Article:

Cardenas Gomez, Karla, Narino Rojas, Daniel, James, Sophie A. et al. (3 more authors) (2024) The Epidemiology and Management of Ralstonia solanacearum and Ralstonia pseudosolanacearum in Central and Northern Europe. Plant health cases. phcs20240028. ISSN 2959-880X

https://doi.org/10.1079/planthealthcases.2024.0028

The epidemiology and management of *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum* in Central and Northern Europe

Summary

Ralstonia solanacearum is a widespread plant pathogen, responsible for severe crop losses worldwide. In Central and Northern Europe, there have been sporadic outbreaks of potato brown rot caused by cool-adapted strains of this bacterium since the 1970s. Recently, tropical strains have also been isolated around these areas, highlighting its potential range expansion. This case study revisits *R. solanacearum* and *R. pseudosolanacearum* epidemiology in Northern and Central Europe, and discusses new diagnostic and environmentally-friendly biocontrol approaches for the management of this pathogen.

Abstract

The brown rot of potato and bacterial wilt in solanaceous plants are caused by *Ralstonia solanacearum*, one of the most important plant pathogens in the world, which is present in multiple locations globally, capable of infecting hundreds of plant species and resulting in severe economic losses. This soil-borne bacterium enters the roots of the plant and proliferates in the xylem, causing the characteristic wilting symptoms. It is taxonomically grouped into the *Ralstonia solanacearum* species complex (RSSC), formed by: *R. solanacearum, R. pseudosolanacearum*, and *R. syzygii*. In Central and Northern Europe, potato brown rot has been historically caused by cool-adapted *R. solanacearum* strains since the 1970s. However, a 2015 outbreak in the Netherlands of warm-adapted *R. pseudosolanacearum* in greenhouse *Rosa* spp. has highlighted the risk of potential geographical and host range expansion of tropical RSSC species. Continuous surveillance of host plants and surface waters using reliable identification tests needs to be maintained to prevent new outbreaks. Until now, no method has been able to efficiently limit the spread of RSSC species, nor to eradicate the bacterium in the field. Biological control methods, including the use of antagonistic bacteria or bacteriophages have however shown great promise, potentially providing an environmentally-friendly approach to control this pathogen.

Learning outcomes

1. Describe the characteristics of bacterial wilt and potato brown rot caused by RSSC, focusing on their impact in Central and Northern Europe.
2. Explain the potential range expansion of the RSSC in light of climate change, agricultural activity and transport of contaminated plant materials.
3. Discuss diagnostic and control methods available to manage and control RSSC.

Introduction

Ralstonia solanacearum: plant hosts, distribution and economic significance

Phytopathogenic *Ralstonia solanacearum* bacterium is considered the second most destructive crop pest in the world (Mansfield et al, 2012). It is responsible for lethal wilts and rots in a wide range of plants globally, including the tropics of the Americas and several countries in Africa, Europe, South East Asia and Oceania. In Europe, the pathogen is present in Belgium, Bulgaria, France, Germany, Hungary, Italy, Poland, Portugal, Romania, Russia, Slovakia, Spain and the United Kingdom, and is transient in the Czech Republic and Ukraine (Figure 1). The unusually broad host range comprises over 250 plant species of different monocot and dicot families of trees, weeds and shrubs, including economically important solanaceous crop plants such as tomatoes and potatoes (Genin & Denny, 2012). Other crop hosts are banana, groundnut plantain, ginger, cotton, eggplant, tobacco, peppers and cucurbits (Osdaghi, 2020). The host range is constantly expanding and new hosts are continuously reported (Genin & Denny, 2012). This bacterium can also survive asymptomatically in secondary plant hosts, and the weed bittersweet (*Solanum dulcamara*), commonly found in riverbanks across North America and Europe, is an important environmental reservoir for this pathogen (Wenneker et al, 1999).

Bacterial wilt is usually a disease of warm temperate regions such as the tropics and subtropics, whereas brown rot commonly occurs in cool temperate regions (Persley, 1985). These diseases rarely occur in regions where the monthly mean temperature is less than 10°C, since the optimal growth temperature range for these bacteria is between 25 to 37°C (Huerta et al, 2015).

Crop losses, as well as disease surveillance and eradication in affected zones, result in considerable costs for growers (Elphinstone, 2001). It is estimated that this pathogen is responsible for an annual global loss of 1 billion USD per year related to potato production alone (Allen et al, 2005), while bacterial wilt accounts for 20 to 100% of yield crop losses over 1.5 million ha in different parts of the world, affecting around 3 million farmers (Elphinstone, 2005; Wang et al, 2023). The economic impact in a particular area depends on many factors, including the local climatic conditions, cropping practices, soil characteristics, crop of choice, and the virulence of the specific strains causing the outbreak. Economic losses tend to be higher in zones where the pathogen has quarantine status, as infected potato tubers cannot be sold. For example, after an outbreak in the Netherlands in 1999, it was reported an increase of about £50 per ha for grower levies associated with disease management and eradication (Elphinstone, 2001).

