8th International Bacterial Wilt Symposium

Book of Abstracts

22-26 March 2026

Wageningen, the Netherlands

https://event.wur.nl/ibws2026

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Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Fisheries, Food Security and Nature

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Assigned poster numbers **61**

Poster abstracts **62**

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On behalf of all members of the Organizing Committee, I would like to cordially welcome all participants of the 8th International Bacterial Wilt Symposium (8th IBWS2026) to Wageningen, the Netherlands!

During the last few decades, the International Bacterial Wilt Symposium (IBWS) has become a reference meeting for the scientific *Ralstonia* community worldwide. After the "Conference and Workshop on the Ecology and Control of Bacterial Wilt caused by *Pseudomonas solanacearum*" organized by A. Kelman in North Carolina, U.S.A. in 1976, several editions of the International Bacterial Wilt Symposium have been organized. The first edition was held in Taiwan in 1992, after which the IBWS was hosted in different locations around the world, including Guadeloupe (1997), White River (2002), York (2006), Wuhan (2011), Toulouse (2016) and Montevideo (2023).

We are honored to host the 8th IBWS2026 edition in Wageningen! I am convinced that the 8th IBWS2026, similarly to previous editions of the IBWS, will play an important role in promoting collaborative research within the *Ralstonia* scientific community, particularly by engaging many young researchers, and by encouraging innovative research ideas that may shape bacterial wilt research in the future. Both fundamental and applied research topics on Ralstonia have been included in the 8th IBWS2026 program, covering the majority of topics in the current research field. Next to the *Ralstonia* topics, a special session on plant microbiome research in the Netherlands will provide insights into how plant health can be improved by making use of the functional diversity of the plant microbiome and of chemical signaling in the rhizosphere. This can lead to new insights regarding *Ralstonia* prevention and control.

The organization of the 8th IBWS2026 has been made possible through close collaboration between Wageningen University & Research (WUR), the Dutch General Inspection Service for Agricultural Seeds and Seed Potatoes (NAK), the Netherlands Inspection Service for Horticulture (Naktuinbouw) and the Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP-NVWA). Regarding plant pathogenic *Ralstonia* species, these four organizations are collaborating closely on inspections, diagnostics, certification and research activities, thus jointly ensuring effective *Ralstonia* control in the Netherlands. I am deeply indebted to these four organizations for their enthusiastic support and commitment.

My appreciation also extends to all invited speakers and the members of the scientific committee, for their participation and contributions to the scientific program. I would also like to acknowledge all sponsoring organizations for their generous financial support, making this conference possible and supporting a number of travel awards for students and young researchers from around the world. Lastly, I would like to thank all conference participants for their contributions.

Welcome to Wageningen and enjoy the symposium!

Maria Bergsma-Vlami

8th International Bacterial Wilt Symposium

22-26 March 2026, Wageningen, the Netherlands

Program

	Sun 22	Mon 23	Tue 24	Wed 25	Thu 26
8:00		8:00 Registration and coffee		8:15 Excursion	
8:45		8:45 Welcome	8:45 Theme 2		8:45 Theme 3
9:00		9:15 Special session			
10:00		10:15 Coffee break	10:15 Coffee break		10:15 Coffee break
10:45		10:45 Special session (continued)	10:45 Theme 2 (continued)		10:45 Poster session
11:00		11:15 Opening lecture	11:25 Theme 5		11:35 Theme 3 (continued)
11:45		11:45 Theme 1			
12:00		12:35 Lunch break	12:35 Lunch break		12:35 Lunch break
13:00		13:35 Theme 1 (continued)	13:35 Theme 5 (continued)		13:35 Theme 2 (continued)
14:00			14:55 Theme 4		
15:00		15:15 Coffee break	15:25 Coffee break		15:15 Coffee break
15:45		15:45 Poster session	15:55 Theme 4 (continued)		15:45 Closing lecture, Awards and closing
16:00	16:00 Drinks and registration				
17:00					
18:00					
19:00					19:00 Conference dinner
20:00					
21:00					

Themes and special session

The scientific programme will consist of five diverse bacterial wilt related themes as shown in the list below. In addition, a broader special session on the plant microbiome will be organized.

Theme 1
Diversity, genomics, and evolution of the *Ralstonia solanacearum* species complex (e.g. phylogeny, pangenomics, population genetics, evolution)

Theme 2
Molecular plant-microbe interactions (e.g. effectors, quorum sensing, plant responses/gene expression, genetics and metabolomics)

Theme 3
Ecology and epidemiology (e.g. pathogen survival, range expansion, plant host range, microbiome interactions)

Theme 4
Host resistance and crop improvement (e.g. resistance genes, resistance markers, breeding, genetic engineering)

Theme 5
Disease control, prevention, and diagnostics (e.g. detection, identification, biological control, containment, ICM)

Sunday March 22

Hotel Wander - Restaurant

16:00 - 18:00 Welcome drinks and registration
For those of you who are already in Wageningen. An opportunity to get registered and talk to your colleagues while enjoying a drink and some small snacks.

Monday March 23
Hotel Wander - HUGO TECH

8:00 - 8:45 Registration (lobby of the hotel)
If you could not register on Sunday, you can register now.

8:45 Opening of the IBWS2026 Maria Bergsma-Vlami

Special session on plant microbiome research in the Netherlands
Chair: Florien Gorter
9:15 Keynote: Jos Raaymakers | NIOO + Leiden University | The Netherlands
A walk on the wild side
9:45 Keynote: Roeland Berendsen | Utrecht University | The Netherlands
From soil-borne legacies to predictive microbiomes: phenotyping of plant-microbe interactions

10:15 - 10:45 Coffee break (restaurant)

10:45 Keynote: Lemeng Dong | University of Amsterdam | The Netherlands
Chemical signalling in plant-microbe-nematode interactions

Opening lecture
11:15 Keynote: Stephane Genin | INRAE | France
The R. solanacearum expanded family: expanding geographical areas, expanding host range, and expanding knowledge

Theme 1: Diversity, genomics, and evolution of the *R. solanacearum* species complex
Chair: Tiffany Lowe-Power
11:45 Keynote: Honour McCann | Max Planck Institute | Germany
Antagonism and emergence: genomics of historic and contemporary Ralstonia spp. outbreaks
12:15 Poliane Alfenas Zerbini | Universidade Federal de Viçosa | Brazil
Emergence of Ralstonia syzygii subsp. syzygii (Phylotype IV) in Brazil: evidence for a Myrtaceae-associated lineage expansion

12:35 - 13:35 Lunch break (restaurant)

13:35 Karla Cardenas Gomez | University of York | United Kingdom
Can temperature adaptation drive Ralstonia solanacearum strain K60 range expansion and virulence?
13:55 Emma Sheriff | University of Helsinki | Finland
Understanding phage defence system synergy in the plant pathogen Ralstonia
14:15 Shuo Wang | University of Helsinki; Nanjing Agricultural University | Finland
Prophages drive the recombination of anti-phage defence systems within and between Ralstonia species
14:35 Fe Dela Cueva | University of the Philippines Los Baños | Philippines
Genetic diversity and population structure of Ralstonia solanacearum phylotype II in Philippine bananas
14:55 Alice Guidot | INRAE | France
Changes in DNA methylation, a form of epigenetic modification, contribute to rapid adaptation of Ralstonia pseudosolanacearum to the host plant

15:15 - 15:45 Coffee break (HUGO TECH)

15:45 - 17:30 Poster session 1

Tuesday March 24
Hotel Wander - HUGO TECH

Theme 2: Molecular plant-microbe interactions
Chair: Alice Guidot
8:45 Keynote: Zhong Wei | Nanjing Agricultural University | China
Harnessing the rhizobiont to control Ralstonia solanacearum invasion
9:15 Mercedes Rocafort Ferrer | CRAG; Universitat de Barcelona | Spain
The phenylacetic acid pathway is required for R. solanacearum rhizosphere establishment
9:35 Rebecca Schomer | University of Arizona | USA
Dissecting the bacterial-plant chemical conversations that drive Ralstonia phytopathogens towards host roots
9:55 Caroline Baroukh | INRAE | France
What mathematical modelling has revealed about bacterial wilt disease

10:15 - 10:45 Coffee break (restaurant)

10:45 Yasufumi Hikichi | Kochi University | Japan
A novel transcriptional regulator RalT contributes to the fine-tuning of the quorum sensing-dependent production of major exopolysaccharide EPS I in Ralstonia pseudosolanacearum strain OE1-1
11:05 Tiffany Lowe-Power | University of California Davis | USA
The EPS-I exopolysaccharide transforms Ralstonia wilt pathogen biofilms into mobile viscoelastic fluids for rapid dissemination in planta

Theme 5: Disease control, prevention, and diagnostics
Chair: David Norman
11:25 Keynote: Ville Friman | University of Helsinki | Finland
Using phages to biocontrol Ralstonia solanacearum
11:55 Boshou Liao | OCRI-CAAS | China
Development of high-throughput resistance screening approaches and utilization of diverse resistant germplasm for effective control of bacterial wilt in peanut
12:15 Martijn Vogelaar | Wageningen University and Research | The Netherlands
Development of a new TaqMan PCR for simultaneous detection and distinction of R. pseudosolanacearum and R. solanacearum in surface water

12:35 - 13:35 Lunch break (restaurant)

13:35 Viola Kurm | Wageningen University and Research | The Netherlands
From Pangenomics to Diagnostics: Resolving Diversity in Ralstonia syzygii
13:55 Daniel Narino-Rojas | University of York | United Kingdom
Genome-wide identification of Ralstonia solanacearum genes conferring resistance to diverse bacteriophages using RB-TnSeq
14:15 Manigundan Kaari | Dong-A University | South Korea
Actinobacterial synthetic community alters the rhizosphere microbiota and enhance the tomato health
14:35 Can-Hua Lu | Yunnan Academy of Tobacco Agricultural Sciences | China
Six-year field evaluation of cropping systems for management of bacterial wilt and root knot nematode of tobacco

Theme 4: Host resistance and crop improvement
Chair: Boshou Liao
14:55 Keynote: Marc Valls | Universitat de Barcelona | Spain
Characterisation and engineering of the mechanisms conferring vascular resistance to bacterial wilt in tomato

15:25 - 15:55 Coffee break (restaurant)

Program for Tuesday continues on next page

Tuesday March 24 (continued)
Hotel Wander - HUGO TECH

15:55 Nathalie Aoun | University of California Davis | USA
Natural variation in a NLR pair confers thermostable resistance to a devastating bacterial pathogen
16:15 Bo Li | Huazhong Agricultural University | China
The mechanism of receptor-mediated immunity to bacterial wilt in tomato
16:35 Huaiyong Luo | OCRI-CAAS | China
Identification of key loci associated with resistance to bacterial wilt in peanut through whole-genome resequencing
16:55 Paola Gaiero | Universidad de la República | Uruguay
Genetic architecture of bacterial wilt resistance in potato wild relatives from Uruguay and new source for introgression
17:15 Mary Mwangi | International Potato Center | Peru
Development of bacterial wilt resistant potato using Efr, Jim2 and Roq1 genes

Wednesday March 25
Excursion

8:15 Gather in front of Hotel Wander
8:45 Departure of bus

Visit to NPEC and UNLOCK
9:15 Tour NPEC or UNLOCK (Wageningen University and Research Campus)
Guided tour through the Netherlands Plant Eco-phenotyping Centre (NPEC) facilities or the UNLOCK microbial research facilities at the Wageningen University and Research campus

~10:30 - 11:00 Coffee break (Unifarm)

11:00 Tour NPEC or UNLOCK (Wageningen University and Research Campus)
Guided tour through the Netherlands Plant Eco-phenotyping Centre (NPEC) facilities or the UNLOCK microbial research facilities at the Wageningen University and Research campus

12:30 - 13:30 Lunch break (Omnia)

13:30 Group picture (outside Omnia)

13:45 Departure of bus (outside Omnia)

Visit to the city center of Wageningen
14:15 Tour Wageningen historical center
Guided tour through the historical part of the center of Wageningen
15:45 Drinks at Visum Mundi
Catch up with colleagues while enjoying some drinks and small snacks

Thursday March 26
Hotel Wander - HUGO TECH

Theme 3: Ecology and epidemiology
Chair: Yasufumi Hikichi
8:45 Keynote: Gilles Cellier | ANSES | France (Réunion)
How population genetic signatures revealed contrasted outbreak histories of bacterial diseases?
9:15 Amandine Cunty | ANSES | France
Tracing the origin of Ralstonia solanacearum in mainland France
9:35 Maria Bergsma-Vlami | NIVIP | The Netherlands
Exploring the genetic diversity of recent Ralstonia pseudosolanacearum phylotype I findings in Europe
9:55 Robert Vreeburg | NIVIP | The Netherlands
Ralstonia in Dutch surface water, looking back and looking forward

10:15 - 10:45 Coffee break (HUGO TECH)

10:45 - 11:35 Poster session 2

Theme 3: Ecology and epidemiology (continued)
11:35 Florien Gorter | Wageningen University and Research | The Netherlands
Quantifying the dissemination of Ralstonia pseudosolanaceraum via free water in soil using a custom laboratory set-up
11:55 Kenji Kai | Osaka Metropolitan University | Japan
Ralstonia parasitizes not only plants but also some fungi
12:15 Virginia Ferreira | Universidad de la República | Uruguay
Bacterial wilt resistance is correlated with rhizosphere bacterial communities in potato genotypes

12:35 - 13:35 Lunch break (restaurant)

Theme 2: Molecular plant-microbe interactions (continued)
Chair: Marc Valls
13:35 Anjali Iyer-Pascuzzi | Purdue University | USA
A type III effector from Ralstonia solanacearum interacts with the plant cytoskeleton and is required for virulence
13:55 Corri Hamilton | University of Missouri | USA
Ralstonia solanacearum R3B2 strain UW551 overcomes inhibitory xylem chemistry to break tomato bacterial wilt resistance
14:15 Qinghong Li | Zhejiang University | China
Related type 2C protein phosphatases LOPP and CIPP1 negatively regulate immunity to Ralstonia solanacearum in tomato
14:35 Kouhei Ohnishi | Kochi University | Japan
Sucrose and malic acid in the tobacco plant induce hrp regulon in a phytopathogen Ralstonia pseudosolanacearum
14:55 Milka Kezimana-Oculi | Université de Toulouse | France
Origin and role of putrescine in bacterial wilt disease

15:15 - 15:45 Coffee break (restaurant)

Closing session
15:45 Award ceremony
Hayward-Prior travel grant winners and Best student contribution awards
16:05 Keynote: Caitilyn Allen | University of Wisconsin | USA
How plant pathogenic Ralstonia respond to a host – the story of RprR
16:50 Official closing of the IBWS2026 - Maria Bergsma-Vlami

19:00 - 22:00 Conference dinner (restaurant)

A walk on the wild side

Jos M. Raaijmakers
Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

Microorganisms living on and inside eukaryotes – the microbiota - are key drivers of host health. For plants, microbiota can greatly expand their genomic capabilities by enhancing immunity against bacterial and fungal pathogens, nutrient acquisition, and tolerance to environmental stresses. More than ever, plant microbiota are being considered as a lever to increase the sustainability of food production under climate change conditions. Emerging from this global interest to harness the largely unexplored functional potential of microbiota, the microbiome rewilding hypothesis posits that plant growth and health can be improved by reinstating key members of the diverse (ancestral) microbiota that were depleted or even lost through domestication and industrialization, including changes in fertilization, breeding and the (over)use of antibiotics and pesticides. In my talk, I will go '*Back to the Roots*' of wild relatives of crop plants grown in their center of origin and describe how domestication affected the taxonomic and functional diversity of the microbiome and the reciprocal effects on stress resilience of modern crop cultivars. This fundamental knowledge provides a scientific basis for the design of practical applications: *M*-breeding programs can integrate genetic plant traits associated with beneficial microbial recruitment, while microbial inoculants or synthetic communities bring back lost functionalities.

From soil-borne legacies to predictive microbiomes: phenotyping of plant–microbe interactions

Roeland L. Berendsen
Plant–Microbe Interactions, Institute of Environmental Biology, Department of Biology, Science4Life, Utrecht University, Utrecht, the Netherlands.
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Microbiomes are integral to plant health and resilience, yet their potential for sustainable agriculture is only beginning to be realized. Our work shows that plants under pathogen attack actively recruit protective microbes, establishing a soil-borne legacy that enhances the resistance of subsequent plant generations. In parallel, we demonstrated that the seed tuber microbiome predicts potato vigor, providing a novel quality parameter for crop production. These studies highlight the dual role of the plant microbiome as both a dynamic defense partner and a predictive marker of performance. To move from correlation to mechanism, we use high-resolution phenotyping at the Netherlands Plant Eco-phenotyping Centre (NPEC). These platforms enable controlled, time-resolved analyses of plant performance in relation to microbiome composition, allowing us to uncover how plants orchestrate microbial recruitment under stress. This integrative approach opens new avenues to understand and harness plant–microbe interactions for resilient agroecosystems.

Chemical signalling in plant-microbe-nematode interactions

Davar Abedini[1], Kangning Wang[1], Atharv Ambekar[1], Alessandra Guerrieri[1], Fred White[1,2], Yuting Yang[1], Mario Schilder[1], Lieke E. Vlaar[1], André Machado Bertran[3], Aska Goverse[3], Harro Bouwmeester[1], Lemeng Dong[1]
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[2]Biosystems Data Analysis Group, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands
[3]Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Wageningen, The Netherlands

Plants actively shape their rhizosphere through the release of diverse chemical signals, with root exudates accounting for up to 20–40% of photosynthetically fixed carbon entering the soil. These exudates play a central role in structuring microbial communities, regulating nutrient cycling, and mediating interactions with both beneficial and harmful organisms. A striking example of rhizosphere signalling is the production of eclepins, highly potent hatching factors that trigger cyst nematode egg hatching at extremely low concentrations.
Here, we focus on solanoeclepin A (SolA), a key hatching factor for potato cyst nematodes, and address the long-standing question of why plants would release such signals to their parasites. We show that SolA is produced through a coordinated plant-microbe process under non-sterile conditions and is tightly regulated by nitrogen availability. Using transcriptomics, metabolomics, and functional genetics, we identify plant cytochrome P450 enzymes as candidate genes involved in SolA biosynthesis. Integration of multi-omics datasets further reveals a strong positive association between SolA levels and the abundance of plant growth-promoting bacteria that enhance plant performance under nitrogen-limited conditions. Interestingly, by exploring natural variation, we uncovered potato cultivars and wild relatives that either produce novel eclepins or no detectable eclepins, providing a unique opportunity to investigate the biological relevance and diversification of eclepin signalling in plant–microbe-nematode interactions.
Together, our findings support a model in which SolA represents part of a hybrid chemical language co-created by plants and microbes, inadvertently exploited by cyst nematodes but evolutionarily shaped to recruit beneficial microbes and mitigate nutrient stress. This work opens new avenues for sustainable cyst nematode control and improved nutrient-use efficiency in agriculture.

The *R. solanacearum* expanded family: expanding geographical areas, expanding host range, and expanding knowledge

Stephane Genin
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Ralstonia solanacearum is present all around the globe, essentially in the intertropical zone, and it tends to spread beyond in temperate regions, probably under the combined effects of intensified international trade, global warming and its ability to adapt to many local environments. What we refer to here as *Ralstonia solanacearum* encompasses a vast set of strains, whose diversity has been progressively defined by approaches of molecular and then genomic taxonomy. The characterization of this diversity (geographic, phenotypic, genetic, pathogenic) has coincided in recent years to a taxonomic reclassification with a division of the *Ralstonia solanacearum* species complex into three distinct species. Nevertheless, the term species complex remains relevant if we consider that this group of strains, besides all being plant pathogens, possess major common traits, such as the tropism to reach and multiply in the xylem vessels and, for a large majority, their ability to reside in the soil and carry out root infection.
In my presentation, I will discuss some of the challenges this pathogen poses for understanding the mechanisms underlying its pathogenicity, as well as the implications for managing the disease. I will also illustrate how new conceptual and methodological approaches have renewed our knowledge of bacterial wilt and its causal agent.

Antagonism and emergence: genomics of historic and contemporary *Ralstonia* spp. outbreaks

Honour McCann
Max Planck for Biology, Tuebingen, Germany.

Recurring outbreaks of *Ralstonia* spp. pose a threat to crop cultivation at regional and global scales. I will present recent work from two collaborations describing the origins and genomic mechanisms linked with disease emergence in a historic outbreak in Martinique and a contemporary outbreak in Indonesia. Despite being caused by different species of *Ralstonia* and affecting different hosts, we observe shared features that provide insight into the antagonistic interactions shaping pathogen evolution.

