOPs? With whom?
- What organisms do/could you use them for?
- How about communications?
- Have you had issues with SOPs? Can you foresee problems? Benefits?

CPDN

**Standard Operating Procedure for
Plant Diagnostic Laboratories**

Disease Common Name
Disease Scientific Name

Version 1.0

--	--	--	--	--

Authors, date

CL Harmon, UF/IFAS and SPDN

CPDN Lab Network SOPs

Keep in mind...

- Every SOP will have many Method SOPs within it – one for every type of activity (shipping, sampling, ELISA, PCR, etc)
- Put step-by-step instructions for everything!
 - Sanitation of field and lab equipment
 - Collection and handling of samples
 - Recipes for culture media
 - DNA extraction and primer info
 - Explicit directions for all procedures
 - Hints to make things work better for your situation/crop/lab
- Make it so easy to follow so anyone could walk into your lab and do it as long as they follow the SOP to the letter

Now it's your turn

- Break into path/ento groups
- 10 minutes: decide which 5 organisms or procedures you need CPDN SOPs for
- Assign each person one procedure or organism
- 15 minutes: Develop an outline for the information you will need in the SOP
- 5 minutes each: present your outline to the group