Taxonomic classification

The numerous and heterogenous group of strains comprising *R. solanacearum* has been grouped into the *Ralstonia solanacearum* species complex (RSSC)(Fegan & Prior, 2005). Historically, the RSSC was divided into races, then biotypes and later into four phylotypes, that roughly correspond to the strain's geographical origin: phylotype I originated from Asia, II from the Americas, III from Africa, and IV from Indonesia (Genin & Denny, 2012). Phylotype II has two subclusters: IIA and IIB (Castillo & Greenberg, 2007), the latter can survive at lower temperatures and has been responsible for important outbreaks of potato brown rot in Northern Europe (Sebastià et al, 2021). More recently, comparative genomic analysis conducted on representative strains from each of the four phylotypes, divided the RSSC into three species: *R. solanacearum* comprising phylotype II, *R. pseudosolanacearum* grouping phylotypes I and III, and *R. syzygii* corresponding to phylotype IV and *R. syzygii* (Prior et al, 2016; Safni et al, 2014). Onwards in this case study, when referring to a particular species, it will be named by their species name (e.g. *Ralstonia syzygii*), while the term RSSC will refer to the three species collectively.

Transmission and life cycle

The RSSC spreads mainly through contaminated soil and water, where pathogens can survive for several years (Mansfield et al, 2012). The pathogen can also be transmitted by infected seed potatoes, plant debris and contaminated farming tools. After entering the roots of the susceptible host, the bacterium colonises the root cortex, invades the xylem vessels, and using the vascular system of the plant, spreads to the aerial parts. Consequently, the water flow within the vessels gets constricted and the plant loses its turgidity (Denny, 2007). The symptoms of bacterial wilt resemble water stress (Figure 2), while the symptoms of potato brown rot consist of browning and necrosis of the vascular tissue of the potato tuber (Elphinstone, 2001). The infected plant eventually dies and infectious bacteria remain as saprophytes in the soil, but once in contact with a new host plant, the disease cycle can start again. Irrigation water and farming tools can facilitate the further dispersal of the bacterium (Figure 3). River water contamination is facilitated by run-off from contaminated soil or plant materials coming from infested cropping fields or processing plants. Secondary hosts such as the weed bittersweet (*Solanum dulcamara*) (Figure 4), the perennial stinging nettle (*Urtica dioica*) and the annual black nightshade (*Solanum nigrum*) inhabiting river banks across North America, and Central and Northern Europe, can get infected when they come into contact with contaminated river water (Elphinstone et al, 1998). These environmental reservoirs usually remain asymptomatic, only showing wilting symptoms at temperatures exceeding 25°C (van der Gaag et al, 2019). Once infected, these plants can host the bacterium in their roots for prolonged periods of time and spill-off releases them back into the river, facilitating their transmission and geographical range expansion.

Bacterial wilt and brown rot of potato in Central and Northern Europe

Ralstonia solanacearum phylotype IIB potato

In Europe, *R. solanacearum* phylotype IIB sequevar 1 (historically denominated race 3, biovar 2) is the causative agent of potato brown rot (Parkinson et al, 2013). This disease is considered a major threat to potato crop production in temperate areas, due to the pathogen's capacity to survive at low temperatures in primary hosts without visible disease symptoms, but also overwinter in environmental reservoir hosts (Elphinstone et al, 1998). It is highly probable that these cool-adapted strains originated in potato plants in the temperate region of the Andes in South America (Parkinson et al, 2013), and have spread over the past decades by infected seed potatoes around the globe, especially across Europe (Janse, 2012). The prevalence of *R. solanacearum* phylotype IIB in Central and Northern Europe, particularly in the Netherlands, the UK and Sweden, has been correlated with asymptomatic infections in *S. dulcamara* (Williamson et al, 2002). In the UK, bacterial population numbers in river water vary depending on the season, with undetectable levels during winter, and higher numbers around summer, when the water temperature is above 15°C (Elphinstone & Matthews-Berry, 2017). In contrast, the pathogen can be detected all year round in the aquatic roots of *S. dulcamara,* in which the bacterium remains viable even during winter, given the plant's ability to restrict bacterial movement inside the xylem and limit bacterial root colonisation (Sebastià et al, 2021). The mechanisms used by *R. solanacearum* phylotype IIB to overwinter in secondary hosts are not fully understood and require further research.

Currently, *R. solanacearum* phylotype IIB and all the RSSC members (with the exception of *R. syzygii subsp. syzygii*) are classified as quarantine pests in many countries in the EU and outbreaks caused by this pathogen imply significant political and economic repercussions, including statutory control measures (Parkinson et al, 2013). For instance, the UK and the Netherlands have been conducting annual surveys over the last decades to monitor the bacterium's presence in waterways, in order to restrict the pathogen's spread and prevent new outbreaks (Elphinstone, 2001; Vogelaar et al, 2023). Over the course of those surveys, some rivers have been demarcated as contaminated in England, and their use for host crop irrigation has been banned (Elphinstone & Matthews-Berry, 2017).

