14th ICPPB ASSISI 2022

14th International Conference
on Plant Pathogenic Bacteria
July 3-8, 2022-Assisi-Italy

"The Impact of Plant Pathogenic Bacteria on Global Plant Health"

BOOK OF ABSTRACTS

Regione Umbria
Giunta Regionale

Un ringraziamento speciale all'Assessore alle Politiche agricole e agroalimentari, alla tutela e valorizzazione ambientale dell'Umbria, Roberto Morroni, per la cortese disponibilità e collaborazione ai fini della stampa di questo libro

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Dear Colleagues,

we are honored to welcome you to the 14th International Conference on Plant Pathogenic Bacteria (ICPPB 2022) in Assisi, Italy.

Since the last ICPPB conference in Shanghai eight years ago, basic and applied research in phytobacteriology has seen extraordinary progress, stimulated by advances in technology and bacterial phytosanitary emergences during this period. This wordwide progress is confirmed through the numerous innovative and interesting studies submitted to the Conference: 230 abstracts from 37 countries across all continents. We also perceived the great desire to meet colleagues face to face. ICPPB 2022 will be an excellent opportunity for PhD students, post-docs and early career researchers to look for career opportunities, to meet colleagues that have common interests and to create professional ties.

It was hard work to organize the Conference, with two postponements due to covid-19 and the recent departure from life of our friend and conference co-chairman Nicola Sante Iacobellis: a tragic event that has left us feeling sad and dismayed. Your closeness, ensuthiasm and his memory are giving us the strength to provide you with a successful Conference, and we would like to dedicate our Conference to Nicola; a conference that he strongly desired with all his heart.

Please enjoy this exciting week filled with scientific, cultural and social activities. You are what makes this meeting a success.

The Organizing Committee

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GENERAL INFORMATION

Conference Hall
The conference will take place in the Conference Hall (San Francescuccio) situated 900 m from Hotel Valle di Assisi (see map at page 2.)

Covid-19 prevention measures
The covid situation in Italy is being kept under control. As of May 1, the masks remain mandatory only on public transportation and in hospital or healthcare settings. In all public places a mask is recommended. Nevertheless, to ensure your and our safety during the 14th ICPPB in Assisi (Italy), the following rules will apply:
Masks, FFP2 grade, will be mandatory during the conference;
We will ensure a strong safe distance during the sessions;
Delegates will be invited to chat out of the Conference Hall in the surrounding garden.
Currently, travelers are free to visit Italy provided that they have the Covid 19 vaccination certificate card and, likely, after May 31st rules will be more relaxed.
The organization will help you to book antigen or molecular covid-19 tests if required, which will be paid for by the delegates.

Poster instructions
Posters are numbered as indicated in the Book of Abstracts and a corresponding numbered poster board will be available for attaching your poster/s (maximum size 80 x 100 cm) in the poster area (see map at page 3) of the Conference Hall.
Concerning the display time, the posters will be divided into two groups:

Group	Session n.	Mounting	Removal
1	1, 2, 3 and 4	3 July (14:00-) - 4 July (10:00)	5 July (19:00-)
2	5 and 6	6 July (8:00) - 7 July (10:00)	8 July (14:00-)

Presenting authors are asked to be in attendance at their posters on July 4 and July 5, from 13:30 to 14:00 (Sessions 1, 2, 3 and 4) and on July 7 and July 8, from 13:30 to 14:00 (Sessions 5 and 6).

Instructions for speakers
Speakers are required to bring their presentation in Power Point (slides in 16:9 aspect ratio) to the Slide technicians present in the Conference Hall at least 1 hour before their scheduled talks.

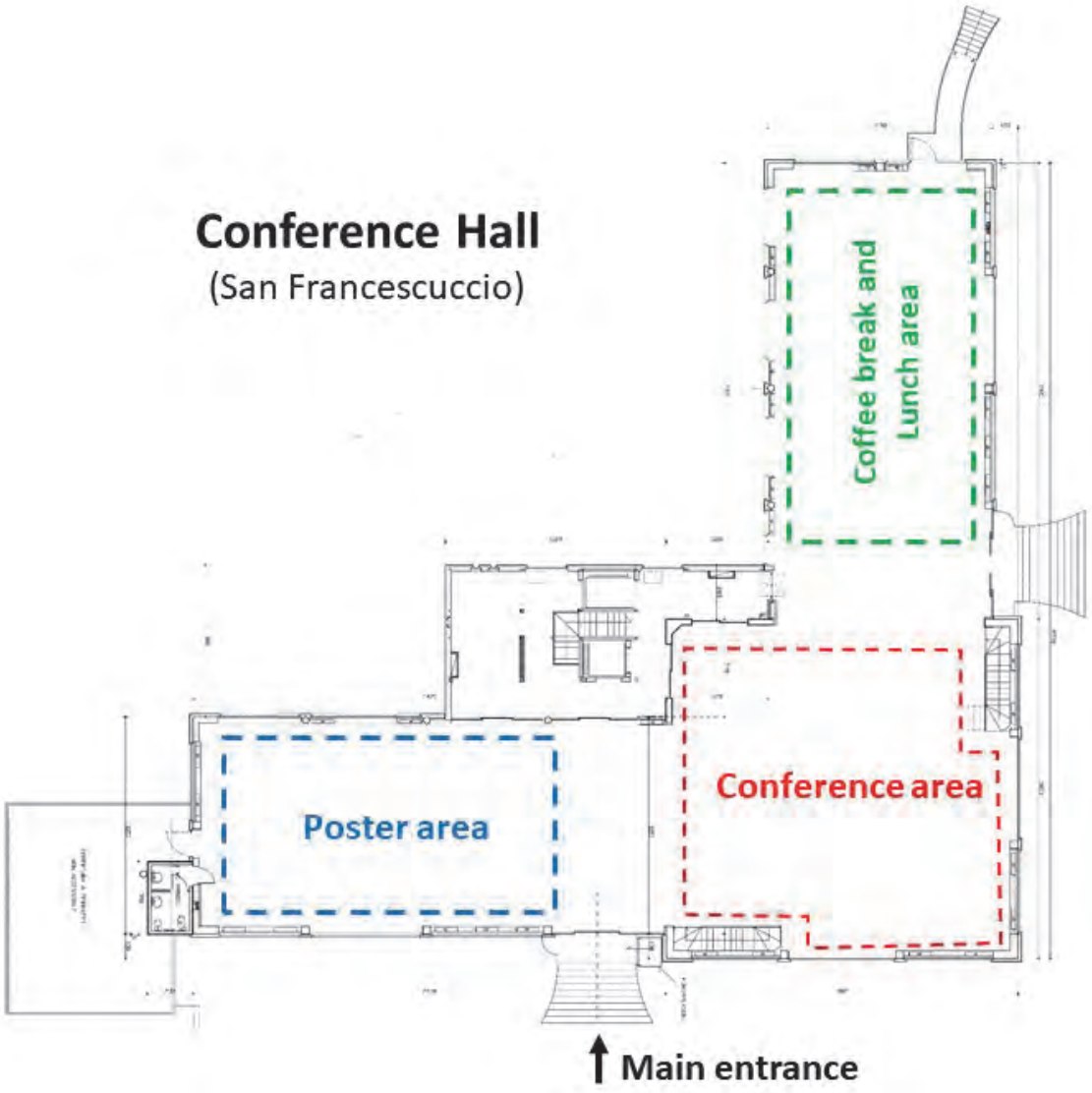
Badge
All participants are kindly asked to wear the provided name badge in the Conference Hall. Coffee breaks and lunches will take place in the Lunch area of the Conference Hall.

Internet connection
A free WiFi internet connection is available in the Conference Hall area and Hotel Valle di Assisi.

Awards
There will be Awards for best posters. Please plan to attend the Closing Ceremony to find out if you are a winner!

Notice Boards
Notice Boards are positioned in the Congress Hall where you can post/receive messages.


Valle di Assisi
RESORT SPA GOLF
Secondo Ingresso
1: LA CHIESETTA - THE LITTLE CHURCH
2: VILLA DEL FATTORE
3: SAN FRANCESCUCCIO
4: VILLA GIRASOLE (401-402)
5: VILLA GRAPPOLO (da 501 a 514)
6: VILLA SPIGA (601-602)
7: VILLA ULIVO (da 701 a 713)
8: VILLA PAGLIAIO (801-802-803-804)
9: LA CANTINA- THE WINE CELLAR
10: SALA DEGUSTAZIONI // TASTING ROOM
11: PISCINA RESORT - RESORT SWIMMING POOL
12: IL LAGHETTO - THE LITTLE LAKE
13: SALA VELA
14: SALA LIMONAIA
15: HOTEL // RECEPTION // RECANTO RESTAURANT
16: COLONNINE RICARICA AUTO ELETTRICHE - ELECTRIC CAR CHARGING STATION
17: AREA GIOCHI // PLAYGROUND
18: BIKE ROOM + OFFICINA BIKE
Conference Hall (San Francescuccio)
resort area
Via Delle Bonifiche
Golf Yard
Golf Yard
hotel RECEPTION
Hotel Valle di Assisi
Via Delle Bonifiche
Calcetto Tennis Padel
Ingresso Hotel Valle di Assisi
Via Porziuncola
Route to reach the Conference Hall from the Hotel Valle di Assisi (about 900 m)


Conference Hall
(San Francescuccio)
Coffee break and
Lunch area
Poster area
Conference area
Main entrance

Program at a glance ICPPB 2022

Sunday 3 July, 2022

14.00-19.00 Participant arrival, registration and accommodation, poster setup
19.00- Welcome reception at the Hotel Valle di Assisi to taste Italian foods and wines

Monday, July 4, 2022

OPENING
Co-Chairs: Iacobellis Nicola Sante and Buonaurio Roberto

8.30-9.10 Opening ceremony
9.10-9.40 Opening lecture (OL1) He Sheng-Yang

Session 1A. Molecular Plant - Bacteria (and Insect) Interactions
Chairs: Sundin George W., Ichinose Yuki

9.45-10.10 S1A-KN1
10.10-10.25 S1A-O1
10.25-10.55 Coffee break
10.55-12.10 S1A-O2…S1A-O6
12.10-12.30 S1A-P1… S1A-P10
12.30-14.00 Lunch break and Poster session

Session 2A. New Tools in Disease Diagnostics and Pathogen Identification
Chairs: Konstantinidis Kostas, Fischer-Le Saux Marion

14.02-14.27 S2A-KN1
14.27-15.27 S2A-O1… S2A-O4
15.27-15.47 S2A-P1… S2A-P10
15.47-16.15 Coffee break

Special session A. Nanotechnology in Disease Control
Chairs: Balestra Giorgio M., Paret Mathews L.

16.15-16.40 SSA-KN1
16.40-17.55 SSA-O1… SSA-O5
17.55- Poster reviewing

Tuesday, July 5, 2022

Session 3A. Disease Epidemiology and Pathogen Ecology
Chairs: Morris Cindy, Catara Vittoria

8.32-8.57 S3A-KN1
8.57-9.57 S3A-O1… S3A-O4
9.57-10.17 S3A-P1… S3A-P11
10.17-10.45 Coffee break

Session 4. Bacterial Pathogens and the Phytobiome
Chairs: Berg Gabriele, Leach Jan E.

10.45-11.10 S4-KN1
11.10-12.10 S4-O1… S4-O4
12.10-12.28 S4-P1… S4-P10
12.28-14.00 Lunch break and Poster session

Session 1B. Molecular Plant - Bacteria (and Insect) Interactions
Chairs: Burdman Saul, Moretti Chiaraluce

14.00-14.25 S1B-KN1
14.25-15.40 S1B-O1… S1B-O5
15.40-16.15 Coffee break

Special session B. New insights on *Xylella fastidiosa*
Chairs: De La Fuente Leonardo, Hopkins Donald

16.15-16.40 SSB-KN1
16.40-17.55 SSB-O1… SSB-O5
17.55- Poster reviewing

Wednesday, July 6, 2022

8.30-19.00 Excursion to Gubbio

Thursday, July 7, 2022

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Chairs: Potnis Neha, Vicente Joana

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9.55-10.15 S5A-P1… S5A-P10
10.15-10.45 Coffee break

Session 6A. Disease Control and Prevention
Chairs: Montesinos Emilio, Yang Ching-Hong

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11.10-12.10 S6A-O1… S6A-O4
12.10-12.30 S6A-P1… S6A-P11
12.30-13.55 Lunch break and Poster session

Session 7. Natural and Engineered Plant Disease Resistance
Chairs: Zipfel Cyril, Jones Jeffrey B.

13.55-14.20 S7-KN1
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15.35-16.10 Coffee break

Session 3B. Disease Epidemiology and Pathogen Ecology
Chairs: Toth Ian, Czajkowski Robert

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20.00- Gala dinner

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Session 6B. Disease Control and Prevention
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10.10-10.45 Coffee break

Session 2B. New Tools in Disease Diagnostics and Pathogen Identification
Chairs: Vinatzer Boris A., Bull Carolee T.

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Session 5B. Disease Emergence and Pathogen Evolution
Chairs: Jackson Robert W, Scortichini Marco

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14.25-15.45 S5B-O1… S5B-O5
15.45-16.15 Coffee break

Session 6C. Disease Control and Prevention
Chairs: Hopkins Donald, Van der Wolf Jan

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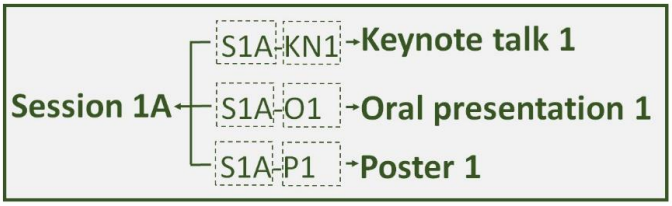
16.40-17.40 S6C-O1… S6C-O4
17.40 Closing ceremony



Conference program

(Opening lecture, Keynote talks, Oral presentations, Poster presentations)

Monday, 4th July, 2022

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Memorial of Professor Nicola Sante Iacobellis
(1949-2022)

Perseverance, passion for research and scientific rigor: here is the synthesis for our collegue and friend Nicola Sante Iacobellis, who suddenly and prematurely left us on May 22nd 2022. The 14th ICPPB 2022 Organizing Committee would like to remember a talented plant pathologist, whose scientific production extends from 1975 to February 2022. In his last paper, showing the same constant and irreducible desire, he was still working to unveil the biology and epidemiology of *Pseudomonas savastanoi*, the bacterial causal agent of olive and oleander knot disease. *P. savastanoi*, which has been a lifelong companion for Nicola, together with several other phytopathogenic pseudomonads. Many of his studies have been carried out together with his friends Giuseppe Surico and Dino Varvaro, who had the opportunity to meet Nicola over 50 years ago when he was a resolute biology student already working as a technician at the National Reasearch Council. After that, they continued sharing personal and professional friendship and collaboration, including when Nicola moved to the University of Basilicata as a full professor of plant pathology until his retirement in 2015. Nicola's international scientific activity dates back many decades, as a visiting scientist at the University of California in Davis, when only a few Italian researchers were having stages abroad.

