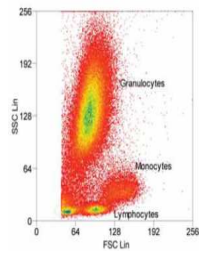

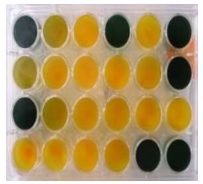

Description and strategies for best management of *Ralstonia solanacearum* Race 3 biovar 2 as a potential incitant of bacterial wilt of tomato

P.G. Champoiseau, J.B. Jones, C. Allen, and T. Momol

24th Annual Tomato Disease Workshop

November 3-5, 2009 ◆ State College, Pennsylvania

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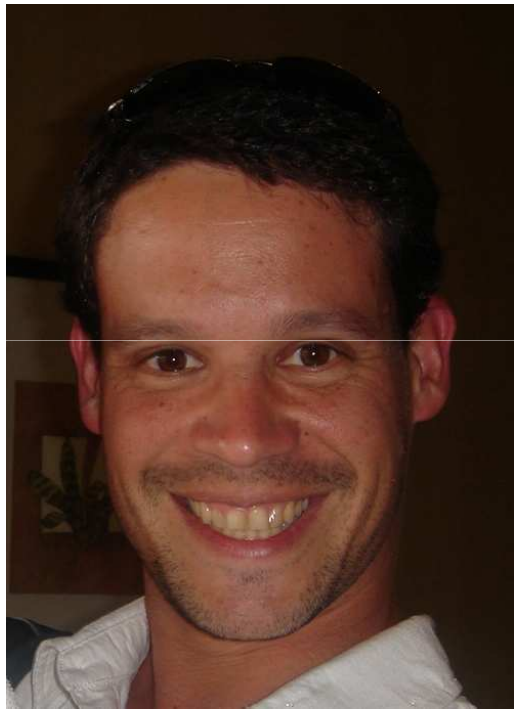
Who was your speaker?



Patrice G. Champoiseau

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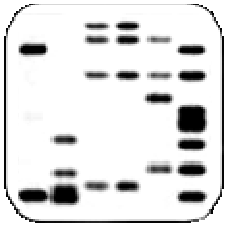
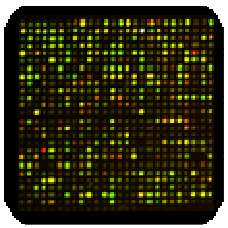
Introduction

- USDA-NRI Project: *Ralstonia solanacearum* race 3 biovar 2: Detection, exclusion, and analysis of a select agent pathogen.



Aim 1. Develop rapid, robust, and reliable diagnostic assays for *Ralstonia solanacearum* race 3 biovar 2, using both immunological and DNA-based approaches.



Aim 2. Identify *Ralstonia solanacearum* race 3 biovar 2 genes involved in cold adaptation and growth in plant hosts, using a microarray-based post-genomic approach.

Aim 3. Develop and deliver a package of optimized education and management training modules.
Develop evaluation tools to assess program effectiveness.

Outlines

- Introduction
- Description of the disease
- Description of the pathogen
- Disease management strategies
- Sample submission procedure
- Acknowledgements

Introduction

- *Ralstonia solanacearum* is a plant pathogenic bacterium.
- Formerly known as *Pseudomonas solanacearum*.
- Causes bacterial wilts on a wide range of crops, ornamentals, and weeds.
- Worldwide distribution from tropical to warm-temperate areas.
- Considered a “species complex” due to significant variation within the group.

Introduction

- Race 1: endemic to the Southern US and causes bacterial wilt on potato, tobacco, and tomato.

Most severe infections can result in 100% production loss.

Photo: T.M. Momol, UF-IFAS

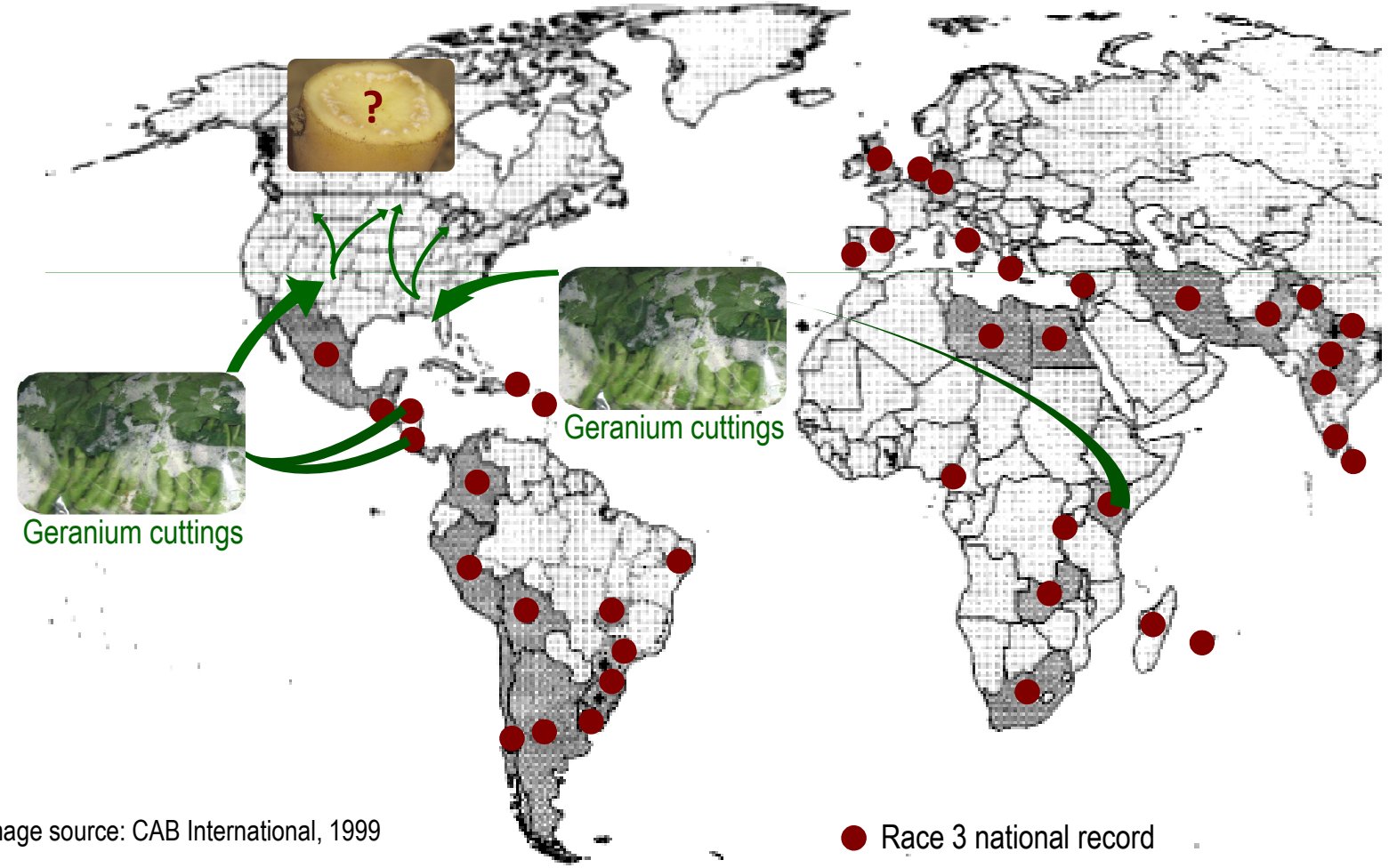
Introduction

- Race 3 biovar 2 (R3b2) subgroup: Select Agent pathogen in the US.
- Probably originated in the tropic highlands (Andes).
- Worldwide distribution except US and Canada.
- Primary hosts: potato and tomato.
- Potato: estimated 1$ billion losses yearly.
- Also pathogenic on pepper, tobacco and geranium.
- Risk for re-introduction of R3b2 in the US.

