Brown Rot of Potato

Original webpage (see link at the end of the document)

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- See definitions of red-colored words in the glossary at the end of this document -

Brown rot, also known as bacterial wilt, is one of the most destructive diseases of potato. The disease has been estimated to affect about 3.75 million acres in approximately 80 countries throughout the world with global damage estimates currently over $950 million per year. The disease is known to occur in the wet tropics, sub-tropics and some temperate regions of the world. In the United States, brown rot is limited to the Southeast from Maryland to Florida.

The disease is caused by the bacterium *Ralstonia solanacearum*, previously known as *Pseudomonas solanacearum*. It is one of the most damaging plant pathogens. Strains of this pathogen affect more than 200 plant species in over 50 families throughout the world, including a wide range of crop plants, ornamentals and weeds.

Strains of *R. solanacearum* have conventionally been classified as races and biovars (see the causal organism section for more details). Brown rot of potato is caused by either race 1 or race 3 of *R. solanacearum*. In the United States, race 1 is endemic and can cause bacterial wilt on several major crops such as eggplant, pepper, potato, tobacco and tomato. Although several introductions of race 3 to the United States have already occurred through importation of infested geranium cuttings from off-shore production sites, this race has been eradicated so far and is not considered to be established in North America. However, because of the risk of its possible re-introduction and its potential to affect potato in the northern United States, *R. solanacearum* race 3 biovar 2 is considered a serious threat to the United States potato industry. It is of quarantine importance and has been listed as a Select Agent plant pathogen under the Agricultural Bioterrorism Act of 2002.

Symptoms and signs

At the early stages of disease, the first visible symptoms of brown rot are usually on the foliage of plants. These symptoms consist of wilting of the youngest leaves at the ends of the branches during the hottest part of the day (**Photo 1**).

Photo 1. Symptom of brown rot of potato caused by *R. solanacearum* showing wilting of youngest leaves of plant.
(Photo courtesy of D.P. Weingartner – IFAS, University of Florida, Hastings)

Photo 2. Symptom of brown rot of potato caused by *R. solanacearum* showing wilting and stunting of plant.
(Photo courtesy of David Thurston, Cornell University)

At this stage, only one or half a leaflet may wilt, and plants may appear to recover at night, when the temperatures are cooler. As the disease develops under favorable conditions, all leaves in a hill may wilt quickly and desiccate, although dried leaves remain green, leading to general wilting and yellowing of foliage and eventually plant death. Another common symptom that can be associated with brown rot in the field is stunting of plants (**Photo 2**). These symptoms may appear at any stage of plant growth.

In young potato stems, infected vascular bundles may become visible as long, narrow, dark brown streaks. In young, succulent plants of highly susceptible varieties, collapse of the stem may also be observed. In well-established infections, cross-sections of stems and stolons may reveal brown discoloration of infected tissues (**Photo 3**).

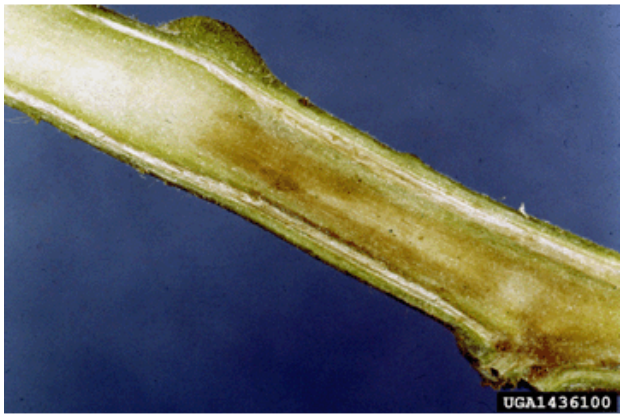

Photo 3. Brown discoloration of stem tissues caused by *R. solanacearum* *(Photo courtesy of Clemson University - USDA Cooperative Extension Slide Series,Bugwood.org)*

Brown rot symptoms may also be present in potato tubers at the later stages of disease. Cross-section of infected potato tubers may reveal a grey-brown discoloration of vascular tissues, also called the vascular ring (**Photo 4**). As infection progresses, the discoloration may extend into the pith or cortex of the tuber. A milky-white sticky exudate (ooze), which indicates the presence of bacteria cells, might also be observed from freshly-cut sections of infected tubers (**Photo 4**).