We would like to remember Nicola, the main leader in the organization of the 14th ICPPB 2022, in particular when he was reproaching us with his simple and spontaneous old fashioned paternal way of speaking. We now remember you and are shocked by an apparent contrast of feelings: although deeply sad, we are now scolding you because you left us a few weeks before the finish line. Yes, finish line is the most suitable expression for this adventure that we have lived together for the last several years. It has been like an endurance test, with you and Roberto Buonaurio as the tireless promoters and the helmsmen of this important and international event that should have been held in 2020, during the International Year of Plant Health, mockingly blocked by the SARSCov2 pandemic. “Ciao” Nicola, we will all miss you, not just as a scientist or a colleague but also as a person with high moral strength and a trustworthy frankness.

(*The 14th ICPPB 2022 Organizing Committee)*

ORAL PRESENTATIONS

14th International Conference on Plant Pathogenic Bacteria
July 3-8, 2022-Assisi-Italy

OL-1

Climate impact on plant-bacterial interactions

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The "Disease Triangle" concept states that plant disease outbreaks require not only a susceptible plant and a virulent pathogen, but also conducive environmental conditions. However, molecular studies in the past three decades on plant-bacterial interactions devoted relatively little effort to understanding why climatic conditions, such as humidity and temperature, have a profound effect on host susceptibility and bacterial virulence *in vivo.* Moreover, current studies often ignore the potentially pervasive effect a plant's endogenous microbiome may have on host-pathogen interactions. In this talk, I will give an example of interplay between disease, environment and microbiota during *Pseudomonas syringae* infection of host plants. Results suggest that future studies should increasingly consider the multi-dimensional nature of "disease-environment-microbiome" interactions, which are likely more reflective of what occur in natural ecosystems.

Memorial of Professor Alan Vivian

9th December 1941 – 2nd June 2021

Alan Vivian was a microbiologist who trained at the University of Reading, Glasgow University and John Innes Centre, before taking academic jobs first at Thames Polytechnic and then at Bristol Polytechnic, now UWE Bristol. At UWE he quickly rose to Reader then Professor and during his career he was an editor for *Microbiology* and became General Secretary for the Society of General Microbiology (now the Microbiology Society). He retired in 2004.

Alan's early career studied fertility variants and plasmids in *Streptomyces* with David Hopwood and then as an establishing group leader, plasmids in *Acinetobacter*. He then started working with the plant pathogenic bacterium, *Pseudomonas syringae* pv. *pisi* studying its interaction with its plant host, pea. Encouraged by Mike Daniels, Alan established fruitful collaborations with John Taylor at HRI and John Mansfield at Wye applying functional cloning strategies to identify genes for avirulence in *Pseudomonas syringae*. Alan's group focused on the pea blight system and were among the first to show that *avr* genes controlled not only race/cultivar interactions but also resistance in non-host plants. He was fascinated by the role of plasmids in *P. syringae* and this led to the cloning of plasmid-borne *virPphA* (now known as *hopAB1*), as the first gene encoding an effector protein to be discovered by its virulence function.

Alan was a regular contributor to conferences. A particular highlight at a *P. syringae* meeting in Berlin was the way he galvanised the UK participants to form an impromptu folk group in the singing contest organised by Klaus Rudolph, the conference organiser. Amongst the traditional national tunes, Alan's choice for GB was –"My old man's a dustman" and the group came second overall! Needless to say Alan will be remembered for his quick wit and sense of humour.

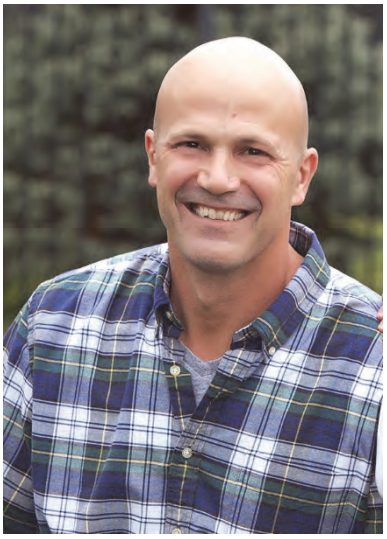
Alan was a prolific and very generous mentor. Along the years, many foreign students, postdocs and professors made visits to his laboratory and benefited from his ideas, biological material and expertise, as well as from the hospitality and friendship of the members of his laboratory. He instilled a sense of collaboration in all of his lab members, encouraging his staff and students to visit other labs, as well as hosting visitors like Satoshi Yamamoto (Kamaishi, Japan), Stefania Tegli (Florence, Italy) and Vittoria Catara (Catania, Italy).

The Vivian family loved the outdoors and Alan was in his element walking the coastal regions of his native Somerset, often with family and friends, enjoying good banter along the way. He, his wife May, daughter Kate and son Robert, were always open-hearted, friendly and welcoming to visiting scientists, who were normally accommodated in his own house, where they were treated as additional members of the family. Battling illness in his final years, he maintained a glint of mischief in his eye and a strong sense of humour, right to the end. We remember Alan as a wonderful colleague and friend.

(*Robert Jackson, Dawn Arnold, Jesus Murillo and John Mansfield*)

Memorial of Professor James "Jim" Robert Alfano

2nd June 1963-21st November 2019



Many attendees of ICPPB 2022 not only know James R. Alfano from his over 60 outstanding research articles on the molecular biology of bacterial plant pathogens, but also as simply Jim, our dear colleague and friend whom we always looked forward to seeing at meetings such as ICPPB. Jim, born in Burbank, California on June 2, 1963, passed away in Lincoln, Nebraska, on November 21, 2019. Jim accomplished so much in his life that any short tribute cannot do him justice. However, we will highlight a number of his most important contributions to our understanding the molecular mechanisms employed by pathogens to cause disease in plants.

Jim obtained his B.S. from San Diego State University in 1986 and Ph.D. in Microbiology in 1993 from Washington State University, where he studied metabolism in *Rhizobium melilotii*. He then joined Alan Collmer's laboratory at Cornell University for a postdoc, where he made break-through advances in our understanding of type III secretion in the model plant pathogen *Pseudomonas syringae*. Contributing to a 1996 *Plant Cell* paper and leading a 1997 *J. Bacteriology* paper, Jim showed that plant pathogen type III secreted effector proteins act within plant cells in a manner analogous to what had been found for animal pathogens.

Jim started his own lab at the University of Nevada, Las Vegas in 1997, and then moved to University of Nebraska - Lincoln in 2000. During this time he continued to work with the Collmer group to expand our understanding of *P. syringae* in general, and the type III secretion system in particular. In 2000 he published his seminal *PNAS* paper on the *Pseudomonas syringae* Hrp pathogenicity island, which he found to have a tripartite structure composed of genes encoding the type III secretion apparatus flanked by an exchangeable effector locus and a conserved effector locus. This paper was the most extensive sequencing effort in *P. syringae* at the time, but more importantly, set the stage for many future studies on effector diversity and function. Additionally, it also helped launch the *P. syringae* pv. *tomato* DC3000 genome sequencing project and the identification of the complete repertoire of this strain's effector profile. Following these studies, Jim focused on deciphering the molecular mechanisms used by type III effectors to promote disease and suppress plant immunity. Jim's group performed beautiful functional analyses on numerous effector families, identifying a range of new mechanisms of function. One such family was HopU1, which his group found to encode a mono-ADP-ribosyltransferase that ADP-ribosylates *Arabidopsis thaliana* RNA-binding proteins; the first identification of a pathogen effector targeting host RNA-binding proteins.

In addition to Jim's path-breaking science, he was also proponent and caring mentor to undergraduate students, graduate students, and postdocs. At the University of Nebraska – Lincoln Jim started the undergraduate program in Microbiology, of which he was also director. His work and contributions to science was recognized by many awards, including promotion to the Charles Bessey Distinguished Professor in the Center for Plant Science Innovation, and election as Fellow in the American Phytopathological Society, the American Association of Advancement of Science, and the American Academy of Microbiology.

Jim passion for science was only exceeded by his passion for his family, of whom he spoke frequently and lovingly. Jim leaves behind his daughter Isabella (Izzy), born in 2007, wife Karin van Dijk, sister Maryann, and niece Keturah.

As Alan Collmer so perfectly stated in his tribute to Jim published in *MPMI*, Jim was a giant in his field as well as a giant in his humanity. He was a true friend and great colleague who valued collaboration, openness, and simply having a few drinks and a good laugh with colleagues over the rough and tumble of science. Jim, we miss you.

(*Boris A. Vinatzer, David S. Guttman and Robert W. Jackson*)

S1A-KN1

Strategic spatio-temporal control of transitions in pathogenesis are dependent on cyclic-di-GMP and small RNAs

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Erwinia amylovora is the causal agent of the devastating fire blight disease which now occurs in most apple and pear-growing regions of the world. Infection of apple shoots is initiated by *E. amylovora* cells actively expressing the type III secretion system (T3SS). Shortly thereafter, *E. amylovora* cells migrate to and form biofilms within xylem vessels. The regulatory reprogramming associated with the transition from T3SS-mediated pathogenesis to biofilm formation is controlled by the induction of cyclic-di-GMP (c-di-GMP) synthesis via the diguanylate cyclase EdcE. We have shown that c-di-GMP is required for attachment, cellulose biosynthesis, and controllable synthesis of the exopolysaccharide amylovoran. All three of these traits are critical for biofilm formation by *E. amylovora*. Global transcriptomic shifts during the transition to the biofilm state are also dependent on c-di-GMP. Dispersal of cells from biofilms within xylem vessels is required for rapid systemic movement in addition to the parallel migration of *E. amylovora* cells through trees via T3SS-mediated pathogenesis in the cortical parenchyma cell layers. We have shown that the small RNA RprA plays a major role in effecting this transition. I will also highlight the importance of both c-di-GMP and sRNA signaling in pathogenesis in other plant pathogen genera.

S1A-O1

Identification of chemoreceptor proteins for amino acids involved in host tobacco infection in *Pseudomonas syringae* pv. *tabaci* 6605

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Pseudomonas syringae pv. *tabaci* 6605 (*Pta*6605) is a causal agent of wildfire disease in host tobacco plants. *Pta*6605 wild-type (WT) strain is highly motile, and we have clarified flagella-defective mutants were reduced the virulence. *Pta*6605 has two major chemotaxis gene clusters including *cheA*, a gene encoding a histidine kinase and *cheY*, a gene encoding a response regulator. Mutant analysis of Δ*cheA1*, Δ*cheY1*, Δ*cheA2* and Δ*cheY2* revealed that Δ*cheA2* and Δ*cheY2* mutants were unable to swarm and to perform chemotaxis, whereas Δ*cheA1* and Δ*cheY1* mutants retained chemotaxis ability almost equal to that of the WT strain. The Δ*cheA2* and Δ*cheY2* mutants did not cause severe disease symptoms on host tobacco leaves, indicating that chemotaxis cluster II containing *cheA2* and *cheY2* is required for optimal chemotaxis and host plant infection[1]. *Pta*6605 harbors more than fifty genes for methyl-accepting chemotaxis proteins (*mcp*), but almost all are functionally uncharacterized. Recently, we identified four genes encoding dCache_1 type Mcps as chemoreceptors McpG for γ-aminobutyric acid (GABA)[2] and PscA, PscB and PscC2 for proteinaceous amino acids[3]. *Pta*6605 WT strain was attracted to GABA and 19 proteinaceous amino acids except for tyrosine. However, Δ*mcpG* mutant abolished chemotaxis to GABA, and Δ*pscA*, Δ*pscB* and Δ*pscC2* mutants failed to respond or showed weak response to 14, 14 and 12 amino acids, respectively. Furthermore, virulence of Δ*mcpG*, Δ*pscB* and Δ*pscC2* was largely reduced. These results indicate that McpG, PscB and PscC2 are involved in the effective infection of *Pta*6605.

This work was supported in part by Grants-in-Aid for Scientific Research (19H02956) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

[1]Tumewu, S.A. et al., 2021. Mol. Genet. Genomics 296, 299-312.
[2]Tumewu, S.A. et al., 2020. Microbes Environ 35, ME20114.
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S1A-O2

Extracellular Vesicles of *Pectobacterium* as vehicles for virulence factors

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Pectobacterium is the causative agent of soft rot diseases of various plants species. The ability to macerate plant tissue and cause disease is governed by the presence of plant cell wall degrading enzymes, small virulence proteins or flagella that are secreted through Type II, III and VI secretion systems [1]. Moreover, it has also been shown that the production of membrane vesicles (MVs) enables the transfer of various virulence factors outside the cells of pathogenic bacteria.
In this study, we have shown that various *Pectobacterium* species secrete MVs [2, 3]. The morphology and yield of MVs production depend on growth medium composition. The highest MVs production was in the medium supplemented with PGA, which appeared to be a strong inductor of vesiculation. The proteomic analysis revealed that bacteria could pack into MVs cytoplasmic proteins that are not native to the cell, such as a GFP protein and β-lactamase. The presence of gfp protein in MVs was confirmed by western blotting and confocal microscopy. The results of the MIC assay confirmed that the concentration of β-lactamase in MVs allows the growth of other susceptible bacteria in the presence of ampicillin. Moreover, the MVs carry pectate degrading enzymes, and their activity was confirmed by plate assay, while the ability of MVs to macerate plant tissues was assessed with the pathogenicity test on Calla lily leaves.
We can conclude that MVs and their cargo might be an essential mechanism of *Pectobacterium* fitness and survival in various habitats and participate in the quorum quenching mechanism.

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[3]Jońca *et al.* 2021. Int. J. Mol. Sci. 2021,22, 12574.

S1A-O3

Functional analysis of gene families in plant pathogenic bacteria through CRISPRi

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CRISPR has dramatically improved genome editing efficiency and specificity in most living organisms but bacteria. CRISPR-interference (CRISPRi) uses a modified Cas9 unable to cleave DNA (dCas9) that interferes with transcription leading to silencing. This tool can be used in functional analysis of almost identical, multigene families since a single guide RNA (sgRNA) can silence several genes at once. Here, we created a CRISPRi platform to target virulence multigene families in pathogenic *Xanthomonas* bacteria, which infect over 400 plant species. We examined the cryptic biological activities of highly homologous Transcription Activator-Like Effector (TALE) family, which encode major virulence factors in many *Xanthomonas* species. Two sgRNAs were designed based on *Xanthomonas phaseoli* pv. *manihotis* (*Xpm*) *tales* at conserved promoter and 5'-UTR sequences. Both constructs efficiently silenced expression of Xam668 *tales,* prevented activation of the susceptibility host cassava gene *MeSWEET10a*, which thus decreased symptoms and bacterial population. Complementation with plasmid-borne *tales* lacking the sgRNA-targeted sequence restored molecular and virulence phenotypes. This system was adapted to other *Xanthomonas* pathogens of cassava, rice, citrus, and Brassica. Remarkably, we were able to observe silencing of up to 16 *tales* with one sgRNA in strain. CRISPRi-mediated *tale* silencing evidenced that *Xanthomonas campestris pv. campestris* CN08 *tale* genes are relevant for symptom development in cauliflower and determined *MeSWEET10a* as a conserved target in cassava for virulence. This latter finding is the second example of TALE evolutionary convergence between phylogenetically-distant *Xanthomonas*. Overall, this novel technology provides a platform for discovery and rapid functional understanding of highly conserved gene families.

