

A pair of hands is shown from a top-down perspective, cupping a small, vibrant green plant with several leaves. The plant is growing out of a mound of dark, rich soil. The background is a dark, textured surface, possibly a table or a mat. The overall lighting is soft, highlighting the texture of the soil and the freshness of the plant.

# Standardizing Diagnostics through SOPs

CPDN Diagnostic Training  
June 2010

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# 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.

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# 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

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# 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?



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# 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**

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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