Introduction

- Pathways for introduction of R3b2 in the US.

Introduction of R3b2 in the US is possible through infected geranium cuttings that are produced in geographical areas where the pathogen is present.



Introduction

- Previous introductions resulted in $ millions losses due to regulatory eradication protocols.
- Critical to prevent re-introduction and spread of R3b2 in the US.
- Requires early detection and application of effective field sanitation practices.
- **Objective**:

  Provide description of R3b2 and tomato bacterial wilt.

  Describe current diagnosis methods and strategies for best management of the disease.

Outlines

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Description of the disease

- Common symptoms

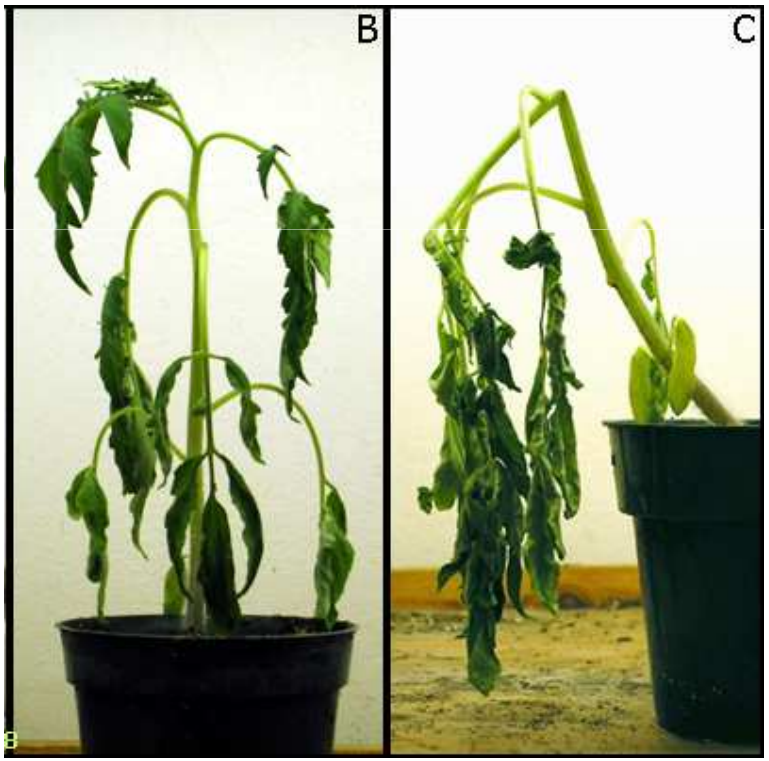
- Wilting of youngest leaves

- Wilting and yellowing of foliage, stunting

- Plant death

Description of the disease

- Other symptoms
  - Brown discoloration of vascular tissue

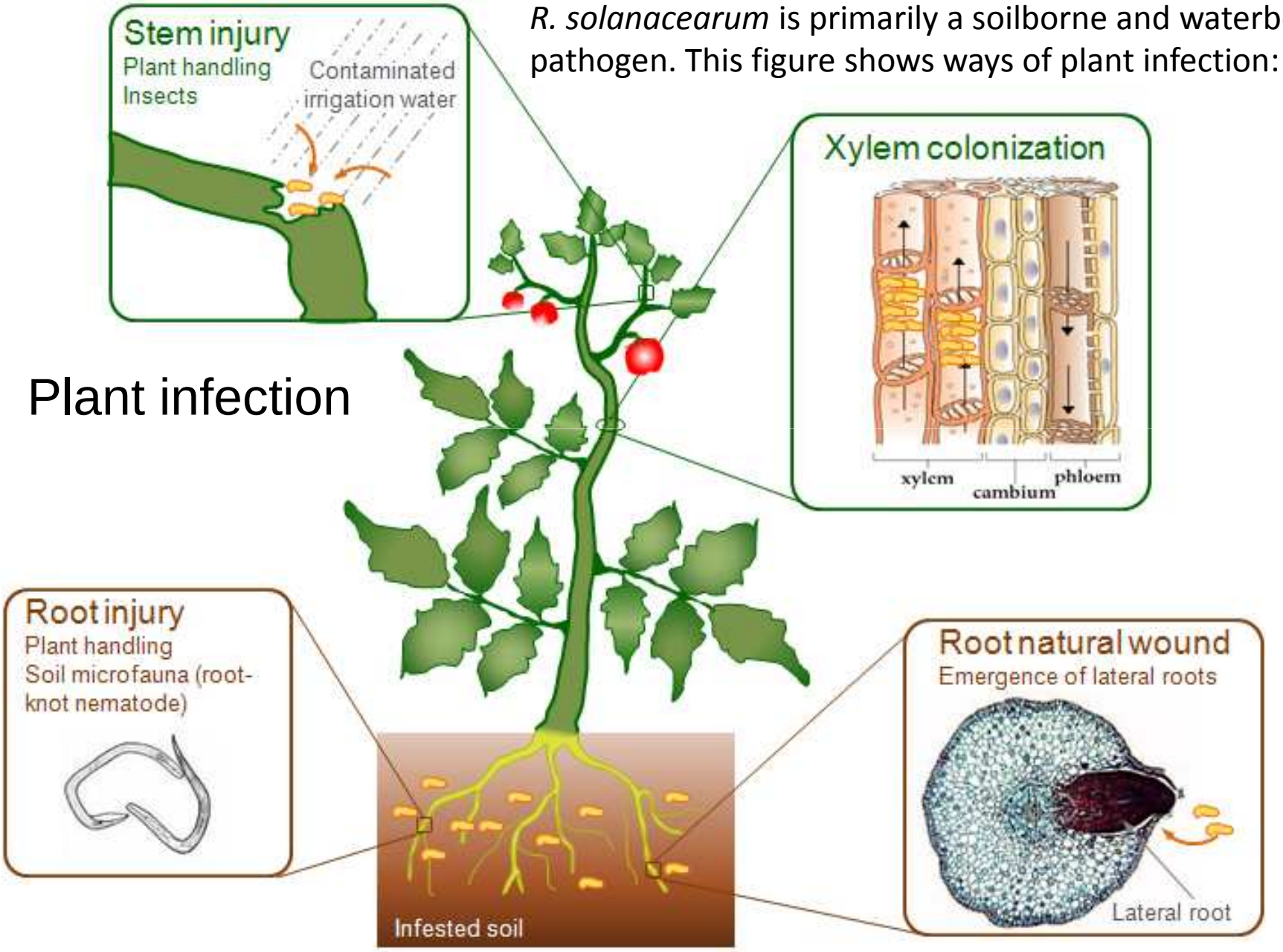


- Stem collapse (young succulent plants)