S1A-O4
Novel aspects of virulence mechanisms of HopBF1, a bacterial effector that targets host HSP90 protein

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HopBF1 is a type three effector secreted by worldwide spread *Pseudomonas syringae* strains. Our previous studies have contributed to the finding that HopBF1 adopts a minimal and atypical kinase fold, and targets eukaryotic HSP90 protein in a "betrayal-like" mechanism[1]. First, the effector mimics HSP90 client to undergo maturation. Subsequently, the activated HopBF1 phosphorylates a highly conserved serine residue of the chaperone and thereby inhibits its ATPase activity[1]. We further showed, that HopBF1 interferes with NB-LRR signaling. Transient expression of the wild-type HopBF1, but not the variants mutated in the predicted active site, evokes tissue collapse in all plant species tested. Unexpectedly, we have noticed that expression of wild-type HopBF1 in lower parts of *Nicotiana benthamiana* plants leads to abnormalities in upper (systemic) parts. New leaves develop distorted and crinkled, and later on display epinasty. We checked that, as expected, neither *Agrobacterium* used to establish expression in local leaves nor HopBF1 itself are present in the systemic leaves. Similarly, in plants inoculated locally with *P. syringae* expressing wild-type HopBF1, but not its kinase dead variants, the systemic leaves develop symptoms, and are free of the bacteria. Currently, we analyze this phenomenon with a special emphasis on the nature of the molecule that relays the systemic signal.

This work was supported by the Polish National Science Centre 2020/37/B/NZ1/03603

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S1A-O5
Regulation of the virulence of pectinolytic bacteria by RsmC protein

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Pectinolytic bacteria are responsible for soft rot disease in plants. At the beginning of the infection, these bacteria multiply while evading the plant's immune system. When they are in sufficient numbers, they produce enzymes such as pectinases to digest the plant cell wall[1]. The timing of the onset of pectinases secretion is decisive and is finely regulated: if it occurs too early, the infection is not efficient. Motility is also crucial for host colonization an immobile mutant does not cause symptoms. The flagellum is also a target of the plant immune system, so its synthesis is highly controlled. Deciphering these regulatory mechanisms is important for understanding the development of virulence in these bacteria. A previous work from my laboratory showed that a protein named RsmC would play a role in the control of virulence in *Dickeya dadantii*, a model organism of pectinolytic bacteria[2]. The *rsmC* gene is conserved only in *Pectobacteriaceae* and has been poorly studied.The structure of RsmC is unknown and only one study describes this protein as an "anti-FlhDC protein", i.e. a repressor of the FlhDC regulator, a complex regulating both motility and secretion of pectinases[3].
The objective of my work is to understand the role of RsmC in the regulation of virulence in *D. dadantii* using two approaches: 1) Defining the interactome of RsmC, to understand by which intermediary this protein exerts its function; 2) Deciphering the expression conditions of the *rsmC* gene to study when and how RsmC intervenes in the life cycle of *D. dadantii.*

[1]Hugouvieux-Cotte-Pattat N. *et al.*, 2020. eLS John Wiley & Sons, Ltd : Chichester
[2]Royet K. *et al.*, 2019. Molecular Plant Pathology, 20,287-306.
[3]Chatterjee A. *et al.*, 2009. Journal of Bacteriology, 191, 4582-4593.

S1A-O6

Pseudomonas syringae flagella display phenotypic heterogeneity

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Pseudomonas syringae is a plant-pathogenic bacteria that infects a large number of plant hosts including economically relevant crops. It is ubiquitous in nature, colonizing a wide range of niches including water, soil and plant phyllosphere and rhizosphere. Its life cycle is related to the water cycle and when it reaches the plant can colonize the leaf surface and enter into the apoplast through stomata or wounds present in the leaf. For all these processes motility is considered a very important trait. Flagellar motility has been shown to confer epiphytic advantages to *P. syringae,* however, expression of flagellar genes within the apoplast implies the recognition of flagellin, the main component of the flagellar filament and a very well described pathogen-associated molecular pattern (PAMP), by pattern recognition receptors (PRRs) , which leads to activation of PAMP triggered inmunity (PTI). Using single-cell techniques such as flow cytometry and confocal microscopy we have observed that flagella is expressed heterogenously when *P. syringae* is growing within the apoplast and it is cross-regulated with the type III secretion system (T3SS), an key element in suppressing PTI . We have previously described that the T3SS expression is also heterogeneous when *P. syringae* is growing in the apoplast [1], thus the formation of FlagON/OFF, T3SSON/OFF subpopulations is relevant for the pathogenic process. In addition, expression of these two elements has an impact in bacterial fitness so, as in others animals pathogen, we propose their heterogeneous expression a division of labour strategy for *P. syringae* plant adaptation.

[1]Rufián JS. *et al.,* 2016. Environ Microbiol, 18(10):3593-3605

S1B-KN1

Uncovering the arsenal of type III effectors of the cucurbit pathogenic bacterium *Acidovorax citrulli*

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The *Acidovorax* genus includes several plant-pathogenic species. Among them, *Acidovorax citrulli*, which causes bacterial fruit blotch of melon, watermelon and other cucurbits, is one of the most agronomically important species. Pathogenicity of *A. citrulli* relies on a functional type III secretion (T3S) system that translocates effector proteins into the host cell[1]. Type III-secreted effectors (T3Es) promote virulence by altering the host cell metabolism and/or suppressing defence responses. As similar as in *Xanthomonas* spp. and in *Ralstonia solanacearum*, in *A. citrulli*, the AraC-type transcriptional activator HrpX (HrpB in *R. solanacearum*) is a key regulator of T3S and T3E genes[2,3]. In contrast to the wide knowledge on T3Es of model plant-pathogenic bacteria, the pool of *Acidovorax* T3E effectors is largely uncharacterized. In-depth sequence analysis of the genome of the *A. citrulli* model strain, M6, in combination with a machine learning approach for identification of putative T3E genes and expression analysis of HrpX-regulated genes revealed that *A. citrulli* possesses an arsenal of at least ~60 T3E genes, being much larger than previously estimated. This study also uncovered the HrpX-regulon of *A. citrulli*, and revealed seven novel T3E genes that could be detected only in plant-pathogenic *Acidovorax* spp, and could be thus involved in the pathoadaptive evolution of phytopathogenic bacteria belonging to this genus. In this presentation I will report on ongoing research in our lab focusing on characterization of selected T3E genes, and their contribution to virulence and to host-preferential association towards different cucurbit crops.

This study was supported by research grant IS-5023-17C from the United States-Israel Binational Agriculture Research and Development (BARD) Fund.

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[2]Zhang X.X. *et al*., 2018. Front. Microbiol., 9, 507.
[3]Jiménez-Guerrero I. *et al.*, 2020. Mol. Plant Pathol., 21, 17-37.

S1B-O1

Pangenomics facilitated with structural analysis reveals host NAD manipulation as a major virulence activity of bacterial effectors

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The metabolite and redox agent nicotinamide adenine dinucleotide (NAD^+) is involved in diverse cellular processes. Recent findings that Toll/interleukin-1 receptor (TIR) proteins function as NAD^+ hydrolases (NADase) directly link NAD^+ metabolism with immune signaling. In this study we investigated how NAD^+ metabolism can be manipulated by pathogens as a potential virulence mechanism. Utilising the pangenome of the model bacterial pathogen *Pseudomonas syringae* including 531 strains, we conducted structure-based similarity search from 35,000 orthogroups for proteins with potential NAD^+ hydrolysis activities conferred by seven enzyme families. From these proteins, we further identified 15 Type III effectors (T3Es) including nine known effectors and five novel effectors. Our results show that most *P. syringae* strains encode at least one NAD^+-manipulating T3Es, suggesting that it is an important virulence strategy. We experimentally confirmed the protein OG18056, which harbors an ADPR cyclase domain, as a true T3E and found that it induced cell death in planta through its enzymatic activity. In addition, we analyzed bacterial proteins with putative TIR domains for NAD^+-binding residues at the catalytic site using AlphaFold2 structural prediction and *in silico* molecular docking. These analyses revealed that residues at the same NAD^+-interacting positions are divergent, suggesting that variation in the catalytic site may explain the diverse role of these enzymes in pathogenicity and immunity.

S1B-O2

A Na^+/Ca^{2+} exchanger is critical for the virulence of *Pseudomonas savastanoi* pv. savastanoi and *Pseudomonas syringae* pv. tomato

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Calcium (Ca^{2+}) represents an important environmental clue, as reported for bacteria infecting mammalians. We, here, demonstrate that Ca^{2+} entry in *Pseudomonas savastanoi* pv. savastanoi (*Psav*) and *Pseudomonas syringae* pv. tomato (*Pst*) is mediated by a Na^+/Ca^{2+} exchanger critical for virulence. Using a fluorimetric method[1], we demonstrate that Ca^{2+} enters *Psav* and *Pst* cells foremost when they experience low levels of energy, a situation mimicking the apoplastic fluid. In fact, Ca^{2+} entry was suppressed in the presence of high concentrations of glucose, fructose, sucrose or ATP. Since Ca^{2+} entry was inhibited by nifedipine and LiCl, we conclude that the channel for Ca^{2+} entry is a Na^+/Ca^{2+} exchanger. *In silico* analysis of the *Psav* and *Pst* genomes revealed the presence of a single gene coding for a Na^+/Ca^{2+} exchanger (*cneA*), which is a widely conserved and ancestral gene within the *P. syringae* complex based on gene phylogeny. Mutation of *Psav-cneA* inhibited the hypersensitive response in tobacco leaves and disease symptom development in olive while mutation of *Pst-cneA* markedly reduced swarming motility and the ability to develop disease symptoms in tomato and Arabidopsis plants but not compromised the hypersensitive response in tobacco. In *Psav-cneA* mutant the expression of both pathogenicity (*hrpL*, *hrpA* and *iaaM*) and virulence (*ptz*) genes was reduced. Complementation of the *Psav-cneA* mutation restored both Ca^{2+} entry and pathogenicity in olive plants, but failed to restore the HR in tobacco leaves[2]. In conclusion, Ca^{2+} entry acts as a 'host signal' that allows and promotes *Psav* and *Pst* pathogenicity.

[1]Trabalza S. *et al.*, 2021. Bio-protocol, 11(6), e3949.
[2] Moretti C. *et al.*, 2019. Mol. Plant Pathol., 20(5), 716-730.

S1B-O3

The Complex and Compartmentalized Cyclic di-GMP Signaling Network is a Global Regulator of Phase-transition and Host Colonization in *Erwinia amylovora*

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The bacterial second messenger cyclic-di-GMP (c-di-GMP) is a regulator of several virulence factors in the fire blight pathogen, *Erwinia amylovora*. The presence of multiple (12) formative (diguanylate cyclases encoded by *edc* genes) and degradative (phosphodiesterases encoded by *pde* genes) enzymes that control the intracellular levels of c-di-GMP in *E. amylovora* elevates the complexity of this signaling system. In order to study regulatory contribution of each individual Edc and Pde enzyme, we constructed a c-di-GMP null strain by deleting all 12 *dgc* and *pde* genes in *E. amylovora* strain Ea1189Δ12. Ea1189Δ12 was unable to colonize xylem vessels in apple shoots due to an impairment in surface anchoring and biofilm initiation dependent on both the flagellar filament and the type IV pilus. An RNA-Seq study comparing the transcriptomic profiles of Ea1189Δ8 (lacking the 5 Edcs and 3 Pdes that are enzymatically active) and Ea1189Δ8 overexpressing each of the 5 *edc* genes highlighted that a small subset of genes involved in metabolism and transcriptional regulation were regulated by all 5 Edcs. However, a majority of the regulatory targets of each of the Edcs were unique. Further, our study revealed that the c-di-GMP generated by EdcE can regulate the transcription of *hrpL* through a LysR family transcriptional regulator (CdrZ) in conjunction with the c-di-GMP binding protein YajQ. Thus, our study highlights that c-di-GMP is critical for host colonization and that this process is mediated by a dual modality of compartmentalized and global regulatory frameworks in *E. amylovora*.

S1B-O4

On the deciphering the molecular chat occurring between *Curtobacterium flaccumfaciens pv. flaccumfaciens* and plants

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A wide range of data are available since a long time ago on the molecular mechanisms involved in the interaction between Gram-negative plant pathogenic bacteria and their host and non-host plants. Conversely, very little is known about these same molecular aspects on Gram-positive plant pathogenic bacteria, as occurring for the EU quarantine plant pathogen *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*), causal agent of the bacterial wilt of bean and of the soybean tan spot. Although the recent availability of several *Cff* genome sequences in the last couple of years, no functional studies have been ever carried out and nothing is still known on molecular interactions between *Cff* and its plant hosts. In addition to their scientific importance, studies aiming to unveil the molecular strategies played by *Cff* to attack plants, as well as the plant defences triggered by *Cff*, provide data that are pivotal to develop highly targeted and effective control methods against this destructive Gram-positive phytopathogenic bacterium[1]. In this frame, here for the first time metabolomics via 1H NMR (Nuclear Magnetic Resonance) and gene expression studies were developed and used for *Cff*. Several low molecular weight metabolites were identified by 1H NMR, produced by *Cff in vitro*, in newly developed “naturalised” culture media and in a xylem mimicking medium, and *in planta* into the bean apoplast. In the same experimental conditions, gene expression studies were designed and carried out, and the most suitable housekeeping genes for *Cff* as well as of some candidate pathogenicity and virulence genes have been identified.

[1]Tegli *et al.*, 2020. Microorganisms*, 8, 1705*

S1B-O5

Lipid signals in phytopatogenic bacteria *Xylella fastidiosa*

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Lipids can modulate the pathogens virulence as well as the plant defences. Free Fatty Acids (FFA) and oxylipins, might serve as signals in several process, e.g. in cell-to-cell communication as autocrine and paracrine signal. In this study, we investigated FFA and oxylipins in *X. fastidiosa* (Xf) associated to the the Olive Quick Decline Syndrome (OQDS). A metabolomic approach was used to unveil the lipid profile of Xf, of *Nicotiana tabacum* Petite Havana SR1 artificially infected with Xf and *Olea europea* var Ogliarola salentina and Leccino naturally infected with Xf and treated or no with treated or not with the zinc copper-citric acid biocomplex Dentamet®. The lipidome profile highlighted some lipids entities as hallmark of Xf lifestyle (i.e. plancktonic *versus* biofilm stages) and plant infection and we combined HPLC-MS-based targeted lipidomics with supervised learning algorithms (Random Forest, Support Vector Machine and Neural Networks) to classify olive tree samples from Salento. The dataset included samples from either OQDS positive or OQDS negative. The classifiers are able to discriminate olive tree samples: i) infected and non-infected; ii) belonging to different cultivars; iii) treated or untreated with Dentamet®. Lipid entities emerging as predictors of the thesis are: free fatty acids (C16:1, C18:1, C18:2, C18:3); the LOX-derived oxylipins 9- and 13-HPOD/TrE; 31 the DOX-derived oxylipin 10-HPOME; diacylglyceride DAG36:4(18:1/18:3)It is noteworthy that in *Xfp+* plants, ten lipid entities are more abundant than in *Xfp-* plants: FFAs (e.g. 18:1), diglyceride (36:2) and oxylipins (e.g. 9-HODE). The ability of these 10 lipids to modulate the dual state of *Xfp* was tested: oleic and linoleic acid and some oxylipins can control the switch planktonic *vs* biofilm. The lipoxygenase (LOX)-derived 9-HODE induced biofilm formation whereas the dioxygenase-derived oxylipins such as 7,10-diHOME induced the plancktonic growth. Moreover, the deletion of a gene orthologue for a bacterial DOX confirmed the role of 7,10 diHOME in the biofilm formation in *X. fastidiosa* subsp. *fastidiosa*. Taken together these data suggest that lipids modulate *Xf* lifestyle and plant-pathogen cross talk.