Central and Northern Europe outbreaks and potato infections

Commercial potato cultivars cropped in Northern and Central Europe show low levels of resistance to *R. solanacearum* phylotype IIB, which has resulted in sporadic outbreaks of potato brown rot since the 1970s, including Sweden in 1972, Belgium in 1989 and the Netherlands in 1995 (Williamson et al, 2002), with the majority of positive testing coming from asymptomatic infected tubers. There have been seven confirmed outbreaks in the UK: two in the Thames Valley in 1992 and 1995, one in Northamptonshire in 1999, one in Kent in 2000, one in Nottinghamshire in 2005, one in Somerset and another in Cornwall in 2010 (Elphinstone & Matthews-Berry, 2017). *R. solanacearum* phylotype IIB also caused bacterial wilt in tomato crops in Bedfordshire in 1997 and 1998 (Elphinstone & Matthews-Berry, 2017). All of these outbreaks have been related to contaminated river water used for crop irrigation, except for the 2010 outbreaks that were linked to infected imported seed potatoes. It is possible that agricultural practices such as monoculture, and the centralization and intensification of potato production have facilitated the spread of *R. solanacearum* across many countries in Central and Northern Europe (Parkinson et al, 2013).

Eradicating *R. solanacearum* phylotype IIB from Central and Northern Europe might be difficult, since it would involve the decontamination of rivers and irrigation sources, as well as the removal of environmental reservoirs (Williamson et al, 2002). In the UK, small scale removal of *S. dulcamara* from small water courses was proven to be successful in restricting the spread of the pathogen in river water downstream. However, host weed removals at large scale have proven impractical, given the complexity of vast river networks (Elphinstone & Matthews-Berry, 2017).

Potential range expansion and introduction of *R. pseudosolanacearum* in Central and Northern Europe

Human agricultural activity has played a key role in the dissemination of RSSC species, facilitating the presence of multiple strains in a single region (Huerta et al, 2015). During the last decades, warm-adapted strains that usually inhabit the highland tropics of South America, Africa and Asia have been accidentally introduced via infected plant material into Europe and North America (Remenant et al, 2010; Sebastià et al, 2021). In 2015, the warm-adapted *R. pseudosolanacearum* (phylotype I) was detected in greenhouse rose in the Netherlands (EPPO, 2015; Tjou-Tam-sin et al, 2017), an ornamental plant that prior to this outbreak was not a declared host of RSSC. The infected greenhouse roses presented the classic bacterial wilt symptoms, with necrosis on pruned branches, cholorosis, early drop of leaves, and in a few cases, bacteria oozing from the stems (Tjou-Tam-sin et al, 2017). Water surveys conducted in 2020 and 2021 in two different geographical regions in the Netherlands, also reported the presence of *R. pseudosolanacearum* (phylotype I) in surface water at a minimum temperature of 15°C. Genetic studies on both incursions suggested that the isolated strains were similar to *R. pseudosolanacearum* (phylotype I) isolates found previously in the Netherlands on greenhouse rose with the ability to overwinter in *S. dulcamara (Vogelaar et al, 2023)*. The phylogenetic assignments concluded that the *R. pseudosolanacearum* (phylotype I) isolates from rose clustered with an isolate that originated from eggplant in India (Bergsma-Vlami et al, 2018). In 2021 and 2022, there were additional detections of *R. pseudosolanacearum* in ginger plants in Germany and in watercourses in Hungary respectively (EPPO, 2021; 2022b). Later in March 2023, this bacterium was also detected rhizomes of ginger (*Zingiber officinale*) imported for consumption in Germany (EPPO, 2023). These reports suggest that occurrences of *R. pseudosolanacearum* across Central and Northern Europe are increasing, posing a serious threat to crop production in this region. Climate change could also play a significant role in the distribution of RSSC species, since changes in climatic conditions might promote bacterium survival and help it spread to higher latitudes. Experiments in controlled environment chambers have demonstrated that the severity of wilting in resistant tomato and tobacco lines significantly increases at high temperatures (Bittner et al, 2016; Krausz & Thurston, 1975), which suggests that global warming could make RSSC outbreaks more severe in the future.

Monitoring and diagnostic tools

Diagnostic tools with high efficiency, specificity, and sensitivity are crucial for detecting RSSC in the environment. In the UK, statutory controls mandate the Scottish Agricultural Science Agency and the Department for Environment, Food & Rural Affairs to annually survey rivers, test plant stocks, and provide advice to keep potatoes free of quarantine pathogens (SASA, 2023). Imported material carrying pathogens is prohibited, and potatoes are inspected and tested by Fera Science Ltd in England and similar bodies in Scotland and Northern Ireland. Similarly, local Food and Consumer Product Safety Authorities conduct regular surveys for RSSC across Europe (NVWA, 2023).

Detection methods for RSSC species include selective plating, serological methods, and molecular assays (EPPO, 2022c). Plating methods are based on the use of selective media to isolate bacteria from infected plant material or contaminated soil or water. Triphenyl Tetrazolium Chloride (TTC) and semi-selective SMSA media (Elphinstone et al, 1996) are the gold standard for isolating RSSC species. Bacteria metabolize the TTC in the media producing fluidal-creamy colonies with pink or red centres, depending on whether they are virulent or non-virulent, respectively. This technique is widely used in the UK for sampling waterways, but it can be time-consuming, and detects any viable cells without suppressing the growth of other Gram-negative bacteria from environmental water samples.

Molecular techniques, such as PCR and qPCR, offer higher specificity by targeting conserved genetic regions of the RSSC, like the flagellum subunit FliC protein (Schönfeld et al, 2003) for RSSC identification and quantification. However, these techniques require dedicated equipment and expertise. Loop-mediated isothermal amplification (LAMP) is gaining popularity due to its simplicity, rapid results, and high sensitivity. LAMP can detect the pathogen in various types of samples within an hour and is easier to use than other molecular assays (Notomi et al, 2000). Whole genome sequencing has also become more feasible and is cheaper, allowing analysis of different outbreak strains at the whole genome level (Wang et al, 2024).

