

Food and Agriculture Organization of the United Nations



International Plant Protection Convention





Survey Guidance for Ralstonia solanacearum

# Survey Guidance for Ralstonia solanacearum

#### **Scientific Name**

*Ralstonia solanacearum* (Smith, 1896) Yabuuchi et al., 1996 emend. Safni et al. 2014

#### **Common Names**

**Bacterial wilt** 

# **Type of Pest**

Bacterium

### **Taxonomic Position**

Superkingdom: Bacteria, Class: Betaproteobacteria, Order: Burkholderiales, Family: Burkholderiaceae



Figure 1. Wilting by *R. solanacearum* on tomatoes. (Image courtesy of Clemson University – USDA Cooperative Extension Slide Series, Bugwood.org)

## **Known Hosts**

## Preferred hosts

Major hosts include Capsicum annuum (pepper),

*Nicotiana tabacum* (tobacco), *Solanum* spp. (solanaceous crops), *Solanum lycopersicum* (tomato), *Solanum melongena* (eggplant), *Solanum tuberosum* (potato) and *Musa* spp. (banana).

Other hosts include *Cyphomandra betaceae* (tamarillo), *Pelargonium* spp. (geranium), *Physalis angulate* (cutleaf groundcherry), and *Portulaca oleracea* (little hogweed). The following solanaceous weeds are also considered hosts: *Solanum cinereum* (Narrawa burr), *Solanum dulcamara* (climbing nightshade), *Solanum nigrum* (black nightshade), and *Urtica dioica* (stinging nettle).

## **Survey Protocol**

## **Survey Site Selection**

Surveys should target production areas where host plants are grown. Other areas to survey include areas where water accumulates or throughout the field and plants near drainage canals or irrigation rigs. If water sampling, target any water near host fields or water used for irrigation.

#### Time of year to survey:

When host plants are in peak vegetative growth. Typically, for potatoes, aim to survey around 4 to 6 weeks after planting, or when potato plants have emerged from the ground and have developed foliage. For tobacco, aim to survey 6 to 8 weeks after planting, this is when plants have established a healthy growth and are reaching their peak vegetative stage. For tomatoes, aim to survey 6 to 8 weeks after transplanting, during the vegetative growth and early flowering, make sure plants have developed a strong canopy of leaves and are starting to produce flowers. Generally, these surveys should take place during the day when temperatures are the hottest and wilting symptoms are most obvious (**Figs. 1, 2A, 4, 6**).

# Visual Survey

Conduct a visual inspection by searching for plants with typical wilting symptoms (see *Signs and Symptoms* section). The absence of symptoms, however, does not confirm an absence of *R. solanacearum* in the inspected area.

# Symptoms and signs:

While scouting in the field, surveyors should look for common *R. solanacearum* symptoms. These symptoms in potatoes include:

- Wilting (Fig. 2A)
- The vascular ring of symptomatic potato tubers will show grey-brown discoloration and ooze (Fig. 2B)
- Bacterial ooze is present in freshly cut tubers (Fig. 9A) and on potato eyes (Fig. 9B).
- Yellowing
- Vascular discoloration of the stem; appears to be grey or brown



**Figure 2. Symptoms of brown rot caused by** *R. solanacearum* **on potato.** (Images courtesy of D. Thurston, Cornell University (A) and P. Champoiseau, University of Florida (B)

The following symptoms are shown in tobacco:

- Unilateral yellowing, stunting and leaf distortion (Fig. 3)
- Wilting (Fig. 4)
- Reddish to brown discoloration of the vascular system, and stem lesions (Fig. 5)
- Root rot



Figure 3. Unilateral yellowing in tobacco caused by *R. solanacearum* (Image courtesy of R. García, North Carolina State University)



Figure 4. Wilting by *R. solanacearum* on tobacco (Image courtesy of R. García, North Carolina State University)



Figure 5. Reddish to brown discoloration of the vascular tissue on tobacco stem caused by *R. solanacearum* (Image courtesy of R. García, North Carolina State University)

The following symptoms are shown in tomato:

- Wilting during the hottest time of the day (**Fig. 6**). Plants may appear to recover following rain or when temperatures cool down at night.
- Stunting occurring at any stage of growth (Fig. 7A).
- The entire plant may decline rapidly (as quickly as 4-7 days) under favorable conditions, starting with wilt, leaf chlorosis and ending with death (**Fig.7B**).
- Infected stems may collapse, revealing vascular dark brown necrotic streaks (Fig. 8) with white bacterial ooze.