Funding information: Regione Puglia thought the agreement "Strategie di controllo integrato per il contenimento di Xylella fastidiosa in oliveti pugliesi ed analisi epidemiologiche del complesso del disseccamento rapido dell'olivo (CoDiRO; Italian Ministry of Agriculture, Food Forestry and Turism Policies Salvaguardia e valorizzazione del patrimonio olivicoloitaliano con azioni di ricerca nel settore della difesa fitosanitaria (SALVAOLIVI)"and Olivicoltura e difesa da Xylella fastidiosa e da insetti vettori in Italia (Oli.Di.X.I.It)

Memorial of Dr David Eric Stead

(3rd June 1949 – 28th May 2019)

Dr David Stead died peacefully at York Hospice on 28th May 2019 after several years of illness with oesophageal cancer. David was an internationally renowned plant bacteriologist who from an early age knew he wanted to go into science and more specifically to be a plant pathologist despite at school being more gifted in the arts. Alongside this he developed an early love for the natural world inspired by "mooching walks" with his great uncle Walt.

He pursued both this love of science and of nature in his career and life and inspired others in both. He was a gifted teacher and all through his career his mentoring and encouragement was appreciated by a great number of his colleagues.

After gaining a 1st class BSc in microbiology at Chelsea College, London, he went on to do a PhD at Silwood Park the field station of Imperial College, London. It was while he was there that he met African students and the idea of working in Africa was born. He took up a post as biology lecturer at Chancellor college, University of Malawi and spent some fascinating years there. In his spare time, he pursued his love of nature by exploring the national parks and doing scientific recording studies on elephants and birds, and gathering memorable stories of Africa to be regaled for years to come.

He returned to the UK in 1981 to start his career as a government scientist in the UK plant quarantine service. He loved his job. His work brought him into contact with scientists from around the world and he welcomed the opportunity both for scientific collaboration and to share different cultures, often with singing thrown in. I had the joy of working alongside him as a colleague and then later as his wife enjoying a shared passion in biology, the natural world and in meeting folk from across the world.

He continued his hobbies of Morris dancing and singing in his retirement and also took up a new science challenge moving into entomological activities (the 'dark side'). He became proficient in moth identification including micro moth identification - a step too far was the thought from some friends - and became a county recorder.

His presence is sadly missed by his family, his son James and wife Alison, but his legacy of love of nature and warmth in meeting people lives on.

(*Dr Alison J Stead (nee Wright) wife and colleague*)

Reflections from John Elphinstone

It was my pleasure and honour to work for David Stead for 16 years in his Plant and Environmental Bacteriology team, initially at the Central Science Laboratory (CSL) in Harpenden UK, which subsequently became the Food and Environment Research Agency (Fera) following relocation to York. The primary aim of these laboratories was to provide scientific support to Defra (the UK Government Department for Environment, Food and Rural Affairs) on matters of statutory importance. Here David led major investigations into the detection, exclusion, epidemiology and control of several important emerging plant pathogenic bacteria, including *Erwinia amylovora*, *Pseudomonas syringae* pathovar pisi, *Ralstonia solanacearum* and *Clavibacter sepedonicus*.

David's long career in plant bacteriology started with his PhD in 1974, working on soft rots of potato under the guidance of the esteemed Prof. R.K.S. Wood. During the following years at the University of Malawi, he collected many stories of his experiences in Africa with which he would entertain his colleagues for years to come. On return to the UK in 1981, he joined the Ministry of Agriculture, Fisheries and Food (MAFF) Plant Pathology Laboratory at Hatching Green, Harpenden (later to become CSL) under renowned plant bacteriologist Dr. Ron Lelliott. Their joint publication "Methods for the diagnosis of bacterial diseases of plants" (Lelliott, R. A. and Stead, D. E., 1987 Blackwell Scientific Publications, Oxford. 216 pp.) is, to this day, a standard textbook for diagnosticians worldwide. David also took on the curation and development of the National Collection of Plant Pathogenic Bacteria (NCPPB), creating a world class biochemically characterised reference collection of almost all known variants of species causing plant disease. He also pioneered the use of fatty-acid profiling in the UK for identification of bacterial plant pathogens, still routinely used in diagnostic work.

In addition to his enviable publication record and extensive knowledge of plant bacteria and wider biology, David will also be remembered for his warmth of character and skills in connecting with people on all levels. He served for many years on the EPPO Panel on Diagnostics in Bacteriology and was an active member of the International Society for Plant Pathology in the panel of experts responsible for publishing updated lists of valid names for all taxonomically distinct pathogens. His many overseas collaborations included study tours in INRA Angers and the University of Delaware, consultancies on plant health matters for the European Commission and Chinese Government and many collaborative research projects with colleagues around the world, including the EU, USA, Canada, Egypt, Mauritius and Georgia. He was a welcome and entertaining speaker at many international conferences and in 2006 organised the 11th International Conference on Plant Pathogenic Bacteria. He was always keen to start up a folk song and could be seen folk dancing with his Morris Men most Mondays in the city of York. A wonderful person to work with, he was always a very caring leader of his team and openly shared his unique knowledge. His profound love of the natural world was infectious to all around him and surpassed only by the love for his family. We all miss him greatly.

(*Dr J Elphinstone Plant Bacteriologist, Plant Protection Programme, Fera Science Ltd*)

S2A-KN1

Metagenome-based identification of the causative agent of (diarrheal) disease

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Diagnosis of microbial infections currently relies on culture-based methods, which are not rapid enough for real-time disease surveillance and frequently fail to identify the causative agents. Culture-independent metagenomic analyses of disease samples could overcome these limitations. In this talk, I will summarize our recent efforts to validate metagenomics for foodborne outbreak characterization, and the bioinformatics approaches we have developed to deal with several challenges associated with metagenome-based analysis of human stool samples such as how to detect and quantify target species (e.g., pathogens) and genes (e.g., toxins) in complex, short-read metagenomes, and how to identify and genotype new pathogens with no previously sequenced representatives. Application of these tools to samples from foodborne diarrheal outbreaks showed that the traditional culture-based and PCR methods misidentified the causative agent (pathogen) in about 50% of the cases. By using a novel approach that combined isolate-based sequencing with metagenomics analysis of stool samples and epidemiological data, we identified the causative agent in these cases, show that in many cases -but not all- the disease and healthy states of the gut microbial community can be distinguished from each other, opening new possibilities for rapid diagnostics. The relevance of these findings and technologies for plant pathogens and diseases will also be discussed.

Recommended reading.

[1]Meziti *et al*., 2021.Appl Environ Microbiol. 87(6):e02593-20. 2021.
[2]Pena-Gonzalez *et al*., 2019. Appl Environ Microbiol. Nov 27;85(24). pii: e01820-19.

S2A-O1

Highlighting genomic marker sets to predict plant pathogenicity of bacteria

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In numerous situations, scientists face the question: is this bacterial strain a plant pathogen? Answering this question is not straightforward. Pathogenicity tests might be unfeasible because host is unknown or unavailable. In addition, pathogenicity is not always related to species classification. Comparative genomics emerges as a promising method to check *in silico* the presence of genomic markers to distinguish pathogenic from non-pathogenic microorganisms. A workflow[1] based on with *a priori* and without *a priori* approaches has been developed to detect genomic markers associated to pathogenicity on plant. Binary logistic regressions were used to identify markers able to predict the different phenotypic classes. Our approach was tested using 147 *Ralstonia* spp. genomes, 108 classified as plant pathogens and 39 as not pathogenic on plants. This workflow and the parameters settings have allowed to create exclusive datasets of markers that could be used as predictors of the potential pathogenicity on plant of *Ralstonia* spp. It has also revealed common traits that could be linked to ecological adaptation. An allele of a class D β-lactamase-encoding gene was exclusively detected in all genomes from species responsible for nosocomial infections. Specific markers of plant pathogens have been found in type II and type III secretion systems. Non-ribosomal peptide synthetase and homoserine lactone clusters were exclusively retrieved in plant pathogenic *Ralstonia* spp. The beta version of a tool designed to help users to classify unknown *Ralstonia* genomes as plant pathogen or not, using the highlighted marker datasets, is available in the CIRM-CFBP Galaxy instance (https://iris.angers.inra.fr/galaxypub-cfbp/).

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement n. PCOFUND-GA-2013-609102, through the PRESTIGE programme coordinated by Campus France. This research was conducted in the framework of the regional program "Objectif Végétal, Research, Education and Innovation in Pays de la Loire" supported by the French Region Pays de la Loire and Angers Loire Métropole. It was supported by University Bretagne Loire Programme d'Attractivité Post-Doctorale 2017-2018.

[1]https://github.com/felipelira/PrediPath

S2A-O2

Comparative genomics, refined taxonomy, and genome-informed diagnostics for differentiation of the *Xanthomonas hortorum – Xanthomonas hydrangeae* species complex

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The *Xanthomonas hortorum – Xanthomonas hydrangeae* species complex, comprises of the seven pathovars of *X. hortorum*[1] and *X. hydrangeae*[2], and causes disease on a multitude of plants, including crops, ornamental and wild plants. Cross-pathogenicity was proven for some of the strains within this species complex. It is thus important to have highly specific and fast diagnostics methods for members of this species complex. Genomics was first used to fill the knoweldge gap regarding representative members of this species complex in terms of genomic diversity, taxonomy, and molecular diagnostics. Comparative genomics revealed a set of conserved genomic features (e.g., type III secretion system) and that genomic diversity within the species complex was mostly related to the type 3 effectome and plasmid content. Singletons identified by comparative genomics were used to develop seven loop-mediated isothermal amplification (LAMP) diagnostics assays detecting six *X. hortorum – X. hydrangeae* species complex clades, in addition to one assay specific for the entire species complex. These seven assays are promising for use as diagnostic tools for various members within the *X. hortorum – X. hydrangeae* species complex, and for assigning new and historical isolates to this species complex and, when possible, to lower taxonomic levels. The results of the assays revealed that the host range of members of this species complex may be more diverse than described to date.

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Based upon work from COST Action CA16107 EuroXanth, supported by COST (European Cooperation in Science and Technology).

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[2]Dia N.C. *et al*., 2021. Int. J. Syst. Evol. Microbiol., 71, 005163.
[3]Dia N.C. *et al*., 2022. Front. Agron., under revision.

S2A-O3

***Effectidor*: an automated machine-learning based web server for the prediction of type-III secretion system effectors**

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Type-III secretion systems are utilized by many Gram-negative bacteria including plant pathogens such as *Xanthomonas*, *Acidovorax*, and *Ralstonia*, to inject type 3 effectors (T3Es) to eukaryotic cells. These effectors manipulate host processes for the benefit of the bacteria and thus promote disease. They can also function as host-specificity determinants through their recognition as avirulence proteins that elicit immune response. Identifying the full effector repertoire within a set of bacterial genomes is of great importance to develop appropriate treatments against the associated pathogens. Nevertheless, unmasking the full effector repertoire is a difficult task, due to the availability of strain specific effectors and the ambiguity of the secretion signal. To tackle this task, we developed Effectidor - a user-friendly web server that harnesses several machine-learning techniques to predict T3Es within bacterial genomes. Using dozens of features of various domains that use for strain specific training, it manages to identify known effectors in the genome as well as potential novel effectors. It shows high accuracy (AUPRC above 0.98 in all tested cases) on labeled data, as well as unlabeled data. Several novel effectors, some of which are strain specific, were identified so far using the Effectidor algorithm in several pathogens.
Availability and implementation: Effectidor is available at: https://effectidor.tau.ac.il, and the source code is available at: https://github.com/naamawagner/Effectidor.

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S2A-O4

Elucidation of the chemical structures of the O-specific polysaccharides within the lipopolysaccharides of diverse soft rot *Pectobacteriaceae*

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Lipopolysaccharide (LPS) consists of lipid A, core oligosaccharide, and a variable O-antigen built of repeating oligosaccharide units. This molecule belongs to the Pathogen-Associated Molecular Patterns (PAMP) and plays a role in the virulence of soft rot *Pectobacteriaceae*, especially during colonization and overcoming the defence mechanisms of the plant host. We aimed at examination of the variability within the chemical structure of O-specific polysaccharides (OPS) of the LPS of diverse *Pectobacterium* and *Dickeya* spp. strains with the use of NMR spectroscopy and chemical methods. The OPS within the *Dickeya* genus turned out to be conserved and simple as it contained 6-deoxy-d-altrose homopolymers in terms of 4 *D. solani* strains (IFB0099, IFB0158, IFB0223, IPO2222^{TS}), *D. dadantii* 3937, *D. dianthicola* IFB0485 and *D. zeae* IPO946 [1, 2]. Solely *D. aquatica* IFB0154 possessed a different OPS structure, *i.e.* linear disaccharide →3)-α-d-Fucp-(1 → 4)-α-l-Rhap-(1→, partially acetylated at O-2 or O-3[2]. On the contrary, intraspecies variation, exhibited by three diverse OPS molecules, was observed among 6 investigated *P. parmentieri* strains[3]. Interestingly, a rare pseudaminic acid residue was discovered in the OPS of *P. parmentieri* SCC3193 and IFB5432[3]. All the above-mentioned OPS structures were different than the previously described O-antigen molecules of *P. atrosepticum* SCRI1039 and GSPB 9205 in addition to *P. carotovorum* FERM P-7576 and GSPB 436. Broadening knowledge on the OPS of diverse soft rot *Pectobacteriaceae* not only reveals important details of the interplay between the pathogen and the plant host, but also might be used for to-species identification purposes.