Despite advancements in molecular assays, ongoing development is needed for quick detection and differentiation of pathogen species in environmental samples. Once identified, government agencies will report the detection, detailing the pathogen's characteristics, diagnostic tests used, and infection extent. Recent findings of *R. pseudosolanacearum* in the Netherlands and elsewhere in Europe highlight the importance of regular surveys and the use of robust diagnostic tests to prevent outbreaks.

Control methods for RSSC management

RSSC bacteria has proven to be extremely hard to control. Nonetheless, several approaches aimed at managing bacterial wilt have been employed, such as crop rotation, the use of bioorganic fertilisers, and breeding of resistant cultivars (Ahmed et al, 2022). Furthermore, chemical pesticides are frequently used to control pathogens and manage bacterial diseases, but they are often ineffective and their use has serious negative effects on the environment, as well as leading to bacterial resistance and disturbance of the native microbiome (Nicolopoulou-Stamati et al, 2016).

Crop rotation involves growing different types of crops in the same area each year, and this approach is widely used in agriculture to control many diseases caused by soil-borne pathogens. However, the ability of RSSC species to survive for long periods of time in soil (Niu et al, 2017) and to infect a wide range of hosts makes this control method unsuitable. Similarly, the breeding of resistant plant cultivars is a highly desirable control approach that has achieved significant progress over the years to generate resistant varieties of some economically important crops, such as tomatoes and potatoes (Huet, 2014). However, based on genetic analyses conducted on some Solanaceaous plants, resistance to pathogens seems to involve multiple genes, which might make resistance difficult to breed (Salgon et al, 2017). Some promising results have recently been obtained by genetically engineering resistance genes in potato from *Arabidopsis* and wild potato relatives (Boschi et al, 2017).

Biological control

Biological control, or biocontrol, is the use of living organisms to control pests and diseases (Pal & Gardener, 2006). Insects and microorganisms, such as bacteria, fungi and viruses, act as natural enemies of pathogens and reduce their densities, minimizing the harmful effects of pathogens in their hosts and managing the incidence of disease. Some microorganisms, such as bacteria, might produce substances or compounds that inhibit the growth of pathogens and outcompete them for shelter and nutrients (Pohjanen et al, 2014).

The use of different bacteria with potential biocontrol effects against RSSC species is currently being explored. Mohammed et al (2020) isolated and identified seven *Pseudomonas* species from the rhizosphere of tomato plants, and they found that six of them inhibited the growth of *R. solanacearum*, including *P. aeruginosa*, *P. syringae*, and *P. fluorescens*, and also promoted the growth of tomato plants (Mohammed et al, 2020). Soliman (2020), isolated rhizobacterium *Paenibacillus polymyxa* from the soil of a potato field and reported on its efficacy (80%) in suppressing the incidence of potato brown rot by producing antimicrobial compounds that inhibited *R. solanacearum* growth and competitiveness (Soliman, 2020). Wang et al (2019), isolated the rhizospheric bacterial strain *Bacillus cereus* AR156 from forest soil, and they investigated the antagonistic effect of this bacterium against *R. solanacearum*. They showed that *B. cereus* AR156 was able to reduce bacterial wilt disease in tomato and showed an efficacy of 51% relative to the control (Wang et al, 2019a).

Interestingly, some species of bacteria display a mutualistic relationship with plants and provide protection against pathogens (Ahmed et al, 2022). Yang et al (2023a), reported on how plants can modify the microbial community composition via root exudation, as particular root exudate profiles led to the recruitment of microbiota able to suppress *R. solanacearum* (Yang et al, 2023a). Overall, these studies provide evidence of the beneficial effects that some bacterial species may offer as biocontrol agents of phytopathogenic bacteria. Growth inhibition of plant pathogenic bacteria due to antagonistic bacteria outcompeting them, plant growth promotion and suppression of disease incidence are some important benefits of microbial biocontrol agents. The challenge of using bacteria as biocontrol agents is that they might face difficulties in colonising and establishing a population in an already crowded microbiome.

Bacteriophages (phages), viruses that infect and kill bacteria, could provide better success by specifically targeting the pathogen (Harper, 2021). During infection, phages can enter in a lytic cycle, where they adsorb to a receptor on the bacterial cell surface, replicate inside the bacterium, and finally lyse the cell, resulting in the release of new virions and the death of the bacterium (Mirski et al, 2019). Alternatively, phages can enter a lysogenic cycle, in which the bacterial cell is not destroyed and the phage's genome integrates into the bacterial chromosome (Harper, 2021). Lytic phages can be used as biocontrol agents, by reducing bacterial population densities after each infection cycle. Thus far, the efficacy of a wide variety of lytic phages against RSSC bacteria has been described and reported in the literature (Alvarez et al, 2019; Elhalag et al, 2018; Wang et al, 2019b; Yang et al, 2023b). Elhalag et al (2018), isolated a soilborne lytic phage from Egyptian soil to assess the effect on *R. solanacearum* phylotype IIA *in vitro* and under greenhouse conditions. They found that bacteriolytic activity of the phage was detected at different multiplicity of infection (MOI) and the phage was able to reduce and prevent the formation of exopolysaccharides of *R. solanacearum* during the exponential growth phase. Moreover, the phage proved to be effective in suppressing the bacterium growth under greenhouse conditions, as all phage-treated tomato plants showed no wilting symptoms, whereas all untreated plants wilted after 10 days of infection (Elhalag et al, 2018). Likewise, Alvarez et al. (2019) also reported on the use of phages as a biocontrol method against *R. solanacearum* in irrigation water and host plants. Phages proved to be highly specific as to which bacterial species they infect, without damaging non-target bacteria, and were able to reduce bacterial densities when a range of environmental conditions, such as pH, temperature and salinity, were varied (Alvarez et al, 2019).

