BACTERIAL WILT



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BACTERIAL WILT

Bacterial wilt is known as one of the most destructive bacterial plant disease in the world and due to the pathogen's virulence, survival rate, wide host range and geographic distribution in South Africa, it has been given quarantine status (Agricultural Pests Act, 1983). The pathogen is widely distributed in the Lowveld, as well as the coastal regions of KwaZulu-Natal, especially where tobacco and vegetable crops are cultivated in warm subtropical conditions.

Bacterial wilt in potatoes is also known as "vrotpootjie" or brown rot and is caused by different strains of the pathogen Ralstonia solanacearum (initially known as Pseudomonas solanacearum and subsequently as Burkholderia solanacearum).

The pathogen is a seed and soil-borne bacterium that causes

bacterial wilt disease in a variety of crops. The fact that the disease is carried in seed potatoes and has a wide range of hosts makes the control of this disease particularly difficult. The latent (not visually observable) infections in seed potatoes allow the disease to spread unnoticed from one potato field to another and from one production region to another. Once infected, it is extremely difficult to free the soil of the pathogen.

A 2005 study estimated that global economic losses as a result of bacterial wilt in potatoes amount to approximately 950 million American dollars per year. In Africa this disease has an enormous economic impact on potatoes, as well as crops such as tomato, pepper and tobacco.

DEVELOPMENT OF DISEASE



Symptom expression is not the same for all cultivars

Plant

Wide host range

R. solanacearum

- Wet and warm conditions are favourable for the development of disease
- Able to survive in the moist soil for many years
- Temperatures >40°C are lethal to bacterial cells

The pathogen typically enters the roots of the host plant through wounds caused by various factors. The role of rootknot nematodes should not be underestimated. The pathogen can also access the plant's roots at the points where secondary roots form. However, it is only with a high concentration of bacteria that such root infection will occur.

The initial colonisation of the host tissue does not occur in the xylem vessels as expected, but rather in the surrounding small cells.

The incubation period varies widely and depends on factors such as host species, environmental conditions, age of the host and the resistance of the host.

MANAGEMENT OF BACTERIAL WILT (for producers of table potatoes)

	RISK	MANAGEMENT
PLANTING TIME	Warm, wet time of the year	Wet, warm soil creates the ideal conditions for bacterial wilt disease. - If the disease occurs repeatedly, consider planting at a cooler time of the year, if possible.
CHOICE OF LAND	Contaminated soil	 Ralstonia solanacearum has the ability to survive in the soil for many years. Maintain a rotation programme of at least four years, but preferably longer. Ensure that rotation crops are not hosts of bacterial wilt. Maize, sorghum, wheat, other grain crops and one year and multiple year grasses are the preferred rotation crops. Any land in the Lowveld that is leased for winter planting must be considered high risk. If the preceding crop was affected by bacterial wilt, postpone the planting of potatoes by at least four years. Control volunteer plants and weeds that serve as hosts, in order to maintain the effectiveness of the rotation programme.
	Heavier clay soils	 Extend the rotation programme to longer than four years, since the pathogen has the tendency to survive for longer in heavier clay soils than in light sandy soils. Livestock grazing on infected land can spread the pathogen via their hooves.
	Distribution of the disease across potato fields	 Bacterial wilt can be spread via vehicles and people travelling between different farms, depots and markets. Wet and muddy conditions are ideal for the spreading of the pathogen. Strict access control to sensitive areas of the farm is recommended. Parking areas should preferably have cement surfaces to allow for the thorough disinfection of vehicles on site. All visitors' shoes, the soles in particular, must be disinfected before being granted access to pack houses and potato fields. The loading surfaces of trucks and trailers must be disinfected, particularly where there are signs of wet exudation in the potato load. The installation of a permanent dipping trough for vehicles at the exit point of an infected site can serve to effectively combat the disease, provided all soil and mud is sprayed off the vehicle before it passes through the dipping trough and provided that the sanitizer is kept at the proper concentration. Disinfect all implements and equipment prior to entering other sites. Dispose unmarketable plant debris from infected potato fields in a responsible manner. Erect fences around potato fields to reduce the risk of the disease being spread by wildlife and stray livestock.
CHOICE OF CULTIVAR	Susceptible cultivars	- There are no potato cultivars that are resistant to bacterial wilt, however, the symptom expression of different cultivars can differ.