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S2B-KN1

Computational approaches to address the challenges inherent to genome-based metagenome-based identification of plant pathogens

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Fast and precise pathogen identification is key to successfully controlling new disease outbreaks. While it is possible to use pathogen-specific probes to detect known pathogens, there are no probes available to identify newly emerging pathogens. Therefore, sequencing all microbes associated with a symptomatic organism, i.e., metagenomic sequencing, and then identifying the sequences of putative pathogenic agents is a promising approach. These sequences can then be used to design probes for high throughput detection assays. The main challenge inherent to genome-based plant pathogen identification is that adaptation to new plant hosts often occurs within the same pathogen species and that identification thus needs to go beyond species-level resolution. We previously developed the Life Identification Number (LIN) system for genome-based bacterial classification and identification that approaches strain-level resolution and we implemented the approach in the LINbase web server, which we are currently upgrading to a type of genome preprint server, called genomeRxiv. We have also shown that the LIN approach can be adapted to fungal plant pathogen identification and we combined the popular Kraken 2 tool used for taxonomic characterization of metagenomes with the LIN approach to take the resolution of Kraken 2 from the species level towards the strain level. Finally, we are exploring machine learning as a complement to database-dependent pathogen identification. We will discuss the power of these tools and approaches to contribute to faster and more precise identification of emerging plant pathogens by plant disease diagnostic clinics.

S2B-O1

Novel race-specific detection method for the lettuce bacterial leaf spot pathogen, *Xanthomonas hortorum* pv. *vitians*.

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Lettuce industries worldwide are threatened by the sporadic yet devastating bacterial leaf disease caused by *Xanthomonas hortorum* pv. *vitians* (*Xhv*). Infection causes water-soaking, chlorosis, and small black spots on the lettuce leaves that later coalesce to form large lesions. Major outbreaks can cause 100% crop loss; even minor outbreaks significantly reduce the crop's sellable yield. The pathogen can survive in weeds and debris long enough to serve as an inoculum source in subsequent planting seasons[1]. Three pathogenic races have been described based on the distinct lettuce cultivar in which each elicits a hypersensitive response[2]. Prior to this study, a rapid test to differentiate *Xhv* from all other *X. hortorum* pathovars, or to distinguish the *Xhv* races, had not yet been developed successfully. Here we report a novel conventional PCR method to address this gap. Whole genome sequences of 25 *Xhv* strains representing the three races were aligned using the anvi'o pangenomic workflow[3]. *Xhv*-specific and race-specific gene clusters were identified, and primers were developed for amplification within these clusters. The specificity of the single-plex reactions was tested using a collection of greater than 100 *Xhv* strains of known races and a set of *Xanthomonas* type strains. The reactions will be multiplexed and specificity will be confirmed in a blind study with a subset of *Xhv* strains representing the three races. This procedure will allow for rapid pathogen detection, better tracing of the pathogenic races, and precise cultivar recommendations to avoid subsequent outbreaks.

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S2B-O2

EURL Proficiency Tests and Beyond: Towards Comparable Detection of *Xylella fastidiosa* in Diagnostics and Research

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Detection of plant pathogens represents a cornerstone of plant health. Non-comparable test performance and interpretation can lead to erroneous validation data, research conclusions and missed opportunities to prevent disease outbreaks. In 2019, the EU designated EU reference laboratory for pests of plants on bacteria[1] providing an opportunity for systematic inter-laboratory studies among the EU national reference laboratories (NRLs) over time. Between 2019 and 2022 the National Institute of Biology, Slovenia, focused on *Xylella fastidiosa* (Xyf) and organized two proficiency tests (PTs) on molecular detection tests. Overall, results of the PTs showed that cell densities of Xyf as commonly expected in plant material are readily detected by most of the participating laboratories. A plethora of variable approaches to detection proved successful including choice and sequence of tests, DNA extraction, instruments, reagents and other technical details, as well as different interpretation of the results. This demonstrates that knowledge and competence overrule the need for strict standardization[2]. However, several laboratories needed to improve their performance. To efficiently support them, it was necessary to identify the cause of underperformance and plan the panel of samples accordingly. This, together, with a systematic approach to collecting structured data and metadata in turn proved useful far beyond assessment of proficiency of individual laboratories leading to new insights into the interplay of tests, host plants and Xyf subspecies.

The EU Reference Laboratory for pests of plants on bacteria is a consortium led by the the Netherlands Food and Consumer Product Safety Authority, National Reference Centre Plant Health (NVWA-NRC, The Netherlands) and is also composed of the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO, Belgium), and the Research Centre for Plant Protection and Certification (CREA-DC, Italy), funded by the European Commission (grant S12. 809508) and co-funded by the Slovenian Ministry of agriculture, forestry and food. The work was also supported through ERA-NET Euphresco project 2016-A-215, COST Action CA16107 – EuroXanth (STSM reference number 39497), and the Slovenian Resaerch Agency (P4-0165, J2-2488).

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S2B-O3

Evaluation of the MALDI-TOF technique for plant-pathogenic bacteria and construction of the reference spectra database at CIRM

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The French Collection for Plant-associated Bacteria CIRM-CFBP preserves resources strategic for plant health and holds plant-pathogenic bacteria representing most of the known diversity of these pests. These resources, available for the whole scientific community (https://cirm-cfbp.fr), can help plant pathologists to better understand the relationships between plant and bacteria, improving taxonomy or permitting the development of reliable diagnostic tools. Among diagnostic techniques, the use of MALDI-TOF (Matrix Assisted Laser Desorption Ionisation - Time of Flight) is developing. After ionization, the peptides and small proteins of the analysed bacteria are separated according to their mass-to-charge ratio and measured by the analysis of their times of flight. The produced spectra are compared to a database of reference spectra. This technique is today widely used in the human medicine sector and is powerful for rapid and reliable diagnostic[1]. However, for plant-pathology the reference database does not exist yet, hampering the development of this promising technique[2]. During the EUPHESCO project "MALD-ID", CIRM-CFBP, along with 6 partners, CIRM-BP and the Pixanim platform[3], produced the reference mass spectra necessary to develop a database for plant-pathogenic bacteria identification. MALDI-TOF appears to be reliable for efficient identification at the genus level or even species in some cases. However, it is not precise enough to differenciate closely related species or species sub-divisions. As the taxonomic level relevant for plant pathology is often at the sub-specific level, the MALDI-TOF technique appears to be useful as a pre-screening tool, permitting to determine the most suitable technique for precise identification.

EUPHRESCO project MALD-ID

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INRAE Centre Val de Loire, UMR Physiologie de la Reproduction et des Comportements, UMR INRAE 85-CNRS 7247-UFR-IFCE, 37380 Nouzilly France.

S2B-O4

Rapid and precise pathogen identification using metagenomics in combination with an exclusively genome-similarity-based classification system

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Metagenomics provides an unbiased and culture-independent method for pathogen surveillance. With the recent advances in long-read sequencing technology, such as nanopore sequencing, a sample of a symptomatic plant of unknown etiology can now be sequenced in almost any setting simply with a computer and limited lab equipment. However, accurately identifying the potential pathogen sequences within the so obtained metagenomic sequences is still a challenge. Numerous metagenome classification tools have been developed, but most of these have low resolution. One reason is that these tools depend on NCBI taxonomic classification, which considers species as the lowest rank of classification. To overcome these issues and improve the resolution and sensitivity of analysis, we have employed the LIN (Life Identification Number) system to build a database independent of classical rank-based taxonomy. The LIN database relies on clustering genomes on increasing thresholds of ANI (Average Nucleotide Identity), which ranges from 70%-99.999% similarity. This system offers 20 levels of classification, out of which 14 are above the 95% similarity threshold, which corresponds to the species rank. These added thresholds can be utilized to approach outbreak strain-level resolution without compromising speed when determining the taxonomic composition of a metagenome. Therefore, this study aims to develop a metagenomics classification tool that overcomes the limitations of current tools and pipelines by combining the popular taxonomic classification tool Kraken2 with the LIN database. We tested the tool with metagenomes of naturally infected plants, artificially inoculated plants, and mock communities of known composition.

S2B-O5

Xanthomonas euroxanthea-specific DNA Markers for Genotyping and Multiplex PCR-based Detection

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Xanthomonas euroxanthea is a new bacterial species encompassing both pathogenic and nonpathogenic strains[1] frequently found colonizing the same host plants as the pathogen *Xanthomonas arboricola*[2], a species with which *X. euroxanthea* is frequently mistaken for[3]. The present study aimed to develop a PCR method to specifically detect *X. euroxanthea* strains, and distinguish these from the closely related *X. arboricola*. Eight DNA markers (XEA1-XEA8) were selected by comparative genomics, and validated for specificity and genomic context by *in silico* approaches prior to lab validation. No determinants linked to genomic-plasticity, were found in the vicinity of any of the markers XEA1 to XEA8. Furthermore, synteny analysis, GC content and CAI/eCAI values suggest that the eight DNA markers are in conserved genomic regions of *X. euroxanthea*. Five out of eight DNA markers (XEA4, XEA5, XEA6, XEA7, XEA8) were unfailingly present in the genome of the studied *X. euroxanthea* strains. A multiplex PCR targeting DNA markers XEA1, XEA5 and XEA8 was shown to be efficient and specific for seven *X. euroxanthea* strains analyzed. For genotyping potential evaluation, maximum-likelihood trees were generated with the concatenated sequences of three DNA markers (XEA5, XEA6, XEA8) and four housekeeping genes (*gyrB, rpoD, fyuA* and *acnB*) from 11 *X. euroxanthea* strains. The topology of both trees underlines the suitability of these DNA markers to detect and simultaneously discriminate *X. euroxanthea* lineages. Overall, this data provides a method conciliating detection and genotyping of *X. euroxanthea*-strains, contributing to monitor for the presence of *X. euroxanthea* in *X. arboricola*-colonizing habitats.

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Memorial of Prof. Philippe Prior
(1957-2018)

Philippe began his great passion for Plant Pathology in the mid-1980s and devoted his professional life to reducing global crop losses to bacterial wilt disease. Combined with his love for the South (Philippe was born in Gabon), his extensive fieldwork gave him deep sympathy for the world's farmers, especially those working in the tropics. He loved learning from them and, in turn, they responded to his warm personality and lively humor. As a molecular epidemiologist, he occupied a unique intellectual niche among bacterial wilt researchers and was unquestionably one of the world's experts in his area. Starting in Guadeloupe, he extended his hunting ground to the whole planet by even seeking to return to the sources of the disease in his last travels to Peru.

Philippe began his scientific romance with his favorite bacterium, *Ralstonia solanacearum*, the source of his incessant questions about the strategies to adopt to fight against the terrible diseases associated to a large number of cultivated plants, especially in intertropical areas. He focused on the microbe's adaptations and its astonishing diversity, much of which he discovered himself. His work is documented in his many books and papers, beginning with his graduate research in the Antilles and continuing over 30 years into whole-genome phylogenetic analyses that he helped develop. Philippe's carefully curated collection of thousands of "*Ralstonia*" strains is a precious gift that will live on by advancing the science he loved even though he is no longer with us.

Defending his doctoral thesis at the University of Orsay (France) in 1990 under the attentive and friendly eye of Michel Dron, Philippe continued his work in the French West Indies until 1998, when he began a close interaction in Brisbane with Mark Fegan, an Australian specialist in this disease. During this Australian sabbatical period, Philippe interacted with many of the most influential specialists of *Ralstonia* including Luis Sequeira, Caitilyn Allen, Christian Boucher, Tim Denny, and Chris Hayward.

This was followed by a stay of a few years at INRA in Avignon; nevertheless, his attraction to tropical regions remained intact, which led Philippe and Evelyne, along with their childen: Juliette, Mathilde and Alexandre, to settle in Reunion Island in July 2005, an island that Philippe loved intensely for paragliding. Philippe was a man of challenges and lead the organization the 12th International Conference on Phytopathogenic Bacteria in Saint Denis on Réunion Island. As always, Philippe invested all his energy in this adventure with the imperative desire that it be a congress of high scientific quality but obviously by making an important place for human exchanges, and gave an opportunity to many researchers from the countries bordering the Indian Ocean and Africa to actively participate in this international scientific event.

A pioneer in bacterial plant pathology, Philippe understood very early on the importance of the natural diversity of the bacterial genus *Ralstonia*. He wanted to embrace the diversity of this group of bacteria as an indicator of the recent adaptive history of this bacterium to our agricultural systems and as a reservoir for future epidemics.

Beyond the scientist, it is the man who will be sorely missed. Philippe was marked by his unconditional love for his wife Evelyne and for his children Juliette, Mathilde and Alexandre. He was a pillar for some because of his intellectual honesty, his moral strength, and his frankness, combined with an astonishing open-mindedness. He respected every colleague regardless of rank. He had a real sense of friendship and listening, and a desire to help others. Philippe was one of those passionate and exciting people, intimately convinced of his ideas and positions.

This warm, visionary, deeply human scientist will be much missed and long remembered.

(*Cindy Morris and Cellier Gilles*)

S3A-KN1

Pervasive reservoirs, long distance aerial spread, variable host range: integrating the challenges of anticipating disease caused by *Pseudomonas syringae*

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Pseudomonas syringae is more frequently reported as causing new diseases than are any other group of plant pathogenic bacteria and even certain fungi[1]. Management of emerging plant diseases has been, up to present, a post-hoc effort involving development of diagnostics deployed in agricultural contexts to find traces of the emerging strains. Many plant pathogens can survive and multiply as saprophytes, although little research has been devoted to understanding the extent to which saprophytic phases impact pathogen evolution and disease emergence. *P. syringae* is found in a multitude of habitats within and beyond agricultural contexts. Habitats outside of agriculture harbor the greatest genetic diversity including strains with the greatest potential for aggressiveness as plant pathogens[1]. All these strains are disseminated by water – including major rivers used for irrigation – and by wind until they are deposited with precipitation[2]. To develop a surveillance system that accounts for a more comprehensive scope of *P. syringae* reservoirs and dissemination we have i) produced maps of the trajectories of its air and water dissemination in a French river basin based on network analyses of meteorological and hydrological data that we superimposed on land use for this region, and ii) conducted comprehensive pathogenicity tests of strains in the *P. syringae* complex to identify indicators of its host range within and beyond angiosperms. Detection of environmental reservoirs and natural long distance movement of *P. syringae* raises questions about agronomic practices for management of plant health that will be discussed in the presentation.

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S3A-O1

Unravelling contributions of transposon Tn*6212* to ecological success of *Pseudomonas syringae* pv. *actinidiae* (Psa)

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Global dissemination of the kiwifruit canker pathogen *Pseudomonas syringae* pv. *actinidiae* (Psa)[1] was instrumental in the spread of a complex and highly conserved transposon (Tn*6212*)[2] that hitchhiked within a diverse array of integrative and conjugative elements (ICEs)[3]. Tn*6212* comprises 17 genes, none of which are obvious determinants of virulence or ecological success. Here we report a genetic analysis of Tn*6212*, its origins, distribution, contribution to host fitness and effects on host transcriptome. Although a Tn*6212* knockout showed no effect on Psa growth in leaf-based pathogenicity assays, Tn*6212* enhances bacterial fitness on TCA-cycle sugars. RNA-seq performed on a set of nested Tn*6212* mutants show striking succinate-dependent genome-wide effects with the majority of effects being attributable to a single LysR regulator. In the presence of succinate, transcription of genes involved in central metabolism are elevated, including tRNA synthetases, oxidative phosphorylation and parts of the TCA cycle, while genes involved in flagella synthesis and chemotaxis are repressed. Effects on metabolism and ATP synthesis likely derive from up-regulation of the RNA degradosome, with two genes of the complex being encoded by the transposon. Phenotypic tests of growth, motility and chemotaxis confirm results from the RNA-seq data. These findings suggest Tn*6212* contributes to host fitness in planta by redirecting all resources to ATP synthesis and growth in a succinate-dependent manner. On-going work using glass capillary tubes as proxy for the plant vascular system tests the hypothesis that elevated growth caused by Tn*6212* leads to capillary blockage thus connecting the transposon to severe wilting symptoms observed during the Psa pandemic.