Phage biocontrol is seldom perfect and one reason behind this could be the rapid evolution of phage resistance, which has been shown to evolve both in the lab and tomato rhizosphere (Wang et al, 2019b). Phage resistance can be highly costly to bacteria, often compromising bacterial fitness and virulence via trade-offs (Burmeister & Turner, 2020). For example, resistance to phages in *R. solanacearum* can result in mutations in the type IV pili or type II secretion systems, which are used as likely receptors by the phage (Wang et al, 2024). However, type IV pili and type II secretion systems are also important virulence factors, and mutations in these genes also result in reduced virulence (da Silva Xavier et al, 2022; Narulita et al, 2016). Spatial heterogeneity of the soil and the presence of competitors might constraint the evolution of phage resistance (Wang et al, 2017), and our understanting of RSSC species phage resistance in natural environments remains limited. Nevertheless, phages remain a promising control strategy against RSSC bacteria but more research is needed, especially at the field scale to assess its efficacy in agricultural systems.

Future research

- Obtaining control strategies that stop *R. solanacearum* diseases in the field is a global priority and future research should focus on finding environmentally-friendly and efficient approaches to combat this devastating pathogen. Combined approaches, like cocktails of inhibitory bacteria and phages, should be prioritized, instead of just single biocontrol agents. These combinations would reduce pathogen levels, boost protective bacteria in the soil, and potentially help to activate plant defences. This should also be linked to basic research to understand the molecular and genetics mechanisms of phage resistance, phage-bacteria interactions and the processes causing competition between bacteria. This research could help discover new biocontrol agents by allowing studies to focus on microorganisms with specific traits that make them effective against *R. solanacearum*, even under stressful environmental conditions caused by climate change.

- Developing resistant plant cultivars is crucial for managing bacterial wilt and potato brown rot. While tolerant cultivars are used in some areas, they do not reduce bacterial levels in the soil. Identifying genes linked to resistance is essential, however no fully resistant cultivars yet exist, due to the complex interaction between plant, bacteria, and environment. Future research should focus on finding new resistance genes, engineering them into commercial cultivars, and exploring wild plant relatives for potential resistance mechanisms.

Discussion Points

- Why the RSSC is one of the most important bacteria plant pathogens in the world?
- How human agricultural activities and climate change can facilitate the host and geographic range expansion of RSSC species?
- Given that it has been difficult to control the spread of RSSC species, which are the best alternatives for disease diagnosis, management and eradication?
- How effective are the biological control methods to eradicate RSSC species? And, what are their limitations?

Conclusions

The spread of warm-adapted *R. pseudosolanacearum* in riverways and wild and crop plants in temperate countries such as the Netherlands, Hungary and Italy (EPPO, 2022a) poses a new threat to food security, highlighting the ability of this pathogen to potentially adapt to new environmental conditions. Climate change could worsen this situation and new outbreaks with different RSSC species can be expected in new areas in the future. Reliable, quick, cheap and easy-to-use diagnostic tests are required to stop the spread of this phytopathogen. Until now, there is no completely efficient method to eradicate this pathogen, due to its massive host range, and its capacity to survive in soil, water, seeds and farming tools. Biocontrol methods have proved environmentally-friendly and efficient. These control methods require further efficacy, and their use combined with resistant plant cultivars, might attain improved disease control.

Further reading

- Updated distribution of *R. solanacearum* in the world: https://gd.eppo.int/taxon/RALSSL/distribution
- Plant Protection Service with the last findings of *R. solanacearum*: https://english.nvwa.nl/topics/pest-reporting/pest-reports
- EPPO Diagnostic tools: https://gd.eppo.int/download/standard/145/pm7-021-03-rev-en.pdf

Figures

Figure 1. Distribution of the *Ralstonia solanacearum* species Complex in Europe (RSSC) (information from EPPO: https://gd.eppo.int/taxon/RALSSO/distribution on 6th December 2023)

Figure 2. Bacterial wilt in tomato caused by *R. solanacearum* strain UW551 a model strain of phylotype II in the tomato cv Moneymaker. A) healthy plant, B) bacterial wilt symptoms at 21 days post-infection.

Figure 3. A schematic figure of RSSC life cycle.

Figure 4. *Solanum dulcamara* plants growing in a glasshouse.

References

Ahmed, W., Yang, J., Tan, Y., Munir, S., Liu, Q., Zhang, J., Ji, G. & Zhao, Z. (2022) *Ralstonia solanacearum*, a deadly pathogen: Revisiting the bacterial wilt biocontrol practices in tobacco and other Solanaceae. *Rhizosphere*, 21, 100479.