MANAGEMENT OF BACTERIAL WILT (for producers of table potatoes)

	RISK	MANAGEMENT
SEED POTATOES	Contaminated seed potatoes	 Ensure that only certified seed potatoes are planted on the premises, including land leased from other producers, and in all vegetable gardens situated on the farm. Obtain copies of certification reports from suppliers and keep the certification labels of all seed lots for reference purposes. Do not purchase any potatoes at fresh produce markets and use them for planting. According to the specifications of the South African Seed Potato Certification Scheme, seed potato growers are entitled to sell the potatoes harvested from infected potato fields on fresh produce markets. Avoid the dipping or cutting of seed potatoes. The chemical treatment of seed potatoes potentially infected with the pathogen is not recommended, since it has a very limited chance of succeeding.
CROP MAINTENANCE	Infection of a potato field through irrigation water	- Avoid the use of a potentially infected water source, since it is physically impossible to disinfect an infected water source.
	Spread of the disease through infected plants	 Wilted plants in commercial plantings (not seed potato production) must be thoroughly inspected for typical symptoms of bacterial wilt, to be confirmed by a representative of Potatoes South Africa. In the case of commercial plantings (not seed potato production), removing the wilted plants together with all their tubers can help to limit the spread of the pathogen. In the case of commercial plantings, the plants must be incinerated (not applicable to seed potato production).
	Infection between plantings	 Help prevent the spread of the pathogen by washing all tractor tyres and implements with a high-pressure hose, leaving them to dry, and then treating them with a sanitizer. Cultivate new and uncontaminated fields before cultivating any fields that are already contaminated. Run-off water can also infect the surrounding potato fields, dams and streams.
	Spread of the disease within a potato field	 The mechanical spread of the pathogen can be controlled by limiting activity in the infected potato field to a minimum. The spread of the pathogen can be limited by certain practices, e.g. to ridging potatoes early and to follow controlled spraying programmes. Water is one of the primary ways in which the disease can be spread across a potato field. Do not over-irrigate to avoid run-off.
HARVEST- ING	Contaminated tubers serve to increase the disease pressure	 Unmarketable tubers left on the potato field raise the inoculum level even further. Bury them deep underground, or leave them lying above ground to bake in the sun for a few months. Infected tubers can be fed to cattle, but their movement must be limited.
PACK HOUSE	Infection of tubers during the harvesting and sorting process	 All sorting, pack house and potato handling equipment must be disinfected at least once a year. Sell the potatoes of infected potato fields as soon as possible.

SYMPTOMS

The disease has the ability to survive in a symptomless (latent) form in potato tubers, with the type of visual symptoms being potentially influenced by climate conditions. Conditions associated with symptomless infection include cool and dry growing seasons, or infection late in the growing season. Infected tubers left on the ground continue to rot, and secondary organisms access the tuber through cracks in the skin, culminating in the tuber rotting completely into a slimy mass.

Above ground symptoms

- The rapid green wilting of the foliage, even where the soil is wet, is the most typical above ground symptom visible.
- The foliage sometimes turns yellow over time and then eventually turns brown until total die-off occurs.
- On the main stem, the vascular bundles appear brown in colour when the infection is at an advanced stage.
- The lower leaves sometimes turn yellow before the leaves start wilting. Sometimes wilting happens so quickly that no yellowing of the lower leaves occurs.
- Sometimes wilting is evident without all the tubers displaying visual symptoms.

Below-ground symptoms

- A cross-section of the tuber on the stolon end reveals a visible ring symptom in the vascular bundle.
- Slight pressure on the tuber causes pale-white drops of

bacteria to collect in the vascular bundle ring.

- In the case of heavily infected tubers, a white/cream coloured substance (bacteria) can sometimes be seen leaking from eyes on the surface of the tuber.
- Tubers can show symptoms without there being any sign of wilting in the foliage.

Confirmation



Potentially infected tubers or seed potatoes can be sent to Plantovita (formerly known as the Coen Bezuidenhout Seed Test Centre) for confirmation. Contact Plantovita regarding packing and distribution requirements, costs, etc. Tel: 012 819 8123. Physical address: Plot 20, Zeekoegat, Roodeplaat, Pretoria.

The milk test. A stem clipping of an infected plant is suspended in a glass

of water. Within a few minutes, fine milky white threads, consisting of masses of bacteria, should start flowing from the stem. This test serves to differentiate bacterial wilt from other fungal diseases that result in a similar wilting effect.

THE PATHOGEN

The outbreaks of bacterial wilt in the early 1980s led to an investigation into the distribution and identification of the different biotypes of the pathogen *Ralstonia solanacearum*, with 45 isolates of the pathogen being collected from across South Africa.