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S3A-O2

Molecular epidemiology of Ralstonia pseudosolanacearum Phylotype I strains in the South West Indian Ocean region and their relatedness to African strains

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Long-distance traveling of human beings and movement of goods drastically increase over time in relation with globalization of trade and exchanges, and so is the spread of bacterial pathogens and associated infectious diseases across the globe. Key for improved disease control lies into acquiring a thorough knowledge on factors shaping pathogen populations at fine scales and how they interact with their environment. Bacterial lineage-centered molecular genotyping techniques, such as multilocus variable number of tandem repeats analysis (MLVA), are of interest especially when they provide high throughput, a sound phylogenetic signal, and a resolution fitting the spatiotemporal scale investigated.
Bacterial wilt caused by the *Ralstonia solanacearum* species complex represents one of the most important plant pathogenic disease in tropical and sub-tropical regions; regularly reported in the literature as a disaster both in terms of economical and sociological impact resulting from its wide host range and geographical distribution.
Several studies achieved molecular characterization of outbreak strains during the last decade mostly using multiplex PCR phylotyping, *egl* partial sequencing or multilocus sequence typing (MLST). Africa and islands bordering it on its southeastern side made no exception and reports of active epidemics suggested the same tendency.
Herein, our objectives were to (i) evaluate the performance of the newly revamped MLVA scheme dedicated to Phylotype I[1] in terms of discriminatory power and phylogenetic coherence, and (ii) decipher the population structure of this Phylotype in Africa and the SWIO region with a special emphasis on Eastern African countries (Uganda, Kenya, Tanzania) where alarming reports were produced.

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S3A-O3

Interspecies microbial interactions can intensify disease severity caused by plant-pathogenic bacteria.

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The ability to move on solid surfaces provides ecological advantages for bacteria, yet many bacterial species lack this trait. We found that *Xanthomonas* spp. overcome this limitation by making use of proficient motile bacteria in their vicinity. Using *X. perforans* and *Paenibacillus* sp. YH6 as models, we show that *X. perforans* cells attract *Paenibacillus* sp. YH6 and use it as a "ride" for dispersal. Examination of the compound mediating this interaction indicated that it is an airborne substance, which increases the environmental pH value[1]. Using fluorescent stained *X. perforans* cells, we show that this hitchhiking strategy also occurs on tomato leaves[2], implicating an important role for epiphytic survival, colonization and host infection. Indeed co-inoculation experiments of tomato plants with *X. perforans* with *Paenibacillus* YH6 resulted in aggravated symptoms compared to inoculation with *X. perforans* alone. The described interaction was observed between several xanthomonads and additional *Paenibacillus* species, thus suggesting that this hitchhiking strategy might be widespread and ecologically important. This study provides an example as to how bacteria can rely on the skills of their neighboring species for their own benefit, signifying the importance of a communal organization for fitness.
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S3A-O4

Removal of plant pathogenic bacteria during managed aquifer recharge and associated risks of recycling irrigation water

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Freshwater availability can restrict agricultural production in coastal areas where groundwater is brackish and surface water not suitable for irrigation. A natural solution to provide safe irrigation water is managed aquifer recharge (MAR) for agriculture where excess rain water is collected via the tile drainage system and stored in the subsurface. This results in a fresh water 'bubble' giving farmers access to sufficient irrigation water even in times of drought. As the injected drainage water may be contaminated with plant pathogens, changes in microbial water quality during MAR were investigated. We focused on three plant pathogenic bacteria, namely *Ralstonia solanacearum, Dickeya solani*, and *Pectobacterium carotovorum.* The soil passage of the MAR acts as natural sand filter where bacterial pathogens are removed by die-off in the water phase, die-off on the solid surface of a soil grain, (ir)reversible attachment or straining. To predict changes in water quality during MAR, the bacterial inactivation in water microcosms and their transport and removal in anoxic saturated sediment columns was analysed.
In aerobic natural water microcosms, 3-$\log_{10}$ reduction by die-off was observed within 19 days while the die-off in anaerobic microcosms took more than 50 days. Soil columns filled with clean quartz sand achieved little bacterial removal (0.4-2.7 log/m) as this sand type offers less attachment sites for the bacteria. In columns filled with natural MAR sand high removal rates (18-40 log/m) were observed. Experimental results will be implemented in quantitative microbial risk assessment to determine the treatment efficiency of agricultural MAR.

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S3B-KN1

Working together to tackle bacterial threats to plant health in Scotland and the wider UK

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Bacterial threats to plants in the UK are a significant challenge to our biosecurity. These include pathogens that are endemic as well as those that threaten our borders. This presentation describes two initiatives that are helping to combat bacterial plant diseases. Scotland's Plant Health Centre provides evidence on plant health issues to inform policy decisions and support stakeholders across forestry, horticulture, environment and agriculture to help fill evidence gaps in plant health and coordinate knowledge, skills and activities across Scotland. Two of our biggest bacterial threats are from *Candidatus* Liberibacter solanacearum and *Xylella fastidiosa,* and we have commissioned projects to help determine the likelihood of entry, spread and survival of these pathogens. Such initiatives have also allowed us to build teams of experts to improve knowledge transfer and coordination activities. A second initiative is the UK-wide Bacteria Plant Diseases Programme. This is a €21 million interdisciplinary research consortium that supports nine research projects addressing a range of bacterial threats to plants. The pathogens (and their vectors) threaten the sectors described above and have the potential to cause widespread and severe economic, environmental and social impacts on landscapes and ecosystems, both rural and urban. The initiative is helping to bring together researchers with a focus on bacterial plant diseases and encourages others with an interest in this area to get involved in wider discussions. This presentation will describe how such initiatives are helping to bring people together to tackle important biosecurity issues and to identify evidence gaps in the fight against these pathogens.

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S3B-O1

Receptors used by lytic bacteriophages to interact with Soft Rot *Pectobacteriaceae* bacteria – from genes to phenotypes and back

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Pectinolytic Soft Rot *Pectobacteriaceae* (SRP – *Pectobacterium* spp. and *Dickeya* spp.) cause soft rot diseases on a great variety of crops and ornamentals worldwide, leading to losses of up to 250 million Euro annually in crop production. In their natural habitats, SRP bacteria are often exposed to lytic bacteriophages and therefore may be frequently and repeatably infected. However, little is known about receptors used by these viruses to interact with SRP bacteria. Likewise, the molecular mechanisms governing phage-SRP host interactions remain poorly understood. Therefore, this study aimed to: (i) identify and characterize those SRP genes encoding bacterial structures required for lytic phages ΦD5 and ΦA38 attachment and susceptibility to infection and (ii) to evaluate whether disruption of such genes in *D. solani* and *P. parmentieri* may result in altered phenotypes of the bacterial mutants both *in vitro* and *in planta*. Using random transposon mutagenesis, we identified and characterized *D. solani* and *P. parmentieri* loci that encode structures required for ΦD5 and ΦA38 attachment to host cells. Our main finding is that the resistance to viral infection causes ecological costs for phage-resistant bacterial variants. Even though phage resistance did not affect most of the phenotypes of SRP bacteria *in vitro*, compared to the wild-type SRP strains, all phage resistant mutants exhibited a reduced ability to colonize and to cause symptoms in growing potato (*S. tuberosum* L.) plants. This suggests that phage-resistance in the natural environment has side effects for SRP bacteria and may lead to their compromised ecological fitness.

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S3B-O2

Elucidating the phylogeny and host adaptation of *Pseudomonas* strains associated with the bacterial canker of stone fruit trees in Western Cape, South Africa

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The stone fruit industry in South Africa consists of approximately 22% of cultivated land with the majority occurring in the Western Cape. Pests and diseases on stone fruit production are of major concern and economic significance. Previously, a multilocus sequence analysis of four housekeeping genes revealed that *Pseudomonas* strains isolated from symptomatic *Prunus* hosts, i.e. apricot, peach and plum, associated with bacterial canker, belonged to two phylogenetic groups in the *Pseudomonas syringae* species complex, namely, *P. syringae sensu stricto* (PG2) and *P. viridiflava* (PG7)[1]. Here, we used a comparative genomic approach to determine the position of these strains in the *P. syringae* species complex and to provide insight into the underlying mechanisms of host adaptation of these strains on P*runus* spp. A phylogenetic tree constructed using genome-wide single nucleotide polymorphisms, and pairwise nucleotide comparisons of the coding gene regions (CDSs) between *Pseudomonas* strains isolated from symptomatic stone fruit trees, and reference strains in the *P. syringae* species complex highly supported the delineation of *Pseudomonas* strains in *P. syringae sensu stricto* and *P. viridiflava*, collectively. Specific T3SS effector proteins, such as AvrE, HopM1 and HopAA1-1 found in the conserved effector locus (CEL) of the canonical T3SS together with HopBE1, HopBH1, HopH3 and HopZ5 were among a few T3SEs detected in the *Pseudomonas* strain. Additionally, there were no differences in the pathogenicity of the *Pseudomonas* strains on both apricot and plum seedlings. Altogether, these findings suggest a lack of host specificity among *Pseudomonas* strains known to infect *Prunus* hosts.

[1]K. Bophela, Y. Petersen, C. T. Bull and T. A. Coutinho., 2020. Plant Dis., Vol. 104, Pages 882-892.

S3B-O3

***Erwinia amylovora*-induced volatile organic emission: effects on plant resistance, pathogen metabolism and honeybees-mediated dispersal**

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Volatile organic compounds (VOCs) emitted during the infection of apple plants by *Erwinia amylovora* were characterised by gas chromatography-mass spectrometry and proton transfer reaction-mass spectrometry. Infection altered volatile VOCs emission both in leaves[1] and flowers[2]. Disease-specific VOCs include several bio-active compounds, such as ethylene, methyl salicylate, β-caryophyllene, hexenal isomers, phenylethyl alcohol, and 2,3-butanediol. Plants exposed to VOCs from *E. amylovora*-infected plants showed a higher resistance to the pathogen and a lower bacterial growth and migration in host tissues. Resistance induction was mediated by the activation of salicylic acid synthesis and signal transduction suggesting an induction by methyl salicylate. However, in infected plants, the emission of hexenal isomers and ethylene repressed salicylic acid response by activating jasmonic acid (JA)- and ethylene-dependent responses. VOCs may also influence pathogen growth and ecological interaction. Phenylethyl alcohol is produced by bacterial metabolism and may aid the pathogen in microbial competition. Hexenal, terpenes, β-caryophyllene and isoprenoids may reduce pathogen growth either directly or by the production of ozone and reactive oxygen species under the exposure of sunlight. Finally, VOCs have a direct effect on honeybee-mediated dispersal of *E. amylovora*. VOCs from infected flowers reduce the pollinator visit rate. However, as the deterrence effect is not complete, occasional honeybee visits on infected flowers may allow the pathogen to be vectored to the more frequently visited healthy flowers facilitating pathogen dispersal and leading to local epidemic burst.

[1]Cellini A., *et al.*, 2018. Mol. Plant Pathol. 19(1), 158-168.
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S3B-O4

New haplotypes found among the clonal population of *Pseudomonas syringae* pv. *actinidiae* isolates, biovar 3, obtained from Portuguese orchards

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Kiwifruit bacterial canker has been causing of economic loses in the kiwifruit agri-chain. The etiological agent, *Pseudomonas syringae* pv. *actinidiae* (Psa) is a quarantine phytopathogenic bacteria included in the EPPO A2 list. The disease is characterized by angular necrotic spots in leaves, bacterial ooze and cankers in trunks and branches. Aiming to address the epidemiology, thirty-seven isolates of Psa isolated between 2013 to 2019 from 20 geographically distinct orchards in the north and centre of Portugal were genotyped by MultiLocus Sequence Analysis (MLSA) and Multi-Locus VNTR Analysis (MLVA) and characterized for their carbon metabolism and susceptibility to different subtracts using BIOLOG GEN III. The identity of the isolates as Psa was carried out according the EPPO guidelines. Regarding MLSA the concatenated sequences (1858bp) of four housekeeping genes (*gapA* [564bp], *gltA* [435bp], *gyrB* [425bp] and *rpoD* [434bp]) were used to construct a neighbour-joining phylogenetic tree (Geneious, USA). Psa biovar 3 (CFBP 7286, Italy - 2008) and Biovar 1 (CFBP 4909, Japan – 1889, type strain) were used as reference strains. To further discriminate the Psa isolates, polymorphism of 13 tandem-repeat sequences were assessed by MLVA. The results showed that all Psa isolates studied revealed an identical BIOLOG pattern, that is aligned with strain CFBP 7286, a Psa biovar 3, with exception for their incapacity to metabolize Glycyl-L-Proline. As expected, further differences of BIOLOG metabolic profile were observed with CFBO 4909, a Psa biovar 1 strain. Biovar 1 type strain did showed other differences in the BIOLOG pattern compared to all biovar 3 strains identified in this study. Regarding MLSA genotyping, no polymorphisms were found between our isolates, neither with strain CFBP 7286, suggesting a high clonal population structure and further confirming the identification of the isolates as members of biovar 3. Regardless the clonality found MLVA profiling revealed different haplotypes that will help to identify the most prevalent strains, contributing to make phytosanitary risk assessments important to adopt the best practices for disease containment.

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S3B-O5

River water survey of soft rot *Pectobacteriaceae* at the scale of a large watershed

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Irrigation water can potentially serve as a source of plant pathogens. Detection and monitoring of pathogens in water sources used for irrigation and near crop fields is therefore important for disease management. Most water surveys lack a broad temporal and spatial coverage that provides clues about pathogen diversity and circulation. We conducted a two-year survey of the diversity of soft rot *Pectobacteriaceae* (SRP) species in the Durance River, a mixed-use watershed of 323 km long in France. SRP isolated strains (582) were characterized by amplification and sequencing of the *gapA* housekeeping gene. The abundance of SRP isolated strains correlates with the agricultural gradient along the watershed with a positive correlation found with temperature, nitrate and dissolved organic carbon water concentration. *Pectobacterium* spp. were far more abundant (549 isolates) than *Dickeya* spp. (33 isolates). *Dickeya* isolates were only observed in the lower part of the river when water temperature was above 19°C. *D. oryzae* dominates the *Dickeya* spp. and *P. versatile*[1] and *P. aquaticum*[2] dominates the *Pectobacterium spp*. but their repartition along the watershed was different, *P. versatile* was the only species regularly recovered all along the watershed. Excepting *P. versatile*, *Dickeya* and *Pectobacterium* spp. responsible for disease outbreak on crops were less abundant or rarely detected. This work sheds light on the various ecological behaviors of SRP species in stream water and indicates that SRP occupation is geographically structured.