Harnessing the rhizobiont to control *Ralstonia solanacearum invasion*

Zhong Wei
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A common preventive approachagainst *Ralstonia solanacearum* invasion is the inoculationof probiotics intothe rhizosphere. However, such inoculations often fact practicalchallenges, including poor colonization, weak persistence, and unstable disease suppression. To address these challenges, we introduce the concept of the rhizosphere holobiont—an integrated ecological system that establishesbeneficial interactions between plants and probiotics. Within this framework, plants secrete specific prebiotics that promote probiotic colonizationand activity, forming synbiotics. In turn,these probiotics enhance plants resistance by suppressing pathogen invasion. Here, we explored therepresentative synbiotics cases driven by plant-derived vitaminsand hormones.Specifically, we found that root-exuded riboflavin (vitamin B_2) recruits beneficial*Streptomyces* sp. in the rhizosphere, effectively reducing tomato bacterial wilt. Furthermore, a strigolactone-deficient tomato mutant activated the ABA–flavonoid regulatory axis, which synergistically enhances*Pseudomonas*-mediated niche competition and antibiotic biosynthesis, thereby suppressing*R. solanacearum* invasion.Together, these findings reveal that plant-derived signaling molecules and nutrients act as natural prebiotics, linking beneficial microbes to form syntioticsfor disease suppresion. Harnessing rhizobionts offers a promising strategy for thetargeted and sustainable control of soil-borne pathogens.

Using phages to biocontrol *Ralstonia solanacearum*

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Bacterial viruses - phages – are ubiquitous and shape the structure of natural bacterial communities. Phages can also be used to biocontrol plant pathogenic bacteria to improve agricultural production and cut crop losses to plant diseases. In this talk, I will review our recent findings on using phages to suppress the notorious *Ralstonia solanacearum* plant pathogenic bacterium. I will first present how *R. solanacearum*-specific phages can be used to efficiently precision engineer rhizosphere microbiomes without harming beneficial bacteria. I will also discuss the potential problem of phage resistance evolution, underlying genetic mechanisms, and how phage selection can be used as an evolutionary tool to steer pathogen virulence through costly life-history trade-offs. Finally, I will discuss how rhizosphere phages that do not target the pathogen can select for disease suppressive bacterial species and activate the disease suppressiveness of resident microbiota, resulting in improved disease control.

This research was financially supported by BBSRC, NERC, Research Council of Finland and Novo Nordisk Foundation

Characterisation and engineering of the mechanisms conferring vascular resistance to bacterial wilt in tomato

A. Jimenez-Jimenez[1], L. Quinteiro[1], L. Gruetz[1], L. Hurtado[1,2], N.S. Coll[1*] and M. Valls[1,2*]
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Vascular pathogens cause devastating diseases in a wide range of economically important crops, posing a serious threat to global agriculture. Some plant varieties have evolved strong defense responses that result in resistance to these pathogens. Using the tomato–Ralstonia solanacearum pathosystem as a model, we have shown that resistant cultivars have the capacity to block bacterial spread within the xylem and to the neighbouring tissues. We show that structural constraints are key for resistance to bacterial wilt both in root and shoot tissues. We have identified ligno-suberin coatings and feruloyl-tyramine as the key components of these vascular barriers induced upon infection with R. solanacearum. In agreement with these findings, we demostrate that both genetic and pharmacological manipulation of this metabolic pathway significantly increases resistance to infection. Our findings offer a promising avenue for engineering broad-spectrum resistance to vascular pathogens through precise modification of plant cell wall metabolism.

How population genetic signatures revealed contrasted outbreak histories of bacterial diseases?

G. Cellier
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The management of agricultural epidemics relies heavily on identifying whether a pathogen is a recently introduced exotic threat or a long-standing endemic population undergoing a resurgence. While traditional field observations can track disease progression, they often fail to capture the underlying evolutionary mechanisms that drive bacterial emergence in crops.
In the case of complex plant pathogens, such as the *Ralstonia solanacearum* species complex (RSSC), *'Candidatus Liberibacter spp.'* (associated with Huanglongbing), and *Xanthomonas citri* pv. *citri*, several studies over the last decade have achieved molecular characterization of outbreak strains and shed light on their epidemiological features. Effective disease management is often hindered by the difficulty of determining whether an outbreak stems from a localized resurgence of endemic strains or the cryptic introduction of exotic, highly aggressive lineages through contaminated plant materials.
Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) can hence be performed to decode the microevolutionary history of bacterial populations, by targeting hypervariable tandem repeats (VNTR). Due to high mutation rates, MLVA achieves higher discriminatory power than traditional MLST, allowing for the differentiation of nearly clonal strains and uncovering evolutionary events often invisible to broader genomic methods. Nevertheless, genomic approaches, based on a different marker type and underlying evolutionary mechanisms, remain the most – and the sole - frequently used pipelines for molecular epidemiology and evolutionary research.
This discussion explores the selection of proper genetic markers and analytical algorithms through examples of epidemiological results produced within the framework of three pathosystems. We will also highlight proper sampling and methodological approaches, alongside the adoption of complementary multiscale analyses - from gene to genome - to comprehend epidemiological complexity as a whole.

How Plant Pathogenic *Ralstonia* Respond to a Host – the Story of RprR

C. Allen[1], B. S. O'Banion[1], M. D. Carter[2], C. D. Hamilton[3], T. M. Tran[4], and J. A. Sanchez Gallego[1]
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Plant pathogenic *Ralstonia* behave very differently when they grow in culture than when they live in association with a host plant. How do *Ralstonia*integrate signals to transition from soil to their pathogenic niche in host xylem tissue? Mutating a putative sensing/signaling protein gene had little transcriptomic effect on *R. pseudosolanacearum* GMI1000 in culture. However, when the mutant grew in tomato over 180 genes were differentially expressed relative to wild type. The gene was named *rprR* for *Ralstonia*plant-responsive regulator. *In planta*, the Δ*rprR* mutant dysregulated genes for diverse traits including stress response, degradation of phenolic compounds, motility, attachment, and production of extracellular polysaccharide (EPS), a key virulence factor. Quantifying EPS by ELISA found increased levels in stems of plants infected with Δ*rprR* as compared to wild-type GMI1000. Functional assays showed Δ*rprR* is defective in root attachment, stem colonization, and bacterial wilt virulence, especially on wilt-resistant Hawaii7996 tomato. In rich medium, Δ*rprR* formed biofilm normally, but the mutant formed less biofilm in tomato stem homogenate and in tomato xylem sap under flow. This phenotype correlates with the mutant's altered expression of EPS biosynthetic genes and aberrant extracellular matrix. When grown in tomato stem homogenate, Δ*rprR* produced 57% more of the bacterial signal cyclic di-GMP (c-di-GMP) than wild type. This is consistent with the presence in RprR of predicted c-di-GMP modulating domains. Together these findings reveal that RprR, which is highly conserved across plant pathogenic *Ralstonia*, modulates several bacterial wilt virulence traits in response to the plant host.

Monday March 23
Hotel Wander - HUGO TECH

Theme 1
Diversity, genomics, and evolution of the *Ralstonia solanacearum* species complex (e.g. phylogeny, pangenomics, population genetics, evolution)

Emergence of *Ralstonia syzygii* subsp. *syzygii* (Phylotype IV) in Brazil: Evidence for a Myrtaceae-Associated Lineage Expansion

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The *Ralstonia solanacearum* species complex (RSSC) comprises diverse lineages with distinct geographic and ecological adaptations. Phylotype IV, once considered confined to Southeast Asia, encompasses *Ralstonia syzygii* subspecies with narrow host ranges, including pathogens of clove (*R. syzygii* subsp. *syzygii*), solanaceous plants (*R. syzygi* subsp. *indonesiensis*), and banana (*R. syzygii* subsp. *celebesensis*). Here, we report, for the first time, the occurrence of *R. syzygii* subsp. *syzygii* in the Americas. Strains were isolated from diseased *Eucalyptus urophylla* and clove plants cultivated in southern Bahia, Brazil. Both hosts belong to the Myrtaceae family, raising the possibility of a recent spillover from clove to eucalyptus. Clove trees aged between 2 and 60 years were examined: younger plants were asymptomatic, whereas trees older than 12 years exhibited severe decline symptoms. Whole-genome sequencing of all isolates and phylogenomic analyses revealed that the Brazilian strains cluster tightly with Asian *R. syzygii* subsp. *syzygii* isolates, sharing 98-99.9% average nucleotide identity (ANI). Pathogenicity assays confirmed that Brazilian strains infect *E. urophylla*, inducing wilt symptoms less aggressive than those caused by phylotype I and II strains. The detection of *R. syzygii* subsp. *syzygii* in Brazil expands the known geographic and host range of RSSC phylotype IV and suggests that this lineage possesses greater ecological plasticity than previously recognized. The close phylogenomic relationship with Asian isolates and the association with two Myrtaceae hosts in Brazil raise important questions about introduction routes, host adaptation, and the evolutionary history of phylotype IV in tropical ecosystems.

Can temperature adaptation drive *Ralstonia solanacearum* strain K60 range expansion and virulence?

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In recent years, tropical strains of phytopathogenic *Ralstonia solanacearum* species complex (RSSC) have been detected in cool-temperate regions of Europe. A 2015 outbreak of warm-adapted *Ralstonia pseudosolanacearum* in greenhouse rose in the Netherlands emphasises the potential risk of geographical range expansion of this pathogen. Here, we used *in vitro* experimental evolution, genomics, and *in planta* virulence assessments to determine whether RSSC strain K60 isolated from tomato in the warm-temperate region of North Carolina (US) can adapt to colder climates. Ancestral K60 was experimentally evolved in rich liquid media at four different temperatures (15, 20, 28 and 34 °C) for 148 generations after adaptation was measured in individual clones isolated from all treatment replicates. K60 rapidly adapted to the 15 °C environment, and the improved growth at this temperature was especially clear in evolved non-mucoid colony types, indicative of trade-off between exopolysaccharide production and growth. Variant calling analysis revealed that non-mucoid clones had overall more mutations than the mucoid. Mucoid clones evolved at 15 °C had mutations in genes *hprK* (related to ATP catalysis),and *mprA* (associated withstress adaptation), while non-mucoid clones had variations in *rluB* (encoding pseudouridine synthase) and *Int* (important for membrane integrity). Virulence assessments in tomato plants showed that ancestral K60 was more pathogenic than mutants evolved at 15 °C, however, mucoid clones evolved at this temperature were more virulent than the non-mucoid. These results suggest that while K60 adapted *in vitro* to the 15 °C environment, its virulence was reduced, indicating low potential for range expansion.

Understanding phage defence system synergy in the plant pathogen *Ralstonia*

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The *Ralstonia solanacearum* species complex (RSSC) is a group of soil-borne pathogens which are causative agents of bacterial wilt disease in many agriculturally relevant crops. As RSSC continues to compromise global food production, new treatment methods are needed. Recent research has driven interest in the use of bacteriophages (phages) as biocontrol agents. Previous *in planta* studies show that treatment with phages can effectively limit bacterial wilt disease caused by RSSC. However, these species harbour many diverse phage defence systems capable of inhibiting phage infection and preventing effective phage treatment. To better understand the function of these systems and their potential for synergistic protection, we generated a library of defence system deletions as well as a total defence-null mutant. In the absence of phage, these deletions have no effect on *in vitro* growth or infectiveness in a tomato plant model. A survey of over 60 genetically diverse phages revealed changes in infectivity associated with single- and multi-system deletions. We also show the first experimental evidence for phage protection by a predicted novel phage defence system, PDC-S35. Further research is in progress to determine PDC-S35's mechanism of action and its protective capacity against phage biocontrol in a tomato plant model. We hypothesize that understanding the range of protection provided by each phage defence system alone and in tandem will allow for selection complementary phages to evade these systems. Altogether, these results will inform the design and implementation of the most effective phage mixtures in the development of rational phage biocontrol methods.

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Prophages drive the recombination of anti-phage defence systems within and between *Ralstonia*species

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Anti-phage defence systems play important roles in phage resistance and they are widely distributed in bacterial genomes including *Ralstonia* species. However, the recombination and horizontal movement of defence system within and between *Ralstonia* species is still poorly understood. Here we asked if prophages could potentially drive horizontal transfer of phage defence system movement within and between *Ralstonia* species. To study this, we first constructed a global *Ralstonia solanacearum* species complex (RSSC) repository by combining 329 new Chinese isolates with existing RSSC strains in Refseq database. We could identify over 1000 anti-phage defence gene clusters that belonged to 79 types of defence systems and over 4,000 prophages in RSSC genomes. While the most common 14 defence systems were similarly distributed between *Ralstonia solanacearum* (RS) and *Ralstonia pseudosolanacearum* (RPS) species, RS contained 12 unique and RPS 17 unique defence systems. Sharing of defence system was common and 63.29% of all detected defence systems (50) were shared between both species. Interestingly, several defence systems were not chromosomal but located within or nearby prophages. Crucially, over half of the shared defence gene clusters between RS and RPS were associated with prophages, indicating that prophages could have horizontally transferred defence systems between the species. To verify this, we induced prophages from RPS strains and infected one recipient RS strain with the derived prophage mixture. By using metagenomics, we could observe the integration of two RPS prophages into RS genome and one of them included a complete RM and an incomplete MZA phage defence system cluster. Together these results suggest that phage defence systems are highly diverse and abundant in RSSC and that they are frequently recombining within and between species along with temperate phages.

Genetic Diversity and Population Structure of *Ralstonia solanacearum* Phylotype II in Philippine Bananas

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Bugtok, a unique manifestation of *Ralstonia solanacearum* Phylotype II infection, is a destructive bacterial disease of cooking bananas in the Philippines related to Banana blood disease occurring in Indonesia and peninsular Malaysia. Despite its economic importance, updated information on pathogen distribution and intraspecific genetic diversity remains scarce. This study investigated the current geographic distribution of Bugtok and assessed the genetic diversity of Phylotype II isolates collected from Luzon, Visayas, and Mindanao. Symptomatic banana samples were obtained from major production areas, and bacterial isolates were cultured for DNA extraction using a modified lysis and precipitation protocol. Two genetic loci were targeted for PCR amplification: the virulence-associated *egl* gene and the DNA mismatch repair gene *mutS*. Maximum-likelihood phylogenetic reconstructions using PhyML were generated from the resulting sequences and compared with reference strains from CIRAD Dataverse to establish the placement of 72 Philippine isolates within the broader *R. solanacearum* species complex. Examination of the *egl* gene showed that the Philippine isolates clustered within Phylotype IIB/3. In a separate analysis, the *mutS*-based phylogeny also placed the Philippine isolates within Phylotype IIB/3.These isolates formed a distinct subclade characterized by short branch lengths, indicating low genetic divergence and suggesting a recent common ancestry. Comparison with global sequences confirmed the placement of the Philippine isolates within the established *R. solanacearum* species complex and indicated a distinct phylogenetic clustering relative to other accessions. The findings demonstrate the genetic homogeneity of Phylotype II isolates in the Philippines, thereby contributing to ongoing efforts to develop precise diagnostic tools, and offer insights that may guide future resistance-breeding strategies.

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Changes in DNA methylation, a form of epigenetic modification, contribute to rapid adaptation of *Ralstonia pseudosolanacearum* to the host plant

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In the present work, we tested the hypothesis of the role of DNA methylation, a form of epigenetic modification, in adaptation of *Ralstonia pseudosolanacearum* GMI1000 strain to the host during experimental evolution. Using SMRT-seq technology, we analyzed the methylomes of 31 experimentally evolved clones obtained after serial passages on 5 different plant species during 300 generations. Comparison with the methylome of the ancestral clone revealed a list of 50 differential methylated sites (DMSs) at the GTWWAC motif. Gene expression analysis of the 39 genes targeted by these DMSs revealed limited correlation between differential methylation and differential expression of the corresponding genes. Only 1 gene showed a correlation, the RSp0338 gene encoding the EpsR regulator protein. Using site-directed mutagenesis, we demonstrated the contribution of the 2 DMSs upstream RSp0338 in host adaptation. As these DMSs appeared very early in the experimental evolution, we hypothesize that such fast epigenetic changes can allow rapid adaptation to the plant stem environment. In addition, we found that the change in DNA methylation upstream RSp0338 remains stable at least for 100 generations outside the host and thus can contribute to long-term adaptation to the host plant. To our knowledge, this is the first study showing a direct link between bacterial epigenetic variation and adaptation to a new environment

Reference:
Gopalan-Nair R, Coissac A, Legrand L, Lopez-Roques C, Pécrix Y, Vandecasteele C, Bouchez O, Barlet X, Lanois A, Givaudan A, et al. 2024. Changes in DNA methylation contribute to rapid adaptation in bacterial plant pathogen evolution. PLoS Biol. 22:e3002792.

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Tuesday March 24
Hotel Wander - HUGO TECH

Theme 2
Molecular plant-microbe interactions (e.g. effectors, quorum sensing, plant responses/gene expression, genetics and metabolomics)

The phenylacetic acid pathway is required for *R. solanacearum* rhizosphere establishment

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The rhizosphere is a highly competitive environment where soil-borne plant pathogens must adapt to infect their host. Despite its importance for infection outcomes, research has mainly focused on pathogen strategies for host colonization. Here, we present the first genome-wide mutant fitness screen of a plant pathogen in the rhizosphere using the bacterial wilt pathogen *Ralstonia solanacearum* as a model. We applied barcoded transposon sequencing (RB-TnSeq) to track bacterial gene fitness in the tomato rhizosphere under greenhouse, non-sterile conditions. We identified 108 genes required for rhizosphere survival and validated 10 candidate genes involved in motility and metabolism. Notably, two of these genes belong to the phenylacetic acid degradation pathway. Disruption of this pathway markedly reduced *R. solanacearum* survival in the rhizosphere, leading to delayed symptom development in tomato plants. Importantly, mutation of the pathway did not affect bacterial fitness inside the host, revealing that the phenylacetic acid metabolism is specifically required for rhizosphere colonization. We further show that *R. solanacearum* cannot use efficiently phenylacetic acid as a carbon source and instead, this pathway helps to cope with environmental stressors such as oxidative and antibiotic exposure. Together, our findings demonstrate that colonization of the rhizosphere is a decisive stage for *R. solanacearum* infection and highlight the phenylacetic acid pathway as an important mechanism for bacterial survival and disease initiation.

Dissecting the bacterial-plant chemical conversations that drive *Ralstonia* phytopathogens towards host roots

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To cause infection from the soil, the *Ralstonia solanacearum* species complex must locate the host root within a complex chemical environment. An established mechanism in locating host plants is chemotaxis, or the ability to swim directionally along a chemical gradient. Root-exuded chemicals serve as beacons of the host. These chemical beacons are sensed by an array of membrane-bound chemoreceptor proteins. Pangenomic analyses reveal 19 broadly conserved, core chemoreceptors in *Ralstonia* pangenome. We target uncharacterized core chemoreceptors to link them to ligands present in host-root exudate profiles. To screen chemoreceptor specificity, we use hybrid chemoreceptor-two component systems (HyChemosensors) which fuse ligand-binding domains of *Ralstonia* receptors to the signal transduction domain of the two-component system, NarQ. HyChemosensors activate transcriptional readouts within heterologous *E. coli* reporter strains, allowing us to screen individual receptors-ligand pairs in isolation. Using this approach, we identify receptors with specificity for primary metabolites, and secondary aromatic metabolites. We confirm receptors' role in *Ralstonia* behaviour and infection using deletions in wild-type and chemotaxis-enriched genetic backgrounds. Intriguingly, we find that receptors with overlapping chemical specificity can have distinct roles *Ralstonia* infection via soil soak. Further, we observe chemotaxis-enriched *Ralstonia* isolates have reduced success locating hosts from the soil. Using quantitativ tracking analyses and a bioinformatic pipeline to identify mutations in these isolates, our data supports a model that motility is quantitatively optimized for host discovery in wild-type strains. In the long-term, understanding how complex chemotaxis drives host discovery from the soil reservoir could translate into strategies for pathogen control.

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What mathematical modelling has revealed about bacterial wilt disease

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Mathematical modelling has emerged as a powerful approach to decipher the complexity of biological systems, enabling the integration of heterogeneous datasets, the testing of hypotheses, and the identification of mechanisms that remain difficult to capture experimentally. Since 2016, such approach has increasingly been applied to unravel the key processes underlying bacterial wilt disease caused by the *Ralstonia solanacearum* species complex.

Different classes of models can be used to represent the behavior of a pathosystem. A first category includes macroscopic models, which focus on physiological traits such as pathogen nutrient-dependent proliferation within the plant or bacterial mortality. These models are dynamic and allow the testing of ecological hypotheses. For example, they can estimate how many bacteria enter the plant at the onset of infection or determine how *R. solanacearum* proliferates in xylem vessels under resource limitation (Baroukh et al., 2024). Their simplicity makes them useful for exploring infection scenarios and identifying key drivers of disease progression.

A second category includes molecular-scale models, which describe intracellular processes such as gene regulation or metabolism (Peyraud et al., 2016). These models provide mechanistic insights by linking genotype to phenotype and can quantify trade-offs, such as how virulence expression affects bacterial growth. They also help identify essential metabolic pathways or regulatory nodes. However, they are often challenging to construct, require extensive input data, and are usually static, limiting their ability to capture temporal dynamics.

Although these approaches differ in scale, methodology, and data requirements, they provide complementary insights into bacterial wilt biology. In this overview, I will compare their respective strengths and limitations and illustrate how each contributed to a deeper understanding of *R. solanacearum* infection. I will also discuss future directions toward predictive modelling frameworks to support disease management.