The pathogen is classified into subgroups of either strains or boivars. Five strains and five biovars have thus far been identified internationally. The strain is classified according to the type of crop infected, while the biovar is classified according to the pathogen's ability to oxidise eight carbohydrates. Four biovars can affect potatoes, but only biovar 2 (strain 3) and biovar 3 (strain 1) have thus far been confirmed in South African potatoes. In South Africa, biovar 2 is primarily isolated from potatoes, while biovar 3 is mainly associated with tomato and tobacco. Biovar 2 is better adapted to a mild, cooler climate than biovar 3, and has already spread to almost all potato production regions in South Africa. Molecular investigations into the characterisation of and possible variation amongst different isolates served to differentiate between biovar 2 and biovar 3, but could not determine correlations between isolates and the regions from which they had originated.

SPREADING

Tubers. The disease is spread from farm to farm and from one production region to another primarily via seed potatoes, especially those with latent infection. Bacteria occur on the external surfaces of tubers, in the lenticels and in the vascular tissue. Surface bacteria can be controlled by means of chemical treatment, but internal infections remain problematic. There is evidence that an epiphytic phase can occur in the life cycle of *R. solanacearum* (for example in the case of certain peppers), which can aid in the survival of the pathogen and produce other sources of inoculum. There is no evidence, however, of the pathogen having the ability to survive on the leaves of plants.

Water. Water is one of the primary ways in which the disease is spread throughout a potato field. Run-off water can also infect the surrounding potato fields, dams and streams.

Hosts. *Ralstonia solanacearum* has a wide host range, affecting more than 450 plant species, including a variety of vegetables, tropical fruits and weeds. Table 1 of the South African Seed Potato Certification Scheme identifies the following crops as hosts of the pathogen: groundnut, the cabbage family, cabbage, pepper, watermelon, pumpkin, soybean, cotton, sunflower, tomato, tobacco and eggplant. These crops may not be planted in rotation with potatoes cultivated for purposes of registered seed potato production.

The presence of host plants (crops or weeds) will encourage the survival, propagation and spread of the disease in the region. Table 1 lists the following weeds: pigweed, Spanish blackjack, large and common thorn apple, wild tobacco, wild gooseberry, castor oil plant and nightshade. It is extremely important that these weeds are properly controlled regulary during the potato growing season, as well as during the crop rotation period, since these weeds encourage the development of the pathogen in the soil. Certain weeds and crops are also symptomless carriers of *R. solanacearum*.

Soil. The pathogen can survive for eight years or even longer in the soil, depending on factors such as the quantity of bacteria in the soil (inoculum levels), soil type, temperature, water status, soil depth and the presence of host plants. The bacteria cannot survive in hot, dry above ground layers (20 cm) for long (a few months to a few years), but has the ability to survive for many more years in deeper (40 to 60 cm) soil layers.

Infected soil and bacterial slime adhering to wheels, implements, crates, boots and animal hooves can carry the bacteria to other locations and lead to new infections.

The survival of *R. solanacearum* in the soil is influenced by various factors, such as the initial inoculum concentration, whether the land is left fallow or if non-host crops are planted, as well as the biological, chemical and physical characteristics of the soil. It is almost impossible to predict the survival period.

On loamy clay soil sites at Pretoria University, biovar 2 in some cases survived up to nine years of fallow and maize monoculture. Biovar 3 has a better survival rate than biovar 2.

Some experts argue that *R. solanacearum* typically exists in the antivirulent form in order to preserve energy and cellular resources for a greater chance of survival. As soon as host matter becomes available the bacteria multiply, and once sufficient cell mass has been achieved, the extracellular virulence factors are produced.

Soil characteristics affecting the survival of the pathogen in soil:

• Soil temperature – Biovar 2 (strain 3) has a lower optimum temperature for development than isolates of strain 1.

Disease development in respect of wilting and visible tuber infection occurs at a lower temperature $(14 - 16^{\circ}C)$ in the case of biovar 2 than in the case of biovar 3. Depending on the time of exposure, *R. solanacearum* can be destroyed at temperatures higher than 40°C.

- Soil moisture The impact of soil moisture takes place in interaction with soil temperature. Soil moisture is essential for the survival of *R. solanacearum*, but excess moisture in the soil is detrimental to the survival of the bacteria due to the resulting shortage of oxygen. Drying out of the soil helps to destroy the pathogen, but the bacteria can filter down into the deeper layers of the soil where the moist environment allows it to survive.
- Soil type The pathogen typically survives for longer in heavier clay soils than in lighter sandy soils. More intense microbial activity is typically encountered in heavier soils, and in such cases the survival rate of the bacteria may diminish more rapidly than in lighter soils, since they have to compete with the soil microbes for nutrients. Soil type determines soil moisture, which in turn affects the pathogen's ability to survive.
- Soil depth Most results indicate that *R. solanacearum* survives for longer in deeper soil layers. The deeper-lying roots of host plants are less disturbed, with less microbial activity in deeper soil layers. Deeper soil layers are less likely to dry out, aiding in the survival of bacteria.
- Oxygen status of the soil R. Solanacearum is an aerobic organism, and conditions that limit the availability of oxygen will be detrimental to the survival of the bacteria. Flooding (drowning) of the soil dramatically reduces the bacterial population.
- Soil pH Both strain 3 and strain 1 were found to suffer total loss of virulence at a pH of 4.5 and lower. At a pH of 8.5, the growth and virulence of strain 3 was diminished, while strain 1 continued to develop steadily, although the virulence of strain 1 was reduced. It was also found that at low pH levels, strain 3 remains more virulent than strain 1.