Symptoms induced by R3b2 cannot be distinguished from those induced by race 1 strains

Description of the disease

R. solanacearum is primarily a soilborne and waterborne pathogen. This figure shows ways of plant infection:



- Plant infection

Description of the disease

- Pathogen survival

Once established in a field or in the plant, the bacterium can survive for days to years in:

Description of the disease

- Disease epidemiology

Spread of the pathogen from infested to healthy fields or plants can occur by:

Outlines

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Description of the pathogen

- Diversity: a “species complex”

- 5 races: based loosely on host range.
- 5 biovars: differential ability of strains to produce acid from a panel of 5 to 8 carbohydrate substrates.
- 4 phylotypes: major genetic groups based on gene sequence analysis. Reflect the and ancestral relationships geographical origins of the strains.

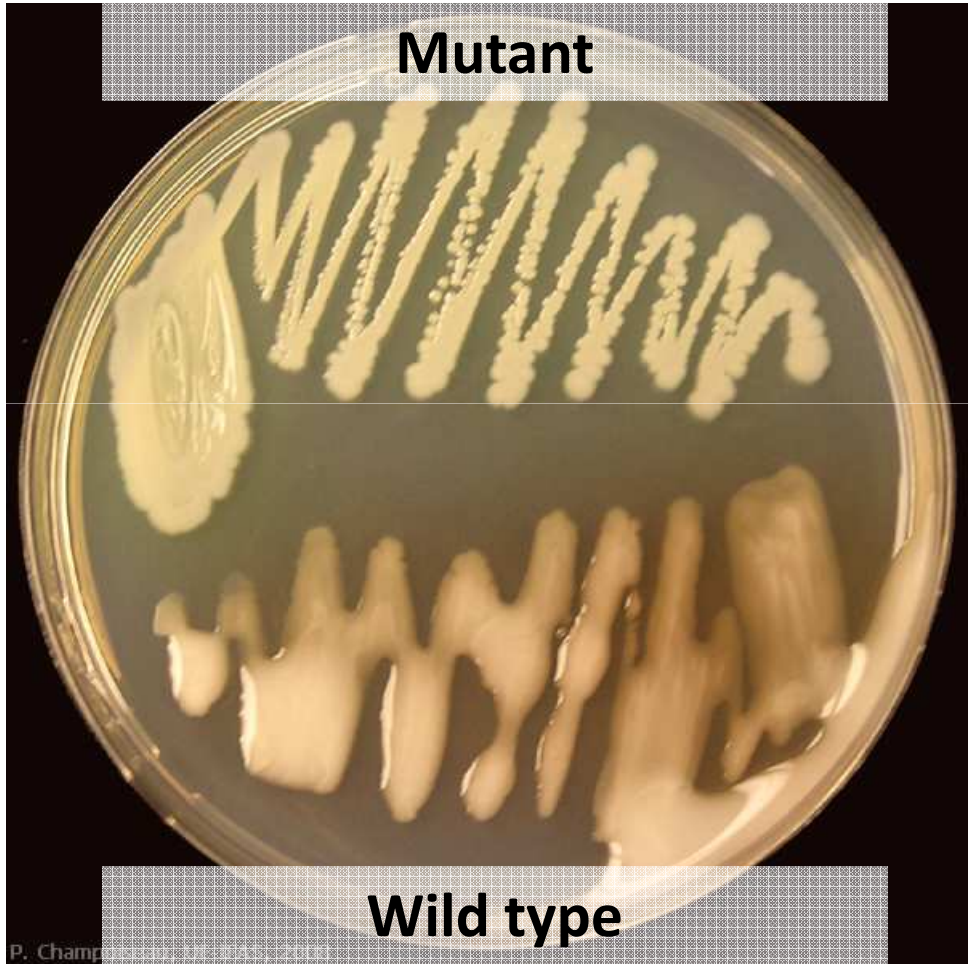
- R3b2 strains belong to phylotype II (sequevars 1 and 2)

See Table 1

Description of the pathogen

- Culture and identification

- Easy to culture
- Optimal growth Temp is 82-90°F
- Individual colonies after 36-48 hours.
- Two colony types:
  - ✓ Wild type (virulent)
  - ✓ Mutant (non-virulent)



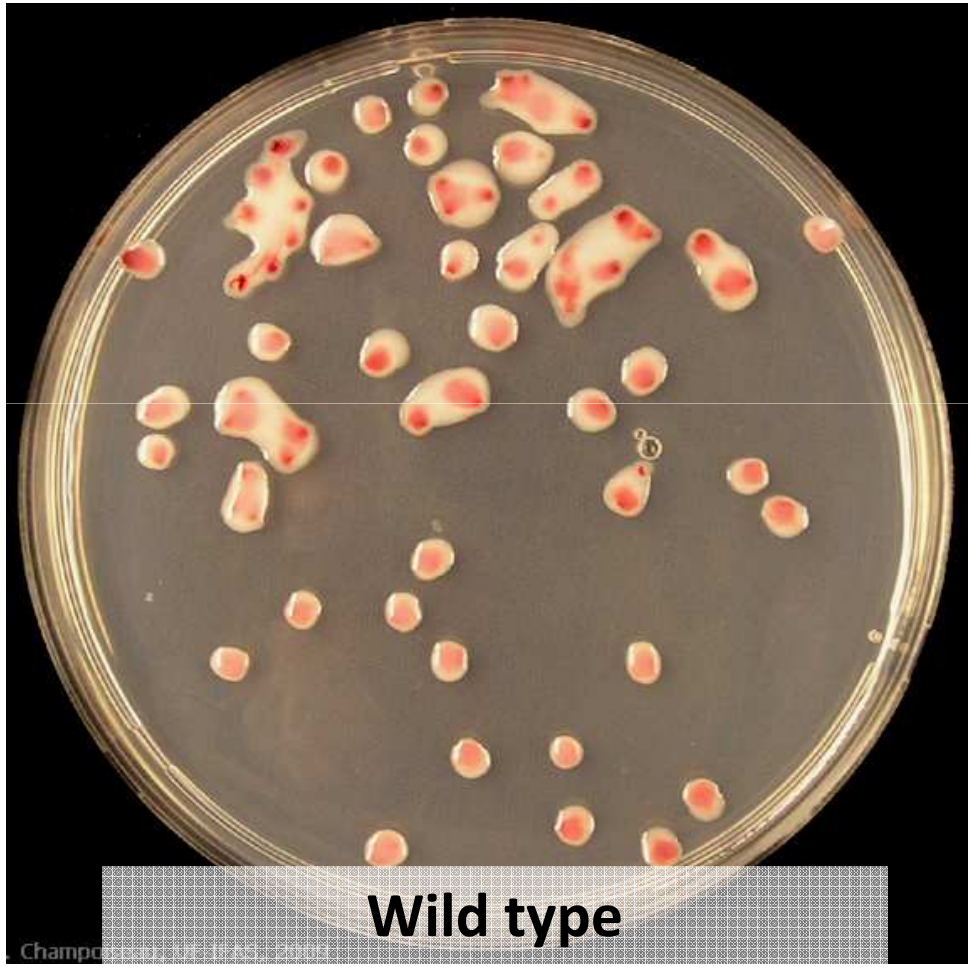
Description of the pathogen

- Culture and identification

- Tetrazolium chloride (TZC) medium

  ✓ Wild type (pink center)