Peyraud R, Cottret L, Marmiesse L, Gouzy J, Genin S. 2016. A resource allocation trade-off between virulence and proliferation drives metabolic versatility in the plant pathogen *Ralstonia solanacearum*. PLoS Pathogens 12: e1005939.

Baroukh, C., Gerlin, L., Escourrou, A., & Genin, S. 2025. Unraveling the in planta population dynamics of the plant pathogen *Ralstonia pseudosolanacearum* by mathematical modeling. New Phytologist, 246(1), 251-261.

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A novel transcriptional regulator RalT contributes to the fine-tuning of the quorum sensing-dependent production of major exopolysaccharide EPS I in *Ralstonia pseudosolanacearum* strain OE1-1

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During the quorum sensing (QS)-active state, *Ralstonia pseudosolanacearum* strain OE1-1 activates the LysR-type transcriptional regulator PhcA, inducing the major exopolysaccharide EPS I production-related genesand the ralfuranone production-related genes. Both virulence-related secondary metabolites contribute to the feedback loop in the QS. To elucidate the regulation mechanisms underlying thesesecondary metabolites, we first identified RSp0599 gene (*ralT*) encoding a novel TetR/AcrRfamily transcriptional regulator RalT; the expression level of *ralT* reduced significantly in ralfuranone-deficient (Δ*ralA*) but not *phcA*-deletion (Δ*phcA*) mutants relative to expression level in strain OE1-1. The promoter assay showed that RalT represses *ralT* expression. Furthermore, the transcriptome data of *ralT*-deletion mutant (Δ*ralT*) showed RalT contributes to the regulation of 77.7% of positively PhcA-regulated genes including the EPS I production-related genes such as *xpsR*, which encodes the transcriptional regulator XpsR that positively regulatesthe *eps* operon, and the ralfuranone production-related genes such as *ralA* and *ralD*. However, the *ralT*-deletion led to asignificantly increasing EPS I production, and the tomato plants inoculated with Δ*ralT* had a significantly lower survival rate, compared to those with OE1-1. The EPS I production of Δ*phcA* transformant with *xpsR* fused the constitutively activated *RSc0900* promoter suggested that EPS I production depends on PhcA, which controls the XpsR-regulated pathwayand an alternative pathway that is negatively regulated by RalT. Collectively, our findings suggest that RalT contributes to not only the positive regulation of the XpsR-regulated pathway but also the negative regulation of the alternative pathway, fine-tuning EPS I production and subsequent virulence of the strain OE1-1.

The EPS-I exopolysaccharide transforms *Ralstonia* wilt pathogen biofilms into mobile viscoelastic fluids for rapid dissemination in planta

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Ralstonia solanacearum species complex (RSSC) pathogens rapidly spread through the water-transporting xylem where they grow prolifically and produce abundant biofilm that clogs xylem vessels. To understand RSSC biofilm behavior *in planta*, we examined their complex fluid mechanics. Rheological analyses revealed that unlike all previously analyzed microbial biofilms, RSSC biofilms are shear-thinning, viscoelastic fluids at physiologically relevant shear forces. To determine which factors confer these unique mechanics, we analyzed biofilms of bacterial mutants with altered biofilm components: absence of EPS-I (*epsB* mutant), increased extracellular DNA (*nucAB* mutants), fewer lectins (*lecF*, *lecX*, and *lecFX* mutants). Genetic analysis demonstrated that development of the viscous-dominant biofilms required production of EPS-I, an amphiphilic exopolysaccharide that is a major virulence factor for all RSSC pathogens. We show that EPS-I confers "biofilm mobility", which allows wildtype RSSC colonies to passively expand when deformed. Despite its high metabolic cost, bioassays demonstrated that EPS-I production conferred a net fitness benefit where biofilm mobility allowed the pathogen to spread and access more nutrients in complex environments like xylem vessels or physically structured environments like agar surfaces. The RSSC are a monophyletic lineage of aggressive plant wilt pathogens, and our evolutionary hypothesis testing suggests the origin of the *eps* biosynthetic gene cluster coincides with the emergence of wilt pathogenesis in the RSSC ancestor. Furthermore, comparative physiological assays demonstrated that biofilm mobility is unique to the RSSC within the genus *Ralstonia*. In summary, EPS-I production is a key evolutionary innovation that enables RSSC dispersal and virulence by conferring unique biofilm mechanics.

Tuesday March 24
Hotel Wander - HUGO TECH

Theme 5
Disease control, prevention, and diagnostics (e.g. detection, identification, biological control, containment, ICM)

Development of High-throughput Resistance Screening Approaches and Utilization of Diverse Resistant Germplasm for Effective Control of Bacterial Wilt in Peanut

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Breeding and planting of resistant cultivars is the core component for integrated management ofbacterial wilt (BW) in peanut caused by *Ralstonia pseudosolanacearum*. Traditionally, natural nurseries were mostly used in screening BW resistance in peanut, but the accuracy and repeatability of resistance screening were often influenced by environmental factors and some fungi diseases such as root rot and stem rot. In recent years, novel high throughput screening strategies for characterizing BW resistance have been developed withenhanced efficiency and accuracy. A hydroponic inoculation method was developed, in which, the culture platform and nutrition solution, age of plants for inoculation (~30 days), concentration of bacterial suspension (1×10^7 CFU/mL) and optimal time-point for disease scoring (~12 days after inoculation) have been empirically optimized. The hydroponic method possessed higher efficiency, accuracy, reduced experiment costs with less interaction with other fungi wilt diseases. Innatural nursery screening, field management was improved to prevent root rot and stem rot, and thus the interferenceof these fungi wilt diseases to BW scoring was much reduced.The available diagnosticmarkers for BW resistance have been used in assisting the resistance scoring and verification. Meanwhile, approaches for scoring resistance to root rot and stem rot have been deployed in evaluating the diverse BW-resistant peanut germplasm and breeding lines, from which, several elite genotypes with combined resistance both to BW and fungi wilt have been identified and consequently utilized in breeding. The above progress has contributed to the improved management of peanut BW in China.

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Development of a new TaqMan PCR for simultaneous detection and distinction of *R. pseudosolanacearum* and *R. solanacearum* in surface water

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Ralstonia pseudosolanacearum and *R. solanacearum* are two of three *Ralstonia* species causing bacterial wilt in many plant species, such as potato, tomato, and rose. Because of their aggressiveness and persistence in the environment, both *Ralstonia* species are listed as Quarantine organisms in the EU. To control both *Ralstonia* species, plant protection organisations in the EU rely on rapid and highly sensitive diagnostic methods. However, detection in surface water currently relies on the isolation of *Ralstonia* on SMSA medium, which cannot easily distinguish among the two *Ralstonia* species. Therefore, we developed a new multiplex TaqMan PCR for the detection of *R. pseudosolanacearum* and *R. solanacearum* in surface water. New primer pairs were designed to specifically detect *R. solanacearum* (phylotype II) or *R. pseudosolanacearum* (phylotype I and III). The specificity of the primers was evaluated with 20 target and 8 off-target species, and further checked *in silico* against 234 available *Ralstonia* genomes. The designed TaqMan primers and FAM- or CY5-labeled probes were specific for the target *Ralstonia* species and do not detect other *Ralstonia* species. The assay can detect DNA quantities up to 100 fg and cell concentrations of 10^4 colony forming units/mL concentrated extract. Enrichment of surface water samples in CIPEB medium further increases the detection limit to 10 colony forming units/mL. The assay was validated by four Dutch organisations, routinely testing on *Ralstonia*, to verify the results and robustness of the test. Results will be discussed addressing the use of this multiplex TaqMan for routine diagnostics.

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From Pangenomics to Diagnostics: Resolving Diversity in *Ralstonia syzygii*

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Ralstonia syzygii is a bacterial plant pathogen causing wilting disease in several economically important crops. Although not yet reported in Europe, the emergence of strains adapted to temperate conditions and ongoing climate change raise concerns about its potential spread. Effective and specific detection methods are therefore essential. *R. syzygii* is currently divided into three subspecies with distinct host ranges. Previous studies have suggested even greater diversity, though based on limited genome collections.
The project "Detection methods for *Ralstonia syzygii*" aims to refine the phylogenetic classification of the species, assess virulence across hosts, and develop specific diagnostic tools for relevant subgroups.
A comprehensive pangenomic analysis of an extensive set of whole-genome sequences revealed a revised classification, identifying several distinct and well-supported subgroups. Subsequent virulence assays confirmed biological differences among these groups, emphasizing the need for subgroup-specific detection. The pangenome data also enabled identification of specific gene loci suitable as targets for diagnostics.
Here, we present our workflow from strain and sequence collection through pangenome analysis and virulence testing to the development of subgroup-specific detection assays (currently ongoing). Our results provide new insights into the diversity, host adaptation, and detection of this understudied bacterial wilt pathogen, supporting improved surveillance and management strategies.

This research was financially supported by LVVN.

Genome-wide identification of *Ralstonia solanacearum* genes conferring resistance to diverse bacteriophages using RB-TnSeq

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Ralstonia solanacearum (Rs) is a devastating plant pathogen, which causes bacterial wilt and brown rot disease, leading to huge economic losses worldwide. As there are no effective plant protection measures against this pathogen, development of novel control approaches are urgently required. Bacteriophages (phages) are promising biocontrol agents for Rs: they are target specific, propagate rapidly, and efficiently reduce bacterial densities. Phages start their infection cycle by adsorbing to structures on the bacterial host membrane surface, known as receptors. Mutations in receptor genes provide resistance to phage infection, mainly by hindering adsorption. However, knowledge on receptors and phage-bacteria interactions in Rs is still limited, hindering the production of effective phage cocktails. Here we used random barcode transposon site sequencing (RB-TnSeq) in two model Rs strains (GMI1000 and UY031) to identify host genes involved in resistance to three phylogenetically different phages. Gene ontology enrichment analysis of high-fitness genes revealed eight metabolic pathways that were enriched, including cellular polysaccharide and lipopolysaccharide (LPS) biosynthetic processes. Glycosyltransferase and O-antigen ligase mutants obtained high fitness scores in both bacterial strains, while a DNA-binding protein increased only GMI1000 fitness in one phage treatment. Mutant lines were subsequently generated by knocking out high fitness genes to validate phage resistance phenotypes observed in the RB-TnSeq screen. These mutants will also be used in plant disease assays to test if mutations in LPS conferring fitness benefits during phage infection could lead to trade-offs with pathogen virulence, as LPS plays a major role in tolerance to plant immunity and virulence processes.

Actinobacterial synthetic community alters the rhizospheremicrobiota and enhance the tomato health

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Actinobacteria are spore-forming microbes in competitive environments and enhance plant health. Secondary metabolites production and the interactions between soil and plant makes them more crucial. However, the mechanism behind the interaction and their linkage to the disease suppression are unclear. We first characterized 106 actinobacterial strains and selected fourteen with distinct functions to construct an actinobacterial synthetic community (ActSynCom) for suppressing tomato bacterial wilt in upland and forest soil systems. Second, 16S rRNA gene amplicon sequencing revealed that five strains were key in altering the tomato rhizosphere microbiome and promoting the colonization of a specific ActSynCom member *Arthrobacter* sp. in the rhizosphere before and after pathogen inoculation. This simplified ActSynCom and individual *Arthrobacter* sp. FR-101 significantly suppressed bacterial wilt in both soil system. Third, we show that simplified ActSynCom and *Arthrobacter* sp. FR101 alone modulate tomato root exudation, directly recruiting disease-suppressive taxa. Lastly, root exudate metabolites, protect tomato plants by recruiting beneficial bacteria such as *Arthrobacter* sp. FR-101 and by enhancing their growth, biofilm and secondary metabolite production. Together, these results indicate that *Arthrobacter* sp. FR-101 mediated secondary metabolism, triggered by root exudate signaling, enhances tomato health and provides new insights into their molecular antibacterial mechanisms.

This research was financially supported by National Research Foundation of Korea (NRF) grant (RS-2020-NR049596, MOE), Republic of Korea.

Six-year field evaluation of cropping systems for management of bacterial wilt and root knot nematode of tobacco

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A six-year (2020–2025) field experiment was conducted in Yunnan Province of China (acidic soil, 1470 m altitude) to systematically assess the impacts of different cropping systems on the incidence of tobacco bacterial wilt (BWT), root-knot nematode (RKN) disease, andtobacco agronomic traits. Thirteen distinct treatments were designed, including four continuous cropping treatments (e.g., T01, T02), seven annual rotation treatments (e.g., T06: tobacco–rape–rocket–chili; T10: tobacco–wheat–soybean–wheat), two biennial rotation treatments (e.g., T09: tobacco–wheat–corn–wheat–soybean–wheat; T13: tobacco–rape–corn–rape–soybean–rape).The results showed that under continuous cropping systems, the population of *Ralstonia*spp. in soil increased annually, all exceeding the critical pathogenic threshold of 10^3–10^4 genomes/g. The incidence of BWT peaked in the 4th year of continuous cropping, accompanied by a significantly high base population of RKN. In contrast, annual and biennial rotation systems not only significantly reduced soil pathogen load and disease incidence but also improved tobacco agronomic traits, with T06 and T09 exhibiting the best control effects. These optimal rotation systems further enhanced the richness and stability of soil microbial communities, improved soil physicochemical properties, and promoted the compliance and coordination of main chemical components in tobacco leaves. Notably, winter potato cultivation under continuous cropping (T02) further aggravated disease severity and soil degradation.These findings provide comprehensive insights into the role of cropping systems in tobacco soil-borne diseases management and leaf quality improvement, highlighting the identified optimal rotation systems as sustainable agricultural strategies to mitigate the challenges associated with tobacco continuous cropping.

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Tuesday March 24
Hotel Wander - HUGO TECH

Theme 4
Host resistance and crop improvement (e.g. resistance genes, resistance markers, breeding, genetic engineering)

Natural variation in a NLR pair confers thermostable resistance to a devastating bacterial pathogen

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Climate change, particularly heat stress, is reshaping host-pathogen interactions by increasing pathogen aggressiveness and weakening plant immune responses. Recent studies have shown that some nucleotide-binding leucine-rich repeat immune receptors (NLRs), key players in pathogen recognition, can be negatively affected under elevated temperatures in plants, posing a major challenge to disease resistance. In *Arabidopsis thaliana*, the well-characterized RPS4/RRS1 NLR pair confers broad spectrum disease resistance to the worldwide devastating plant pathogenic *Ralstonia pseudosolanacearum*, the causal agent of bacterial wilt. However, we found that this resistance is compromised at elevated temperatures. To identify genetic factors underlying thermostable resistance, we screened a large panel of Arabidopsis accessions at permissive (27°C) and elevated (30°C) temperatures and identified one accession that exhibited fully thermostable disease resistance response to bacterial wilt. By integrating natural variation analysis, genetic mapping, polymorphism analysis, structural modeling, and functional complementation, we demonstrate that the paucity of fully thermostable resistance is mainly associated with a unique RPS4/RRS1 haplotype carrying key substitutions in their leucin-rich repeat domain, which are predicted to stabilize the immune function of these paired NLRs at 30°C. Our findings uncover natural genetic diversity as a source of thermostable resistance and highlight promising opportunities for engineering climate change-resilient plants.

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The mechanism of receptor-mediated immunity to bacterial wilt in tomato

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Bacterial wilt caused by *Ralstonia solanacearum* (*Rs*) is a devastating vascular disease that threatens global production of Solanaceous crops such as tomato and potato. Plants detect pathogens through diverse immune receptors, yet how the tomato immune system perceives Rs invasion and transduces defence signals remains obscure. Extracellular polysaccharide (EPS) secreted by *Rs* not only occludes xylem vessels but also elicits tomato immunity. Here we identified the receptor-like kinase SlLYK4 as a candidate EPS sensor. SlLYK4 assembles into a trimeric complex with SlLYK1 and SlLYK13 that additionally mediates recognition of fungal chitin. C-terminal phosphorylation sites in SlLYK4 differentially regulate EPS- and chitin-triggered immunity, revealing how one receptor kinase achieves signal-specific outputs.
Solanaceae plants specifically recognise *Rs* cold-shock protein and its conserved peptide csp22. We found that the tomato RLCK SlCAK1 participates in csp22-mediated immunity and resistance to *Rs*. Upon csp22 perception, SlCAK1 is recruited by the SlCORE–SlBAK1a receptor complex; SlBAK1a phosphorylates SlCAK1 at Ser60, a residue conserved only among Solanaceae orthologues. Phospho-Ser60 modulates interaction with the SlBAK1a kinase domain, enabling full SlCAK1 activation and downstream signalling. This work delineates the csp22-triggered immune pathway in tomato and provides a new mechanism for RLCK phosphorylation activation.
Additionally, we showed that the *Rs* exo-polygalacturonase PehC is specifically recognised by tomato roots. Collectively, these studies expand our understanding of tomato immune receptor recognition of *Rs*, elucidate phosphorylation-based activation mechanisms, and provide theoretical support and genetic resources for breeding resistance against bacterial vascular diseases

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Identification of Key Loci Associated with Resistance to Bacterial Wilt in Peanut through Whole-genome Resequencing

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Peanut (*Arachis hypogaea* L.), also known as groundnut, is an important oilseed crop worldwide, in which, bacterial wilt (BW) caused by *Ralstonia pseudosolanacearum* is a major constraint in several countries. Application of host-plant resistance is the most effective approach to control BW in peanut, hence identification of loci/genes for BW resistance is crucial for breeding. In this study, we identified genomic variations based on whole-genome resequencing of 415 diverse peanut accessions and characterized their reaction to BW in four environments. A total of 87.29 billion paired-end reads of 150 bp in length (13.03 Tb of bases) were generated based on the illumine platform and mapped to the reference peanut genome. Through genome-wide association studies (GWAS), five significant marker-trait associations (MTAs) were identified in a 1.50 Mb genomic region on chromosome B02 and were stably identified in all four environments. Three of the five MTAs were in a strong LD block and located within a gene encode for an NBS-LRR disease resistance protein. The resistant haplotype was repeatedly detectable in Xiekangqing (a well-known peanut line with durable BW resistance) and its progenies derived from various crossing combinations with different parental genotypes. Based on the identified loci, Kompetitive Allele-Specific PCR (KASP) markers were developed and have been used as marker-assisted selection approach in breeding programs for BW resistance with improved selection efficiency.

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Genetic architecture of bacterial wilt resistance in potato wild relatives from Uruguay and new sources for introgression

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Potato wild relatives *Solanum commersonii* (cmm)*, S. malmeanum* (mlm) and *S. chacoense* (chc) show resistance against bacterial wilt (BW), although their response can vary widely within and across species. We screened a core collection from Uruguay, finding a wider response than previously reported. We identified new sources of resistance for introgression, but without knowledge on the genetic architecture underlying their response. To explore the genetic basis of resistance to BW in potato wild relatives from Uruguay, three mapping approaches were undertaken. First, we worked with 190 full-sibs (moderately resistant cmm x moderately susceptible cmm), then with a diversity panel (181 accessions from Uruguay) and currently with 190 full-sibs (highly resistant mlm x highly susceptible mlm). We screened the populations with aggressive strain UY031 at 10^{6-1} soil, with root damage. Disease progression was registered regularly and resistance level was calculated by the area under disease progress curve (AUDPC). We genotyped through GBS to identify high-confidence SNPs using available reference genomes. The cmmxcmm full-sib population allowed genetic mapping and displayed transgressive segregation for AUDPC. The panel showed some genetic structure, with three genetic groups and differential responses to BW. Higher resistance was found in mlm accessions. Some resistant accessions showed no latency. No resistant chc accessions were found. Preliminary results identified QTLs and associations in some genomic regions. Further exploration may pinpoint species-specific loci to be combined for introgression into potato. These efforts, currently underway on the mlmxmlm population, will shed light on the differential architecture of mlm BW resistance.

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Development Of Bacterial Wilt Resistant Potato Using *Efr, Jim2* And *Roq1* Genes

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In Africa, potato (*Solanum tuberosum* L.) is mainly grown by smallholder farmers. In Kenya, it is the 2nd most important crop, estimated to employ more than 2.5 million people. Bacterial wilt (BW) caused by *Ralstonia solanacearum* is a devastating disease for ~74% of farms in Kenya. Current management strategies have limited efficacy. We hypothesize host resistance using pattern recognition receptors (PRR) and Nucleotide-binding Leucine rich repeat (NLR) proteins could confer resistance to BW by inducing pathogen triggered immunity (PTI) and effector triggered immunity (ETI) respectively. *Efr* (Elongation Factor Tu receptor) from *Arabidopsis thaliana* has been shown to enhance resistance against bacterial wilt. *Roq1* TIR-NLR gene and *Jim2*, a receptor like cytoplasmic kinase family XII (which signals via *Zar1*) from *Nicotiana benthamiana* have been shown to mediate recognition of *RipB* and *RipP1* Ralstonia effectors, respectively. In this study we bioengineered the Kenyan varieties 'Unica' and 'Victoria' using a stack of *Efr, Roq1* and *Jim2* genes through *Agrobacterium*-mediated genetic transformation of internodal potato explants. Regenerants were selected on chlorsulfuron to produce putative transgenic events. Molecular characterization selected events positive for *Efr, Roq1* and *Jim2* genes, negative for left and right border backbone vector sequences and that carries a single T-DNA copy. Reactive Oxygen Species (ROS) assays confirm *Efr* gene activity. Transient expression of *XopQ* and *XopJ* (*Xanthomonas* homologues of *RipB* and *RipP1* effectors in *R.solanacearum* respectively*)* in transgenic events confirmed functional *Roq1* and *Jim2* genes. 6/8 'Unica' and 4/7 'Victoria' transgenic events evaluated under greenhouse bioassays using a local strain showed no wilting in all reps inoculated in comparison to the non-transgenic equivalents .To predict the durability of BW resistance provided by the stack we have conducted WGS *R. solanacearum* isolates from Kenya to assess the occurrence of the target effectors *(RipB* and *RipP1)*. These results and latent infection in emerging plants from tubers from symptom-less events, will be presented.