Cold storage does not destroy the pathogen. The storage of tubers or seed potatoes with latent infection at low temperatures does not destroy the pathogen. The bacterial population is decreases after storage at 4 to 6°C, but as soon as the seed potatoes are removed from cold storage, the bacteria multiply extremely quickly.

MANAGEMENT OF BACTERIAL WILT

There is a significant difference in the way in which bacterial wilt disease is managed in the case of seed potato production and in the case of commercial cultivation of potatoes for the table market, especially following the confirmation of infection. In the case of seed potato production, the certification authority has instituted some strict and very specific measures in respect of affected farms. This includes the consideration of possible further seed potato production on the farm and, if so, on which specific potato fields, fencing requirements, isolation requirements, handling of harvested crops, etc.

Choice of cultivar. Although there are some wild potato species that are resistant or tolerant to *R. solanacearum*, there is no commercial cultivar available that can be recommended as such.

Certified seed potatoes. Because bacterial wilt in potatoes is a disease with quarantine status, all plantings registered as seed potato plantings and due to be certified by Potato Certification Service, are sampled and tested for the presence of *R. solanacearum* by the Potato Laboratory Services. Any seed potato plantings that test positive for the presence of bacterial wilt are withdrawn from certification. For this reason, and also due to the potato industry's dedication to the certification requirements, there has been no significant increase in the incidence of the disease in potato plantings over recent years.

The incidence of the disease in seed and table plantings shall always remain a threat and can only be avoided if each and every potato producer takes heed of the threat and the risk it poses to the potato industry and its continued existence.

Chemical control. Various chemical formulations have been evaluated for the control of bacterial wilt disease. Limited success has been achieved. Many of the substances are also harmful to the environment. Certain formulations did

provide a reasonable degree of control, but their effectiveness in killing bacteria in deeper soil layers was insufficient. In the case of the production of registered seed potatoes, any form of control that does not entail total control is not acceptable.

Disinfection. The annual cleaning and disinfection of storage and handling equipment and machinery as a means to prevent the transfer of disease causing organisms to work surfaces, is essential for proper potato health management, even where there were no disease problems encountered during previous seasons. Waste tubers, broken containers, old bags, etc. should be incinerated or buried. After removing all waste and residue all floors, containers and walls must be cleaned using a high-pressure hose and a solution of hot water and soap. Since water can carry pathogens, a suitable disinfectant must be added to the water. After being washed and rinsed, all implements, floors, walls and containers must be disinfected according to the product specifications. Soil and organic material can reduce the effectiveness of disinfectants. Workers should be provided with disinfectants and washing facilities.

Implements and other equipment can be cleaned in a number of ways. Effective disinfectant measures include, high-temperature steam sterilisation at 80°C, cleansing with a general disinfectant and exposure to direct sunlight for a few days.

Effective disinfectants are characterised by their rapid germkilling action, their effectiveness in the presence of organic matter and hard water, their low toxicity for humans, long shelf life, non-corrosive nature, and ease of use. The use of 0.5% carbolic acid (Jeyes Fluid), Jik and formalin is recommended for the cleaning and disinfection of implements and machinery. A sanitizer is only effective if left in contact with the treated surface for at least 15 minutes.

Disinfection is less effective in the case of porous surfaces such as concrete and wood.



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SYMPTOMS OF BACTERIAL WILT



Rapid green wilting of the foliage is the most typical visible above ground symptom (1). Sometimes signs of wilting in weeds, e.g. the thorn apple, is the only sign of bacterial wilt (2). When looking at a cross-section of the tuber on the stolon end, a ring symptom is visible in the vascular bundle ring. Slight pressure on the tuber causes pale-white drops of bacteria to collect in the vascular bundle ring (3). The vascular tissue sometimes rots, forming pits (4). On the main stem, the vascular bundles appear brownish in colour when the infection is at an advanced stage (5). In the case of heavily infected tubers, a white/cream coloured substance (bacteria) can sometimes be seen leaking from eyes on the surface of the tuber (6).

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