Current Status of Bacterial Wilt Disease in Plants

Dr. Dilip Kumar Sharma
Director, Vardhman Mahaveer Open University (VMOU), Kota, Rajasthan, India

Received 9th Jul 2022, Accepted 10th Aug 2022, Online 12th Sep 2022

Abstract: Bacterial wilt is a complex of diseases that occur in plants such as Cucurbitaceae and Solanaceae (tomato, common bean, etc.) and are caused by the pathogens Erwinia tracheiphila, a gram-negative bacterium, or Curtobacterium flaccumfaciens pv. flaccumfaciens, a gram-positive bacterium. Cucumber and muskmelon plants are most susceptible, but squash, pumpkins, and gourds may also become infected. Erwinia tracheiphila is spread between plants by two species of insect vectors, striped cucumber beetles (Acalymma vittatum) and spotted cucumber beetles (Diabrotica undecimpunctata). The beetles acquire E. tracheiphila by feeding on infected plants, then carry the bacteria in their digestive tracts. The disease may be spread to susceptible plants through feeding wounds, by way of infected mouthparts or frass. The bacteria is capable of overwintering in the gut of its insect vectors. Bacterial wilt is a disease of the vascular tissue. When a plant is infected, E. tracheiphila multiplies within the xylem, eventually causing mechanical blockage of the water transport system. The first sign of infection, which appears about five days after acquisition, is the wilting of individual leaves on a single stem. However, the disease will soon spread down the runner and then infect the whole plant, causing it to shrivel and die. There is a diagnostic test for bacterial wilt that can be done in the field. The presence of the E. tracheiphila causes the sap to become a milky color and acquire a sticky consistency. If the stem is cut near the crown and the ends are slowly pulled apart, the sap should form a viscous string. Once a plant is infected, there is no way of stopping the spread of the disease. Some cucurbit cultivars are less susceptible than others, so it is beneficial to plant these cultivars. However, since wilt-resistant plants have not yet been developed, the most effective way to prevent the disease is to keep beetle populations at a minimum. While various methods of beetle control have been tested, the most effective preventative measure is to keep beetle populations as low as possible through careful field monitoring and insecticide sprays. Cultural control can also be effective, thus this means one should apply the direct methods.

Keywords: bacterial wilt, disease, status, infection, whole plant, beetle, insecticide, bactericide.

Introduction

Bacterial wilt is one of the major diseases of tomato and other solanaceous plants. The disease is known to occur in the wet tropics, subtropics and some temperate regions of the world.[1]

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). Bacterial wilt of tomato is caused by either race 1 or race 3 of *R. solanacearum* and, rarely, by race 2. Race 1 is endemic in the United States 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 occurred as a result of importation of infected geranium cuttings from production sites off-shore, this race has been eradicated so far and is not considered 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.[2,3]

At the early stages of disease, the first visible symptoms of bacterial wilt are usually seen 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).

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, the entire plant 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 bacterial wilt in the field is stunting of plants (Photo 2). These symptoms may appear at any stage of plant growth, although in the field it is common for healthy-appearing plants to suddenly wilt when fruits are rapidly expanding. [4,5]

![Photo 1. Symptom of bacterial wilt of tomato caused by *R. solanacearum* showing wilting of leaves at the end of plant branch](image-url)
Photo 2. Symptom of bacterial wilt of tomato caused by *R. solanacearum* showing wilting of foliage and stunting of plant

In young tomato 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. (Photo 3). In well-established infections, cross-sections of stems may reveal brown discoloration of infected tissues (Photo 4).

Photo 3. Symptom of bacterial wilt of tomato caused by *R. solanacearum* showing collapse of young stem after artificial inoculation of the plant
Symptom expression is favored by high temperatures (85-95°F) 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 bacterial wilt of tomato 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 in the xylem (Photo 5). Another common sign of the disease can be observed when the cut stem sections are placed in clear water as shown in Photo 6. 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 6). This “stem-streaming” test is easy to conduct and can be used as a valuable diagnostic tool for quick detection of bacterial wilt in the field.[6,7]

*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.[8,9]

The bacterium usually infects tomato 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 (85-95°F) 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 antagonist microorganisms.[10,11]

![Photo 6. Bacterial streaming in clear water from stem cross-section of plant infected by *R. solanacearum*](image)

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, tomato plants infected with *R. solanacearum* may not show any disease symptoms. In this case, latently infected plants can play a major role in spread of the bacterium. In the United States, the southern states (Georgia and Florida) are a major source of tomato transplants for the north-eastern states and southern Canada and as a result bacterial wilt of tomatoes is occasionally found in the north via infected seedlings. The organism does not overwinter in the north, however. Transplants are either field-grown (not common anymore) or container-grown in greenhouses. Cultural practices at either field production (high plant density, use of irrigation several times a day, multiple clipping, or plants undercutting before harvest) or greenhouse production (overhead irrigation or plant handling) may favor plant infection and spread of the pathogen from infected tomato transplants production sites to healthy tomato growing sites.