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Thursday March 26
Hotel Wander - HUGO TECH

Theme 3
Ecology and epidemiology (e.g. pathogen survival, range expansion, plant host range, microbiome interactions)

Tracing the origin of *Ralstonia solanacearum* in mainland France

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Since 1990, cases of *Ralstonia solanacearum* have been reported in several European countries. Despite all RSSC species being classified as quarantine pests in the European Union, few studies have focused on the genotypic diversity of European RSSC strains, particularly French ones.
Between 1994 and 2023, more than 360 RSSC strains were collected in mainland France in the framework of territory surveillance and research projects. Phylotype and sequevar characterisation revealed that all strains belonged to phylotype IIB sequevar 1 (IIB-1). Two approaches were taken to investigate the genotypic and genomic diversity of these strains.
Multiple Loci VNTR Analysis (MLVA) was first explored as a means of discriminating closely related *R. solanacearum* strains. A novel MLVA scheme was therefore specifically designed for *R. solanacearum* phylotype IIB-1. A total of 46 VNTR profiles were identified among the near-clonal French strains. Adding non-French *R. solanacearum* phylotype IIB-1 strains provided deeper insights into the origins of outbreaks in mainland France.
The genomes of 230 French and seven non-French (from neighboring or from Euro-Mediterranean region countries historically among the first ones to be affected by *R. solanacearum*, such as Belgium, Cyprus, Israel, Spain, Sweden, Turkey, and United Kingdom)*R. solanacearum* IIB-1 strains were then sequenced. In addition, 68 publicly available non-French genomes of *R. solanacearum* IIB-1 were considered. Tip-dating analysis was used to infer the dates at which several phylogenetic clades separated.
These genomic data complement the genotypic data obtained by MLVA and provide further evidence for the origins and dates of introduction of *R. solanacearum* in mainland France.

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Exploring the genetic diversity of recent *Ralstonia pseudosolanacearum* phylotype I findings in Europe

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The increasing prevalence of *Ralstonia pseudosolanacearum* phylotype I (phy I) in agricultural regions across Europe highlights the need for awareness and preventive measures. Recent detections in multiple European countries span a range of agricultural systems, host plants, and natural aquatic environments. The findings have been linked either to infected plant material at import (interceptions) or to plants cultivated in specific European regions, for which the origin of the infections can in many cases be linked to the imported propagation material. These findings, in combination with the pattern of dissemination of this pathogen through irrigation water, crop debris, common agricultural practices and latent presence in traded plant products, pose a serious threat to agricultural production in temperate climates. As this group of bacteria occurs frequently as non-symptomatic infections when plants are growing under a temperate climate, the presence of this disease on known and new host plants, might be easily overlooked.
The genetic diversity of these emerging populations was investigated by analysing whole-genome sequence (WGS) data from a collection of 62 *R. pseudosolanacearum* phy I isolates, including those recently obtained in various European countries. The whole-genome sequences were analysed using average nucleotide analysis (ANI) and core genome multilocus sequence typing (cgMLST). Pathogenicity assays were performed on tomato to deduce information on the biological relevance of the genetic diversity observed among these *R. pseudosolanacearum* phy I isolates. Our findings suggest several independent introduction events of virulent *R. pseudosolanacearum* phy I populations into Europe between 2015 and 2024.

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Ralstonia in Dutch surface water, looking back and looking forward

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Ralstonia solanacearum has been found in Dutch surface water since the 1990's and has not disappeared since then. The presence of *R. solanacearum* in surface water has been a threat to the production of seed potatoes and is the likely cause of the large outbreak of potato brown rot in 1995. Since this large outbreak in The Netherlands, policies have been put in place to minimise the introduction of *R. solanacearum* into seed and ware potatoes. These measures have proven to be effective in keeping the number of infected seed potato lots to a minimum. Annual surveys for the presence of *R. solanacearum* in the Dutch waterways have led to the recent findings of *R. pseudosolanacearum* in Dutch surface water as well. The introduction of this new species allowed us to monitor its spread which currently seems to be restricted.. An overview will be given based on historical data, the measures taken and what the introduction of *R. pseudosolanacearum* in Dutch surface water might hold for the future.

Quantifying the dissemination of *Ralstonia pseudosolanaceraum* via free water in soil using a custom laboratory set-up

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Bacteria in the *Ralstoniasolanacearum* species complex (RSSC) are among the most destructive plant pathogens worldwide. In temperate climates, RSSC do not persist long-term in soil or water; instead, they are thought to mainly survive in and be disseminated from reservoir hosts such as bittersweet (*Solanum dulcamara*). Surface and irrigation water may play an important role in the dissemination of RSSC from such inoculum sources, but this remains poorly quantified. We hypothesized that RSSC can spread via free water in and on soil (originating from surface and/or irrigation water) via various mechanisms. Specifically, we propose that horizontal displacement of RSSC may be attributed to diffusion, bacterial motility (in the absence or presence of chemoattractants such as plant-excreted compounds), capillary forces or flooding. Diffusion and motility are expected to operate on spatial and temporal scales that are negligible from an agronomic perspective. By contrast, capillary forces are expected to contribute significantly to the horizontal displacement of RSSC, whereas flooding of saturated soil—even if relatively rare—is expected to be responsible for the largest displacements and consequently be most relevant epidemiologically. We built custom laboratory set-ups to simulate each of these processes and quantify their relative contribution to the horizontal displacement of a rifampicin-marked *Ralstonia pseudosolanacearum* strain in sand and clay soils. This information may be used for risk assessment as well as the evaluation of potential control measures for managing this destructive disease.

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Ralstonia parasitizes not only plants but also some fungi

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Strains of *Ralstonia solanacearum* species complex (RSSC), though known as the causative agent of bacterial wilt disease in plants, induce the chlamydospores of some fungi and invade them through the spores. The lipopeptide ralstonins are the chlamydospore inducers produced by RSSC and are essential for this invasion. However, no mechanistic investigation of this interaction has been conducted. In this study, we report that quorum sensing (QS), which is a bacterial cell–cell communication, is important for RSSC to invade the fungus *Fusarium oxysporum* (*Fo*). Δ*phcB*, a deletion mutant of QS signal synthase, lost the ability to both produce ralstonins and invade *Fo* chlamydospores. The QS signal methyl 3-hydroxymyristate rescued these disabilities. In contrast, exogenous ralstonin A, while inducing *Fo* chlamydospores, failed to rescue the invasive ability. Gene-deletion and -complementation experiments revealed that the QS-dependent production of extracellular polysaccharide I (EPS I) is essential for this invasion. The RSSC cells adhered to *Fo* hyphae and formed biofilms there before inducing chlamydospores. This biofilm formation was not observed in the EPS I- or the ralstonin-deficient mutant. Microscopic analysis showed that RSSC infection resulted in the death of *Fo* chlamydospores. Altogether, we reported that the RSSC QS system is important for this lethal endoparasitism. Among the factors regulated by the QS system, ralstonins, EPS I, and biofilm are important parasitic factors.

Bacterial wilt resistance is correlated with rhizosphere bacterial communities in potato genotypes

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Potato (*Solanum tuberosum*) is one the most important widespread hosts of *Ralstonia solanacearum*, the causal agent of bacterial wilt. In Uruguay is conducted a national potato bredding program focused on development of potato varieties adapted to regional conditions and resistant to bacterial wilt. The strategy adopted involves introgression of resistance from wild native species as *Solanum malmeanum* and *Solanum commersonii*, widely distributed and adapted to our environmental conditions. Germplasm resistant to *R. solanacearum* has been identified and genotypes with contrasting levels of resistance were selected and characterized. The aim of this work was to study the correlation between plant resistance and the structure of rhizospheric bacterial communities in *S. malmeanum* accessions susceptible and resistant to *R. solanacearum* infection. Plants were grown in a hightunnel with soil collected from a potato field, with disease progression monitored and pathogen levels quantified in rhizosphere samples. The impact of plant resistance and pathogen colonization on the structure of rhizosphere bacterial communities were evaluated using high throughput sequencing of 16S rRNA genes. The rhizosphere bacterial communities of infected plants differed significantly from those of healthy controls in both accessions. Control plants of resistant accession presented the highest number of unique ASVs. *Cryseobacterium*, *Sphingobacterium, Komagataeibacter, Gluconobacter, Lactobacillus* and *Dyella* were differential genera and more abundant in the rhizosphere of resistant accession. These genera are potential taxa involved in disease suppression. The results suggest that the rhizosphere bacterial community of the resistant accession contributes to plant protection against *R. solanacearum* infection.

This research was financially supported by Fondo Vaz Ferreira, a national research funding program in Uruguay.

Thursday March 26
Hotel Wander - HUGO TECH

Theme 2
Molecular plant-microbe interactions (e.g. effectors, quorum sensing, plant responses/gene expression, genetics and metabolomics)

A type III effector from *Ralstonia solanacearum* interacts with the plant cytoskeleton and is required for virulence

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Plants are surrounded by microorganisms that can cause disease in the right environment. Our laboratory aims to understand how pathogenic microbes manipulate plant pathways to promote pathogen virulence, and how the plant immune system responds to microbial pathogens across environments. To address these questions, we focus on the tomato-*Ralstonia solanacearum* pathosystem. Like other plant pathogens, *R. solanacearum* secretes type III effector proteins (T3Es) into plant host cells to suppress immune pathways. We found that one of these T3Es, RipU, localized to and interacted with the actin and microtubule cytoskeleton. Transient expression of RipU in *Nicotiana benthamiana* leaves resulted in fewer microtubules within cells and altered actin organization. An *R. solanacearum* K60 mutant that is defective in RipU had decreased virulence compared to wild type bacteria. Expression of RipU in *Arabidopsis thaliana* dramatically altered plant development and lead to above and belowground phenotypes indicative of cytoskeleton defects. Mutational experiments that deleted portions of RipU identified the region that co-localizes and interacts with the cytoskeleton. Thus *R. solanacearum* uses a T3E to target a fundamental plant cellular organelle and promote its *pathogenicity.*

Ralstonia solanacearum R3B2 strain UW551 overcomes inhibitory xylem chemistry to break tomato bacterial wilt resistance.

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Resistance to *Ralstonia sp*.in tomato cultivar H7996 has long been central to breeding programs for bacterial wilt management. However, the globally spread and USDA APHIS select agent strain UW551, which overcomes this resistance, presents a major challenge. This study explores the molecular and metabolic basis of H7996's resistance and UW551's ability to bypass it. Using integrated metabolomics and transcriptomics, we compared host responses in resistant H7996 and susceptible MM cultivars following infection with UW551 and the reference strain GMI1000. Our analyses reveal distinct transcriptional reprogramming and metabolite profiles associated with resistance, including activation of phenylpropanoid and defense-related pathways in H7996. In contrast, UW551 suppresses key defense responses and redirects metabolic fluxes, indicating a sophisticated virulence strategy that circumvents host immunity. These findings highlight how host resistance mechanisms disrupt pathogen homeostasis, triggering adaptive responses that constrain virulence. By elucidating these stress-induced dynamics, this work advances resistance breeding strategies and underscores the complexity of plant–microbe interactions under biotic stress, providing a foundation for developing durable, sustainable disease management approaches.

This research was financially supported by the USDA National Institute of Food and Agriculture (NIFA) Predoctoral Fellowship and the University of Missouri.

Related type 2C protein phosphatases LOPP and CIPP1 negatively regulate immunity to *Ralstonia solanacearum* in tomato

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Plant pattern recognition receptors (PRRs) are essential for plant immunity, recognizing pathogen-associated molecular patterns (PAMPs) from pathogens and activating immune responses. Subsequently, these receptors return to a quiescent state through mechanisms such as dephosphorylation, thereby preventing negative impacts on growth. In *Arabidopsis*, the type 2C protein phosphatase member *At*LOPP dephosphorylates *At*LORE activated after *Ralstonia solanacearum* inoculation. While another member AtCIPP1 dephosphorylates AtCERK1, andthe homolog of *At*CERK1 has been identified as the receptor for the exopolysaccharide (EPS) of *R. solanacearum*in tomato. To investigate this regulatory mechanism, we identified the homologs of LOPP (*Sl*LOPP) and CIPP1 (*Sl*CIPP1) and created knockout mutants using CRISPR/Cas9 technology in Moneymaker background. The mutants exhibited less *R. solanacearum* infection compared to wild type. Interestingly, the *Sllopp* mutants exhibited enhanced vigor relative to the wild type, characterized by longer roots, taller shoots, and larger fruits. These results demonstrate that knocking out *SlLOPP* and *SlCIPP1* confers resistance to bacterial wilt disease in tomatoes without compromising yield. This study provides theoretical and experimental foundations for developing tomato varieties resistant to bacterial wilt disease.

This research was financially supported by the National Natural Science Foundation of China (31970279 and 32270289).

Sucrose and malic acid in the tobacco plant induce *hrp* regulon in a phytopathogen *Ralstonia pseudosolanacearum*

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Genes encoding a type III secretion system in *Ralstonia pseudosolanacearum* are regulated by HrpB as an *hrp* regulon and induced only in plants. This study aimed to identify the plant signals that induce the *hrp* regulon and confirm the signal recognition mechanism. Signaling molecules that induce *hrpB* expression were screened using resting cells of the *hrpB-lacZ* reporter strain. Only the soluble fraction of smashed tobacco seedlings induced *hrpB* expression. The heated soluble fraction retained its *hrpB*-inducing activity, indicating that the signaling molecules were not proteins. When the soluble fraction was fractionated into acidic, neutral, and basic components, both the acidic and neutral fractions induced *hrpB* expression. As neutral compounds, sucrose, glucose, and fructose have been found to induce *hrpB* expression. Sucrose-induced *hrpB* expression was greatly reduced in the *prhA* mutant, indicating that the TonB-dependent receptor PrhA perceives sugars. Among the organic acids found in the acidic fractions, malic acid most efficiently induced *hrpB* expression, which was reduced by the mutation of a hybrid histidine kinase gene of a two-component system, *rsc1598*, indicating that Rsc1598 may sense malic acid. We demonstrated direct binding of Rsc1598 to malic acid using isothermal titration calorimetry.

This research was financially supported by JSPS KAKENHI Grant-in-Aid for Scientific Research(C) Grant Numbers 17K07667 and 20K06054.

Origin and role of putrescine in bacterial wilt disease

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Polyamines have emerged as key regulators of plant–pathogen interactions since they participate in immunity and various pathogens deploy phytotoxins and effectors to manipulate host polyamine levels. In the context of bacterial wilt, the *Ralstonia solanacearum* reference strain GMI1000 was shown to excrete large amounts of putrescine (Peyraud et al., 2016), and its type III effector Brg11 specifically targets host adenine decarboxylase (ADC) promoters, thereby increasing ADC transcript levels (Wu et al., 2019). Metabolomic analyses revealed that infected xylem sap is strongly enriched in putrescine, which may serve as a biomarker of infection (Lowe-Power et al., 2018; Gerlin et al., 2021). An ornithine decarboxylase mutant of GMI1000 is auxotrophic for putrescine and unable to grow in tomato plants, and exogenous putrescine accelerates disease progression (Lowe-Power et al., 2018). However, the origin and functional significance of putrescine during infection remain unclear.
Here, we investigated the source and potential role of putrescine detected in infected xylem sap. *In silico* analyses identified three candidate putrescine transporters, for which we generated single and double deletion mutants. *In vitro* growth assays combined with NMR-based metabolomic profiling showed that Δ*paeA* (ΔRSc2277) was the only mutant unable to excrete putrescine, indicating that *paeA* is the major exporter under these conditions. This mutation did not impair bacterial growth. *In planta* assays on tomato (cv. M82) confirmed the absence of putrescine in xylem sap following infection with Δ*paeA*, demonstrating that the bacterium itself is the source of the putrescine observed during infection. Pathogenicity assays further indicated that *paeA* does not contribute detectably to virulence.

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This research was financially supported by the French Laboratory of Excellence (LABEX) project TULIP (ANR-10-LABX-41 and ANR11-IDEX-0002-02).

Poster presentations

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№ 1

Genomic Diversity and Lineage-Specific Detection Assay Development for the *Ralstonia solanacearum* Species Complex in the Southeastern United States

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The *Ralstonia solanacearum* species complex (RSSC) is prevalent in the Southeastern United States, affecting crops such as tomato, pepper, tobacco, eggplant, and blueberry. Despite its impact, the diversity and distribution of RSSC strains in the region remain poorly characterized. We sequenced 70 genomes from historical and recent collections and integrated them with 631 published genomes to construct a maximum-likelihood phylogeny based on 49 conserved genes. Sequevars were assigned using the egl marker gene. The population is dominated by Phylotype IIC-sequevar 7 (40/70), followed by PhyIIA-seq-38 (19/70), with few PhyI-seq13 isolates, and PhyIIA-seq39 and PhyIIA-seq41-like strains clustering separately. High genetic diversity within sequevar 7 and its limited global distribution suggest an ancient origin in the Southeast, whereas low diversity in sequevar PhyIIA-seq38 and PhyI-seq13 indicate more recent introductions. Host and temporal analysis show PhyIIC strains primarily in tobacco and tomato, while PhyIIA-seq38 and PhyI-seq13 occur sporadically in environmental samples, blueberries, and ornamentals. To enable rapid identification, we are developing PCR assays targeting the PhyIIC and PhyIIA lineages. Pangenome analysis revealed 7 and 22 unique genic regions for sequevars 7 and 38 respectively, suitable for lineage-specific primer design. These findings clarify RSSC population structure in the U.S. Southeast. Because lineages differ in host preferences and regional prevalence, detecting specific strains enables growers to implement crop-specific and location-tailored management strategies.

This research was financially supported by NIFA AFRI Competitive Grant The Role of Water in the Transmission and Distribution of Plant Pathogenic Ralstonia

№ 2

Phenotypic and Genetic Variation of *Ralstonia solanacearum* Isolates Causing Bacterial Wilt of Solanaceous and Cucurbit Crops in the Philippines

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Bacterial wilt (BW), caused by *Ralstonia solanacearum*, continues to limit the production of solanaceous and cucurbit crops. Developing BW-resistant varieties is a sustainable management strategy, but the pathogen's high diversity requires a clear understanding of its pathogenic and genetic variability. We examined the diversity of Philippine *R. solanacearum* strains isolated from various hosts across multiple provinces from 1979 to 2021. Pathogenicity was assessed on tomato, eggplant, pepper, white potato, bitter gourd, cucumber, bottle gourd, and banana seedlings inoculated with 10^8 CFU/mL. Disease progression was monitored up to 15 days after inoculation. Incubation period, percent infection, and AUDPC were analyzed using SRplot with pHeatmap-R. Genetic diversity was evaluated using phylogenetic and haplotype analyses of *egl*, *mutS*, and *hrpB* genes. Results revealed that most *R. solanacearum* isolates remained pathogenic to different crops despite long-term storage. Further, host-specific pathogenicity was observed from banana isolates causing banana bugtok or finger rot disease. The observed pathogenic variation correlated with multi-gene analysis. Banana isolates clustered with reference sequences of Phylotype II strains, with a clear delineation from Phylotype I strains from other crops. This is the first Philippine study to use a multi-gene approach to link pathogenic and genetic variation in *R. solanacearum*, providing valuable insights for breeding and sustainable BW management.

This research was financially supported by: Scientific Career System, Department of Science and Technology (Philippines)

№ 3

Antibacterial Activity and Mechanistic Insights into the Biocontrol Potential of *Burkholderia arboris* Against the *Ralstonia solanacearum* Species Complex

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The *Ralstonia solanacearum* species complex (RSSC) comprises diverseplant-pathogenic bacteria that cause bacterial wiltdisease, yet sustainable management strategies remain elusive. In this study, we identified *Burkholderia arboris* BB417 as a novel biocontrol agent exhibiting strong antagonistic activity against 16 RSSC strains,covering all 8 sequevars from diverse hosts, with inhibition zones ranging from 1.50 to 2.45 cm. In greenhouse assays, pre-inoculation with BB417 reduced tomato bacterial wilt incidence by 62.4%, comparable to the efficacy of the chemical bactericide thiodiazole-copper, while also establishing stable colonization in the rhizosphere and enhancing plant growth (fresh biomass increased by 35.46%). Genomic analysis revealed a three-chromosome architecture encoding 7,361 genes, including 19 biosynthetic gene clusters associated with antimicrobial compound production. Integrated transcriptomic and metabolomic profiling of RSSC antagonized by *B. arboris* indicated that the bacterium induces profound dysregulation of L-glutamate-centered metabolic nodes in RSSC, simultaneously impairing nitrogen assimilation, osmoregulation, and virulence-associated signaling. These alterations culminate in a dual-track inhibition of both metabolic and virulence circuits in the pathogen. This study provides the first mechanistic evidence for pathogens disruption by a *Burkholderia* biocontrol agent, highlighting BB417 as a promising prototype for the development of sustainable biopesticides.