  ✓ Mutant (dark red)



Description of the pathogen

- R3b2 hosts

Primary hosts	**Potato (*Solanum tuberosum*)** **Tomato (*Lycopersicon esculentum*)**
Other cultivated hosts	Bean (*Phaseolus vulgaris*) Beet (*Beta vulgaris*) Bitterground (*Momoridica charantia*) Eggplant (*Solanum melongena*) **Geraniums (*Pelargonium* spp.)** Peppers (*Capsicum* spp.)
Weed hosts	**Bittersweet or climbing nightshade (*Solanum dulcamara*)** Black nightshade (*Solanum nigrum*) Common chickweed (*Stellaria media*) Garden nasturtium (*Tropaeolum majus*) Horsenettle (*Solanum carolinense*) Jimson weed (*Datura stramonium*) Lambsquarters (*Chenopodium album*) Mustards (*Brassica* spp.) Perfoliate blackfoot (*Melampodium perfoliatum*) Pinkhead smartweed (*Polygonum capitatum*) Purslane (*Portulaca oleracea*) Sticky chickweed (*Cerastium glomeratum*) Stinging nettle (*Urtica dioica*) Whitesnow (*Drymaria cordata*)

Description of the pathogen

- Importance of weed hosts

  - *Solanum dulcamara* (bittersweet or climbing nightshade)

  - Semi-aquatic weed

  - In Europe: responsible for outbreaks of bacterial wilt on potato.

Photo: J. Elphinstone, CSL, UK

Outlines

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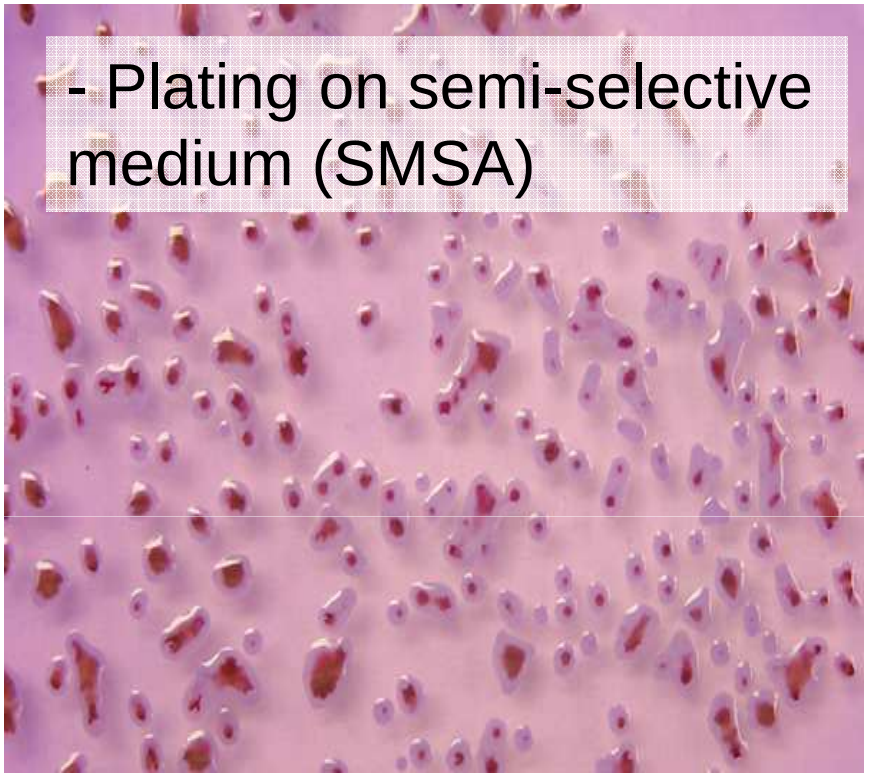
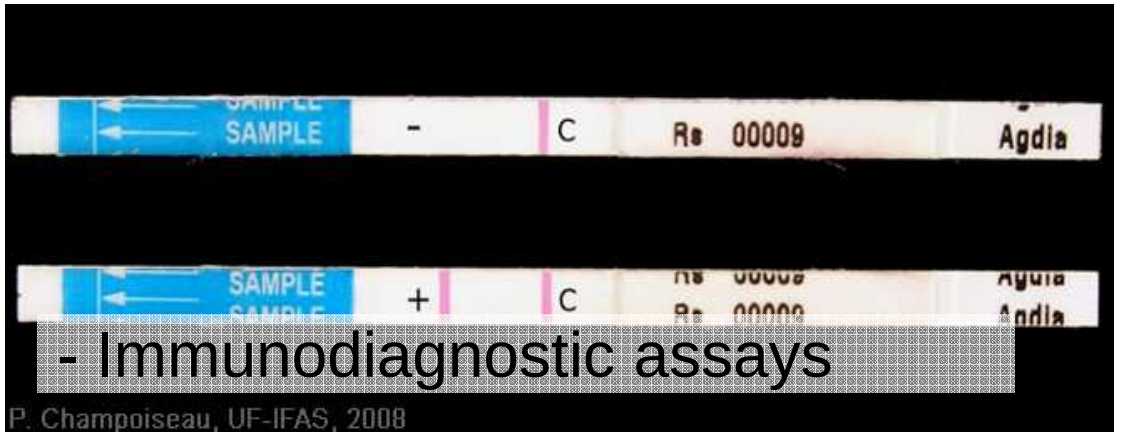
Disease management strategies

- Detection and identification
  - Screening tests:

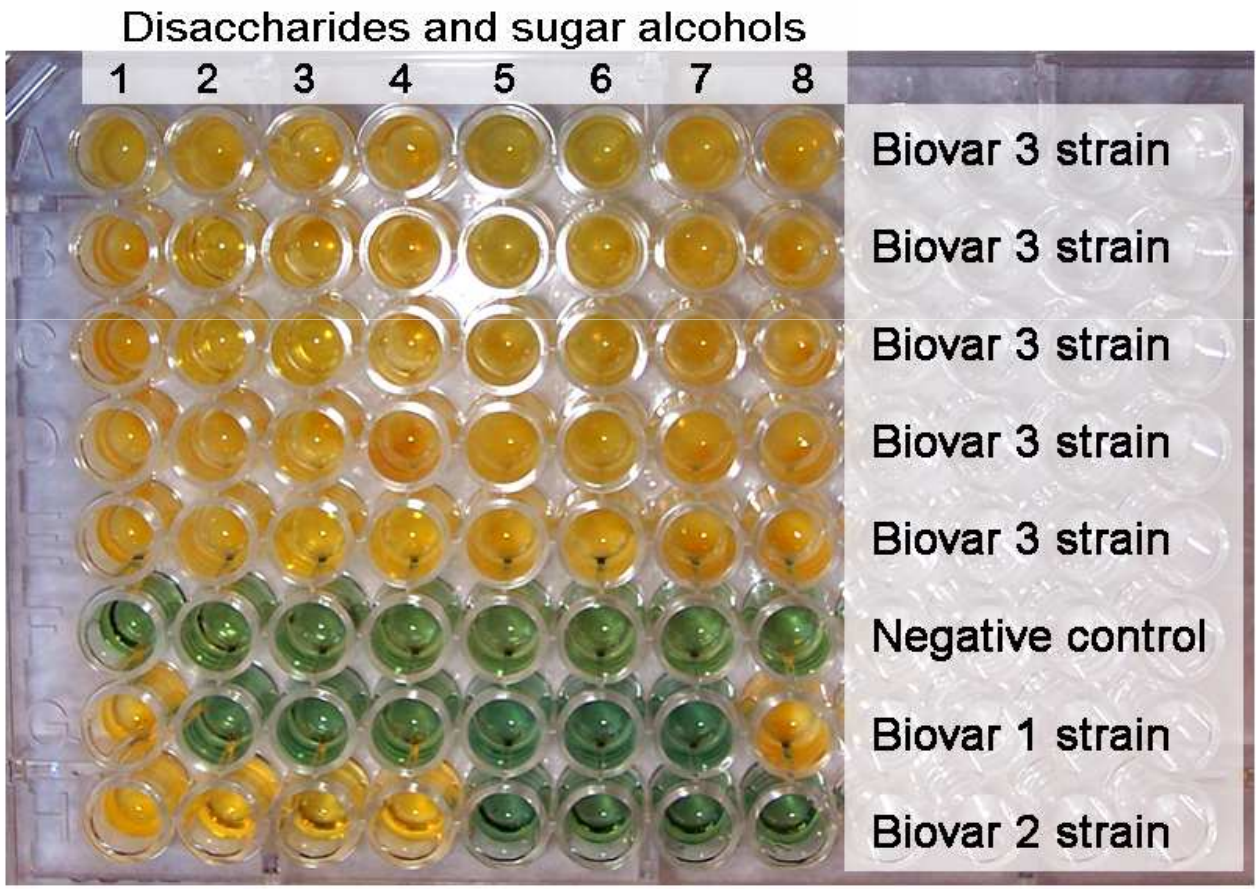




Disease management strategies

- Detection and identification

  - Differentiation of biovars



1 = Glucose ; **2** = Maltose ; **3** = Lactose ; **4** = D-(+)-Cellobiose
5 = Mannitol ; **6** = Sorbitol ; **7** = Dulcitol ; **8** = Trehalose

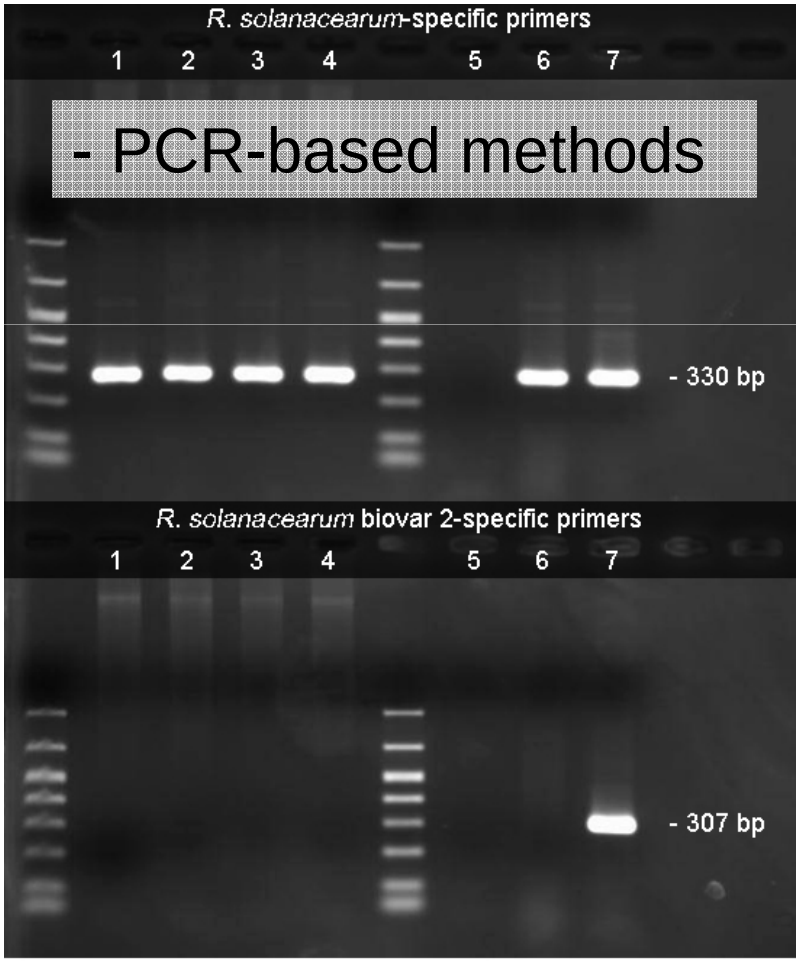
E.N. Twieg, USDA-APHIS-PPQ-CPHST-National Plant Germplasm and Biotechnology Lab., Beltsville, MD

Biovar test is based on differential ability of strains to acidify culture media containing a panel of carbohydrate substrates.