Allen, C., Prior, P. & Hayward, A. (2005) *Bacterial wilt disease and the Ralstonia solanacearum species complex*American Phytopathological Society (APS Press).

Alvarez, B., Lopez, M. M. & Biosca, E. G. (2019) Biocontrol of the Major Plant Pathogen *Ralstonia solanacearum* in Irrigation Water and Host Plants by Novel Waterborne Lytic Bacteriophages. *Frontiers in microbiology*, 10, 2813.

Bergsma-Vlami, M., van de Bilt, J. L. J., Tjou-Tam-Sin, N. N. A., Westenberg, M., Meekes, E. T. M., Teunissen, H. A. S. & van Vaerenbergh, J. (2018) Phylogenetic assignment of *Ralstonia pseudosolanacearum* (*Ralstonia solanacearum* Phylotype I) isolated from *Rosa* spp. *Plant disease*, 102(11), 2258-2267.

Bittner, R. J., Arellano, C. & Mila, A. L. (2016) Effect of temperature and resistance of tobacco cultivars to the progression of bacterial wilt, caused by *Ralstonia solanacearum*. *Plant and soil*, 408(1/2), 299-310.

Boschi, F., Schvartzman, C., Murchio, S., Ferreira, V., Siri, M. I., Galván, G. A., Smoker, M., Stransfeld, L., Zipfel, C., Vilaró, F. L. & Dalla-Rizza, M. (2017) Enhanced Bacterial Wilt

Resistance in Potato Through Expression of *Arabidopsis* EFR and Introgression of Quantitative Resistance from *Solanum commersonii*. *Frontiers in Plant Science*, 8.

Burmeister, A. R. & Turner, P. E. (2020) Trading-off and trading-up in the world of bacteria–phage evolution. *Current Biology*, 30(19), R1120-R1124.

Castillo, J. A. & Greenberg, J. T. (2007) Evolutionary Dynamics of *Ralstonia solanacearum*. *Applied and Environmental Microbiology*, 73(4), 1225-1238.

da Silva Xavier, A., de Melo, A. G., Hendrich, C. G., Tremblay, D. M., Rousseau, G. M., Plante, P. L., Forest, K. T., Alfenas-Zerbini, P., Allen, C. & Moineau, S. (2022) In through the Out Door: A Functional Virulence Factor Secretion System Is Necessary for Phage Infection in *Ralstonia solanacearum*. *mBio*, 13(6), e0147522-e0147522.

Denny, T. (2007) Plant pathogenic *Ralstonia* species, *Plant-Associated Bacteria*. Dordrecht: Springer, 573-644.

Elhalag, K., Nasr-Eldin, M., Hussien, A. & Ahmad, A. (2018) Potential use of soilborne lytic Podoviridae phage as a biocontrol agent against Ralstonia solanacearum. *J Basic Microbiol*, 58(8), 658-669.

Elphinstone, J. (2005) Bacterial wilt disease and the *Ralstonia solanacearum* species complex, in Allen, C., Prior, P. & Hayward, A. (eds), *The current bacterial wilt situation: a global overview*. St. Paul: American Phytopathological Society (APS Press), 9-28.

Elphinstone, J. & Matthews-Berry, S. (2017) *Monitoring and control of the potato brown rot bacterium in irrigation water.* Department for Environment Food and Rural Affairs.

Elphinstone, J. G. (2001) Monitoring and control of the potato brown rot bacterium (*Ralstonia solanacearum*) in the UK: a case study, *2nd European Potato Processing Conference.* Lausanne, Switzerland, 14-15 November, 2001.

Elphinstone, J. G., Hennessy, J., Wilson, J. K. & Stead, D. E. (1996) Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. *Bulletin OEPP*, 26(3-4), 663-678.

Elphinstone, J. G., Stanford, H. M. & Stead, D. E. (1998) Detection of *Ralstonia solanacearum* in Potato Tubers, Solanum dulcamara and Associated Irrigation Water, in Prior, P., Allen, C. & Elphinstone, J. (eds), *Bacterial Wilt Disease: Molecular and Ecological Aspects*. Berlin, Heidelberg: Springer Berlin Heidelberg, 133-139.

EPPO (2015) *Ralstonia solanacearum (race 1) detected in Rosa in the Netherlands*. EPPO Global Database: EPPO Global Database.

EPPO (2021) *First report of Ralstonia pseudosolanacearum in Germany*. EPPO Global Database: EPPO Global Database.

EPPO (2022a) *First report of Ralstonia pseudosolanacearum in Italy*. EPPO Global Database: EPPO Global Database. Available online: https://gd.eppo.int/reporting/article-7425.

EPPO (2022b) *Firts report of Ralstonia pseudosolanacearum in Hungary*. EPPO Global Database: EPPO Global Database.

EPPO (2022c) PM 7/21 (3) *Ralstonia solanacearum*, *R. pseudosolanacearum* and *R. syzygii* (*Ralstonia solanacearum* species complex). *Bulletin OEPP*, 52(2), 225-261.

EPPO (2023) *New finding of Ralstonia pseudosolanacearum in Germany*. EPPO Global Database: EPPO Global Database.

Fegan, M. & Prior, P. (2005), in Allen, C., Prior, P. & Hayward, A. (eds), *Bacterial wilt disease and the R. solanacearum Species Complex* St. Paul, MN: APS Press, 449.