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S4-KN1
The plant microbiota in Health and Disease

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Microbiome research has changed our view on all organisms on Earth; all of them are holobionts and form a functional unit with its microbiome. This has also been proven for plants. The plant microbiota mainly consists of bacteria, archaea and fungi, while the microbiome comprise viruses, mobile genetic elements and the "whole theater of activity" [1]. The microbiota coevolved and specified together with the plant. Bacterial communities are often dominant and characterized by beneficial, intimate interactions with plants. They are vertically transmitted and added by horizontally acquired by environmental communities[2]. Each plant microbiome contains potentially pathogenic microorganisms, and their balance within the microbiome and with host is crucial for health issues. Recent studies indicated a general shift of the plant microbiota characterized by a decrease of evenness and specificity, and an increase of r-strategist and hypermutator prevalence as well as antimicrobial resistance. This typical microbiome signature of the Anthropocene is often followed by a dysbiosis, which leads to outbreaks[3]. Therefore, the plant microbiome is the key to the second green revolution and can provide solutions for sustainable agriculture. Beyond, the plant microbiome is connected across systems and crucial for human and planetary health issues as well. Examples for microbiome research and management (e.g. tomato, apple, sugar beet) will be explained as well as examples for the interconnected plant microbiomes in frame of one/planetary health.

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S4-O1
Bacteria-Insect-Plant Interactions: Microbiomes in Honeydew of Russian Wheat Aphids Increase Aphid Virulence to Wheat

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Phenotypic responses to biotic stresses are often studied as interactions between two species; however, in the phytobiome, these responses likely result from complex interactions involving several organisms as well as the environment. Russian wheat aphid (RWA, *Diuraphis noxia*) is a serious pest that impacts small grains globally. We demonstrated that RWA-induced chlorosis on wheat is partially determined by aphid-associated bacteria[1]. The bacteria themselves are not virulent to wheat or barley, because inoculation of plants did not result in chlorosis, water-soaking or necrosis. Bacteria were not detected in high numbers in the aphid salivary glands or foreguts, but were detected in aphid honeydew, suggesting stylets contaminated during feeding are an avenue for bacterial introduction into leaves. To understand the mechanisms by which bacteria are affecting RWA virulence, we are studying plant defense responses occurring during these tripartite interactions. Aphids with high titers of bacteria induced gene expression and accumulation of salicylic acid (SA), a hormone involved in insect resistance, in wheat. High, sustained expression of SA biosynthetic genes was followed by downregulation of jasmonic acid (JA) biosynthetic genes; JA is associated with insect resistance. Our current hypothesis is that aphid-associated bacteria contribute to aphid virulence by modulating the plant's insect defense mechanisms.

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S4-O2

Ten years of bacterial taxonomy associated with Acute Oak Decline – the path forward

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Symptoms of the current episode of Acute Oak Decline threatening mature native oak species in Great Britain were first observed in the 1980's. However, it has only been in the last decade that the bacteria associated with this syndrome have been identified. From 2008 onwards, bacteria have been consistently isolated from exudate and necrotic lesions on symptomatic oak. The majority of these were found to belong to novel genera, species and subspecies, typically within the order Enterobacterales, using a polyphasic approach[1]. In the last decade, we have described two novel genera, 15 novel species and five novel subspecies, all of which are associated with oak. Cataloguing the bacterial species from symptomatic oak has provided the platform for progress in several different research areas including taxonomy, pathogenicity, genomics, identification and ecology. We now know AOD is a polymicrobial disease with *B. goodwinii* and *G. quercinecans* responsible for the necrotic lesions, along with possible insect involvement from *Agrilus biguttatus*[2]. Metagenomic and proteomic studies have revealed insights into the pathogenic potential of these bacteria[3]; qPCR and HRM analysis can rapidly identify the AOD-associated bacteria; and we are continuously learning more regarding the bacterial interactions, biology and ecology of these bacteria. There are most certainly more novel bacteria to describe from symptomatic and asymptomatic oak, from the tree canopy to the acorns, to the roots and soil. With improving options for data comparison and analysis, along with possible changes to requirements for novel species descriptions, the path forward is exciting and filled with opportunities.

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S4-O3

An antibacterial T6SS in *Pantoea agglomerans* pv. *betae* delivers a lysozyme-like effector to antagonize competitors

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The type VI secretion system (T6SS) is deployed by numerous Gram-negative bacteria to deliver toxic effectors into neighboring cells. The genome of *Pantoea agglomerans* pv. *betae* (*Pab*) phytopathogenic bacteria contains a gene cluster (T6SS1) predicted to encode a complete T6SS. Using secretion and competition assays, we found that T6SS1 in *Pab* is a functional antibacterial system that allows this pathogen to outcompete rival plant-associated bacteria found in its natural environment. Computational analysis of the T6SS1 gene cluster revealed that antibacterial effector and immunity proteins are encoded within three dynamic genomic islands that harbor arrays of orphan immunity genes or toxin and immunity cassettes. Functional analysis demonstrated that the specialized antibacterial effector VgrG contains a C-terminal catalytically active glucosaminidase domain that is used to degrade prey peptidoglycan. Moreover, we confirmed that a bicistronic unit at the end of the T6SS1 cluster encodes a novel antibacterial T6SS effector and immunity pair. Together, these results demonstrate that *Pab* T6SS1 is an antibacterial system delivering a lysozyme-like effector to eliminate competitors, and indicate that this bacterium contains novel T6SS effectors.

S4-O4

Systemic distribution of *Xylella fastidiosa* within olive tree branches and changes on the associated xylem microbiome communities

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Xylella fastidiosa (*Xf*) is a vascular plant pathogen that causes economically important diseases in a wide number of relevant crops such as olive, grapes or almonds. In nature, the plant-to-plant dissemination of *Xf* is driven by xylem-sap feeding insects. After inoculation, the bacterium systemically colonize the xylem vessels by moving towards and/or against the xylem sap flow. This work was focused on mapping *Xf* infection within olive trees and deciphering the changes that the infection can cause in the microbial communities on *Xf*-symptomatic and -asymptomatic branches. Xylem tissue from stems and roots of olives trees of cv. Grappolo growing at Sao Paulo state (Brazil) were sampled during summer. DNA was extracted and used for microbiome community analysis by metagenomic analysis (NGS) and to diagnose the *Xf* presence by specific qPCR and MLST analyses. Sequencing data resulted in a total of 374 bacteria ASVs, distributed in 15 phyla and 155 genera. Proteobacteria was the most abundant phylum (80.56%) followed by Firmicutes (9.95%); whereas *Methylobacterium* and *Bacillus* (68.87% and 9.20%, respectively) were the dominant genera. Results indicated a higher relative abundance (NGS reads) of the pathogen in the branches showing typical *Xf* symptoms (4.65%), in contrast to asymptomatic but *Xf*-infected (1.65%) branches. Furthermore, the relative abundance of the pathogen varied within the same branch. Our results indicate that *Xf* infection induces changes in the microbiome composition of xylem vessels as the bacterium spread trough the tree canopy, irrespective of the presence of symptoms on the branch. These results will help to better understand the changes ocurring in xylem microbiome as a consequence of *Xf* infection and would allow to identify potential bacterial antagonists that react after *Xf* inoculation and vascular spread by increasing their populations to counteract pathogen infection.

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S5A-KN1

Monitoring fine-scale adaptations in plant pathogen populations that contribute towards recurring outbreaks and host jumps

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Increased global trade and intensified monoculture in modern agricultural systems have predisposed agricultural production to a significant challenge of recurring pathogen outbreaks compromising host resistance. A concern of emergence of novel pathogen genotypes, accompanied by host shift, has been noted across different pathosystems. This challenge of host resistance erosion over time or host jump is not only due to emergence or introduction of pathogen variants capable of overcoming the resistance but also due to environmental and anthropogenic factors that can alter evolution of plant-pathogen interactions. Using *Xanthomonas* infecting tomato and pepper as a model system, we have investigated how intraspecific diversity within *Xanthomonas* is structured by environmental parameters, host genotypes and growth dynamics of different pathogen genotypes. Strain-resolved metagenomics allowed us to document differential abundance of pathogen lineages and associated virulence factors across temporal and spatial scales. These dynamic communities showed seasonal succession of two or more pathogen genotypes resulting in more homogeneous pathogen population structure by the end of growing season. We speculate that *Xanthomonas* community dynamics results from the succession of strains with contrasting fitness strategies in response to climatic fluctuations, host defenses and competition with resident flora. Identifying the fine-scale adaptations in *Xanthomonas* lineages that have recently gained ability to infect pepper revealed that recent adaptation to pepper is a multigenic trait, not limited to typical virulence factors, but also involved loci for carbohydrate and amino acid metabolism. This work will advance our understanding into investigation of adaptative traits in the genus *Xanthomonas*.

S5A-O1

Three case studies on *Xanthomonas* spp. diseases that might threaten UK crop production

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Plant diseases caused by *Xanthomonas* species can affect a large number of crops worldwide. In the UK there are frequent outbreaks of black rot of brassicas caused by *X. campestris* pv. *campestris*, but there are only infrequent occurences of angular leaf spot of strawberry caused by *X. fragariae*, and diseases like bacterial leaf streak of maize caused by *X. vasicola* pv. *vasculorum* and black rot of watercress caused by *X. nasturtii* have never been reported. We aim to assess the risk to the UK for these pathogens. Angular leaf spot of strawberry was first identified in the 1960s and is an A2 pathogen in EPPO. We have tested the pathogenicity of a selection of isolates and refined the method for isolation using different media. We will test UK samples and screen popular varieties for disease resistance. Bacterial leaf streak of maize was first reported in South Africa and has now been identified in 10 US states and some regions of Argentina and Brazil, but is currently not present in Europe. We are developing inoculation methods and diagnostic assays to screen UK maize varieties for disease resistance. Black rot of watercress was described in 2017 and is known to be present in Florida, Hawaii, Spain and Portugal. Whole genome sequence comparisons shown genetic diversity amongst the isolates and some evidence for the presence of genes linked to heavy metal tolerance. We are extending our knowledge by characterising these *Xanthomonas* pathogens using different approaches including pathogenicity and genomics to inform future diagnostics.

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S5A-O2

PsICEs, a family of integrative conjugative elements that contributed to the evolution of the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae*

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Horizontal gene transfer allows bacteria to rapidly adapt to novel hosts and abiotic conditions. Integrative and conjugative elements (ICEs) are self-transmissible mobile elements that can transfer functional genetic units across broad phylogenetic distances. The accessory genes shuttled by ICEs can make significant contributions to bacterial fitness, yet ICEs that carry accessory genes encoding functions other than antimicrobial resistance remain poorly characterized. The recent clonal expansion of plant pathogenic *Pseudomonas syringae* pv. *actinidiae* (*Psa*), responsible for a global disease outbreak in kiwifruit, was accompanied by the acquisition and exchange of diverse ICEs among otherwise closely related bacterial host genomes. These ICEs belong to a broad family of 207 ICEs (PsICEs) distributed across multiple phylogroups of the *P. syringae* species complex. Analysis of a reduced set of 53 ICEs representing the diversity of the entire PsICE family shows they have distinct evolutionary histories compared to their bacterial hosts. These ICEs are highly recombinogenic and exhibit a conserved structure punctuated by hotspots of accessory gene integration. The most common cargo carried by this family of ICEs is a transposon (Tn*6212*) which has recently spread across the PsICE family and in the *Psa* outbreak strains. Indipendent acquisition in globally dispersed isolates of diverse ICEs carrying the almost identical Tn*6212* suggests the transposon is involved in the plant-pathogen interaction and contributed to the ecological suggess of *Psa*.

S5A-O3

Genomic acquisitions in emerging populations of Xanthomonas vasicola pv. vasculorum infecting corn in the U.S. and Argentina

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Xanthomonas vasicola pv. vasculorum (Xvv) is an emerging bacterial plant pathogen that causes bacterial leaf streak on corn. First described in South Africa in 1949, reports of this pathogen have greatly increased in the past years in South America and in the U.S. The rapid spread of this disease in North and South America may be due to more favorable environmental conditions, susceptible hosts and/or genomic changes that favored the spread. To understand whether genetic mechanisms exist behind the recent spread of Xvv we used comparative genomics to identify gene acquisitions in Xvv genomes from the U.S. and Argentina. We sequenced 41 genomes of Xvv and the related sorghum-infecting X. vasicola pv. holcicola (Xvh), and performed comparative analyses against all available X. vasicola genomes. Time-measured phylogenetic analyses showed that Xvv strains from the U.S. and Argentina are closely related and arose from two introductions to North and South America. Gene content comparisons identified clusters of genes enriched in corn Xvv that showed evidence of horizontal transfer including one cluster corresponding to a prophage found in all Xvv strains from the U.S. and Argentina as well as in Xvh strains. In this work we explore the genomes of an emerging phytopathogen population as a first step towards identifying genetic changes associated to the emergence. The acquisitions identified may contain virulence determinants or other factors associated with the spread of Xvv in North and South America, and will be the subject of future work.

S5A-O4

Current scenario and EU funded research projects on *Xylella fastidiosa*

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With the discovery of *Xylella fastidiosa* (*Xf*) as the causal agent of one of the most detrimental disease in olive, the olive quick decline syndrome, this bacterium re-emerged as plant pathogen of global importance and of major concern for the Old Continent and the Mediterranean Basin. Not long after its detection in olives in Italy in 2013, several bacterial strains belogning to different *Xf* subspecies have been reported in other outbreaks found in southern Europe, and more recently in Israel. While in the last century, research efforts to tackle *Xf* in crops/forestry/landscape have been centralized essentially in America, the European outbreaks entailed the establishment of research programs in Europe. In 2015, the European phytosanitary autorities funded a first research initiatve (H2020 project POnTE) to fullfill different knowledge gaps in the biology of the bacterium and disease epidemiology in European agro-ecosystems. Subsequently, the research outputs were strengheten through the H2020 Project XF-ACTORS and BBI-H2020 Project BIOVEXO, both focusing on practical sustainable solutions for its containment, while strenghthening preventive measures. In 2022, in the framework of the Horizon Europe program, the project "Beyond *Xylella*, Integrated Management Strategies for Mitigating *Xylella fastidiosa* impact in Europe" (BeXyl) has been approved for funding. BeXyl will capitalize results from the abovementioned H2020 projects, for advancing and extending the currently limited strategies and tools available to counteract the impact of this harmful pathogen. The multidisciplinary workplan is designed to enhance capacities to prevent and find adequate responses to new *Xf* outbreaks under a changing climate scenario, as well as to restore outbreak areas through the use of host plant resistance and innovative biocontrol tools for *Xf* and its insect vectors.