*R. solanacearum* can survive for days to years in infected plant material in soils, infested surface irrigation water, and infected weeds. 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 waters.[12]

At low temperatures (< 39.2ºF), 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 during the winter in semi-aquatic weeds, in plant debris or in the rhizosphere 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. Symptom identification is the first step for early diagnosis of bacterial wilt of tomato. 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. solanacearum specific antibodies, nucleic-acid-based identification using R. solanacearum 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.[13]

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”.

**Discussion**

Bacterial wilt is caused by a soil-borne bacterium named *Ralstonia solanacearum* (formerly known as *Pseudomonas solanacearum*). Potato wilt bacterium mainly inhabits the roots, and enters the root system at points of injury caused by farm tools or equipment and soil pests.

On potato, bacterial wilt is also known as:

- brown rot
- southern wilt
- sore eye
- Jammy eye.

Bacterial wilt is a serious problem in many developing countries in the tropical and subtropical zones of the world. It has been recorded in all Australian states except Tasmania.[14]
Typical 'sore-eye' symptom on infected tuber

Wilting is first seen as a drooping of the tip of some of the lower leaves similar to that caused by a temporary shortage of water. At first only one branch in a hill may show wilting. Affected leaves later become permanently wilted and roll upwards and inwards from the margins. The wilting then extends to leaves further up the stem and is followed by a yellowing of the leaves.

This yellowing, wilting and in-rolling of the leaves makes diseased plants very obvious, especially when surrounded by healthy plants. The leaves finally turn brown and fall off, beginning at the base of the stem and continuing upwards.

*Ralstonia solanacearum* (biovar2, race3) is the causal agent of bacterial wilt and this quarantine phytopathogen is responsible for massive losses in several commercially important crops. Biological control of this pathogen might become a suitable plant protection measure in areas where *R. solanacearum* is endemic. Two bacterial strains, *Bacillus velezensis* (B63) [15] and *Pseudomonas fluorescens* (P142) with in vitro antagonistic activity toward *R. solanacearum* (B3B) were tested for rhizosphere competence, efficient biological control of wilt symptoms on greenhouse-grown tomato, and effects on the indigenous rhizosphere prokaryotic communities. The population densities of B3B and the antagonists were estimated in rhizosphere community DNA by selective plating, real-time quantitative PCR, and *R. solanacearum*-specific fliC PCR-Southern blot hybridization. Moreover, we investigated how the pathogen and/or the antagonists altered the composition of the tomato rhizosphere prokaryotic community by 16S rRNA gene amplicon sequencing. *B. velezensis* (B63) and *P. fluorescens* (P142)-inoculated plants showed drastically reduced wilt disease symptoms, accompanied by significantly lower abundance of the B3B population compared to the non-inoculated pathogen control. Pronounced shifts in prokaryotic community compositions were observed in response to the inoculation of B63 or P142 in the presence or absence of the pathogen B3B and numerous dynamic taxa were identified. Confocal laser scanning microscopy (CLSM) visualization of the gfp-tagged antagonist P142 revealed heterogeneous colonization patterns and P142 was detected in lateral roots, root hairs, epidermal cells, and within xylem vessels. Although competitive niche exclusion cannot be excluded, it is more likely that the inoculation of P142 or B63 and the corresponding microbiome shifts primed the plant defense against the pathogen B3B. Both inoculants are promising biological agents for efficient control of *R. solanacearum* under field conditions.[16]

**Results and Conclusions**

Control of bacterial wilt disease caused by *R. solanacearum* is an important challenge. Many strategies were proposed for controlling bacterial wilt disease. Among them, manipulating soil suppressiveness through organic amendments and managing soil suppressiveness via inoculant strains are considered the most promising and environmentally-friendly alternatives. Our results showed that the strains, *B. velezensis* B63 and *P. brassicacearum* P142, are promising candidates for future biocontrol of *R. solanacearum* under field conditions, through significantly lowered *R. solanacearum* densities in tomato shoots and in the rhizosphere. Amplicon sequencing revealed many dynamic taxa, likely indicating
complex interactions between the inoculant strains, B3B, the prokaryotic community in the tomato rhizosphere and the plant itself. The inoculation with B63 or P142 significantly promoted specific taxa, with potential plant protection and/or growth promotion-related traits, respectively, which might, in turn, affect soil suppressiveness and increase plant defense. For the first time, 16S rRNA gene amplicon sequencing was used to demonstrate *R. solanacearum* reduction through inoculation of in vitro antagonists which were correlated to the reduction of wilting symptoms. Combination between cultivation-dependent and independent methods correlated well and in particular Illumina sequencing of 16S rRNA gene fragments amplified from total community DNA allowed deeper insights into the complex interaction that might lead to pathogen suppression.[16]

References


