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Field survey guidance for *Ralstonia solanacearum*

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Introduction

Regular and systematic monitoring and surveillance are vital for countries to detect the presence or absence of plant pests, particularly those severely impacting food security, the environment, trade, and agricultural productivity. This early warning information is essential for timely rapid response to pest outbreaks, informed phytosanitary decisions, effective risk management, and securing borders against pest invasions.

This field survey guidance provides easy-to-follow guidelines for technical personnel of national plant protection organizations (NPPOs), to survey Ralstonia solanacearum, a key pest commonly called bacterial wilt.

This document provides a protocol to aid in the monitoring, detection, sample collection, and diagnostics of Ralstonia solanacearum, ensuring effective phytosanitary decision-making to manage the pest risk and protect trade in plants and plant products. To simplify identification, this document also provides visuals and guidance on recommended pest traps for the different fruit fly species.

The field survey guidance complements the digital tools available to NPPO plant health inspectors, through the Africa Phytosanitary Programme (APP) mobile application and Geographic Information System (GIS) platforms.

APP is an IPPC initiative designed to transform pest management across Africa by enhancing the capabilities of phytosanitary personnel within NPPOs, to leverage advanced science and modern digital technology for effective and timely pest surveillance, detection, control, and prevention. Some of the African countries involved in APP listed Ralstonia solanacearum as a priority pest in their countries, requiring effective surveillance.

The IPPC implements APP in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the African Union Department of Agriculture, Rural Development, Blue Economy and Sustainable Development, through the African Union Inter-Africa Phytosanitary Council (AU-IAPSC).

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Acknowledgements

This document presents guidance to national plant protection organizations (NPPOs) to support active surveillance, detection, diagnostics, control and prevention of Ralstonia solanacearum. This document was created with financial support from the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). The guidance provided in this document was prepared by subject matter experts from USDA APHIS and reviewed by technical officers at the IPPC Secretariat, in the framework of the Africa Phytosanitary Programme (APP). APP is an initiative of the International Plant Protection Convention (IPPC), implemented in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the African Union Commission on Agriculture, through its technical unit- the African Union Inter-Africa Phytosanitary Council (AU-IAPSC). The IPPC Secretariat and the IPPC community are grateful to all the institutions that contributed to the production of this survey guidance.

Abbreviations

APHIS	Animal and Plant Health Inspection Service
APP	Africa Phytosanitary Programme
AU-IAPSC	African Union Inter-Africa Phytosanitary Council
GIS	Geographic Information System
IPPC	International Plant Protection Convention
NPPO	National Plant Protection Organization
USDA	United States Department of Agriculture

Figure 1: Wilting by *R. solanacearum* on tomatoes.
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Field survey guidance for *Ralstonia solanacearum*

Scientific name

Ralstonia solanacearum (Smith) Yabuuchi *et al.*, Safni *et al.*

Common name

Bacterial wilt

Type of pest

Bacterium

Taxonomic position

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

Known Hosts

Preferred hosts

Major hosts include pepper (*Capsicum annuum*), tobacco (*Nicotiana tabacum*), tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), potato (*Solanum tuberosum*), other solanaceous crops (*Solanum spp.*) and banana (*Musa spp.*).

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

Survey Protocol

Survey-site selection

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

Time of year to survey

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 healthy growth and are reaching their peak vegetative stage. For tomatoes, aim to survey 6 to 8 weeks after transplanting, during 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 (**Figure 1**, **Figure 2 (a)**, **Figure 4**, **Figure 6**).

Visual survey

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

Signs and symptoms

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

These symptoms in potatoes include:

- wilting (**Figure 2(a)**);
- the vascular ring of symptomatic potato tubers showing grey-brown discolouration and oozing (**Figure 2(b)**);
- bacterial ooze present in freshly cut tubers (**Figure 9(a)**) and on potato eyes (**Figure 9(b)**);
- yellowing; and
- vascular discolouration of the stem, which appears to be grey or brown.