Photo 4. Grey-brown discoloration of vascular tissues and bacterial ooze in potato tuber infected by *R. solanacearum.* *(Photo courtesy of K. Tsuchiya)*

Symptom expression is favored by high temperatures (85-95°F / 29-35°C) and symptoms of the disease may progress rapidly after infection. However, under favorable conditions, symptomless plants may remain latently infected for extended periods of time. After infection the pathogen may survive in and be spread from the infected plant.

A common sign of brown rot observed at the surface of freshly-cut sections from severely infected stems is a sticky, milky-white exudate, which indicates the presence of dense masses of bacterial cells in infected vascular bundles, and particularly into the xylem (**Photo 5**).

Bacterial ooze may also be visible at the eyes or at the point where the stolon attached to the tuber (**Photo 6**). These signs may not be visible early in disease development.

Photo 5. Bacterial ooze from freshly-cut section of a geranium stem infected by *R. solanacearum.*
(Photo courtesy of M. Daughtrey, Cornell University)

Photo 6. Bacterial ooze exuding from eye of potato tuber infected by *R. solanacearum*.
(Photo courtesy of Central Science Laboratory, Harpenden Archive, British Crown, Bugwood.org)

Another common sign of the disease is observed when the stem cut sections are placed in clear water as shown in **Photo 7**. It consists of a viscous white spontaneous slime streaming from the cut end of the stem. This streaming represents the bacterial ooze exuding from the cut ends of colonized vascular bundles (**Photo 7**).

This "stem-streaming" test is easy to conduct and can be used as a valuable diagnostic tool for quick detection of brown rot in the field.

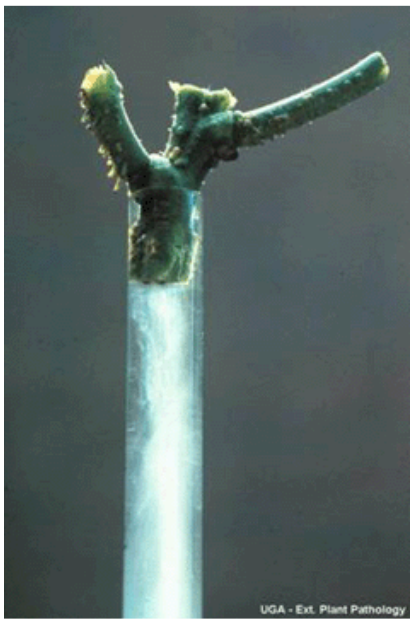

Photo 7. Bacterial streaming in clear water from stem cross-section of plant infected by *R. solanacearum*.
(Photo courtesy of University of Georgia, Plant Pathology Extension)

Causal organism

Ralstonia solanacearum (Smith 1896) Yabuuchi et al. 1996, is a Gram-negative, rod-shaped, strictly aerobic bacterium that is 0.5-0.7 x 1.5-2.0 µm in size. It is very sensitive to desiccation and is inhibited in culture by low concentrations (2%) of sodium chloride (NaCl). For most strains, the optimal growth temperature is between 82 and 90°F (28 and 32°C); however some strains have a lower optimal growth temperature of 80.5°F (27°C).

Liquid and solid (agar) growth media are commonly used for culture of the bacterium. On solid agar medium, individual bacterial colonies are usually visible after 36 to 48 hours of growth at 82.4°F (28°C), and two main colony types differing in morphology can be distinguished: colonies of the normal or virulent type that are white or cream-colored, irregularly-round, fluidal, and opaque; and colonies of the mutant or non-virulent type that are uniformly round, smaller, and butyrous (dry) (**Photo 8**).