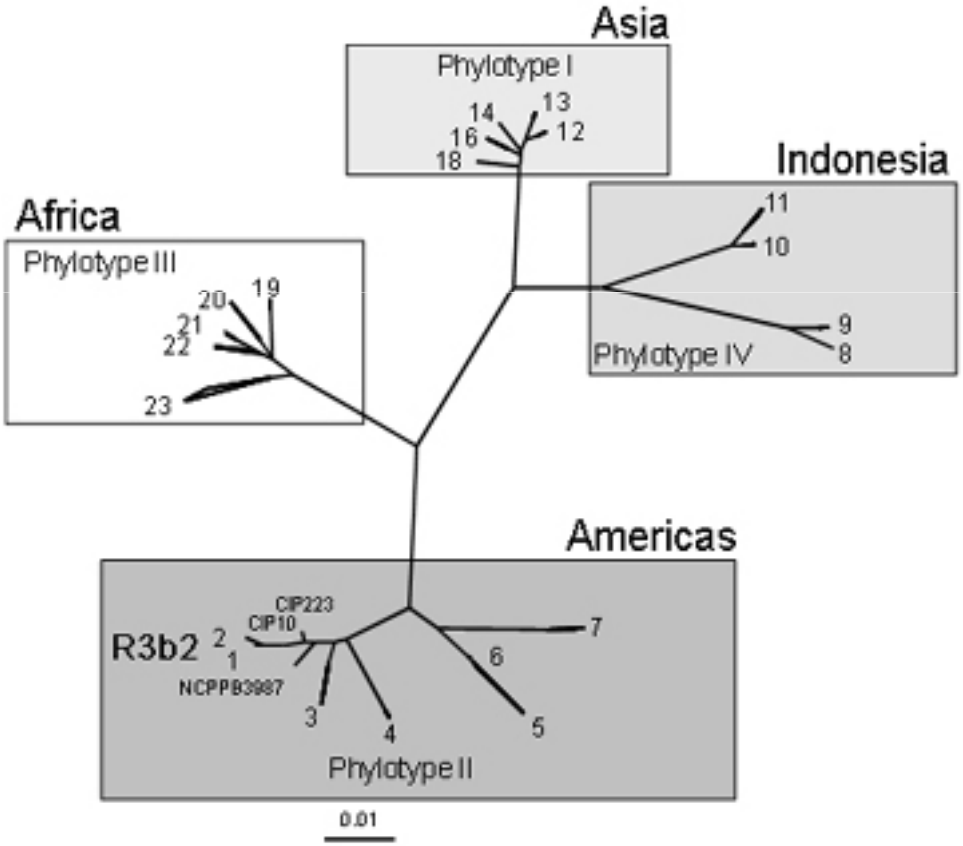
Disease management strategies

- Detection and identification

Several molecular methods can be used to identify *R. solanacearum* at the phylotype, sequevar, and biovar level. These methods include:



1 to 4 = Biovar 1 strains ; 5 = Negative control
6 = Biovar 1 positive control ; 7 = Biovar 2 positive control



- Gene sequencing

Disease management strategies

- In locations where the pathogens is present

  - Some level of bacterial control is possible by using a combination of different control methods in an integrated management strategy:

✓ Host resistance

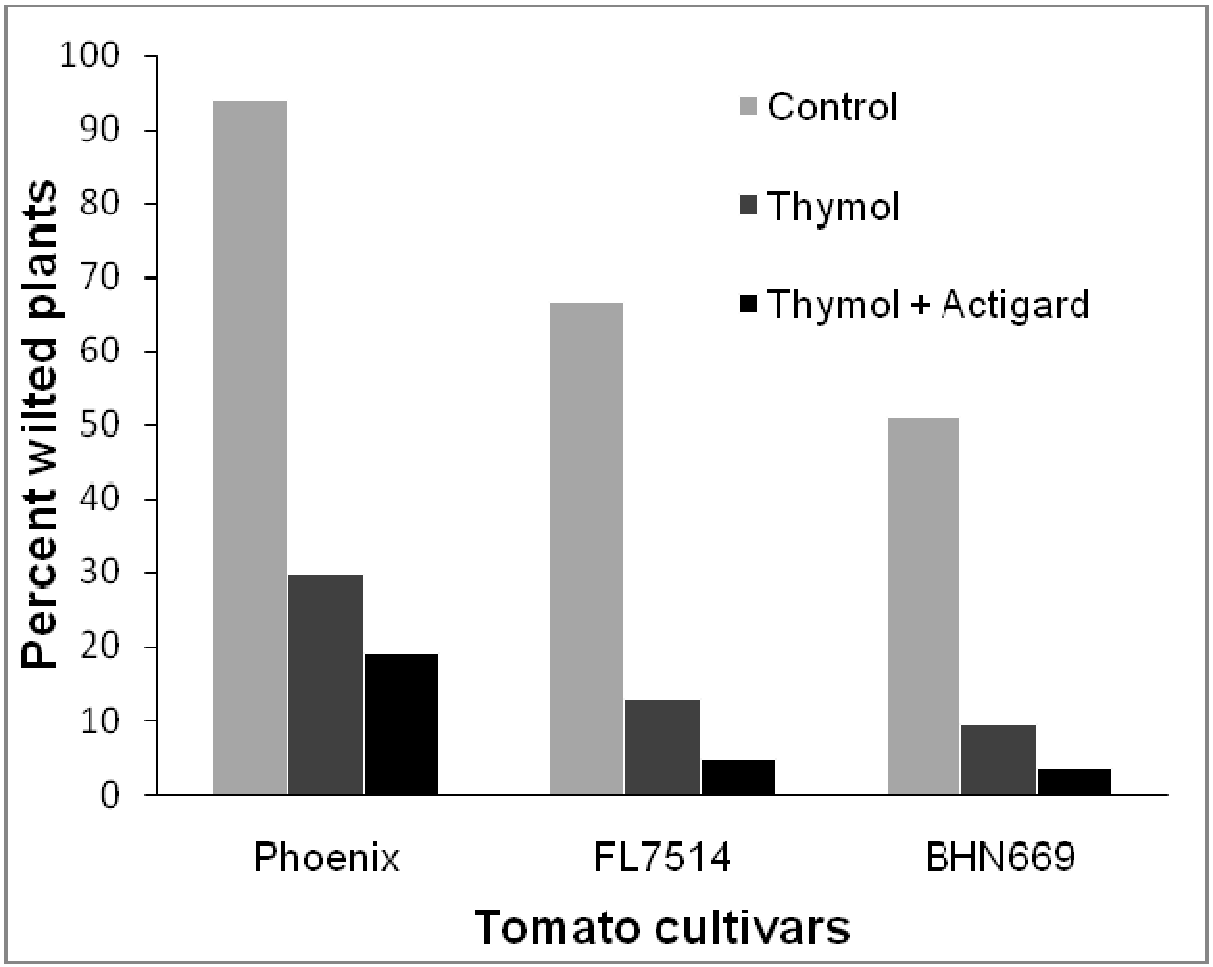
✓ Grafting onto resistant tomato cultivar

✓ Soil treatment (modification of soil pH, solarization, application of stable bleaching powder)

✓ Chemical control (soil fumigation and application of phosphorous acid)

Disease management strategies

- In locations where the pathogen is present
  - ✓ Use of chemicals: Thymol and Actigard



A recent experiment conducted in Quincy in North Florida in 2006 showed that integrated application of Thymol, a plant-derived volatile biochemical, and Actigard, a plant-resistant inducer, resulted in significant reduction in percent of wilted tomato plants on cultivars that showed susceptibility to the disease after artificial inoculation with the pathogen, as shown on this figure.

Disease management strategies

- In locations where the pathogen is present

- Best strategy for controlling bacterial wilt in the field consists primarily of phytosanitation and cultural practices, including:

- Crop rotation with non-host plants such as grasses,
- Intercropping,
- Control of weed and root-knot nematode populations,
- Planting at non-infested production sites,
- Removal of weeds or crop residue where inoculum persists,
- Selection of appropriate planting time to avoid heat,
- Deep plowing of crop residues,
- Satisfactory soil drainage,
- Early- and late-season irrigation management

See Table 4

Disease management strategies

- In locations where the pathogen is NOT present

- It is critical to prevent introduction and, if inadvertently introduced, subsequent movement of the pathogen. This is best achieved with effective cultural sanitation practices :