The following symptoms are shown in tobacco:

- unilateral yellowing, stunting and leaf distortion **(Figure 3)**;
- wilting **(Figure 4)**;
- reddish to brown discolouration of the vascular system and stem lesions **(Figure 5)**; and
- root rot.

Figure 2: Symptoms of brown rot caused by *R. solanacearum* on potato.
© 2(a): D. Thurston, Cornell University; 2(b): P. Champoiseau, University of Florida

Figure 3: Unilateral yellowing in tobacco caused by *R. solanacearum*.

Figure 4: Wilting by *R. solanacearum* on tobacco.

Figure 5: Reddish to brown discoloration of the vascular tissue on tobacco stem caused by *R. solanacearum*.
Figures 3, 4, 5 © R. García, North Carolina State University

The following symptoms are shown in tomato:

- wilting during the hottest time of the day (**Figure 6**) – plants may appear to recover following rain or when temperatures cool at night;
- stunting occurs at any stage of the growth (**Figure 7(a)**);
- the entire plant may decline rapidly (as quickly as in 4 to 7 days) under favourable conditions, starting with wilt, leaf chlorosis and ending with death (**Figure 7(b)**); and
- infected stems may collapse, revealing vascular dark brown necrotic streaks (**Figure 8**) with white bacterial ooze.

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. The test consists in cutting stems of plants with common bacterial symptoms and placing them in water in a clear container. Threads of a viscous white slime can often be observed streaming from the cut end of the stem within 15 minutes (**Figure 10**). Bacterial ooze can also sometimes appear on freshly cut potato tubers (**Figure 9(a)**), on potato eyes (**Figure 9(b)**) or on freshly cut wilted stems (**Figure 11**).

Figure 6: Wilting of tomato plants by *R. solanacearum*. *© Kim Sang Gyu, National Agrobiodiversity Center*

Figure 7: Symptoms of the bacterial wilt of tomato.
© 7(a): C. Allen, University of Wisconsin; 7(b): T.M. Momol, University of Florida

Figure 8. Stem of tomatoes showing brown discoloration caused by *R. solanacearum*. *© Clemson University/USDA, Cooperative Extension Slide Series, Bugwood.org*

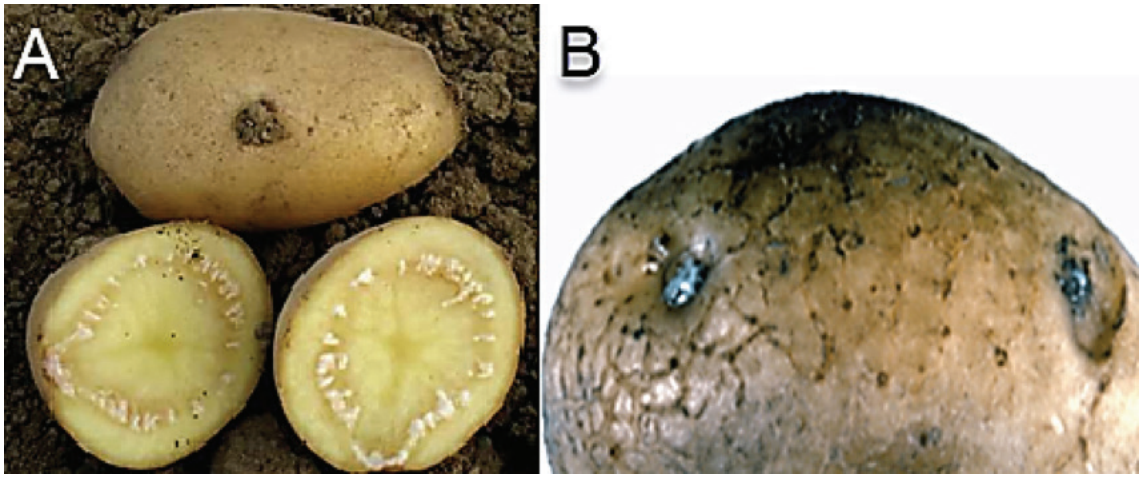

Figure 9. Signs of *R. solanacearum* on potato: (a) Bacterial ooze from freshly cut potato tubers; (b) bacterial ooze from eyes of potato tuber. *© 9(a): P. Champoiseau, University of Florida; 9(b): Central Science Laboratory, Harpenden Archive, British Crown, Bugwood.org*