Photo 8. Virulent (bottom) and non-virulent (top) colonies of *R. solanacearum* on CPG agar growth medium.
(Photo courtesy of P. Champoiseau, University of Florida)

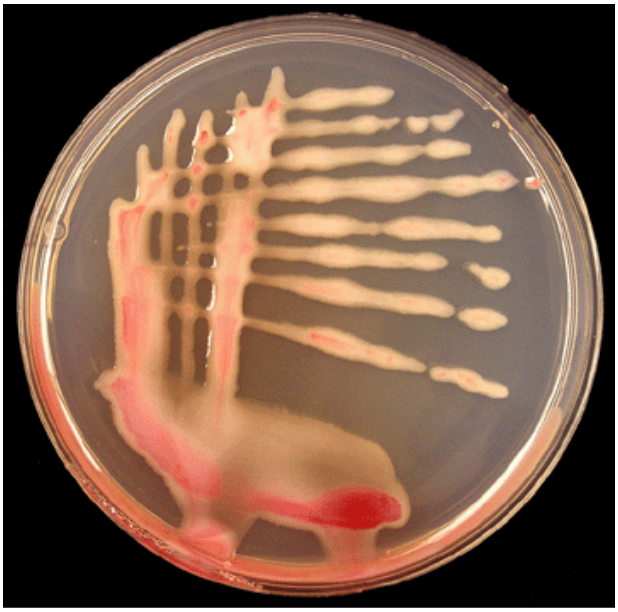

Photo 9. Virulent colonies of *R. solanacearum* on TZC agar medium.
(Photo courtsey of P. Champoiseau, University of Florida)

This shift from virulent to non-virulent bacterial cells occurs during storage or under oxygen stress in liquid media. A tetrazolium chloride (TZC) medium was developed to differentiate between the two colony types, on which virulent colonies appear white with pink centers and non-virulent colonies appear dark red (**Photo 9**). A semi-selective medium, called modified SMSA medium, has been developed for detection of *R. solanacearum* in water and soil samples, and in plant extracts. On this medium, typical bacterial colonies appear fluidal, irregular in shape, and white with pink centers after 2 to 5 days incubation at 82.4ºF (28ºC) (see the detection and identification section).

For long term culture storage, *R. solanacearum* will remain viable for several years at room temperature in sterilized tap, distilled or deionized water.

R. solanacearum is widespread in the tropics and subtropics around the world and many strains of the pathogen have been identified and characterized so far, revealing significant variability within the species. *R. solanacearum* is therefore considered a "species complex". Based on variability in host range and in ability to utilize several carbohydrate substrates, *R. solanacearum* strains were initially subdivided into races and biovars. So far, five races and five biovars have been identified within the species, but this old classification system is unsatisfactory because it is not predictive and some groups (e.g. race 1) contain very large variation. Recently, a new classification scheme has been described for strains of *R. solanacearum*, based on variation of DNA sequences. Four phylotypes were identified within the species that broadly reflect the ancestral relationships and geographical origin of the strains. These phylotypes can be further subdivided into sequevars.

Both race 1 and race 3 can cause brown rot of potato with similar disease symptoms. Race 1 corresponds to biovars 1, 3, and 4 and includes members of all four phylotypes. These strains have a broad host range and may infect many other major economic crops and ornamentals worldwide, such as banana, eggplant, geranium, peanut, pepper, potato, tobacco and tomato. "Race 1" strains are limited to tropical, subtropical and warm-temperate locations and usually cannot survive under cool temperate conditions. In contrast, race 3, which strictly corresponds to biovar 2 (or 2-A), has a limited host range. Initially described as pathogenic on potato and tomato, it was shown to infect and induce symptoms on eggplants, geranium, and pepper. Other solanaceous and non solanaceous weeds, such as the bittersweet or woody nightshade (*Solanum dulcamara*) (**Photo 10**), are considered as alternate hosts. Most of these alternate hosts remain latently infected and may not show any disease symptoms, but they can be epidemiologically important as inoculum sources and refuges.

Photo 10. Bittersweet or woody nightshade (*Solanum dulcamara*). Key features for identification.
(Photo courtesy of J. Elphinstone, Central Science Laboratory, York, UK, Crown Copyright)

Sometimes referred to as the 'cold tolerant' race, *R. solanacearum* race 3 biovar 2 originated in the Andes and was probably disseminated worldwide on potato tubers. It is now known to occur in the highlands of the tropics and in subtropical and temperate areas throughout the world, except in North America. In Europe, it has been introduced on geranium cuttings produced in the African highlands and has been responsible for several outbreaks of brown rot of potato during the last three decades.

Disease cycle and epidemiology

R. solanacearum is a soilborne and waterborne pathogen; the bacterium can survive and disperse for various periods of time in infested soil or water, which can form a reservoir source of inoculum. In potato, the brown rot pathogen is also commonly tuberborne.