№ 4

A genomic and phenotypic overview of four *Ralstonia* strains associated with Costa Rican crops

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In this study, we report the presence of both phylotype I and phylotype II strains of the *Ralstonia solanacearum* species complex (RSSC) in Costa Rica. Four strains were isolated from plant material: three from tomato crops (RALF-MA, RALSA-MA and RALHB-MA) and one from banana (RALBN-MA). All isolates were Gram-negative, motile, rod-shaped bacteria, catalase and oxidase positive, and when grown on TCZ agar, the colonies were irregular and white with a red center.
Using hybrid genome assemblies, we performed a phylogenetic analysis to establish an initial overview of the genetic diversity of RSSC in the country. Strain RALF-MA was identified as *R. pseudosolanacearum* phylotype I sequevar 14, closely related to NCCPB0790 and UW180 from Costa Rica and UW5 from Colombia. The remaining isolates belonged to the *R.solanacearum* species: RALSA-MA was a phylotype IIB sequevar 1 closely related to the strains UW72 (Greece), UW551 (Kenya), RS488 (Brazil) and UY031 (Uruguay); RALHB-MA was phylotype IIB sequevar 4A related to UW171, UW172, and UW174 from Colombia; and RALBN-MA was a phylotype IIA sequevar 53, related to ISBF2570 (Brazil), PD3270 (Costa Rica), CFBP2957 (Martinique), and CIP120 (Perú).
Genomic analyses also revealed the presence of multiple virulence factors, including the enzymes pglA, pemB, phX, cbhA along with a broad repertoire of type III secreted effectors: 71 in RALF-MA, 55 in RALSA-MA, 63 in RALHB-MA, and 53 in RALBN. Additionally, we examined potential biological control agents from soil isolates and microbial culture collections, identifying four bacteria with inhibitory activity: a *Pseudomonas* sp., a *Bacillus* sp., a *Xenorhabdus* sp., and a *Photorhabdus* sp.
The information generated here enhances our understanding of RSSC diversity and dynamics in Costa Rica and supports future efforts aimed at disease management and mitigation strategies.

This research was financially supported by Vicerrectoría de Investigación, Universidad de Costa Rica.

№ 5

Ralstonia solanacearum and *Ralstonia pseudosolanacearum* take diverging evolutionary paths in response to challenge by two lytic bacteriophage biocontrol candidates

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Members of the *Ralstonia solanacearum* Species Complex survive in the environment for long periods, posing a serious challenge to disease control. Bacteriophages (bacteria infecting viruses) can kill *Ralstonia* during this between-host stage, potentially offering a powerful biocontrol tool. However, bacteria have developed a range of strategies to evade phage. *Ralstonia* readily evolve phage resistance in culture and individual strains typically encode multiple phage defense systems. Understanding how individual strains' toolkits driveevolution is vital to phage choice and the design of effective biocontrol strategies. To investigate how diverse *Ralstonia* respond to phage, we exposed two strains to two lytic phages and quantified their eco-evolutionary dynamics using experimental evolution. We included the cool-tolerant *Ralstonia solanaceaurm* strain UW551 and the model *Ralstonia pseudosolanacearum* strain GMI1000. While both are susceptible to the phages, they have different defense system repertoires. Remarkably, we saw vastly different evolutionary outcomes between *Ralstonia*-phage combinations. While GMI1000 drove both phages into extinction, UW551 co-existed with both phages throughout. Evolved UW551 isolates, which were resistant to ancestral phage, gained mutations in the LPS biosynthetic pathway. In contrast, GMI1000 isolates had lower levels of resistance to ancestral phage and lacked genomic mutations. However, one GMI1000 line did develop strong resistance and an LPS mutation, suggesting that this evolutionary pathway is available. These diverging pathways reveal how *Ralstonia* genomic tools influence phage response and demonstrates that varying evolutionary trajectories may have profound effects on the long-term benefits of biocontrol. The capacity to evolve resistance must be considered when choosing phages for application.

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№ 6

Adaptive Evolution of Bacterial wilt pathogen to Microplastics

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Microplastics, as an emerging class of contaminants, have been shown to exacerbate a range of plant diseases, including bacterial wilt in tomatoes. To investigate the impact of microplastics on plant pathogens, we established an experimental evolution system for *Ralstonia pseudosolanacearum* under polyethylene terephthalate (PET) exposure. Evolved bacterial lineages displayed distinct phenotypic changes, including altered colony morphology, attenuated motility, enhanced biofilm formation, and increased root colonization capacity. Genomic analyses identified recurrent mutations in the *aqpZ* gene (encoding Aquaporin Z) across independent evolution lines. Functional characterization of *aqpZ* knockout mutants confirmed its role in modulating bacterial motility, biofilm production, and pathogenicity, while also improving oxidative stress tolerance (H_2O_2) and rhizosphere fitness. Our findings uncover a specific genetic mechanism underlying microbial adaptation to microplastic pollution, with important implications for plant disease ecology, and agricultural sustainability.

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№ 7

Unraveling *Ralstonia* Diversity: Insights from Diverse Vegetable Crops in Benin, West Africa

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Bacterial wilt, caused by *Ralstonia solanacearum* (Rs), is a major constraint to key cash crops in West Africa. In Benin, BW is particularly problematic in traditional vegetables (gboma and amaranth) and on key vegetable crops (tomato and pepper). In this study, the distribution of Rs across the main vegetable-growing regions in Benin was characterized for the first time, which is critical to implement targeted regional management. Overall, 27 localities were surveyed, and 50 strains were isolated and confirmed to be Rs-positive by species-specific primers. Rs diversity was broad, and strains from 3 phylotypes I, II & III were isolated. Most isolates (43) identified by PCR using a phylotype-specific primer were phylotype I and were isolated from tomato, pepper, bitter leaf, African basil, and weed (Mauviase herbe). There were 1 strain in phylo I & II (tomato), 2 in phylo II (tomato), 1 in phylo III (pepper), and 3 in phylo II & III (tomato). The presence of Asian (phylo I) strain may be due to accidental introductions from humans. Sequevar analysis using egl primers showed that most strains belonged to sequences 17 and 18, while few others belonged to sequevars 14, 23, and 31. Given that peanuts are a significant source of protein for people in West Africa, greenhouse trials validated the pathogenicity of the seq. 14 strains on peanuts. The targeted approaches to control Rs, can help protect economically important crops and improve livelihood of subsistence farmers in Benin.

№ 8

Ralstonia solanacearum identification in Ecuador: Questioning methodology and incidence.

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Ralstonia solanacearum is a soil-borne pathogen best known for causing bacterial wilt, leading to significant losses in crops such as potato, tomato, and banana (Seleim et al., 2014). Its isolation from contaminated samples can be hindered by slow growth, competition with associated bacteria, and the presence of cells in a viable but non-culturable (VBNC) state (Imazaki & Nakaho, 2010). This study evaluated the selectivity of an isolation protocol based on culturing samples on SMSA supplemented with antibiotics, catalase and oxidase tests, and molecular confirmation using 16S and whole-genome sequencing (WGS). From a total of 40 samples collected from eight provinces in Ecuador (potato: 32, 2 tomato: 2, banana: 6), 235 bacterial isolates were recovered (potato:194, tomato:8, banana:33), of which 34 exhibited characteristics compatible with *R. solanacearum* growth on SMSA and biochemical tests (oxidase + and catalase +). However, molecular analyses confirmed the absence of *R. solanacearum*, identifying instead *Pseudomonas nitroreducens*, *Pseudomonas rhodesiae*, *Pseudomonas tensinigenes*, and *Klebsiella variicola*. These results are consistent with previous reports describing the interference of fast-growing bacteria with similar morphologies in semi-selective media, highlighting methodological limitations (Obrador et al., 2017). Additionally, due to environmental niche conditions including low temperatures, low precipitation, climate change and potentially suppressive soils, Ecuador may be suboptimal for the establishment of *R. solanacearum*, contributing to its low detection (Ferrer et al., 2025). Overall, these findings suggest the need to optimize microbiological isolation protocols through the incorporation of additional biochemical tests, the use of additives in SMSA, alternative culture media such as B-King, and complementary immunoassays.

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№ 9

A putative genomic fusion of the chromosome and megaplasmid within hemagglutinin gene clusters in phyl. I seq. 14 *R. pseudosolanacearum* strain UW588 from Guatemala

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Like most Burkholderiaceae family bacteria, *Ralstonia solanacearum* species complex (RSSC) have multipartite circular genomes. Typically, RSSC have a ~3.5 Mb chromosome and a ~2.1 Mb megaplasmid, a chromid-like secondary replicon. In the process of assembling chromosome-scale genomes of eleven RSSC strains from Guatemala (nine phylotype I sequevar 14 and two phylotype IIA sequevar 6 genomes), we noticed an anomalous genome. The genome of phylotype I strain UW588 assembled as a 5.6 Mb circular contig. To investigate whether UW588 had a bona fide chromosome/megaplasmid fusion, we mapped the raw nanopore reads onto the assembly using minimap2, performed Mauve alignments with closely related strains, and preliminarily confirmed the fusion with diagnostic PCR and sequencing. To further validate the fusion, pulse-field gel electrophoresis is being optimized. The putative fusion occurred within a >1 kb region of perfect nucleotide homology within large ~8 kb genes annotated as hemagglutinins. The recombinogenic hemagglutinins are located in putative two partner secretion gene clusters. Although single-chromosome RSSC strains have not been reported in the genomics era, Rosenberg et al. 1982 identified clear megaplasmids in only nine of the ten RSSC strains analyzed. We speculate that the mechanism of chromosomal/ megaplasmid fusion in UW588 is homologous recombination.

№ 10

Genetic architecture of bacterial wilt resistance in potato wild relatives from Uruguay discovered through genome-wide association studies (GWAS)

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Potato genetic resources distributed in south-eastern South America, such as *Solanum commersonii* (cmm)*, S. malmeanum* (mlm) and *S. chacoense* (chc), are reported to show resistance against bacterial wilt (BW). Screenings of a core collection from Uruguay identified a wider response than previously reported. However, little is known about the genetic architecture underlying the complex mechanisms they deploy during their interaction with *Ralstonia solanacearum*, which may be different in the three species. To establish the genetic basis of resistance to BW in potato wild relatives from Uruguay, we collected 181 diploid (2x) and triploid (3x) accessions from Uruguay to build a diversity panel. We used a large-scale optimized screening method to phenotype the panel with aggressive strain UY031 at $10^{6\text{-}1}$ of soil, with root damage. Disease progression was registered regularly from the onset of the disease until 31 days after inoculation. Resistance level was calculated by the area under disease progress curve (AUDPC). Genotyping through GBS allowed us to identify 17200 high-confidence SNPs using available reference genomes of the three species. The panel showed some genetic structure, with three genetic groups corresponding to the species and differential responses to BW. Higher resistance levels were found in accessions from mlm. Some resistant accessions showed no latency. Preliminary results identified significant associations in some genomic regions and exploration is underway to pinpoint species-specific loci that can be combined for introgression into potato. Further studies on full-sib populations of cmmXcmm and mlmXmlm will shed light on the differential architecture of their BW resistance.

№ 12

Sourcetrack: emergence, expansion and regress of lineages of the potato brown rot genotype in the demarcated area in Belgium

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Potato brown rot was diagnosed for the first time in Belgium in autumn 1989 and since then 35 findings have been formally recorded. The presence of the bacterium was revealed in a network of canalized and natural water courses connected to a main canal in which the introduction of the bacterium occurred. The diagnosis of *Ralstonia solanacearum* in symptomatic tomato plants in a greenhouse crop in 2020 was linked to water from a canal with contamination history and so isolates from tomato should be connected to isolates from the canal, specifically from the spot of water collection. It triggered the investigation of isolates obtained from potato, water courses and bittersweet over a period of 35 years using forensic methods on bacterial genomes.
Genetic diversity was analysed by (1) gene-by-gene allele calling in cgMLST and agMLST, (2) analysis of repeat copy numbers in genomic loci (MLVA) and (3) analysis of the variations in single nucleotides within the genomic assemblies. Several lineages were identified. Subtypes were already present in the isolates from the earliest findings. Their profiles were no longer found in later isolates. Isolates with identical profiles were distributed over the whole demarcated area. Other isolates showed unique profiles, confirming no link with irrigation. The investigation also connected the isolates from tomato with isolates from the spot of water collection by the grower. MLVA and SNP-analysis are considered appropriate tools for connecting findings but a method for divergence-time dating should be applied for retrospective tracking of the infection source.

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№ 13

ExoRal, a collaborative Euphresco initiative to enhance Europe's preparedness for *Ralstonia pseudosolanacearum* and *Ralstonia syzygii*

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Although *Ralstonia pseudosolanacearum* (RALSPS) has traditionally been considered a tropical pathogen, recent detections of Phylotype I in ornamentals, spice crops, tomato, surface water, and bittersweet in several European countries demonstrate its growing adaptability to temperate climates. Meanwhile, *Ralstonia syzygii* (RALSSY, Phylotype IV) remains largely confined to East and Southeast Asia, but has recently been found in Brazil and Kenya as well. Its future introduction to Europe can therefore not be excluded. Consequently, RALSPS – and potentially RALSSY – pose an emerging threat to European agriculture.

In response, the Euphresco initiative 'ExoRal' established a collaborative platform for the exchange of knowledge, best practices and resources among plant bacteriologists studying these pathogens. The project brings together scientists from 20 laboratories across 13 different countries. Through coordinated research, ExoRal seeks to address key gaps in the diagnostics, pathology, and epidemiology of RALSPS and RALSSY, while advancing our understanding of their genetic diversity, population structure, and evolutionary dynamics. These efforts will contribute to more comprehensive strain inventories, improved sampling strategies, harmonised diagnostic methods and enhanced surveillance. One of the project's main outputs is an open-source interactive tool built via Nextstrain (https://nextstrain.org/), which incorporates genomic sequences and molecular typing data to provide a real-time snapshot of evolving RALSPS / RALSSYpopulations. Additionally, a genome database on *Ralstonia* spp. was established in the clasnip platform (www.clasnip.com) for identification based on Single Nucleotide Polymorphisms. With these activities, ExoRal aims to strengthen Europe's capability to anticipate, monitor and respond to the threat posed by exotic *Ralstonia* species.

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№ 14

Ralstonia solanacearum-Hijacked Host Veratric Acid Reprograms NLR-Mediated Immunity to Facilitate Multi-Pathogen Infection

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Soil acidification is strongly correlated with increased susceptibility to soil-borne pathogens like *Ralstonia solanacearum*, but the underlying molecular mechanisms remain unclear. This study reveals that *R. solanacearum* infection induces the accumulation of specific phenolic acids in tobacco, most notably veratric acid, which systemically suppresses plant immunity, thereby facilitating compound infections. Metabolomic profiling showed a greater than 10-fold accumulation of veratric acid in infected tissues. Subsequent transcriptomic analysis revealed a significant downregulation of Nucleotide-binding leucine-rich repeat (NLR) immune receptor genes, with a key gene (28897/TAO1) being suppressed by over 75%. Functional validation demonstrated that exogenous application of veratric acid dramatically increased disease severity, enhancing TMV susceptibility by 4.27-fold and *Pseudomonas syringae* infection by 3-fold. This disease-promoting effect was linked to the inhibition of flg22-induced ROS burst. Mechanistically, dual-luciferase assays confirmed that veratric acid inhibits the promoter activity of the NLR gene 28897 by 71.9%, and Virus-Induced Gene Silencing (VIGS) of this gene exacerbated TMV susceptibility. Our findings challenge the conventional defensive role of plant metabolites by uncovering that a pathogen-induced phenolic acid acts as an immunosuppressant. We propose a “metabolic preconditioning” model where *R. solanacearum* hijacks host metabolism to produce veratric acid, which targets NLR-mediated immunity, thereby creating a favorable environment for subsequent infections by diverse pathogens. This study provides novel insights into the mechanisms of compound infection and offers potential strategies for crop protection through the activation of NLR pathways.

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№ 15

Getting to the Root of Brg11: the conserved RipTAL from *Ralstonia solanacearum*

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Ralstonia solanacearum, the causal agent of bacterial wilt disease affects over 200 plant species spread globally. Just like *Xanthomonas*, *Ralstonia* releases a Transcription Activator Like Effector or shortly referred to as RipTAL via its Type III Secretion System during infection. RipTALs recognize and bind to a specific region in the host DNA called Effector Binding Element (EBE) and manipulate downstream host gene expression. The RipTAL Brg11 activates transcription of host arginine decarboxylase (ADC) genes. ADCs are the sole target of Brg11. This effector binding element has been conserved across all plant species indicating that Brg11 targets an essential plant metabolic pathway therefore playing a broader functional role that merits further investigation.

However, not much is not known about how *Ralstonia* breaches the root barrier and navigates to the xylem. We suspect that only a subset of root cells receive the RipTAL during early infection, triggering localized cellular changes in the host that aid during early infection. Our results have indicated Brg11 induces a change in the polyamine pool of the root tissues. But polyamines have a plethora of functions in biotic stress responses and plant development. This project aims to pinpoint the specific root cell types *Ralstonia* targets to deliver Brg11 and map the transcriptional changes during the early infection stages that are effector dependent. Moreover, we are also tracing the impact of these host metabolic changes on the bacterial transcriptome to understand how Brg11 activity orchestrates *Ralstonia's* early infection strategy.

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№ 16

Modulation of Temperature-Dependent Transcriptomic Profiles by a LysR-Family Regulator in Cool-Virulent *Ralstonia solanacearum* P673

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Ralstonia solanacearum (RS), the causal agent of bacterial wilt, typically causes disease at temperatures above 25 °C. However, phylotype IIB cool-virulent strains can infect at lower temperatures. The select agent R3B2, absent from the USA, threatens potato production, while Florida strains such as P673 may impact tomato crops. We used P673 as a model cool-virulent strain. Previous comparative genomics identified regions unique to cool-virulent strains. Seven regions containing regulatory genes were selected for functional analysis under the hypothesis that differences in virulence may involve regulatory variation. Deletion of a LysR-family regulator (Tr-Phn), associated with phosphonate metabolism, reduced P673 virulence on tomato at 18 °C but not at 30 °C. In this work, to explore temperature-dependent regulation, we performed comparative transcriptomics during early infection using an in vitro tomato system. Tomato seedlings were co-cultured with ~10^7 CFU of P673 and the deletion mutant at 30 °C and 18 °C for 2 and 5 days respectively. Bacterial RNA was processed with Ribo-Zero Plus and sequenced on an Illumina NovaSeq platform. At 18 °C, P673 showed 236 upregulated and 155 downregulated genes compared with 30 °C, including increased expression of a higher number of pathogenicity related genes. In contrast, the Tr-Phn mutant exhibited only 67 upregulated and 57 downregulated at 18 °C, with phosphonate-related genes downregulated at low temperature. These findings suggest a transcriptional reprogramming of P673 genes to adapt to cooler conditions, with virulence mechanisms enhanced at low temperature. The limited changes in the Tr-Phn mutant transcriptome suggest this regulator may modulate multiple pathways beyond phosphonate metabolism.

№ 17

A *Ralstonia* methyl-accepting chemotaxis protein directly binds two plant hormones

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Plant pathogenic *Ralstonia solanacearum* use flagellar motility and chemotaxis to navigate towards the roots of host plants. Chemical inducers of chemotaxis, or chemoeffectors, are sensed by methyl-accepting chemotaxis proteins (MCPs). There are 19-22 MCPs encoded in a typical *Ralstonia* genome. To date only 9 have known chemoeffectors, most of which are primary metabolites or elemental nutrients. However, secondary metabolites, including the plant defense hormone salicylic acid, have previously been shown to act as chemoeffectors for *Ralstonia*. We constructed hybrid chemosensory proteins with the periplasmic portion of *Ralstonia* MCPs translationally fused to the signalling domain of a model two-component sensor from *Escherichia coli*, NarQ, to screen MCPs for direct binding to chemoeffectors. Here, we identify an MCP that directly binds to two plant hormones (salicylic acid and indole-3-acetic acid, a form of auxin) as well as an array of aromatic chemicals. This is also the first MCP that has been shown to directly bind to auxin. This aromatic-binding MCP is conserved in 101 of 106 analyzed complete genomes in the species complex and therefore one of the core *Ralstonia* MCPs. To investigate the role of this aromatic-binding MCP in *Ralstonia* physiology and virulence, we deleted the gene encoding this MCP from *Ralstonia pseudosolanacearum* GMI1000 and challenged the mutant in chemotaxis and virulence assays. Identifying plant growth and stress hormones as chemoeffectors and pairing them to chemoreceptors will deepen our understanding of how *Ralstonia* hijacks plant signalling to facilitate host discovery from the soil.