Genin, S. & Denny, T. P. (2012) Pathogenomics of the *Ralstonia solanacearum* Species Complex. *Annual Review of Phytopathology*, 50(1), 67-89.

Harper, D. R. (2021) Introduction to Bacteriophages, in Harper, D. R., Abedon, S. T., Burrowes, B. H. & McConville, M. L. (eds), *Bacteriophages*Springer, 3-16.

Huerta, A. I., Milling, A. & Allen, C. (2015) Tropical strains of *Ralstonia solanacearum* outcompete race 3 biovar 2 strains at lowland tropical temperatures. *Applied and Environmental Microbiology*, 81(10), 3542-3551.

Huet, G. (2014) Breeding for resistances to *Ralstonia solanacearum*. *Frontiers in plant science*, 5, 715-715.

Janse, J. (2012) Review on brown rot (*Ralstonia solanacearum* race 3, biovar 2, phylotype IIB) epidemiology and control in the Netherlands since 1995: a success story of integrated pest management. *Journal of Plant Pathology*, 257-272.

Krausz, J. P. & Thurston, H. (1975) Breakdown of resistance to *Pseudomonas solanacearum* in tomato. *Phytopathology*, 65, 1271-1274.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V. & Machado, M. A. (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular plant pathology*, 13(6), 614-629.

Mirski, T., Mizak, L., Nakonieczna, A. & Gryko, R. (2019) Bacteriophages, phage endolysins and antimicrobial peptides - the possibilities for their common use to combat infections and in the design of new drugs. *Annals of Agricultural and Environmental Medicine*, 26(2), 203-209.

Mohammed, A. F., Oloyede, A. R. & Odeseye, A. O. (2020) Biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum* using Pseudomonas species isolated from the rhizosphere of tomato plants. *Archiv für Phytopathologie und Pflanzenschut*, 53(1-2), 1-16.

Narulita, E., Addy, H. S., Kawasaki, T., Fujie, M. & Yamada, T. (2016) The involvement of the PilQ secretin of type IV pili in phage infection in *Ralstonia solanacearum* Bacteriophages. *Biochemical and biophysical research communications*, 469(4), 868-872.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P. & Hens, L. (2016) Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Frontiers in public health*, 4, 148.

Niu, J., Chao, J., Xiao, Y., Chen, W., Zhang, C., Liu, X., Rang, Z., Yin, H. & Dai, L. (2017) Insight into the effects of different cropping systems on soil bacterial community and tobacco bacterial wilt rate. *Journal of basic microbiology*, 57(1), 3-11.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. & Hase, T. (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, 28(12), e63-e63.

NVWA (2023) *Pest reports*, 2023. Available online: https://english.nvwa.nl/topics/pest-reporting/pest-reports [Accessed 24.08.2024].

Osdaghi, E. (2020) *Ralstonia solanacearum (bacterial wilt of potato)*CABI Digital Library.

Pal, K. K. & Gardener, B. M. (2006) Biological Control of Plant Pathogens. *The Plant Health Instructor*, 2, 1117-1142.

Parkinson, N., Bryant, R., Bew, J., Conyers, C., Stones, R., Alcock, M. & Elphinstone, J. (2013) Application of Variable-Number Tandem-Repeat Typing To Discriminate *Ralstonia solanacearum* Strains Associated with English Watercourses and Disease Outbreaks. *Applied and Environmental Microbiology*, 79(19), 6016-6022.

Persley, G. J. (1985) Ecology of *Pseufomonas solanacearum*, the causal agent of bacterial wilt, *Proceedings of an international workshop held at PCARRD, Los Banos, Philippines, 8-10 October 1985*. Los Banos, Philippines: ACIAR Proceedings.

Pohjanen, J., Koskimäki, J. J., Sutela, S., Ardanov, P., Suorsa, M., Niemi, K., Sarjala, T., Häggman, H. & Pirttilä, A. M. (2014) Interaction with ectomycorrhizal fungi and endophytic methylobacterium affects nutrient uptake and growth of pine seedlings in vitro. *Tree physiology*, 34(9), 993-1005.

Prior, P., Ailloud, F., Dalsing, B. L., Remenant, B., Sanchez, B. & Allen, C. (2016) Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. *BMC genomics*, 17(1), 90-90.

Remenant, B., Coupat-Goutaland, B., Guidot, A., Cellier, G., Wicker, E., Allen, C., Fegan, M., Pruvost, O., Elbaz, M., Calteau, A., Salvignol, G., Mornico, D., Mangenot, S., Barbe, V., Médigue, C. & Prior, P. (2010) Genomes of three tomato pathogens within the *Ralstonia solanacearum* species complex reveal significant evolutionary divergence. *BMC genomics*, 11(1), 379-379.

Safni, I., Cleenwerck, I., De Vos, P., Fegan, M., Sly, L. & Kappler, U. (2014) Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *syzygii* subsp. nov., *R. solanacearum* phylotype

IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotype I and III strains as *Ralstonia pseudosolanacearum* sp. nov. *International journal of systematic and evolutionary microbiology*, 64(Pt 9), 3087-3103.