The bacterium usually infects potato plants through the roots (through wounds or at the points of emergence of lateral roots). Soilborne organisms such as the root-knot nematode can cause injury to plant roots and favor penetration of the bacterium. Plant infection can also occur through stem injuries caused by cultural practices or insect damage. In some cases, plant-to-plant spread can occur when bacteria move from roots of infected plants to roots of nearby healthy plants, often via irrigation practices. Spread of bacteria by aerial means and subsequent plant contamination through foliage is not known to occur, thus making *R. solanacearum* a non airborne pathogen. High temperatures (85-95ºF / 29-35ºC) play a major role in pathogen growth and disease development. Several other factors that may affect pathogen survival in soil and water may also favor disease development, including soil type and structure, soil moisture content, organic matter in soil, water pH and salt content, and the presence of antagonistic microorganisms.

The bacterium also has an "exterior" phase (epiphyte) in which it can reside on the outside of the plant. It is of minor importance in epidemiology of the pathogen since bacteria do not survive epiphytically for long periods of time when exposed to hot conditions or when relative humidity is below 95%.

Under favorable conditions, potato plants infected with *R. solanacearum* may not show any disease symptoms. In this case, latently infected tubers used for potato seed production may play a major role in spread of the bacterium from infected potato seed production sites to healthy potato-growing sites. Latently infected tubers were probably responsible for the introduction of the pathogen in Europe. *R. solanacearum* can survive for days to years in infected plant material in soils, infested surface irrigation water, infected weeds, and infected potato washings and sewage. From these sources of inoculum, bacteria can spread from infested to healthy fields by soil transfer on machinery, and surface runoff water after irrigation or rainfall. *R. solanacearum* can also be propagated in infested ponds or rivers and disseminated to non-infested fields through waterways.

Infected semi-aquatic weeds may also play a major role in disseminating the pathogen by releasing bacteria from roots into irrigation water supplies.

At low temperatures (< 39.2ºF / 4ºC) bacterial population densities fall rapidly but the bacteria still can survive, often in a physiological latent state. In natural habitats, *R. solanacerum* race 3 biovar 2 can survive the winter in semi-aquatic weeds, in plant debris or in the rhizophere of non-host plants that act as reservoirs for the pathogen. Bacteria were shown to be increasingly released from semi-aquatic weeds after winter when temperatures start to increase.

Diagnosis and identification

Symptom identification is the first step for early diagnosis of brown rot of potato. Accurate identification of *R. solanacearum* from either symptomatic or asymptomatic plants and from water or soil samples demands multiple microbiological and molecular methods. A battery of complementary tests that differ in their sensitivity and/or specificity should be used for field or laboratory analyses for unambiguous identification of bacteria to species and biovar.

Screening tests can facilitate early detection and identification of bacteria in potentially infected plants or contaminated soil and water samples by *R. solanacearum*. They cannot be used to identify the race or biovar of the organism. These screening tests include stem streaming, plating on semi-selective medium (modified SMSA), immunodiagnostic assays using *R. solanacerum* specific antibodies, nucleic-acid-based identification using *R. solanacerum* specific primers, and pathogenicity assessment using susceptible hosts (e.g. tomato seedlings). Several rapid screening tests, such as immunostrips (Agdia), are available commercially for rapid and field detection of *R. solanacearum*.

A biochemical growth test is used for biovar determination of *R. solanacearum*. This test is based on the differential ability of strains of the pathogen to differentially produce acid from several carbohydrate sources, including disaccharides and sugar alcohols.

At the sub-species level, identification of strains of R. solanacearum can be assessed with several nucleic-acid based methods such as DNA probe hybridization and especially polymerase chain reaction (PCR) amplification with specific probes and primers.

Race determination is not generally possible because *R. solanacearum* strains usually have numerous hosts and do not have race-cultivar specificity on plant hosts. This is why the race sub-classification system has fallen out of favor with scientists, although it still has regulatory meaning because of quarantine rules written for “race 3 biovar 2”.

It is important to understand that unequivocal identification of *R. solanacearum* race 3 biovar 2 must rely on at least two distinct methods, including the biovar test and one of the nucleic acid-based tests that use PCR to amplify one of several specific DNA fragments.