№ 18

Genomic and epigenetic resistance and susceptibility mechanisms of wild nightshades to *Ralstonia solanacearum*

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During unfavourable conditions, *Ralstonia solanacearum* uses wild plants as natural reservoirs: *Solanum dulcamara,* growing alongside rivers where *R. solanacearum* is present, and *Solanum nigrum*, growing around crop fields. These two plants are closely related to important economic crops such as tomato, potato and aubergine, which are commonly affected by the pathogen. Importantly, *S. dulcamara* is partially resistant to *R. solanacearum*, whilst *S. nigrum* shows high susceptibility. We assembled and annotated de novo the genomes of both plants, and using comparative genomics, we identified some defense-related genes shared only with resistant or susceptible plants. In addition, DNA methylation, which can be inherited, encoding a memory of a stress, and regulates gene expression, was assessed in both plant genomes, particularly in defense-related genes. We confirmed *S. nigrum* showed higher DNA methylation frequency across the whole genome, which could be related to adaptive and evolutionary changes. Interestingly, some *S. dulcamara* and *S. nigrum* defence-related genes showed unique and significantly different methylation frequencies. By assessing the gene expression in different plant tissues, including roots, we linked this DNA methylation to a potential tight epigenetic regulation of these genes. Overall, these two species with contrasting susceptibility to this major pathogen had unique genes shared only with resistant or susceptible plants. Their DNA methylation frequencies, especially in defense genes, could indicate that the expression of redundant disease-related genes could be epigenetically regulated. These genomic resources and candidate genes could facilitate further investigations into new defense mechanisms against *R. solanacearum*.

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№ 19

Root exudate-mediated bacterial wilt resistance in the environmental reservoir plant: *Solanum dulcamara*

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In Northern Europe, *Solanum dulcamara* acts as a natural winter reservoir of the major plant pathogenic bacterium *Ralstonia solanacearum*.Importantly, *S. dulcamara* has been identified as partially resistant to this pathogen, as it rarely shows bacterial wilt symptoms, suggesting it could be a useful source of novel resistance mechanisms. Previous work has reported that this plant restricts bacterial movement and colonisation. In this study, we focused on assessing whether the soil microbiome, which is modulated by root exudates, serves as *S. dulcamara*'s first line of defense against *R. solanacearum*.
By combining metabolomics, transcriptomics and soil metabarcoding, we have demonstrated that *S. dulcamara* has a unique root exudate composition compared with a susceptible tomato. We have identified a key root exudate metabolite, 2,6-dihydroxybenzoic acid (2,6-DHBA), which is produced in higher concentration in *S. dulcamara* than in tomatoes, and its production is further boosted only in *S. dulcamara* when the pathogen is present. This compound inhibits *R. solanacearum* growth in-vitro, and when applied to tomatoes, it also reduces bacterial wilt symptoms and changes the soil bacterial composition, increasing the abundance of plant-growth-promoting taxa such as *Burkholderia phytofirmans*, a bacterium that in-vitro also competes with *R. solanacearum*. By assessing root gene expression, we have linked the production of 2,6-DHBA to *S. dulcamara*'s active defense response and identified a hydroxylase gene that could be associated with the production of this compound.Gene editing tomatoes overexpressing this hydroxylase and consequently overproducing 2,6-DHBA will help combat this deadly disease by combining chemical inhibition and soil suppressiveness.

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№ 20

RipTAL-Mediated Induction of Host Arginine Decarboxylase Confers a Competitive Advantage to *Ralstonia solanacearum*

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Ralstonia solanacearum is a soil-borne pathogen causing bacterial wilt in over 300 plant species (Wang et al., 2023). It enters host plants through their roots, colonising the xylem and causing wilting and death (Zhao et al., 2024). To promote virulence, *Ralstonia* injects type-III secreted effector proteins, including RipTALs, which are homologous to transcription activator-like effectors (TALEs) from *Xanthomonas*. RipTALs from all four *Ralstonia* phylotypes target and activate host arginine decarboxylase (ADC) genes, indicating the conservation of the target gene (~180 million years; Gallas et al., 2023). ADC catalyses the conversion of arginine to agmatine, the precursor of polyamines - metabolites with broad roles in plant responses to biotic and abiotic stresses. Polyamines can also conjugate with hydroxycinnamic acids (HCAs) to form phenolamides. RipTAL-induced ADC expression enhances ADC activity, polyamine accumulation, and phenolamide production.

We have demonstrated that ADC overexpression increases host susceptibility to *Ralstonia* infection. We now aim to elucidate how this metabolic shift benefits the pathogen. We initially hypothesised that RipTAL-mediated induction of phenolamides might modulate the rhizosphere microbiome through antimicrobial activity. However, analysis of plant exudates revealed low external phenolamide levels. This suggests that *Ralstonia* may instead exploit altered polyamine metabolism to suppress host defences - potentially through reduced nitric oxide signalling, diminished reactive oxygen species (ROS) accumulation, and enhanced siderophore production. Collectively, these changes may provide *Ralstonia* with a competitive advantage, facilitating successful colonisation and infection.

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№ 21

Response of BW-Resistant Peanut Variety Zhonghua 6 to *Ralstonia pseudosolanacearum*Infection Revealed by Integrated Transcriptome and Metabolome Analysis

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Peanut (*Arachis hypogaea* L.) is an important oilseed and cash crop but has been severely affected by bacterial wilt (BW) caused by *Ralstonia pseudosolanacearum* in various regions. The molecular mechanisms of BW resistance in peanut have not yet been systematically elucidated. In this study, a highly resistant peanut variety Zhonghua 6 against BW was used to investigate the regulatory mechanisms in responses to *R. pseudosolanacearum* infection through integrated transcriptome and metabolome analysis. A total of 24 transcriptome libraries were sequenced and a total of 154.63 Gb of data were obtained, upon which 1122 differentially expressed genes (DEGs) were identified. A total of 1,614 differentially accumulated metabolites (DAMs) were identified in the metabolome of 48 samples. KEGG joint analysis showed that DEGs and DAMs were mainly co-enriched in the pathways such as phenylpropanoid biosynthesis, ABC transporters, and plant hormone signal transduction. A total of 14 hub genes were identified through the co-expression network constructed by WGCNA, including three core regulatory nodes (two P450 genes, *Ah03g095600* and *Ah13g129000*, as well as the UGDH gene *Ah11g339000*), which might play important roles in response of Zhonghua 6 to *R. pseudosolanacearum* infection. Further metabolomics analysis identified some previously-reported metabolites associated with inhibition to *R. pseudosolanacearum* growth including 6-Methylcoumarin and Erucamide. These metabolites would provide important basis for developing novel bio-based anti-bacterial agents which might be valuable in controlling BW of peanut.

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№ 22

A lectin receptor-like kinase confers lipopolysaccharide recognition and disease resistance in tomato

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Pattern-recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs) to activate innate immunity. Lipopolysaccharide (LPS) from Gram-negative bacteria is a major PAMP in both plants and animals, yet the identity of a bona fide plant LPS receptor has remained unresolved. Although LORE was previously proposed as an LPS receptor, it was later shown to recognize medium-chain 3-hydroxy fatty acids (mc-3-OH-FAs) released during lipid A biosynthesis. Here, we identify a lectin-like receptor kinase required specifically for LPS-, but not mc-3-OH-FAs–induced immune activation and antibacterial resistance in tomato (*Solanum lycopersicum*). The dimerized receptor kinase upon LPS perception induces phosphorylationof lysine 560 (K560), which is essential for LPS-mediated signaling. These findings establish lectin-like receptor kinases as central components of LPS-triggered signaling and provide mechanistic insight into plant immune recognition of Gram-negative bacteria.

№ 23

Plants modulate coumarins accumulation to defend *Ralstonia solanacearum* invasion through inducing systemic resistance

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Bacterial wilt caused by soil-borne pathogen *Ralstonia solanacearum* devastates crop production worldwide, yet effective pesticides against disease remain limited. Coumarins is a major phytoalexin that plays a crucial role in disease resistance. In this study, we discovered that the plant metabolic pathway mediated by coumarins contributes to plant resistance to bacterial wilt disease. *Nicotiana tabacum* enhances accumulation of coumarins in response to *R. solanacearum* infection and plants with deficient coumarins biosynthesis are more susceptible to bacterial wilt. Treatment of scopoletin trigger immune response by activating plant defense enzymes, which enhance salicylic acid (SA) and H_2O_2 accumulation and induce pathogenesis-related (PR) genes and calcium signaling expression. Scopoletin exhibits considerable control effect against tobacco bacterial wilt in field condition without affecting *R. solanacearum* growth. Feruloyl-CoA 6-hydroxylase (NtF6'H1) confers broad-spectrum resistance to pathogen by enhancing accumulation of scopoletin and scopolin, which activates systemic resistance by promoting calcium signals and the accumulation of reactive oxygen species (ROS). This work reveals a metabolite pathway-coumarins involved in plant resistance against *R. solanacearum*, highlights the novel elicitor as a sustainable strategy for controlling plant disease.

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№ 24

Ralstonia pseudosolanacearum PhcQ controls quorum sensing-dependent phenotypes by maintaining the protein stability of PhcA

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The quorum sensing (QS) system plays a crucial role in regulating the virulence of the *Ralstonia solanacearum* species complex(RSSC) It is controlled by *phcBSR* operon and *phcA* genes. PhcQ may contribute to activation of PhcA and regulatesexpression of QS-dependent genes. Here, we genetically demonstrate that PhcQ functions as a key component of the QS regulatory pathway by maintaining the protein stability of PhcA in *R. Pseudosolanacearum*OE1-1. Deletion of *phcQ* significantly increased expression of genes encoding the type III secretion system (T3SS), but decreased expression of genes for synthesis of exopolysaccharide (EPS), consistent with the regulatory behavior of PhcA. The *phcQ* mutants exhibited enhanced growth at early stages and retained weak virulence on tobacco plants, although they completely lost pathogenicity on tomato plants. Through genetic analysis using an overexpression system, we positioned PhcQ downstream of PhcB and upstream of PhcA in the QS regulatory cascade. PhcQ did not affect *phcA* transcription, while proteomic analysis and western blot revealed that PhcQ is essential for maintaining PhcA protein stability, with PhcA protein levels reduced to approximately 20% in *phcQ* mutants. Further structural analysis identified a disordered tail in the PhcA C-terminus that is dispensable for function but critical for protein stability, suggesting that PhcQ may prevent proteolytic degradation of PhcA through this region. Additionally, we identified a conserved phosphorylation site D60 that is essential for PhcA function but not stability. These findings reveal a novel regulatory mechanism where PhcQ maintains PhcA protein stability to ensure proper QS-dependent gene regulation in RSSC.

№ 25

Highly transmissible IIB-1 pandemic lineage of *Ralstonia solanacearum* colonizes potato tubers better than other potato-infecting *Ralstonia* strains

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Potato Brown Rot (BR) disease caused by the *Ralstonia solanacearum* species complex inflicts severe losses on tropical highland farmers. The near-clonal IIB-1 (R3bv2) pandemic lineage of *R. solanacearum* from South America has been globally disseminated in infected potato seed tubers. When IIB-1 was recently introduced to Madagascar, it quickly replaced the less destructive endemic African BR strains and is now causing a major epidemic. Is the IIB-1 pandemic lineage more virulent or more effectively transmitted than native African BR strains? To test this hypothesis, we compared *in planta* behaviors of African BR strain RUN2279 from Madagascar with pandemic lineage strain UW551 and a related *R. solanacearum* IIB-4 strain, CFBP6783. These three strains had similar virulence on tomato at 28°C, but on potato at 22°C UW551 was more virulent than either CFBP6783 or RUN2279. Following soil-soak inoculation all three strains reached similar population sizes in infected potato stems, but CFBP6783 and RUN2279 were much less frequently detected in stolons and tubers than IIB-1 strain UW551. Importantly for disease transmission in potato seed tubers, UW551 was far more likely to cause latent (asymptomatic) infections, and UW551 more often colonized tubers from latently infected plants. UW551 consistently out-competed RUN2279 and CFBP6783 in stems, stolons, and tubers of co-inoculated potato plants, although it was not inhibitory in culture. Unexpectedly, only UW551 triggered expression of potato defense gene PR1. Overall, these results demonstrate that the IIB-1 pandemic lineage is both more virulent and more likely to be transmitted in tubers than other potato-infecting *Ralstonia*.

№ 26

Isolation of *Ralstonia pseudosolanacearum* from *Boesenbergia rotunda* and impact of a novel phage on pathogenicity

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In recent years Fera Science Ltd. have isolated *Ralstonia pseudosolanacearum* from a number of hosts including *Curcuma* sp., *Solanum tuberosum* and *Zingiber* sp. and from previously unrecorded hosts such as *Alpinia galanga* and *Boesenbergia rotunda*. This poster presents a case study of the diagnostic process to identify the presence of *R. pseudosolanacearum* from a symptomatic *B. rotunda* sample and the identification of a phage associated with one of the strains isolated.

Traditional techniques were used to isolate *Ralstonia* spp. typical colonies from symptomatic vascular tissue. Isolates with two distinct colony morphologies were observed and both morphologies were identified as *R. pseudosolanacearum* using a range of molecular tests. Whole genome sequencing was then used to identify differences in the genome which could be causing the difference in morphology, with an Autographiviridae *Ralstonia* phage being found to be associated with one of the colony morphologies and not the other.

Host testing showed that the *R. pseudosolanacearum* with the phage appeared less virulent to both aubergine and tomato, causing only localised infection. The isolate with the phage appeared to be unable to move within the plant, only being re-isolated from the inoculation point three days after inoculation. This gives an example of how phages may serve as biocontrol agents if they are able to limit the movement and spread of *Ralstonia* spp. through a host plant. Further work is required to identify the mechanism through which the phage is interacting with the bacterium and the plant.

№ 27

Two partner secretion systems mediate bacterial contact-dependent antagonism in *Ralstonia pseudosolanacearum*

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Pathogens compete with surrounding microorganisms for limited nutrients and access to hosts. Bacterial contact-dependent growth inhibition (CDI) systems facilitate interbacterial recognition and antagonism through the deployment of large extracellular proteins (CdiA) that carry C-terminal toxin domains. Toxin delivery occurs following receptor-dependent binding of CdiA to a target cell, resulting in growth inhibition unless the recipient expresses the corresponding neutralizing immunity protein (CdiI). The genes encoding CdiA and CdiI are organized within a single locus together with the transporter gene *cdiB*. The plant pathogen *Ralstonia pseudosolanacearum* contains six to ten CDI loci per genome, representing a substantial expansion compared to the one or two CDI loci typically found in *Escherichia coli* and *Burkholderia* species, where CDI systems have been extensively characterized. This expansion may facilitate a wider range of interbacterial interactions. The objective of this study was to determine which of the six predicted CDI loci in the model strain *R. pseudosolanacearum* GMI1000 are important for interbacterial competition. A high-throughput competition assay was developed, and pairwise competition experiments were conducted between CDI locus deletion mutants and the wild-type strain. The results demonstrated that two CDI loci confer reduced competitive fitness when deleted, and complementation with the cognate immunity gene restored fitness. These findings provide insight into the molecular mechanisms that drive competitive interactions in both the rhizosphere and phyllosphere.

№ 28

Visualization and functional roles of *Paraburkholderia*–tomato interactions in bacterial wilt suppression

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The *Ralstonia solanacearum* species complex is a globally important bacterial pathogen causing lethal wilt in more than 250 plant species. Owing to the limited effectiveness of existing chemical control options, rhizobacteria-based biological control has emerged as a promising and eco-friendly strategy. In our previous studies, *Paraburkholderia* species rapidly dominated the tomato rhizosphere following synthetic community application, suggesting their ecological competitiveness and disease-suppressive potential. In this study, we isolated eight *Paraburkholderia* strains from tomato rhizosphere soils and evaluated their ability to suppress bacterial wilt (BW) in tomato plants. One unidentified strain, represented by isolate UR125, reduced BW severity despite showing no direct antibacterial activity against *Ralstonia pseudosolanacearum* SL341 *in vitro*. Interestingly, this biocontrol effect was diminished when the strains were introduced into sterilized soil, indicating that interactions with the native microbiome may be essential for their disease-suppressive function. Whole-genome sequencing revealed exceptionally large genome sizes of approximately 10.5 Mb, suggesting high metabolic versatility. Complementary visualization using GFP-tagged strain of *Paraburkholderia*strain UR125 confirmed successful rhizosphere colonization. This suggests that *Paraburkholderia* strain UR125 possess promising but context-dependent biocontrol potential against BW in tomato. Ongoing research aims to elucidate the ecological and molecular mechanisms underlying their disease mitigation, including interactions with the soil microbiome, colonization dynamics, and genome-encoded functional traits.

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№ 29

An interactive dashboard for global reports of the *Ralstonia solanacearum* species complex

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The *Ralstonia solanacearum* species complex (RSSC) pathogens are genetically diverse and the lineages vary in their biogeography and their complex host patterns. Access to pathogen isolation data is vital for understanding pathogen distribution and for improving disease management strategies to safeguard against impactful crop pathogens, but RSSC pathogen metadata remains difficult to access as the information is fragmented across the scientific literature. To better understand these ecological patterns, we previously developed a centralized resource of consolidated pathogen metadata, the RSSC Diversity Dataset / Database, a living pre-print and supplemental Excel spreadsheet with a stable digital object identifier (DOI) and version control (https://www.biorxiv.org/content/10.1101/2020.07.13.189936v5). The RSSC Diversity Dataset / Database catalogs the information of nearly 10,000 strains curated from over 300 sources that report one or more *Ralstonia* strains isolated from over 400 host plant species from over 100 geographic regions. Recently, we developed an interactive web dashboard (https://ralstoniadashboard.shinyapps.io/RalstoniaWiltDashboard/) to visualize the geographic distribution of reported isolates and their reported indicidence on host plants. The dashboard visualizes data in the form of maps, charts, and tables with a variety of user-interactive filters for taxonomic, geographic, and host of isolation specifications. This *Ralstonia* Wilt Dashboard will aid in communicating knowledge to researchers, regulatory scientists, and other stakeholders to improve disease control and regulation.

№ 30

Deciphering molecular specificity in host-associated microbiome-pathogen interactions

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Bacterial pests *Ralstonia solanacearum* and *Clavibacter sepedonicus* pose significant risks to economically important crops. Their infections are difficult to detect early, symptoms can be confused with those of other diseases, and the pathogens can remain latent and environmentally persistent for long periods, thereby enabling undetected spread. The POMATO project is a collaborative consortium project aimed at addressing these challenges in potato and tomato crops by developing early detection systems, biocontrol solutions, and Integrated Pest Management (IPM) strategies to reduce pest incidence by 40-60%.

Under the POMATO project, this work outlines a planned computational approach to explore the molecular mechanisms driving host-pathogen interaction specificity. Complementary models—including genome-scale metabolic models, association networks, and data-driven models—will be used to maximise information captured on molecular determinants of disease. Metagenomics and metatranscriptomics of plant-associated consortia aggregated with different pathogen strains will be analysed across multiple disease stages. A multi-layer network is planned to explore (i) how pathways and functions respond to changes in taxon abundance, (ii) potential interactions between taxa, and (iii) taxa and associated pathways/ functions that vary with pathogen abundance. Multi-omics integration methods such as MOFA, DIABLO, and Random Forest will be evaluated in their ability to integrate heterogeneous data and identify candidate molecular markers of disease. Based on these markers and interactions, dynamic microbiome models are intended to be developed and subsequently validated. By combining complementary methods that capture distinct relationships and optimise for different objectives, this work aims to provide multi-layered insight into host-associated microbiome-pathogen interactions.

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№ 31

A synthetic community derived from rhizosphere microbes enhances cultivar-specific resistance against bacterial wilt in tomato

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Bacterial wilt (BW), caused by the *Ralstonia solanacearum* species complex, threatens *Solanaceae* productivity. The tomato cultivar Hawaii 7996 (H6) displays quantitative resistance, while Moneymaker (MM) is susceptible to BW. We previously identified an upland soil microbiota (Upland) that specifically reduced BW severity in H6 but not in MM. To investigate the mechanisms, we used DESeq2 and Random Forest to identify key cultivar-enriched ASVs. We first assembled a synthetic community (SynCom-H6) using the identified H6-enriched ASVs. As might be expected from conventional approaches, SynCom-H6 broadly reduced disease severity in both the resistant H6 and the susceptible MM cultivars. However, this conventional finding did not explain the cultivar-specific effect of the original Upland microbiota. We observed that Upland transplantation resulted in lower rhizosphere alpha-diversity in H6 than in MM. This led us to hypothesize that ASVs abundant in the susceptible MM rhizosphere may be the key to the specific protection. To test this, we prepared a second synthetic community (SynCom-MM) using the identified MM-enriched ASVs. This SynCom-MM successfully reproduced the original Upland effect by reducing BW severity only in the resistant cultivar H6. These results highlight anunexpected strategy of selecting beneficial taxa from a susceptible host's microbiome, which can uncover microbial consortia that reinforce resistance in resistant cultivars. This approach offers a new perspective for microbiome-mediated resistance research that susceptible cultivar may carry certain beneficial microbes that can be excluded from resistant cultivar.