Figure 6. Wilting of tomato plants by *R.* solanacearum (Image courtesy of Kim Sang Gyu, National Agrobiodiversity Center)



**Figure 7. Symptoms of bacterial wilt of tomato** (Image courtesy of C. Allen, University of Wisconsin (A) and T. M. Momol, University of Florida (B))



Figure 8. Stem of tomatoes showing brown discoloration caused by *R.* solanacearum (Image courtesy of Clemson University – USDA Cooperative Extension Slide Series, Bugwood.org)

Signs of the pathogen include bacterial streaming which is a common diagnostic sign of *R. solanacearum*. The bacterial streaming test is ideal for field practices. This test consists in cutting stems of plants with common bacterial symptoms and place them in water in a clear container. Threads of a viscous white slime often can be observed streaming from the cut end of the stem within 15 minutes (**Fig. 10**). Bacterial ooze can also sometimes be seen from freshly cut potato tubers (**Fig. 9A**), from potato eyes (9B) or from freshly cut wilted stems (**Fig. 11**).

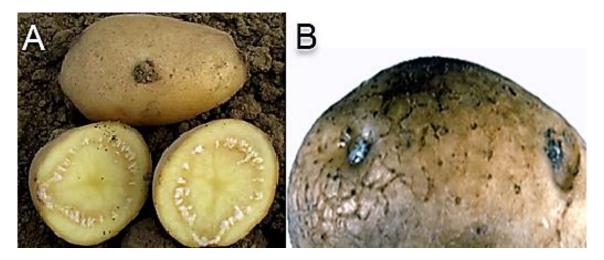


Figure 9. Signs of *R. solanacearum* on potato: Bacterial ooze from freshly cut potato tubers (A) and bacterial ooze from eyes of potato tuber (B). (Image courtesy of P. Champoiseau, University of Florida (A) and Central Science Laboratory, Harpenden Archive, British Crown, Bugwood.org (B))



Figure 10. Bacterial streaming into water from freshly cut wilted tomato stem (Image courtesy of the University of Georgia, Plant Pathology Extension)



Figure 11. Bacterial ooze from freshly cut wilted tomato stem (Image courtesy of Defra, Crown Copyright)

## **Sample Collection**

- 1. Pictures of symptoms from the field can be helpful to identifiers to see the location, overall plant health, and how the samples looked before they were removed from the host.
- Sample should be at least 1 gram of symptomatic root, stem, crown, or tuber tissue. If the host is small enough, the whole plant can be sent as a sample. Stem and crown are likely to have the highest bacteria content and are preferred. Make sure samples are free of soil. Avoid sending completely necrotic (dead or brown) tissue.
- 3. If more than one plant is displaying the characteristic signs or symptoms, take multiple samples that represent the range of what is being observed. It is recommended to wear disposable gloves that can be changed or sanitize between plants and to sanitize any cutting tools used between plants. If sampling between fields where *R. solanacearum* is suspected, clean footwear of soil or use disposable foot covers between fields to avoid spreading the pathogen.
- 4. Double bag the samples and accurately label. Refrigeration of samples is not recommended if samples will be cultured, however if samples will only be sent for molecular testing or cannot be processed immediately refrigeration is recommended. If refrigeration is not an option try to keep cool, at least below 15.6 °C to slow the degradation of the sample.

Since *R. solanacearum* is known to survive in water, water testing can be used to detect presence in irrigation water. At present, there is no approved test that can be used in the field, therefore, all water samples must be sent to a diagnostics laboratory. For sampling of Water Sources:

- 1. Sterilize bottles and;
- 2. Collect ~0.5-liter samples at a depth of 30.5 centimeters (if possible).
- 3. Bottles should be labeled with location and time of sampling.

- 4. Keep samples cool and in a dark location. Do not refrigerate.
- 5. Perform tests within 24 hours of collection. For best results, conduct sampling when water temperatures exceed 15 °C and bacterial populations are highest in water.

### **Pest Identification and Diagnostics**

#### Pest Description

*Ralstonia solanacearum* is a gram-negative soil and waterborne vascular pathogen. Once one positive find is detected, this bacterium might be found in adjacent fields that share water sources or equipment.

### Identification and Diagnostic Resources

This pathogen is usually cultured on yeast extract-peptone-glucose agar (YPGA), non-selective media such as triphenyl tetrazolium chloride (TTC/TZC) agar and casamino acid peptone glucose (CPG), or semi-selective media (SMSA) between the temperatures of 28°C and 29°C. Molecular/ serological techniques are typically needed for accurate diagnostics particularly to the sub species level.

- EPPO Standard on Diagnostics: Ralstonia solanacearum, R. pseudosolanacearum and R. syzygii (Ralstonia solanacearum species complex): <u>Link to Diagnostic Publication</u> <u>with a Key to Species</u>
- 2. Screening Aid for Bacterial Wilt Symptoms: https://download.ceris.purdue.edu/file/1610

### **Easily Mistaken Species**

Ralstonia solanacearum is sometimes confused with Clavibacter sepedonicus, Clavibacter michiganensis subsp. michiganensis, Dickeya spp., Fusarium spp., Verticillium spp., Xanthomonas campestris pv. pelargonii and Xanthomonas hortorum.

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