Currently, for regulatory purposes, the only laboratory with proper registrations for ultimate determination of race and biovar of *R. solanacearum* race 3 biovar 2 is the USDA-APHIS-PPQ National Plant Germplasm and Biotechnology Laboratory in Beltsville, M. D.

USDA-APHIS-PPQ-CPHST
BARC-East, Bldg. 580
Powder Mill Road
Beltsville, MD 20705
Phone number: 301-504-7100
Fax number: 301-504-8539

Management

Control of *R. solanacearum* by planting resistant cultivars has been ineffective for potato, since resistance in this host plant may vary with location and temperature. Similarly, antibiotics (streptomycin, ampicillin, tetracycline and penicillin) have shown little efficiency for suppression of *R. solanacearum* in the field and are also expensive.

As a consequence, cultural methods are the best option for control of *R. solanacearum*. In the regions where the disease is endemic, cultural practices have proven to be effective in some conditions; these practices include use of disease-free seed tubers, crop rotation with non-host plants, intercropping, control of weeds and root-knot nematode populations, planting in uninfested fields, removal of long-term survival sites, selection of appropriate planting time, deep plowing of crop residues and satisfactory soil drainage or early- and late-season irrigation management. Chemical and soil treatments, such as soil fumigation, application of stable bleaching powder, modification of soil pH, heat treatment by solarization, application of plant resistant inducers or phosphorous acid or use of suppressive soils have been shown to reduce bacterial populations or disease severity at the small experimental scale, but these methods are either environmentally destructive, expensive, or both, and still have to be validated in the field. Similarly, biological control, based on use of *R. solanacearum* antagonists, has shown promising results at the small experimental scale, but still needs to be validated at a larger scale.

The most important strategy for protecting potato health is to prevent the introduction and inadvertent spread of the pathogen. This can best be achieved by the establishment of exclusionary and sanitary practices, along with strict governmental regulations. Of major importance in the United States is to prevent introduction or movement of *R. solanacearum* race 3 biovar 2 that might be carried in geranium cuttings imported from off-shore production sites.

A "New Pest Response Guidelines" (USDA-APHIS-PPQ) and a "Recovery plan for *Ralstonia solanacearum* race 3 biovar 2" (USDA-ARS) give the most accurate available information for detection, control, containment, and eradication of *R. solanacearum* race 3 biovar 2.

Exclusionary practices, such as quarantine, testing and visual inspection of imported material of host plants, and regulation and establishment of minimum sanitation protocols for offshore geraniums cutting production can prevent introduction of the pathogen. Practices such as cleaning and sanitizing field and handling equipment, planting seed tubers produced under strict certification procedures, and application of good sanitary cultural practices will prevent movement of the pathogen from infested to pathogen-free fields in case of inadvertent introduction of the pathogen. Even where *R. solanacearum* race 3 biovar 2 is present in soils (as in many parts of the African highlands), use of disease-free seed potatoes can significantly reduce disease incidence and allow growers to harvest a profitable crop.

Along with the establishment of exclusionary strategies, it is critical to monitor potentially infected sites for early detection and further eradication of *R. solanacearum* race 3 biovar 2. Potentially infected sites to be monitored include: soils in which crops infected by the pathogen have been identified; rivers and other surface water used for irrigation, particularly when infected host weeds are present and industrial and domestic waste products (solid and liquid waste, including washing water). In Europe, discharge of liquid effluents directly into waterways has been shown to represent the highest risk of spreading potato brown rot because it can lead to infection of the semi-aquatic weed *Solanum dulcamara*.

Because *R. solanacearum* race 3 biovar 2 is on the Select Agent list in the United States, detection and confirmation of this pathogen by a USDA-APHIS-PPQ recognized authority involves a set of protocols and measures to deal with potential outbreaks of the disease. APHIS is responsible for implementing these measures, in cooperation with states. Immediate reaction might take the form of teams of experts and survey personnel being sent to the site of initial detection in order to place holds, conduct investigations and initiate surveys. Further response activities which may be taken include implementing regulatory measures to quarantine infected or potentially infected production areas, stopping the intrastate and interstate movement of infected or potentially infected articles in commerce, and identifying proper control measures, which may include host removal and destruction, or requiring sanitary practices to eradicate the pathogen.