Salgon, S., Jourda, C., Sauvage, C., Daunay, M. C., Reynaud, B., Wicker, E. & Dintinger, J. (2017) Eggplant resistance to the *Ralstonia solanacearum* species Complex involves both broad-spectrum and strain-specific quantitative trait loci. *Frontiers in plant science*, 8, 828-828.

SASA (2023) *Bacteriology, Brown Rot*, 2023. Available online: https://www.sasa.gov.uk/diagnostics/bacteriology [Accessed 27.08.2024].

Schönfeld, J., Heuer, H., Van Elsas, J. D. & Smalla, K. (2003) Specific and sensitive detection of *Ralstonia solanacearum* in soil on the basis of PCR amplification of fliC fragments. *Appl Environ Microbiol*, 69(12), 7248-56.

Sebastià, P., de Pedro-Jové, R., Daubech, B., Kashyap, A., Coll, N. S. & Valls, M. (2021) The bacterial wilt reservoir host *Solanum dulcamara* shows resistance to *Ralstonia solanacearum* infection. *Frontiers in plant science*, 12, 755708.

Soliman, M. (2020) *Paenibacillus polymyxa* and *Bacillus aryabhattai* as Biocontrol Agents against *Ralstonia solanacearum* In Vitro and In Planta. *Journal of Plant Protection and Pathology*, 11(3), 197-203.

Tjou-Tam-sin, N. N. A., van de Bilt, J. L. J., Westenberg, M., Bergsma-Vlami, M., Korpershoek, H. J., Vermunt, A. M. W., Meekes, E. T. M., Teunissen, H. A. S. & Van Vaerenbergh, J. (2017) First report of bacterial wilt caused by *Ralstonia solanacearum* in ornamental *Rosa* sp. *Plant disease*, 101(2), 378-378.

van der Gaag, D. J., Camilleri, M., Diakaki, M., Schenk, M. & Vos, S. (2019) Pest survey card on potato brown rot, *Ralstonia solanacearum*. *EFSA supporting publications*, 16(2), n/a.

Vogelaar, M., van de Bilt, J., Blom, N., Pel, C., van Doorn, B., Landman, M., Gorkink, P., Raaymakers, T., Vreeburg, R. & Bergsma-Vlami, M. (2023) Presence of *Ralstonia pseudosolanacearum* (phylotype I) in aquatic environments in the Netherlands. *Plant Disease*(ja).

Wang, J., Wang, X., Yang, K., Lu, C., Fields, B., Xu, Y., Shen, Q., Wei, Z. & Friman, V. P. (2024) Phage selection drives resistance-virulence trade-offs in *Ralstonia solanacearum* plant-pathogenic bacterium irrespective of the growth temperature. *Evolution letters*, 8(2), 253-266.

Wang, N., Wang, L., Zhu, K., Hou, S., Chen, L., Mi, D., Gui, Y., Qi, Y., Jiang, C. & Guo, J.-H. (2019a) Plant Root Exudates Are Involved in *Bacillus cereus* AR156 Mediated Biocontrol Against *Ralstonia solanacearum*. *Frontiers in microbiology*, 10, 98-98.

Wang, X., Wei, Z., Li, M., Wang, X., Shan, A., Mei, X., Jousset, A., Shen, Q., Xu, Y. & Friman, V.-P. (2017) Parasites and competitors suppress bacterial pathogen synergistically due to evolutionary trade-offs. *Evolution*, 71(3), 733-746.

Wang, X., Wei, Z., Yang, K., Wang, J., Jousset, A., Xu, Y., Shen, Q. & Friman, V. P. (2019b) Phage combination therapies for bacterial wilt disease in tomato. *Nature biotechnology*, 37(12), 1513-1520.

Wang, Z., Luo, W., Cheng, S., Zhang, H., Zong, J. & Zhang, Z. (2023) *Ralstonia solanacearum*- A soil borne hidden enemy of plants: Research development in management strategies, their action mechanism and challenges. *Frontiers in plant science*, 14, 1141902-1141902.

Wenneker, M., Verdel, M. S. W., Groeneveld, R. M. W., Kempenaar, C., van Beuningen, A. R. & Janse, J. D. (1999) *Ralstonia* (*Pseudomonas*) *solanacearum* Race 3 (Biovar 2) in Surface Water and Natural Weed Hosts: First Report on Stinging Nettle (Urtica dioica). *European Journal of Plant Pathology*, 105(3), 307-315.

Williamson, L., Nakaho, K., Hudelson, B. & Allen, C. (2002) *Ralstonia solanacearum* race 3, biovar 2 strains isolated from geranium are pathogenic on potato. *Plant disease*, 86(9), 987-991.

Yang, K., Fu, R., Feng, H., Jiang, G., Finkel, O., Sun, T., Liu, M., Huang, B., Li, S., Wang, X., Yang, T., Wang, Y., Wang, S., Xu, Y., Shen, Q., Friman, V. P., Jousset, A. & Wei, Z. (2023a)

RIN enhances plant disease resistance via root exudate-mediated assembly of disease-suppressive rhizosphere microbiota. *Molecular plant*, 16(9), 1379-1395.

Yang, K., Wang, X., Hou, R., Lu, C., Fan, Z., Li, J., Wang, S., Xu, Y., Shen, Q., Friman, V.-P. & Wei, Z. (2023b) Rhizosphere phage communities drive soil suppressiveness to bacterial wilt disease.