At the field

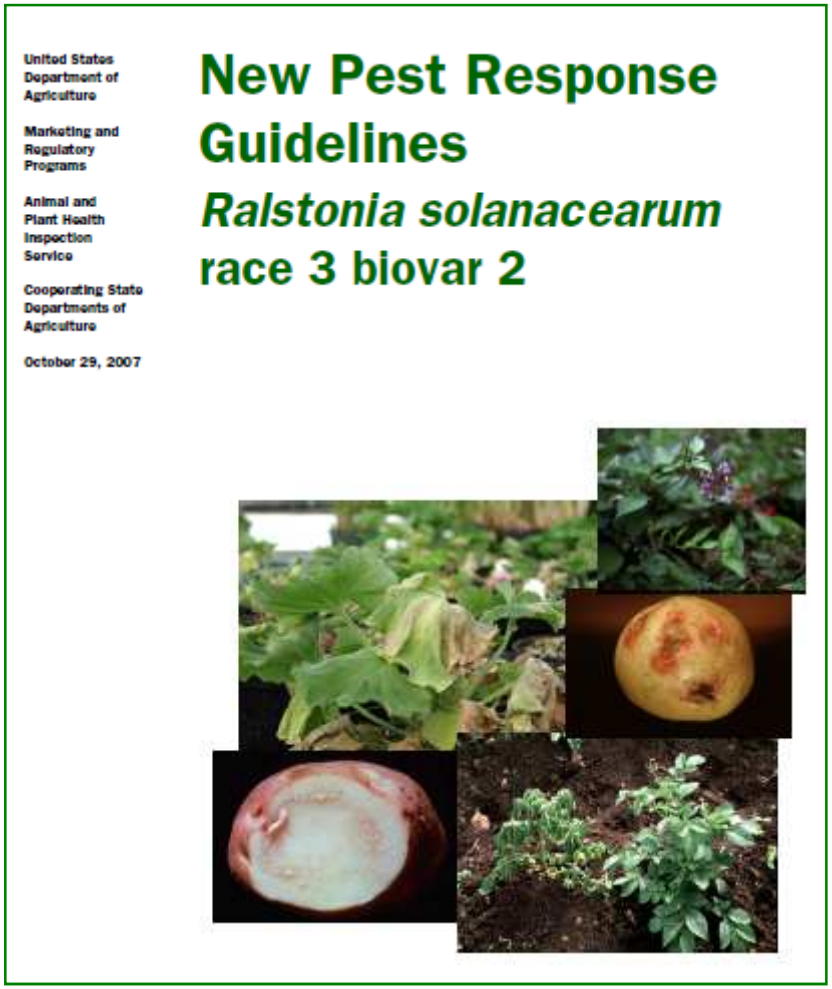
- Planting only certified disease-free plantlets,
- Disinfesting all equipment before moving it between fields,
- Controlling floodwater flow, never using surface water for irrigation.

In the greenhouse

- Avoidance of sub-irrigation,
- Wide separation of greenhouses from field production areas,
- Disinfestations of all frames, trays and tools,
- Use of pathogen-free soils or potting mix,
- Control of weeds, and limited handling of plants.

Disease management strategies

- Disease Management Guide



- Current in formation for detection, control, containment, and eradication of R3b2 according to USDA regulations.

- USDA-APHIS website.
  http://www.aphis.usda.gov

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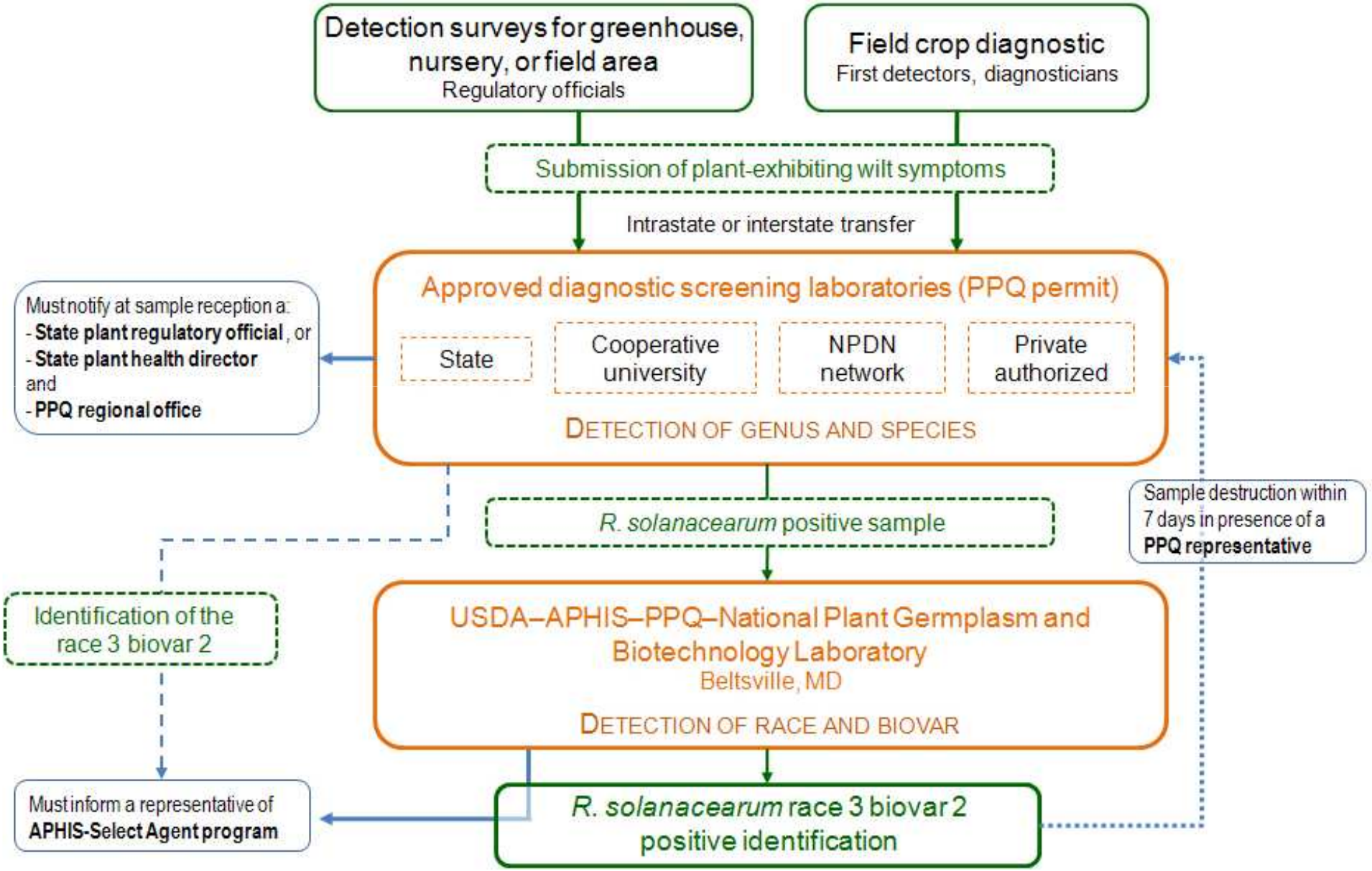
Sample submission procedure