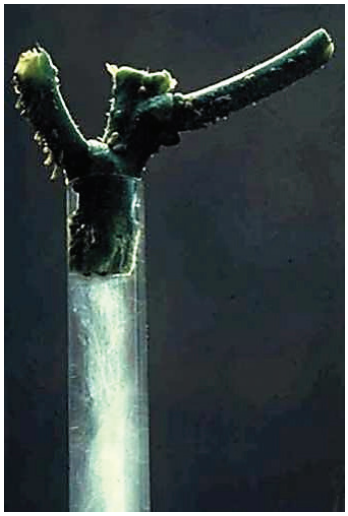

Figure 10. Bacterial streaming into water from freshly cut wilted tomato stem. *© University of Georgia, Plant Pathology Extension*

Figure 11. Bacterial ooze from freshly cut wilted tomato stem. *© Defra, Crown copyright*

Sample collection

1. Photographs of symptoms from the field can help identifiers to determine the location, the overall plant health and how the samples looked before they were removed from the host.
2. The samples should be at least 1 g of symptomatic root, stem, crown or tuber tissue. If the host is small enough, the whole plant can be sent as a sample. The stem and crown are likely to have the highest bacteria content and are preferred. Make sure the 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. Wearing disposable gloves that can be changed or sanitizing hands and any cutting tools used between plants is recommended. If sampling between fields where R. solanacearum is suspected, remove soil from footwear or use disposable foot covers between fields to avoid spreading the pathogen.
4. Place the samples in a double bag and label accurately. The refrigeration of samples is not recommended if the samples will be cultured; however, if the 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 the samples cool, at least below 15.6 °C, to slow down their degradation.

Since R. solanacearum is known to survive in water, water testing can be used to detect its 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 diagnostic laboratory. When sampling water sources:

1. Sterilize the bottles.
2. Collect ~0.5-litre samples at a depth of 30.5 cm (if possible).
3. Label the bottles with the location and time of sampling.
4. Keep the 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 the 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, the 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) at temperatures ranging between 28 °C and 29 °C. Molecular/serological techniques are usually needed for accurate diagnostics, particularly at the subspecies level.

EPPO (European and Mediterranean Plant Protection Organization). 2022. EPPO Standard on Diagnostics. PM 7/21 (3) *Ralstonia solanacearum*, *R. pseudosolanacearum* and *R. syzygii* (*Ralstonia solanacearum* species complex). *EPPO Bulletin*, 52: 225–261. doi.org/10.1111/epp.12837

Sullivan, M., Daniells, E., Southwick, C. & Mackesy, D. 2013. CPHST Pest Datasheet for *Ralstonia solanacearum* race 3 biovar 2. In: *USDA-APHIS-PPQ-CPHST*. [Cited 12 July 2024]. 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*.

IPPC

The International Plant Protection Convention (IPPC) is an international plant-health agreement that aims to protect global plant resources and facilitate safe trade. The IPPC vision is that all countries have the capacity to implement harmonized measures to prevent pest introductions and spread, and minimize the impacts of pests on food security, trade, economic growth, and the environment.

Organization

- There are over 180 IPPC contracting parties.
- Each contracting party has a national plant protection organization (NPPO) and an official IPPC contact point.
- Ten regional plant protection organizations have been established to coordinate NPPOs in various regions of the world.
- The IPPC Secretariat liaises with relevant international organizations to help build regional and national capacities.
- The secretariat is provided by the Food and Agriculture Organization of the United Nations (FAO).

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