Confirmed infestations of potato or other solanaceous crops by *R. solanacearum* race 3 biovar 2 will require quarantine of fields, seed tubers, seedlings or other plant material associated with infested lots, including processing facilities, storage bins,

means of conveyance, soil and irrigation water. Host removal and destruction is required along with disinfection, as well as several years of non-host production in infected fields or associated growing areas before the quarantine can be removed. In case of contamination of water by the pathogen, irrigation with surface water should be prohibited, and water treatments, such as filtration or chemical disinfection, may be applied under control of legal authorities. Any permission to irrigate would be subject to results and testing water samples. Treatment of industrial potato washings requires primary separation of solid and liquid waste. Solid waste will be transported to licensed landfill sites for deep burial. Treatment of liquid effluents can be achieved by several different methods including treatment with heat, by anaerobic digestion, or by filtration or oxidation.

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Glossary

(In order of appearance in the text)

Bacterium. A bacterium is a microscopic organism consisting of individual cells. Bacteria cause diseases in many host plants. They can survive on crop residue, seed, or in soil and water; they may be spread by plant or plant cuttings transfer, mechanical means, insects, and seeds.

Pathogens. A pathogen, or infectious agent, is a biological agent that causes disease or illness to its host. A number of different organisms can cause plant infectious disease. Among them are fungi, bacteria, viruses, nematodes or parasitic plants.

Strains. A strain is a genetic variant or subtype of a microorganism (for example virus or bacterium or fungus).

Species. A species is one of the basic units of biological classification. A species is often defined as a group of organisms capable of interbreeding and producing fertile offspring. Each species is placed within a single genus.

Races. A race is formed by a group of bacterial strains that are differentiated based on the response on a set of host differentials.

Biovars. A biovar is a group of bacterial strains that are distinguishable from other strains of the same species on the basis of their physiological characteristics.

Endemic. Endemic, in a broad sense, can mean "belonging" or "native to", "characteristic of", or "prevalent in" a particular geography, area, or environment; native to an area or scope.

Symptoms. A symptom is a subjective evidence of disease or physical disturbance. It is an evident reaction by a plant to a pathogen, and is not necessarily visible. Different pathogens can induce similar symptoms.

Vascular bundles. Vascular, or conductive, bundles are responsible for long-distance transport of water and nutrients throughout the plant. Highly developed plants have two types of vascular tissues: the xylem and the phloem.

Stolons. A stolon is a slender stem that grows horizontally along the ground, giving rise to roots and aerial (vertical) branches at specialized points called nodes.

Xylem. The xylem is responsible for transportation of raw sap (water and nutrients) from roots to aerial parts of the plant. *R. solanacearum* is a limited xylem-invading pathogen.

Gram-negative. Bacteria have been classified as Gram-negative or Gram-positive regarding structural differences in their cell walls. Many species of Gram-negative bacteria are pathogenic. This pathogenic capability is usually associated with certain components of Gram-negative cell walls.

Aerobic. An aerobic organism requires oxygen for aerobic cellular respiration. Cellular respiration is the mechanism by which aerobic organism require oxygen to utilize substrates (for example sugars and fats) in order to obtain energy.

Bacterial colonies. On the surface of a solid growth medium, individual bacterial cells will grow and multiply to become visible bacterial colonies. All cells within the colony descend from a single ancestor and are identical. Characteristics of bacterial colonies (color, aspect, diameter or growth rate) are commonly used for bacteria identification.

Virulent. Virulence refers to the degree of pathogenicity of a microorganism, or in other words the relative ability of a microorganism to cause disease.

Selective medium. A growth or culture medium is a substance in which microorganisms, such as bacteria, or cells can grow. Selective media are used for the growth of only select microorganisms. They usually contain antibiotics to which the select microorganims is resistant to.

Variability. Variability here refers to variation of a given characteristic from one bacterial strain (or group of strains) to the other.

Species complex. *R. solanacearum* is considered a "species complex" as it includes individual isolates that may not be considered within a single species, as it is the case for the banana blood disease bacterium or *Pseudomonas syzygii*.

Carbohydrate substrates. Carbohydrates are simple organic compounds such as sugars and starch which contain carbon chains. They fill numerous roles in living organisms, such as the storage and transport of energy and structural components. Carbohydrates are differentially used as source of energy by bacteria.