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№ 32

Strain-Level Variation in Pathogenic Invaders Drives Rhizosphere Community Shifts

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The invasion by phytopathogens is often associated with disruption to the resident microbiota. However, the extent to which strain-level variation among pathogens differentially influences these microbial shifts remains poorly understood. Here, we initially conducted a meta-analysis encompassing bacterial, fungal, and nematode pathogens, demonstrating that intraspecific variation within phytopathogen groups is associated with distinct impacts on local microbiota structure. We further validated these findings through inoculation experiments using 38 strains of the bacterial phytopathogen *Ralstonia solanacearum*. These strains were characterized using 10 response traits (e.g., motility, acid/alkali tolerance) and 41 resource utilization metabolisms (e.g., amino and organic acids), which together collectively explained a large proportion of the variation in resident microbial community structure following inoculation. Our results revealed that increasing differences in strain-level traits significantly influences community structural dissimilarity and the contribution of deterministic processes to resident microbial community assembly. Specifically, invaders with similar traits produced similar community changes dominated by deterministic processes, while invaders with divergent traits generated more diverse and heterogeneous effects. Together, these findings underscore the ecological significance of strain-level diversity, from pathogens to beneficial microbial inoculants, offering crucial insights for understanding invader-microbiota interactions and developing strategies for precision microbiota control of diseases.

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№ 33

Influence of rhizosphere microbiota and soil type on tomato hibrids tolerance to bacterial wilt caused by *Ralstonia* Phylotype I and IIB isolated from Costa Rica

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We investigated how interactions between the rhizosphere microbiota of resistant (Acorazado) and susceptible (Gladiador) tomato hybrids, together with soil bacterial communities, influence bacterial wilt severity caused by two RSSC strains isolated in Costa Rica: *Ralstonia solanacearum* RALSA (IIB) and *R. pseudosolanacearum* RALF (I-14). Tomato plants were inoculated in greenhouse conditions by immersing 24-day-old seedlings in a 1.5×10^8 CFU suspension of each strain and planted in two contrasting soils. Disease incidence and severity were recorded for 10 days post-infection, when rhizosphere soil DNA was extracted and sequenced (Macrogen) for the 16S rRNA V3–V4 region. Symptoms appeared by 3 dpi in susceptible plants inoculated with RALF and in all plants inoculated with RALSA. Phylotype IIB caused the highest severity (4.5–5.0), except in Acorazado grown in Soil A, which showed reduced disease (3.5). Infections with phylotype I were severe only in Gladiador–Soil B; all other treatments remained at early disease stages. RALSA-inoculated plants showed greater abundance of *Ralstonia* reads, with maxima in susceptible plants in Soil B. In contrast, RALF generated low read counts except in Gladiador–Soil B. Soil A consistently reduced severity and *Ralstonia* abundance, particularly in the susceptible hybrid. ANOSIM indicated that soil type significantly structured rhizosphere communities ($R=0.41$, $p<0.005$). ASVs affiliated with *Rhodoplanes* correlated with severe infections in Gladiador, whereas *Janibacter*, *Micromonospora*, and *Usitatibacter*. were associated with suppression of both phylotypes. These results highlight role of soil–genotype–microbiome interactions as modulators of wilt severity and identify taxons potentially associated with disease severity.

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№ 34

Ralstonia solanacearum in surface water in the Czech Republic since 2010

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Ralstonia solanacearum is a quarantine pest causing bacterial wilt and brown rot of potatoes. Sources of bacterial inoculum include irrigation and , weeds, infested soil, latently infected propagative plant material and contaminated agricultural tools and equipment. In the Czech Republic, the surface water is important for irrigation of production areas. The first finding of the bacterium in the Czech Republic was in 2010 in a water sample taken from the river Labe. Later on, in 2013, the bacterium was found in samples from the river Dyje. In 2016, *Ralstonia solanacearum* was detected in root system of *Solanum dulcamara* weed sampleemerged in Dyje river. Findings also confirmed a relation between water temperature and presence of bacteria, as it was published by Elphinstone et al. (1998) and Caruso et al. (2005). There is also a correlation between occurrence of bacterium in weed and water samples collected during survey. Detection and identification of the pest was performed according to the EPPO diagnostic standard PM 7/21 *Ralstonia solanacearum*, *R. pseudosolanacearum* and *R*. syzygii (*Ralstonia solanacearum* species complex). *Ralstonia solanacearum* (phylotype II) was confirmed in all suspected isolates. Since 2022, the detection survey according to the COMMISSION IMPLEMENTING REGULATION (EU) 2022/1193 of 11 July 2022 establishing measures to eradicate and prevent the spread of *Ralstonia solanacearum* (Smith 1896) Yabuuchi et al. 1996 emend. Safni et al. 2014, is being conducted.

№ 36

Predicting rhizosphere synbiotics for targeted pathogen suppression

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Ralstonia solanacearum is the causal agent of bacterial wilt and can infect hundreds of crops worldwide. A common preventive strategy is inoculating probiotics that suppress pathogens; however, their efficacy in natural environments is often unstable due to poor colonization, largely driven by the absence of suitable nutrient substrates. Synbiotics combine probiotics with matched prebiotics, offering a targeted strategy to support beneficial microbes through specific carbon sources. Yet, empirical screening for effective synbiotic combinations across diverse plant–soil systems is labor-intensive and inefficient. Here, we integrated soil metagenomics and root metabolomics to construct genome-scale metabolic models capable of predicting optimal matches of plant-derived prebiotics and rhizosphere probiotics for pathogen suppression. Using three Solanaceae crops, both *in vitro* and *in planta* validation demonstrated that our models achieved ~93% accuracy in identifying rhizosphere synbiotics. This study establishes a new framework for harnessing the rhizosphere microbiomes and root exudates to developing synbiotics to control soil-borne pathogens precisely across different plant-soil systems.

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№ 37

Substrate utilization and cross-feeding synergistically determine microbiome resistance to pathogen invasion

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Ralstonia solanacearum, the causal agent of bacterial wilt, leads to severe economic losses worldwide. Yet, an intriguing phenomenon is often observed— even under same field conditions, some plants remain remarkably healthy.Understanding how plant-associated microbiomes resist phytopathogen invasion remains a key challenge in natural ecosystems. Here we combined genome-scale metabolic models with synthetic community experiments, both in vitro and in planta, to unravel the mechanisms driving pathogen suppression. We developed curated genome-scale models for each strain, incorporating 48 common resource utilization profiles to fully capture their metabolic capacities. Trophic interactions inferred from models effectively predicted pathogen invasion outcomes across diverse microbial communities and nutrient environments. Importantly, considering both substrate and metabolite features provided a more holistic understanding of pathogen suppression. In particular, cross-feeding metabolites within the native community emerged as crucial yet often overlooked predictors of community resistance, disproportionally favoring native species over invaders. This study lays the foundation for designing disease-resistant microbiomes, with broad implications for mitigating pathogen exposure in diverse environments.

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№ 38

A Decade of Field Research on Bacterial Wilt Management in Tomato: Challenges, Limitations, and Practical Approaches

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Bacterial wilt caused by *Ralstonia* spp. remains one of the most difficult bacterial diseases to control in global tomato production, particularly in warm climates. Despite decades of research, integrated disease management strategies in the field remain elusive. We conducted over a decade of field research, exploring management tactics including host resistance, grafting technologies, and plant defense inducers. Effective field management requires combining multiple complementary approaches, each with inherent limitations. We evaluated host resistance using global tomato germplasm collected over 40 years at the WorldVeg Center, Taiwan, identifying differential resistance to phylotype I and II strains. Screening global tomato accessions revealed that the *Bwr-12* marker confers resistance to phylotype I but not phylotype II, while *Bwr-6* confers resistance to both phylotypes, providing critical insights for marker-assisted breeding strategies. Grafting using resistant rootstocks provided dual benefits against both bacterial wilt and root-knot nematodes, addressing concurrent production challenges. Transgenic approaches co-expressing disease resistance genes (*Bs2* and *EFR*) demonstrated broad-spectrum field efficacy against bacterial wilt and bacterial spot under field conditions. Systemic acquired resistance (SAR) inducers enhanced plant defense, though application method proved critical for consistent field performance. Our findings demonstrate that bacterial wilt management depends on understanding local epidemiological conditions and production systems. We present practical frameworks for developing region-specific management packages combining available tools-host resistance, grafting strategies, cultural practices, and chemical inducers-while acknowledging local *Ralstonia* populations and farmer constraints.

№ 39

Tomato (*Solanum lycopersicum*) Lines Exhibiting Durable Resistance to Bacterial Wilt Caused by *Ralstonia solanacearum* under Waterlogging Stress

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Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is among the most destructive diseases of tomato (*Solanum lycopersicum*), particularly under humid and waterlogged conditions in the Philippines. This study screened 156 IPB germplasm accessions for BW resistance, evaluated their response under combined BW and waterlogging (BW+WL) stress, and examined associated physiological mechanisms. Disease severity assessment identified 16 resistant and 24 moderately resistant accessions, suggesting a quantitative mode of inheritance. Under BW+WL stress, seven accessions (TM11, TM16, TM46, among others) maintained high greenness (Excess Green Index, ExG), indicating durable resistance. TM20, resistant to BW alone, exhibited reduced tolerance under combined stress. Adventitious root formation (ARF) was significantly correlated with WL tolerance, as ARF-bearing plants had higher ExG (median = 100) than those without (median = 60; $p < 0.0001$; Cohen's $d = 2.94$). Root analysis showed that resistant lines TM20 and TM48 developed more secondary roots under BW challenge, while bacterial quantification confirmed restricted systemic spread beyond the middle stem ($\chi^2 = 19.65$, $p < 0.001$). Resistance appears to be mediated by enhanced root proliferation and vascular blockage. These BW- and WL-tolerant accessions represent promising genetic resources for breeding climate-resilient, high-yielding tomato varieties suitable for off-season production.

№ 40

Development of an *in vitro* bioassay to screen for *Ralstonia* resistance in potato

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Bacteria from the *Ralstonia solanacearum* species complex (RSSC) cause bacterial wilt and are among the most destructive pathogens worldwide affecting potato (*Solanum tuberosum*). We aimed to develop a robust and reproducible bioassay for the rapid evaluation of resistance levels among potato genotypes against different RSSC strains. To optimize the assay conditions, pre-cultivation temperatures of 20°C and 24°C, incubation temperatures of 24°C and 28°C, different concentrations of MS medium (0.86 g/L, 2.15 g/L and 4.3 g/L), growth tube types, and growing media were assessed for their influence on plant development and disease dynamics. Preliminary results suggest that De Wit tubes filled with vermiculite provide a reliable and efficient environment for differentiating resistance responses to RSSC strains. The predictive accuracy of the bioassay was evaluated through comparative greenhouse experiments, which revealed a moderate to strong correlation between disease outcomes under both conditions. *In vitro* bioassays offer several advantages for resistance screening, including low cost, speed, and scalability, enabling large-scale evaluations in a shorter time and at reduced expense. The developed bioassay presents a promising, high-throughput approach for identifying resistance sources within potato germplasm. However, further validation is required to assess the reproducibility and broader applicability of this bioassay.

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№ 41

Phage Therapy as an Efficient and Viable Strategy to Manage Bacterial Wilt in Eucalyptus

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Eucalyptus cultivation represents one of the most important agroforestry industries in Brazil, contributing approximately 1.1% to the national GDP. However, bacterial wilt (BW), caused by members of the *Ralstonia solanacearum* species complex, remains a major constraint to sustainable production. Current management strategies, including chemical treatments and resistance breeding, have shown limited efficacy. In this context, bacteriophages (phages) have re-emerged as a promising biocontrol tool against *Ralstonia*, offering a targeted and environmentally friendly alternative.

In this study, we isolated and characterized four phages from soils of eucalyptus nurseries affected by *Ralstonia*. Their biocontrol potential was evaluated under operational nursery conditions of a commercial forestry company, where eucalyptus seedling roots were treated with phage suspensions prior to planting. Trenches were subsequently irrigated with phage formulations every 15 days for a period of three months. Phage treatments significantly reduced both the incidence of bacterial wilt (by 62–70%) and *Ralstonia* detection in the substrate (by 72%), demonstrating effective suppression of the pathogen in a real-scale production system.

To our knowledge, this represents the first *in situ* demonstration of bacteriophage-mediated control of *Ralstonia* in an agricultural system in Brazil. These results provide strong evidence that phages can be successfully integrated into biocontrol strategies for bacterial wilt management in eucalyptus. Overall, these findings validate the feasibility of implementing phage-based treatments within operational forestry systems, paving the way for large-scale, sustainable disease management solutions.

№ 42

Virulence assessment of *Ralstonia solanacearum* (phylotype II) isolated from ornamental *Rosa* spp. plants

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Ralstonia solanacearum (phylotype II) isolates PD 7421 and PD 7394, found in 2018 in asymptomatic ornamental rose (*Rosa* spp.), were assessed for their virulence in two rose cultivars ("Armando" and "Red Naomi"). After stem inoculation, plants were incubated at 20°C and 26°C for 106 days. Disease severity was assessed during this period and re-isolations were performed from symptomatic plants, in order to confirm the *R. solanacearum* infections. Plants showing no symptoms at 106 dpi were also included for the re-isolations in order to evaluate the presence of *R. solanacearum* in a latent state. The identity of re-isolates exhibiting typical colony morphology was confirmed by MALDI-TOF MS analysis. While rose inoculated with *Ralstonia pseudosolanacearum* (phylotype I) isolate PD 7123, a reference strain previously shown to result in high disease severity in ornamental rose, showed severe symptoms at both temperatures, no typical symptoms were acquired for *R. solanacearum* isolates PD 7421 and PD 7394 on the rose cultivars included in this study, irrespective of the temperature. *R. solanacearum* (phylotype II) is known as a major potato pathogen, causing brown rot in potato. Whole genome multilocus sequence typing analysis demonstrated that the phylotype II isolates from rose were closely related to phylotype II isolates previously found in seed potato and surface water in the Netherlands. Because of this close genetic relatedness, the virulence of PD 7421 and PD 7394 was also assessed in potato plants, where both isolates caused severe disease symptoms on both the above ground plant and the daughter tubers.

№ 43

Validation of a high sample-throughput real-time PCR method for the detection of *Ralstoniasolanacearum, R. pseudosolanacearum* and *Clavibacter sepedonicus* in potato

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The NAK tests more than 25 000 asymptomatic potato samples —consisting of 200 tubers each— per growing season for, *Ralstonia solanacearum*, *R. pseudosolanacearum* and *Clavibacter sepedonicus* using immunofluorescence under EN ISO/IEC 17025:2017 accreditation.
The desire to shift from immuno-based to qPCR-based methods has been increasing over time, considering concerns of occupational health and safety, usage of animal-derived antibodies, and analytical sensitivity and specificity.
Previously, validation experiments of the NYtor real-time PCR (EPPO PM 7/21 – appendix 7) were performed, however, due to the large number of samples which are processed within a 3 to 4 month period, a disproportionate number of implementation challenges had to be overcome.
Therefore, a new validation process was initiated with implementation risk assessments taken into account throughout the relatively short project time. Stakeholders representing the routine laboratory decided for methods that had operational benefits over analytical sensitivity, anticipating a successful implementation.
Here, we present the validation of a method for high sample-throughput that is ready for implementation in the routine laboratory of the NAK and able to detect at least 1×10^4 cells/mL in resuspended pellet from asymptomatic potato tubers in accordance with the Commission Implementing Regulation (EU) 2022/1193 and (EU) 2022/1194.

№ 44

Surveillance and laboratory detection of *Ralstonia solanacearum* in Latvia

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Ralstonia solanacearum (Smith 1896) (RALSSL) is a quarantine pest listed in the EU A2 (Annex II B) and EPPO A2 list. It affects many plants from the *Solanaceae* family, including potatoes (EPPO PM 9/3 (3)). To minimize this risk, Latvia performs regular monitoring and testing for the RALSSL. The National Phytosanitary Laboratory (NPhL) tests approximately 700 potato tuber samples each year, as well as around 25 water samples and a similar number of samples from plants such as tomatoes, roses and wild hosts like bittersweet (*Solanum dulcamara*) and black nightshade (*Solanum nigrum*). According to national requirements (Regulation, Cabinet of Ministers Republic of Latvia No. 572), the standard potato sample contains 200 tubers for every 50,000 kg of seed potatoes or 200,000 kg of potatoes used for food or processing. Water sample must be at least 500 ml.
Until 2025, RALSSL had not been detected in Latvia even host plants are widely present. However, in April 2025, during routine import controls, the Food and Veterinary Service (PVD) took a sample of potatoes originating from Egypt. Further testing performed by NPhL confirmed the presence of RALSSL. Conformation of the result was carried out by the EURL in bacteriology (ILVO, Belgium), which verified that the isolate belongs to phylotype IIB, sequevar 1. This was the first officially confirmed finding of the pathogen in NPhL of Latvia.
This highlights the importance of monitoring and import controls, as climate change may raise the risk of RALSSL spread in Latvia.

№ 45

Assessment of pre-qPCR enrichment approaches to enhance detection of *Ralstonia solanacearum* in asymptomatic geranium plants

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Ralstonia solanacearum race 3 biovar 2 (r3b2), a United States select agent pathogen, causes potato brown rot and has repeatedly entered the country through latently infected geranium cuttings. Reliable molecular detection in asymptomatic plant material is challenging due to low bacterial concentrations, making bacterial enrichment steps potentially useful. This study assessed a 24-hour pre-qPCR enrichment protocol using three media - mCPG, mSMSA, and mCPG supplemented with antibiotics (mCPG+Ab) - to assess their ability to improve detection of the r3b2 strain UW551 and the non-r3b2 strain RUN302 in geranium. Across 79 experimentally inoculated plants, all media performed similarly, indicating that antibiotic amendments did not influence qPCR outcomes when using strain-specific assays. Symptomatic plants consistently produced initial qPCR Cq values < 25, whereas asymptomatic plants showed a broad range from Cq of 20.43 to undetectable. Enrichment markedly improved qPCR sensitivity for samples with initial Cq < 34. However, results were variable for samples with initial Cq ≥ 34: only 45% of these samples exhibited Cq deductions of at least 2, with decreases up to 13.65 for UW551 and 16.43 for RUN3022. Overall, the 24-hour enrichment step significantly enhanced qPCR detection of both r3b2 and non-r3b2 strains in symptomatic and asymptomatic geraniums, although its effectiveness was inconsistent when initial bacterial populations were very low (Cq ≥ 34). These findings highlight the limitations of enrichment for improving detection of very low pathogen levels in geranium.

№ 46

Wood hemicellulose–based spray-dried microencapsulation of a lytic bacteriophage preserves phage viability and improves control of *Ralstonia solanacearum*

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Bacteriophage biocontrol is a promising sustainable management method for bacterial wilt disease. However, for phage therapy to be effective, practical, and scalable, more work is needed to design methods to prepare phage for storage and application. We investigated the use of wood hemicelluloses, glucuronoxylans (GX) and galactoglucomannans (GGM), to encapsulate a lytic phage that infects the pandemic *Ralstonia solanacearum* (RS) strain UW551. Encapsulation via spray drying under two temperature conditions was compared to the conventional excipient maltodextrin (MD). The resulting powders were stored at room temperature or 4°C. Phage titers were measured after drying, and then weekly for 25 weeks. GX yielded the highest titer of encapsulated phage and preserved phage survival effectively at 4°C. GGM had poor results, with low survival after spray drying and low long-term stability at either temperature. *In vitro* experiments demonstrated that encapsulated phages inhibited RS as efficiently as unencapsulated phage. Phage encapsulated in GX and MD also reduced bacterial wilt symptoms on tomato. Strikingly, at low MOIs, phage encapsulated in GX and MD reduced symptoms more than unencapsulated phage, suggesting the excipients themselves could be affecting RS. We found that GX alone could inhibit RS growth *in vitro* and reduce disease progression *in planta* without phage. Together, these results show that the encapsulation of phages in hemicelluloses has great promise for efficient biocontrol methods to combat bacterial wilt disease. Not only are hemicelluloses effective in phage preservation, but also have potential to enhance phage biocontrol efficacy through their antimicrobial activities.

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№ 47

Differences in Suppression of *Ralstonia pseudosolanacearum* Proliferation among Peanut Genotypes

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Bacterial wilt (BW) caused by *Ralstonia pseudosolanacearum* (*Rp*) is a significant threat to peanut production, and utilizing resistant varieties is the most effective measure to control this disease in peanut. In previous studies, a lot ofBW-resistant peanut genotypes were identified and showed variations in root morphology, latency period, and disease resistance loci. This study aims to further compare and analyse the dynamic changes of *Rp* populations in different peanut genotypes post artificial inoculation. A *Rp* strain carrying M-cherry gene was generated, and a linear correlation equation between its CFU and CT values of quantitative real-time PCR (qPCR) was established. The population dynamics of *Rp* were measured in five resistant accessions and one susceptible line at different time points post infection. Comparative analysis of different tissues revealed the highest Rp counts in the roots, followed by the hypocotyl and main stem. The highest bacterial counts in the susceptible genotype, while resistant lines exhibited stronger suppression of bacterial proliferation. Additionally, laser scanning confocal microscopy was used to observe the distribution of M-cherry-labelled *Rp* in the roots of resistant and susceptible peanut genotypes after inoculation and showed consistent results with the qPCR profiling. These findings laida foundation for elucidating resistance mechanisms of peanut against bacterial wilt disease.