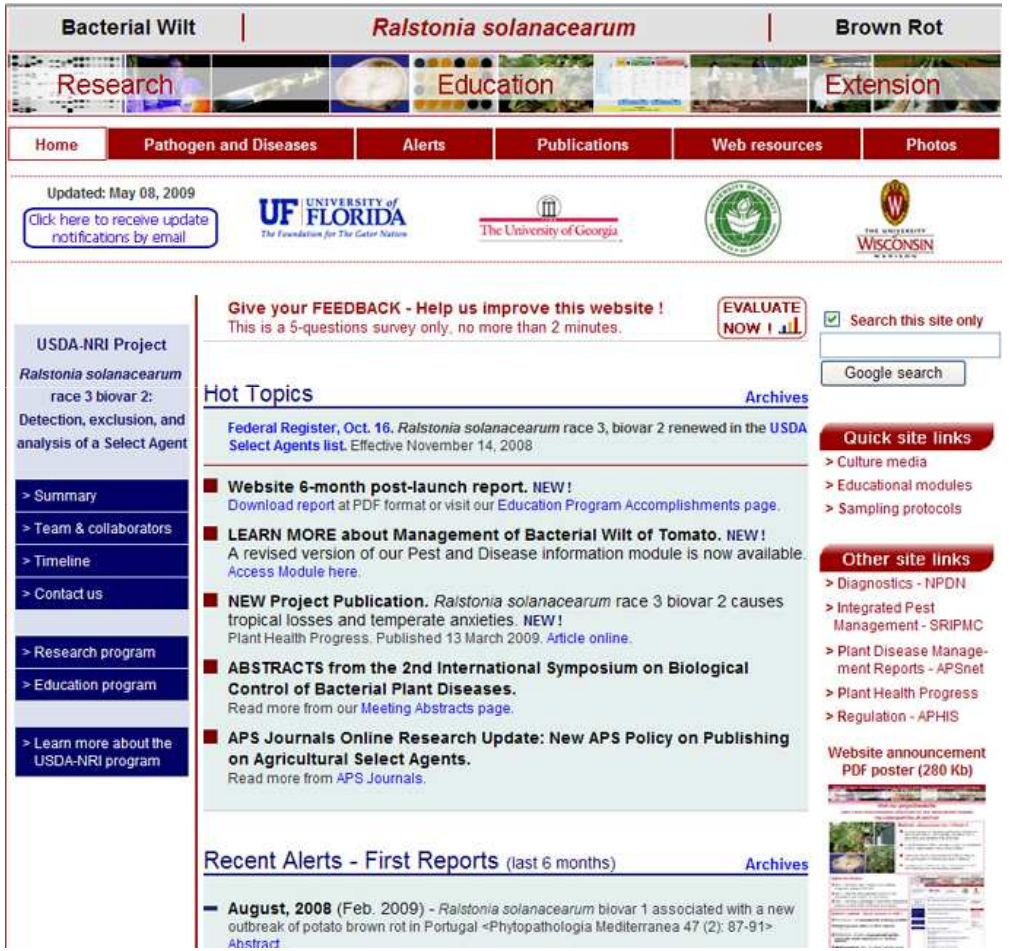


Additional resources

Visit our *Ralstonia*/Bacterial wilt dedicated website:

http://plantpath.ifas.ufl.edu/rsol/

- ✓ Pest and disease management guides
- ✓ Project description, accomplishments
- ✓ Real time pest alerts and first reports worldwide
- ✓ Protocols, book references and journal articles database
- ✓ Web resources
- ✓ Photo galleries
- ✓ Access to Ralstonia-L mailing list



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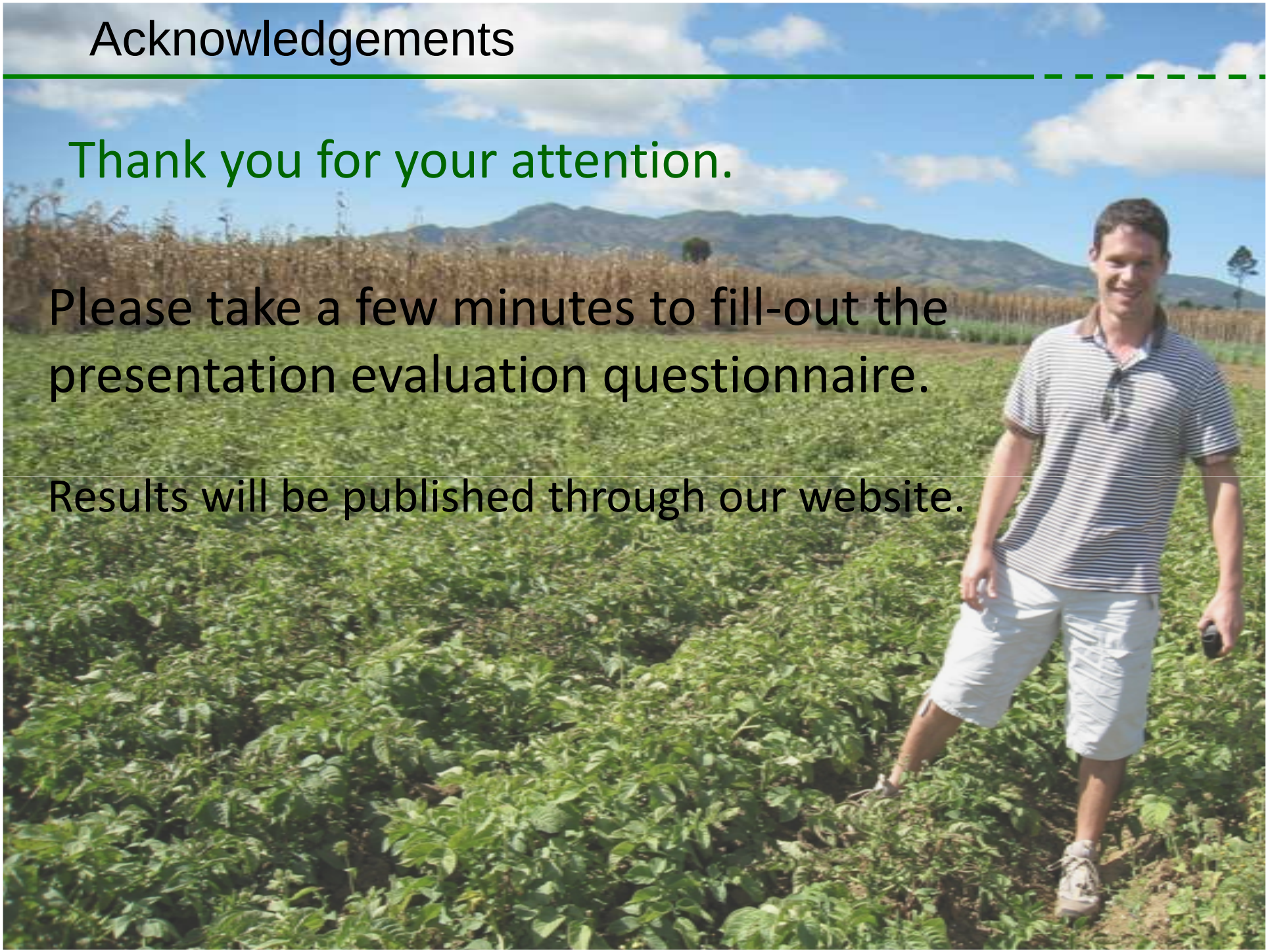

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