Variation of DNA sequences. Comparison of DNA sequences is commonly used for classification studies of strains of microorganisms. It is basically assumed that the higher the homology is between two strains, the more closely related the strains are in terms of evolution. These types of studies are known as phylogenetic studies.

Phylotypes. A phylotype is defined as a group of strains that are closely related based on phylogenetic analysis of sequence data. Each phylotype is composed of a number of sequevars.

Sequevars. A sequevar, or sequence variant, is defined as a group of strains with a highly conserved sequence within the area sequenced.

Pathogenic. Pathogenicity is the ability of an organism to cause disease in another organism.

Root-knot nematode. Root-knot nematodes are plant-parasitic roundworms from the genus *Meloidogyne*. They exist in soil in areas with hot climates or short winters. There are a great many parasitic forms, including pathogens in most plants, animals, and also in humans.

Immunodiagnostic assays. These assays are based on the use of antibiotics in various test formats to detect and identify any molecules or cells (including bacteria). The most commonly used assays for bacteria detection and identification are agglutination, enzyme-linked immunosorbent assay (ELIZA), immunofluorescence, lateral flow strip tests or flow-through assays.

Antibodies. Antibodies (also known as immunoglobulins) are proteins that are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. Due to their specificity, they are commonly used in biology for detection and identification of microorganisms.

Nucleic-acid. A nucleic acid is a molecule composed of nucleotide chains. These molecules carry genetic information. The most common nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acids are universal in living things, as they are found in all cells and viruses.

DNA probe hybridization. DNA probe hybridization uses the ability of two complementary single-stranded nucleic acids to combine into a single molecule. Nucleotide probe of known sequence will be used to bind complementary strand of undetermined organism for identification.

Polymerase chain reaction (PCR) amplification. The polymerase chain reaction is a technique that consists of amplifying a DNA molecule exponentially.

Cultivars. A cultivar is a cultivated plant that has been selected and given a unique name because it has desirable characteristics (decorative or useful) that distinguish it from otherwise similar plants of the same species.

Plant resistance inducer. Plant resistance inducers are natural or synthetic chemical compounds that apparently act by stimulating the natural defense response in the plant.

Suppressive soils. A suppressive soil is one that possesses some level of control of a disease forming organism. All soils have a natural level of disease suppressive activities. In most soils long term management can either reduce or increase this level of suppression.

Biological control. Biological control is defined as the reduction of pest populations (including insects, mites, weeds and plant diseases) by natural enemies. Biological control agents of plant diseases are most often referred to as antagonists.

Internet links

Brown rot of potato original webpage:
http://plantpath.ifas.ufl.edu/rsol/Trainingmodules/BRPotato_Module.html

***Ralstonia solanacearum*/Brown rot-Bacterial wilt website:**
http://plantpath.ifas.ufl.edu/rsol/

***Ralstonia solanacearum* race 3 biovar 2 webpage:**
http://plantpath.ifas.ufl.edu/rsol/Trainingmodules/RalstoniaR3b2_Sptms_Module.html

Southern wilt of geranium webpage:
http://plantpath.ifas.ufl.edu/rsol/Trainingmodules/SWGeranium_Module.html

Bacterial wilt of tomato webpage:
http://plantpath.ifas.ufl.edu/rsol/Trainingmodules/BWTomato_Module.html

USDA-APHIS Select Agents and Toxins list:
http://www.aphis.usda.gov/programs/ag_selectagent/ag_bioterr_toxinlist.shtml

Liquid and solid (agar) growth media for *R. solanacearum*:
http://plantpath.ifas.ufl.edu/rsol/Culturemedia.html

New pest response guidelines: *Ralstonia solanacearum* race 3 biovar 2
http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/nprg-ralstonia.pdf

Minimum sanitation protocols for offshore geranium cutting production
http://www.aphis.usda.gov/plant_health/plant_pest_info/ralstonia/downloads/ralstoniaworkplan.pdf

Recovery plan for *Ralstonia solanacearum* race 3 biovar 2
Causing Brown Rot of Potato, Bacterial Wilt of Tomato, and Southern Wilt of Geranium
http://www.ars.usda.gov/SP2UserFiles/Place/00000000/opmp/Rs3-2RecoveryPlan-v-Oct112006.pdf