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№ 48

Quantitative detection and risk analysis of pathogenic *Ralstonia* spp. in tobacco-planting soils from Yunnan Province of China

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Bacterial wilt of tobacco (BWT) isa critical soil-borne disease in Yunnan Province, China.Four plant pathogenic *Ralstonia* spp. (PPRs), including*Ralstonia nicotianae*,*R. solanacearum*,*R. pseudosolanacearum*, and*R. syzygii*, are known to cause this disease. The overwintering population size of PPRs in soil is a key factor influencing the occurrence and prevalence of BWT. We employed a previously established qPCR detection system toquantitatively determinePPRs in soil prior to flue-cured tobacco transplantation, and we alsomonitored the incidence of BWT from the rapid growth stage to the maturity stage of tobacco plants.The results showed that the population threshold of PPRs inducingBWT was 10^3 to 10^4 genomes per gram of soil (genomes/g). Analysis of 716 soil samples revealed that those with PPR contents ranging from 10^4 to 10^5 genomes/g accounted for the largest proportion (37.43%). The abundance of PPRs insoils from fields under spring rotation with gramineous crops was significantly lower than that in continuously cropped tobacco fields. Compared with winter fallow, soils planted with crops from the Solanaceae, Polygonaceae, and Cruciferaceae families had higher PPR contents. Conversely, soils cultivated with crops from the Fabaceae and Asteraceae families had lower PPR contents, with only Fabaceae cropssignificantly reducingPPR populations in soil. Based on the pathogenic threshold of 10^3~10^4 genomes/g, the risk of BWT in six cities of Yunnan Province is relatively high, requiring close attention to its prevention and control.

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№ 49

Rhizosphere and Endosphere Exploration for Potential Biocontrol Agents against Bugtok Disease in Banana

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The Philippines cultivates one of the most favorite fruits in the world—the banana. However, this crop continues to be affected by recurring diseases. While the fight against Fusarium wilt is not yet fully resolved, another epidemic has emerged: Bugtok disease, caused by *Ralstonia solanacearum* race 2. The pathogen infects the vascular system of banana plants, leading to fruit discoloration, wilting, and eventual plant death. This study focuses on the isolation and identification of *R. solanacearum* from infected banana samples collected in the CALABARZON region, Philippines, and the screening of antagonistic microorganisms with potential biocontrol activity. Diseased specimens were subjected to ooze tests and cultured on Tetrazolium Chloride Agar (TZCA), where typical colonies of *R. solanacearum* exhibiting white centers with pink pigmentation were obtained. Molecular identification through PCR and sequencing confirmed the isolates as *R. solanacearum*. To identify potential biological control agents, bacterial and fungal endophytes isolated from healthy banana and weed tissues were screened using antagonistic assays. Among 115 isolates initially tested, 10 bacterial and 5 fungal isolates showed inhibitory effects against *R. solanacearum*. The study also evaluated preservation methods for biocontrol isolates, revealing that 55% of fungal isolates remained viable using the modified cotton-ball method and 50% on refrigerated PDA slants after one year, while all bacterial isolates stored in sterile distilled water survived for six months compared to 75% under refrigeration. These findings show the potential of naturally occurring endophytes as sustainable and eco-friendly biological control agents against Bugtok disease in bananas.

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№ 50

An interlaboratory comparison of detection and identification methods for *Ralstonia pseudosolanacearum* and *Ralstonia syzygii* as part of the Euphresco ExoRal initiative

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Although *Ralstonia pseudosolanacearum* (RALSPS) has traditionally been considered a tropical pathogen, recent detections of Phylotype I in ornamentals, spice crops, tomato, surface water, and bittersweet in several European countries demonstrate its growing adaptability to temperate climates. Meanwhile, *Ralstonia syzygii* (RALSSY, Phylotype IV) remains largely confined to East and Southeast Asia, but has recently been found in Brazil and Kenya as well. Its future introduction to Europe and other continents with temperate climates can therefore not be excluded. Consequently, RALSPS – and potentially RALSSY – pose an emerging threat to European agriculture.

In response, the Euphresco initiative 'ExoRal' established a collaborative platform for the exchange of knowledge, best practices and resources among plant bacteriologists studying these pathogens. The project brings together scientists from 20 laboratories across 13 different countries. Through coordinated research, ExoRal seeks to address key gaps in the diagnostics, pathology, and epidemiology of RALSPS and RALSSY, while advancing our understanding of their genetic diversity, population structure, and evolutionary dynamics. These efforts will contribute to more comprehensive strain inventories, improved sampling strategies, harmonised diagnostic methods and enhanced surveillance. One of the work packages of the project focused on the organisation of an interlaboratory comparison (ILC) for the detection and identification of *R. pseudosolanacearum* and *R. syzygii*. This ILC allowed the participants to gain experience with diagnostics tests for these organisms and provides insights in the effectiveness of the included tests. The main conclusions of the ILC will be presented. With these activities, ExoRal aims to strengthen Europe's capability to anticipate, monitor and respond to the threat posed by exotic *Ralstonia* species.

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№ 51

BIP: a validated high-throughput sequencing test for the identification of bacteria

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Identification of (quarantine) bacteria at NIVIP-NVWA is performed in different ways depending on the kind of bacteria we are trying to identify. Methods like PCR, DNA barcoding, and MALDI-TOF MS are employed, but these can be limited by their targeted nature, the need for species-specific targets, or reliance on limited databases, respectively. Additionally, these approaches are unable to define the full scope of variation within and between species. To address these limitations, we have developed and validated the Bacterial Identification Pipeline (BIP), an automated, untargeted test based on Illumina high-throughput sequencing (HTS).

The BIP workflow processes raw Illumina sequence reads from pure bacterial cultures through a Snakemake pipeline. This pipeline automates quality control, de novo genome assembly, and annotation. For identification, the assembled genome is compared against the Genome Taxonomy Datase using skani. The pipeline also integrates the NCBI Prokaryotic Genome Annotation Pipeline for genome annotation, a taxonomy check, and to assess genome completeness, thus ensuring high-quality data. Furthermore, BIP provides context by automatically generating a phylogenetic tree based on average nucleotide identity (ANI) scores with type strains of the identified genus or, for quarantine organisms, by using a curated database.

BIP offers a single, powerful, and untargeted test for almost all described bacteria, regardless of prior knowledge. This HTS test provides a comprehensive solution for bacterial diagnostics, delivering a high level of detail, combined with a user-friendly interface, that can be used for identification, pathogen surveillance and more.

№ 52

The NIVIP collection of *Ralstonia solanacearum* species complex (RSSC): safeguard for diagnostic and research purposes in plant health

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Collections of bacterial plant pathogens are vital resources for diagnostic and research purposes, allowing the study of their genetic makeup, relatedness, function and evolution. Especially for plant pathogens belonging to a bacterial species complex, such as the*Ralstonia solanacearum* species complex (RSSC), availability of well-characterized collections is of a much higher significance, contributing to better preparedness for emerging *Ralstonia* threats in Europe and beyond. The Bacteriology group of the Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP) has a long history of testing regulated *Ralstonia* species, dating back to the 1990s. Because isolation has always been, and still is, a key component of the standard diagnostic protocol for *Ralstonia*, the collection has been significantly expanded through the years and includes RSSC isolates obtained from diagnostic samples from national surveillance activities, from imported propagation plant material (consignments) entering the country, and from various research projects. These RSSC isolates cover a broad range of host plants, geographic origins, and years of isolation and are long-term maintained at the NIVIP as freeze-dried specimens. Ongoing characterization of isolates, using microbiological and advanced molecular methods, mostly Next Generation Sequencing techniques, ensures accurate species assignment. The RSSC collection at NIVIP serves as a reliable foundation for accurate diagnostics, method validation and long-term monitoring of strain diversity within the framework of the Dutch National Reference Laboratory (NRL) and the European Union Reference Laboratory (EURL). RSSC isolates can be requested for collaborative research, proficiency testing, and method development.

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№ 53

Chemical warfare in the rhizosphere: unpicking the defensive role of plant root exudates against the bacterial pathogen *Ralstonia solanacearum*.

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Ralstonia solanacearum, the causal agent of bacterial wilt in solanaceous plants and potato brown rot, is considered the second most destructive crop pest in the world. An important environmental reservoir for *R. solanacearum* in the UK is *Solanum dulcamara*, however infection is asymptomatic. *S. dulcamara* produces root exudates which, when applied to the soil, protect tomato plants from severe *Ralstonia* infection. One compound in particular, 2-6 DHBA, has been shown to directly inhibit *Ralstonia* UW551 infection. However, the direct effects of 2-6 DHBA on plant immune gene expression, as well as indirect effects through alterations of the soil microbiome, have yet to be elucidated. Additionally, the efficacy of the use of 2-6 DHBA as a biocontrol agent against a diverse range of *Ralstonia solanacearum* and *pseudosolanacearum* strains has not been tested. Therefore, our research aims to investigate the mode of action by which 2-6 DHBA protects tomato plants from severe *R. solanacearum* infection. To do this, tomatoes are being grown in a hydroponic system supplemented with a tomato soil-derived microbiome, and examined under 4 different conditions: an untreated control, *R. solanacearum*, 2-6 DHBA, and both *R. solanacearum* and 2-6 DHBA. 16S sequencing and transcriptomic analysis of the root will be used to determine how 2-6 DHBA affects both the rhizosphere microbiome composition and plant gene expression. Finally, a diverse collection of *Ralstonia* strains will be used to assess 2-6 DHBA's potential as a broad-spectrum *Ralstonia* biocontrol agent.

№ 54

Development and validation of a curated MALDI-TOF spectral library for plant pathogenic bacterial species.

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Rapid and accurate identification of bacterial plant pathogens is critical for both regulatory and export certification purposes. Matrix assisted laser desorption ionization–time of flight MS (MALDI–TOF MS) provides rapid, cost-effective and accurate alternative to conventional identification methods, such as biochemical, carbohydrate utilization (BIOLOG) and Fatty Acid Methyl Ester (FAME) MS analysis-based test methods. One drawback for bacterial plant pathogens, is the lack of commercial fit for purpose spectral libraries. Development of curated MALDI-TOF spectral libraries requires accurate and reproducible workflows to ensure that the final bacterial proteomic "fingerprints" will produce reliable identifications across all strains of a species/pathovar. A Bruker MALDI Biotyper® sirius system was purchased in 2021, a multi-year research project was initiated to develop and validate a curated signature library for plant pathogenic bacterial species. A stringent stepwise approach was employed to acquire high-quality spectra from each strain/species which involved sample preparation and processing with a specific protocol for library creation to ensure acceptable spectral quality to create a unique Main Spectrum Profile file that is added to the spectral library. Only curated internationally recognized plant pathogenic bacterial strains were used to develop the core for each species/pathovar which increased both the strength and repeatability of the identifications. New species are being added annually, which at the time of writing, includes more than 425 strains representing 186 different species/pathovars. This novel spectral library has now rendered the use of both BIOLOG and FAME MS obsolete in our laboratory for the authoritative identification of plant pathogenic bacteria.

№ 55

The use of MALDI-TOF MS for the identification of *Ralstonia syzygii* subgroups

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MALDI-TOF MS analysis is a fast and reliable method for identification of bacterial species, making it a valuable tool for diagnostic laboratories. Amongst the species that can be identified with MALDI-TOF MS are *Ralstonia solanacearum* (phylotype II), *Ralstonia pseudosolanacearum* (phylotypes I and III)and *Ralstonia syzygii* (phylotype IV). Since reference spectra of these species were not commercially available, we made our own reference spectra at the NIVIP. With these in-house made reference spectra it is not only possible to distinguish between the three species, but even between the two phylotypes of *R. pseudosolanacearum*.

R. syzygii consists of three subspecies (subsp. *syzygii*, *celebesensis* and *indonesiensis*) of which two are considered quarantine organisms by the European Union (subsp. *celebesensis* and *indonesiensis*). Identification of isolates from this species at the subspecies level is therefore of high importance. However, identification of isolates at the subspecies level using MALDI-TOF MS has proven to be more difficult than expected. We have been working on improving our protocols and in-house database to be able to distinguish among the three different *R. syzygii* subspecies. The results of this work will be presented.

№ 56

Close collaboration ensures effective *Ralstonia* control in the Netherlands

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Bacterial wilt is caused by plant pathogenic *Ralstonia* species. Due to its very broad host range and wide geographical distribution, *Ralstonia* is perhaps the most damaging bacterial plant disease worldwide. Many important crops in the Netherlands, such as potatoes and tomatoes, but also ornamentals like rose, anthurium and pelargonium, are highly susceptible to this bacterium, which has a quarantine status in the European Union.

The Netherlands Food and Consumer Product Safety Authority (NVWA), the Dutch General Inspection Service for Agricultural Seeds and Seed Potatoes (NAK), the Netherlands Inspection Service for Horticulture (Naktuinbouw) and the Quality Control Office (KCB), as mandated by the ministry of Agriculture, Fisheries, Food Security and Nature (LVVN), collaborate closely in the phytosanitary inspection program for plant pathogenic *Ralstonia* species in the Netherlands. The goal of this phytosanitary inspection program is to prevent the introduction, spread and establishment of these plant pathogenic *Ralstonia* species into the Netherlands.

This phytosanitary inspection program takes place in many companies involved in the production of healthy propagation material and the cultivation of healthy arable and horticultural crops. Surveillance, certification and testing are required for the phytosanitary inspection program on *Ralstonia* in the Netherlands. This strongly depends on availability of sampling schemes and reliable tests but also on knowledge of epidemiological parameters contributing to the survival, spread and virulence of the pathogen.

In this context, Wageningen University & Research (WUR) significantly strengthens the collaboration between NVWA, NAK, Naktuinbouw and KCB by generating and disseminating the latest *Ralstonia* research breakthroughs to facilitate long-term strategies for effectively controlling bacterial wilt. Together, these coordinated surveillance, regulatory and research efforts form a robust national infrastructure for the effective control of plant pathogenic *Ralstonia* species in the Netherlands.

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2	Fe Dela Cueva	Phenotypic and Genetic Variation of *Ralstonia solanacearum* Isolates Causing Bacterial Wilt of Solanaceous and Cucurbit Crops in the Philippines	
3	Shanwen Ding	Antibacterial Activity and Mechanistic Insights into the Biocontrol Potential of *Burkholderia arboris* Against the *Ralstonia solanacearum* Species Complex	
4	Fiorella Galiano-Murillo	A genomic and phenotypic overview of four *Ralstonia* strains associated with Costa Rican crops	
5	Connor Hendrich	*Ralstonia solanacearum* and *Ralstonia pseudosolanacearum* take diverging evolutionary paths in response to challenge by two lytic bacteriophage biocontrol candidates	
6	Wenjun Jiang	Adaptive Evolution of Bacterial wilt pathogen to Microplastics	
7	Sanju Kunwar	Unraveling *Ralstonia* Diversity: Insights from Diverse Vegetable Crops in Benin, West Africa	
8	Antonio Léon-Reyes	*Ralstonia solanacearum* identification in Ecuador: Questioning methodology and incidence.	
9	Tiffany Lowe-Power	A putative genomic fusion of the chromosome and megaplasmid within hemagglutinin gene clusters in phyl. I seq. 14 *R. pseudosolanacearum* strain UW588 from Guatemala	
10	Nicolas Nuñez	Genetic architecture of bacterial wilt resistance in potato wild relatives from Uruguay discovered through genome-wide association studies (GWAS)	
12	Johan van Vaerenbergh	Sourcetrack: emergence, expansion and regress of lineages of the potato brown rot genotype in the demarcated area in Belgium	
13	Jolien Venneman	ExoRal, a collaborative Euphresco initiative to enhance Europe's preparedness for *Ralstonia pseudosolanacearum* and *Ralstonia syzygii*	
14	Hua Wang	*Ralstonia solanacearum*-Hijacked Host Veratric Acid Reprograms NLR-Mediated Immunity to Facilitate Multi-Pathogen Infection	

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15	Mariam Ataulla	Getting to the Root of Brg11: the conserved RipTAL from *Ralstonia solanacearum*	2
16	Ana Maria Bocsanczy	Modulation of Temperature-Dependent Transcriptomic Profiles by a LysR-Family Regulator in Cool-Virulent *Ralstonia solanacearum* P673	
17	John Dorlon	A *Ralstonia* methyl-accepting chemotaxis protein directly binds two plant hormones	
18	Sara Franco Ortega	Genomic and epigenetic resistance and susceptibility mechanisms of wild nightshades to *Ralstonia solanacearum*	
19	Sara Franco Ortega	Root exudate-mediated bacterial wilt resistance in the environmental reservoir plant: *Solanum dulcamara*	
20	Riëtte Fischer	RipTAL-Mediated Induction of Host Arginine Decarboxylase Confers a Competitive Advantage to *Ralstonia solanacearum*	
21	Huaiyong Luo	Response of BW-Resistant Peanut Variety Zhonghua 6 to *Ralstonia pseudosolanacearum* Infection Revealed by Integrated Transcriptome and Metabolome Analysis	
22	Yaxing Su	A lectin receptor-like kinase confers lipopolysaccharide recognition and disease resistance in tomato	
23	Liang Yang	Plants modulate coumarins accumulation to defend *Ralstonia solanacearum* invasion through inducing systemic resistance	
24	Yong Zhang	*Ralstonia pseudosolanacearum* PhcQ controls quorum sensing-dependent phenotypes by maintaining the protein stability of PhcA	
25	Caitilyn Allen	Highly transmissible IIB-1 pandemic lineage of *Ralstonia solanacearum* colonizes potato tubers better than other potato-infecting *Ralstonia* strains	3
26	Andrew Aspin	Isolation of *Ralstonia pseudosolanacearum* from *Boesenbergia rotunda* and impact of a novel phage on pathogenicity	
27	Sonja Beiter	Two partner secretion systems mediate bacterial contact-dependent antagonism in *Ralstonia pseudosolanacearum*	
28	Minseo Choi	Visualization and functional roles of *Paraburkholderia*–tomato interactions in bacterial wilt suppression	
29	Vienna Elmgreen	An interactive dashboard for global reports of tnhe *Ralstonia solanacearum* species complex	

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31	Joo Hwan Kwon	A synthetic community derived from rhizosphere microbes enhances cultivar-specific resistance against bacterial wilt in tomato	
32	Yarong Li	Strain-Level Variation in Pathogenic Invaders Drives Rhizosphere Community Shifts	
33	Lorena Uribe-Lorío	Influence of rhizosphere microbiota and soil type on tomato hibrids tolerance to bacterial wilt caused by Ralstonia Phylotype I and IIB isolated from Costa Rica	
34	Kristýna Pavelková	*Ralstonia solanacearum* in surface water in the Czech Republic since 2010	
36	Xinrun Yang	Predicting rhizosphere synbiotics for targeted pathogen suppression	
37	Xinrun Yang	Substrate utilization and cross-feeding synergistically determine microbiome resistance to pathogen invasion	
38	Sanju Kunwar	A Decade of Field Research on Bacterial Wilt Management in Tomato: Challenges, Limitations, and Practical Approaches	4
39	Jay-Vee Mendoza	Tomato (*Solanum lycopersicum*) Lines Exhibiting Durable Resistance to Bacterial Wilt Caused by *Ralstonia solanacearum* under Waterlogging Stress	
40	Omer Ozdemir	Development of an *in vitro* bioassay to screen for *Ralstonia* resistance in potato	
41	Poliane Alfenas Zerbini	Phage Therapy as an Efficient and Viable Strategy to Manage Bacterial Wilt in Eucalyptus	5
42	Nathalie Blom	Virulence assessment of *Ralstonia solanacearum* (ph ylotype II) isolated from ornamental *Rosa* spp. plants	
43	Sebastiaan van Kessel	Validation of a high sample-throughput real-time PCR method for the detection of *Ralstoniasolanacearum, R. pseudosolanacearum* and *Clavibacter sepedonicus* in potato	
44	Laura Celma	Surveillance and laboratory detection of *Ralstonia solanacearum* in Latvia	

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46	Connor Hendrich	Wood hemicellulose–based spray-dried microencapsulation of a lytic bacteriophage preserves phage viability and improves control of *Ralstonia solanacearum*	
47	Boshou Liao	Differences in Suppression of *Ralstonia pseudosolanacearum* Proliferation among Peanut Genotypes	
48	Can-Hua Lu	Quantitative detection and risk analysis of pathogenic *Ralstonia* spp. in tobacco-planting soils from Yunnan Province of China	
49	Nicole Angelee Perez	Rhizosphere and Endosphere Exploration for Potential Biocontrol Agents against Bugtok Disease in Banana	
50	Chiel Pel	An interlaboratory comparison of detection and identification methods for *Ralstonia pseudosolanacearum* and *Ralstonia syzygii* as part of the Euphresco ExoRal initiative	
51	Tom Raaymakers	BIP: a validated high-throughput sequencing test for the identification of bacteria	
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