S5B-KN1

Examining the emergence of host-adapted phytopathogens and the role of prophages in transfer of virulence genes

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Pseudomonas syringae (*Ps*) inhabits plant leaves and stems, both on the surface and also within the tissue. Genotypic and phenotypic variation can be observed within populations of the bacterium, for example, variation in the ability to cause disease symptoms as well as quantitative differences in virulence. The genetic basis for these differences is not well understood although Type III protein secretion systems (T3SS) and T3S effectors (T3SE) are key virulence factors. We have used population genetic and phylogenomic analyses of *Ps* strains that live on the surface of cherry leaves and shoots to analyse the frequency of potential pathogens (those carrying a T3SS) within the phyllosphere and have observed regional differences in populations. We have also identified signatures of loss and enrichment of specific T3SE[1]. Effector *hopAB1* is absent from cherry pathogens and we showed that cherry recognises *Ps* expressing *hopAB1* to trigger resistance. Conversely, *hopAR1* is widely dispersed within the *Ps* cherry pathogen and epiphytic population. Genomic analysis indicates many strains harbour *hopAR1* on a prophage suggesting horizontal gene transfer (HGT) may have occurred via the bacteriophage. We used strains with a tagged prophage to demonstrate that stress conditions such as UV light can induce phage DNA harbouring *hopAR1* to excise and circularise from the bacterial chromosome. Moreover, when the strain hosting the prophage was mixed with a recipient strain lacking *hopAR1*, it was possible to observe transfer to the recipient. We have therefore confirmed the bioinformatics-based predictions of phage activity driving evolution through HGT.

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[1]Hulin, M.T. *et al.* (2018) *New Phytologist* 219: 672-696.

S5B-O1

Transcriptome profiling uncovers peculiar responsiveness to apoplast-like conditions among *Pseudomonas syringae* pv. *actinidiae* biovars

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Measures to control *P. syringae* pv. *actinidiae* (Psa), the causal agent of kiwifruit bacterial canker, are mainly based on the use of copper, which may be soon subjected to use restrictions. Moreover, Psa resistance to copper is an event already emerging in several Countries. This context imposes an urgent development of new eco-compatible solutions to control disease epidemics, avoiding at the same time the occurrence of new resistant pathogen genotypes. Promising strategies would rely on pathogen weakening through virulence inhibition, but this requires a deep knowledge about bacterial virulence mechanisms to define efficient targets.

A huge effort has been made to provide advances in the study of bacterial virulence through the comparative analysis of bacterial genomes, in particular to identify effector reportoires. This approach has undoubtedly provided invaluable information on key players in phytopathogenic bacterial aggressiveness. However, this may represent only the tip of the iceberg. Considering that bacteria belonging to the *P. syringae* complex and, a fortiori, strains of the same pathovar, share a high genomic similarity, including pathogenicity and virulence factors, how to explain that very closely-related strains display different behaviors in their potential host plants? In this study, the expression profiles of different Psa biovars grown in apoplast-mimicking conditions reveal a different transcriptional responsiveness to growth conditions among Psa biovars, in particular regarding the activation of the type III secretion system, and provide new insights into Psa virulence molecular mechanisms, including the role of c-di-GMP as a possible switch in Psa signaling.

S5B-O2

Xanthomonad population dynamics and their effect on disease management of bacterial spot of pepper

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Bacterial spot (BS) is an economically important disease of pepper worldwide. Growers rely heavily on copper (Cu) bactericides and host genotypes carrying resistance genes Bs1-5, which drive bacterial evolution. A decade-long survey in the 1990s monitored BS on pepper in North Carolina (NC), USA, and it classified the causal agent into the eleven races of *Xanthomonas campestris* pv. *vesicatoria* (Xcv). However, Xcv was reclassified into four species in 2004: *X. euvesicatoria* (Xe); *X. gardneri* (Xg); *X. perforans* (Xp); and *X. vesicatoria*. Over the past two decades, little is known about the current Xanthomonad populations in NC. Therefore, we started a new survey in 2020. To date, we have collected diseased leaves from 51 cultivars across 22 commercial fields. A total of 105 strains were characterized using qPCR; 85% were Xe, 10% were Xp, and 5% were Xg. Assays on pepper differential lines confirmed representative isolates encompassing races 0, 1, 2, 5, 6, 7, 8, 9 and 10, and the latter accounted for 11% of all collected strains. Strains in race 10 are able to overcome the commonly used BS1-4 resistance genes in the field. Additionally, 49% of all strains were resistant to Cu, and 7% were resistant to both streptomycin and Cu. These findings highlight the complexity of Xanthomonad populations infecting pepper in NC and raise concern to the percentage of resistant isolates in pepper fields. Whole-genome sequencing of these isolates and comparative genomics will soon help elucidate the genetic factors responsible for Cu and streptomycin resistance in the field.

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S5B-O3

Do phages antagonise each other to cause reduced efficiency of killing bacteria?

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Bacterial canker is a major disease of *Prunus* species such as cherry (*Prunus avium*). It is caused by *Pseudomonas syringae* species including *P. syringae* pv. *syringae* (*Pss*) and *P. syringae* pv. *morsprunorum* race 1 (*Psm*1) and race 2 (*Psm*2). Concerns over the environmental impact of, and developing resistance to, copper controls call for alternative approaches to disease management. One method of control could be achieved using naturally occurring bacteriophage (phage) infective to the bacterial pathogens. We have isolated and characterised phages (MR) with host specificity to *Pss*, *Psm*1 and *Psm*2[1]. However, before being used for field applications as a biocontrol agent, it is important to assess their efficacy and robustness as well as changes occuring in the bacterial population. For example, phage resistance can rapidly arise in bacteria and the use of mixtures (cocktails) of phage to overcome this might lead to undesirable phage antagonism with each other and thus failure of the biocontrol. To examine this, killing curves were generated by infecting *Pss* with each MR phage individullay or in a combination of up to five, and optical density (OD) were meastured for 48 h. Our results found that phages used individually had a higher rate of reducing the bacterial population compared to cocktails of phages. Increases in OD was observed after 30-33 h as more phages were added to the combination, suggesting the emergence of resistant bacteria within the population. Our results indicate that phages are likely antagonising one another thus reducing the efficacy of phage biocontrol.

This project has received funding from Biotechnology and Biological Sciences Research Council (BB/T010568/1).

[1]Rabiey M. *et al*., 2020. Microbial Biotech, 13, 1428–1445.

S5B-O5

Building an ecological model of bacteria-bacteriophage interactions using the *Xanthomonas arboricola* pv. *pruni*-peach pathosystem

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Bacteriophage (phage) can impact bacterial populations and are a potential management strategy for bacterial diseases of plants. However, knowledge of ecological impacts of phage-bacteria interactions at the population level is lacking. *Xanthomonas arboricola* pv. *pruni* (Xap) is the causal agent of bacterial spot of *Prunus* and can be endemic to the perennial crop, peach (*P. persica*). The peach-Xap-phage system represents an excellent model to study foliar phage and bacterial pathogen genomics in a dynamic host-parasite spatiotemporal environment. To characterize phage and bacterial phenotypes, we performed overlay host range assays using a panel of Xap isolates from the southeastern United States. Genomic diversity of the phage and bacterial panel was characterized using Illumina NextSeq followed by genome assembly and annotation. Results show wide diversity in bacterial susceptibility to phage infection. The diversity of host range phenotypes parallels variation observed in bacterial gene content and coding sequences. Preliminary annotation of bacterial genomes shows variability in genomic regions possibly associated with phage specificity and bacterial resistance to phage infection. Further analysis of bacterial genome sequences revealed the presence of intact and partial prophage. Prophage are lysogenic, meaning they integrate into the host genome and can be inherited by asexual reproduction and horizontal gene transfer. Integrated prophage have been previously shown to affect bacterial phenotypes including virulence and fitness. Further work is needed to analyze predicted prophage in our Xap strains to deteremine their possible impact. These results are the first step towards building a peach-Xap-phage ecological model that may help predict bacterial evolution.

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S6A-KN1
Bacterial disease prevention and control. Challenges and future prospects

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Diseases caused by plant pathogenic bacteria have different epidemiological trends determined by their three main ways of life (epiphytic, endophytic and rhizophytic). Disease control strategies for preventing economical losses due to bacterial diseases are designed taking into account their disease cycle and epidemiology, and are oriented to short-cut key stages. Prevention of plant bacterial diseases rely on our capacity of monitoring the pathogen and the accuracy and sensitivity of the detection methods, the aggressiveness and host range and the evolutionary potential of the pathogen, the availability of less susceptible or resistant plant material, and of forecasting systems. However, disease control currently rely on chemical products like copper compounds or antibiotics in some countries, a very limited choice in comparison to the great diversity of existing fungicides. Although several microbial biopesticides for bacterial disease control are currently in the market, there is still a lack of novel and effective products. In addition, special attention has to be addressed to the global threats to fruit tree crops of importance in the Mediterranean agriculture posed by emerging and re-emerging bacterial pathogens. Consequently, the challenges are the need for development of novel compounds (e.g. functional peptides, RNAi-based pesticides, nanomaterials), consortia of microbial strains (e.g. syntrophic bacteria), more efficient and sustainable methods of application of treatments (e.g. endotherapy), and reliable and validated disease forecasting systems (e.g. diseases caused by *Xylella fastidiosa*). What we know, how we benefit and future challenges and prospects in prevention and disease control will be discussed.

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S6A-O1
Novel bacterial metabolites to treat citrus Huanglongbing, citrus canker, and fire blight

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Citrus Huanglongbing, citrus canker, and apple fire blight are the most devastating diseases affecting citruses and apples. Despite the tremendous damage posed by these diseases, the control options for growers are extremely limited. Sprays of human antibiotics, streptomycin and oxytetracycline, are the few options that can suppress most of the plant bacterial infections in commercial orchards. However, antibiotic use creates high pressure on farmers: (1) economically, due to high costs and increasing risks of compromised efficacy due to the appearance of antibiotic resistance in the orchards, and (2) societally, due to growing environmental concerns regarding antibiotic use in agriculture and their impacts and risks on human health. Some countries, like those in the E.U., have already prohibited the use of such antibiotics, leaving growers at loss for identifying efficient alternatives. To fulfill this need, we have built the first large collection of microbes from different natural environments in Wisconsin and other US states over a period of five years. A total of ~40,000+ microbial isolates were screened to identify microbial metabolites on disease control. During this study, we have discovered a bacterium *Pseudomonas soli* and its novel metabolites named RejuAgro. From the greenhouse assay or independent field trials, RejuAgro can effectively suppress the citrus Huanglongbing, citrus canker, and apple fire blight when sprayed on the surface of citrus leaves and apple flowers. Our results suggested that applied RejuAgro was taken up by plant leaves and moved systemically where pathogens were located. The overall objective of this study is to develop alternatives to human antibiotics that can effectively control agricultural crop disease. These measures will avoid the risk of losing choices if the use of antibiotics is completely banned.

S6A-O2

Detection of the pathogenic bacteria *Acidovorax citrulli* and *Pseudomonas syringae* in Cucurbit seeds

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The International Seed Health Initiative (ISHI-Veg) brings together seed companies, public sector institutions and private laboratories to develop seed health tests for seed-transmitted pathogens. Recently, two grow-out-based methods for the detection of *Acidovorax citrulli* and *Pseudomonas syringae,* attacking cucurbit species have been developed and validated to assist in the delivery of healthy seeds. A grow-out is an assay in which seeds are sown under disease-conducive conditions, and is an important tool for controlling pathogen viability and pathogenicity, which is a prerequisite for a positive result. *Acidovorax citrulli* is a seed-transmitted bacterium causing bacterial fruit blotch (BFB) on melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) [1]. In favourable environment, the disease can become devastating and causing up to 100% loss of marketable fruits. The initiative has developed a grow-out method based on seedlings grown in sweat boxes as an alternative to the existing grow-out in greenhouses, as a faster, less expensive and less dependent on climate variations method. This method is recognized as reference by the group and can be used in combination with the pre-screen seed extract-qPCR already validated. *Pseudomonas syringae* is a seed-transmitted bacterium causing Zucchini vein clearing disease in squash (*Cucurbita pepo*)[2]. Cool temperatures and humidity are the most favourable conditions for its expression. To reliably test seeds for Pseudomonas syringae causing Zucchini vein clearing disease, the seed industry developed a grow-out based method based on seedlings grown in potting mix in a greenhouse or growth chamber to test squash seed lots for viable, infectious *P. syringae*.

[1]Schaad, N.W. *et al*., 1978. Int. J. Syst. Evol. Microbiol., 28:117-125.
[2]Newberry, E.A. *et al*., 2016. Plant Dis., 100 : 1397-1404.

S6A-O3

Colonization of yeast-like fungi on apple flowers induces host immunity and prevents fire blight infection

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Microbiome on plants is well recognized for its potential to influence plant disease occurrence through impacting the pathogen-host interactions. Fire blight, caused by a bacterial pathogen *Erwinia amylovora*, is a devastating disease of apple and pears. Blossom blight stage of fire blight infection, in which *E. amylovora* mutiplies eiphytically on flower surfaces such as stigma and stamen, prior to entering host through the hypanthium, is a critical step of the disease cycle. Previous research investigating the function of microbiome on fire blight mostly focused on the microbiome-pathogen interactions[1,2], however, to what extent the microbiome interacts with the host, and whether/how such interactions influence disease outcome is less understood. In this study, we characterized the composition and dynamics of the mycobiome on hypanthium of apple flowers. We showed that some members of the mycobiome, namely the yeast-like fungi belonging to the *Aureobasidium* genus, induced the expression of pathogenicity related (PR) genes in the salicilic acid (SA) pathway in apple hypanthium. Some of the fungal isolates that induce host immunity genes also can colonize under cuticles of immature fruits and cause russeting of fruits. Spray-inoculating an *Aureobasidium pullulans* suspension to apple flowers significantly repressed fire blight infection. Our study indicates certain yeast-like fungi, through causing a minor russeting on apple cuticle, induced host immunity and thus primed the host to resist the infection of fire blight, a much more devastating disease that can cause tree mortality.

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[1]Cui Z. *et al*, 2020. The ISME Journal, 15, 318–329
[2]Cui Z. *et al*, 2021. Phytobiomes Journal 5,156-165

S6A-O4

Investigations on the endophytic bacteria taxonomically related to *Xylophilus ampelinus* that may interfere with the PCR based diagnosis of bacterial blight of grapevine

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Xylophilus is a genus of the *Burkholderiales* comprising only one species described to name a xylematic bacterium causing bacterial blight of grapevine, *X. ampelinus*, and one species (yet to be validated) to name the bacterium isolated from the flower of the royal azalea plant, X. rhododendri. Evidence from metagenomic analyses suggests, however, that the phytobiome may be the reservoir for an unexplored yet large diversity laying within the boundaries of this genus.
Recently, we obtained discrepant results when testing grapevines with the two molecular diagnostic protocols for *X. ampelinus* recognized by EPPO. In fact, we evidenced samples collected from asymptomatic rooted grafted plants which resulted positive only with the PCR protocol targeting the ITS region of the pathogen. The incongruity between the results according to the two solid and well-validated protocols prompted us to start this study aimed at unraveling the identity of the microorganisms that acted as the target of the PCR assays. Attempts to isolate in pure culture the unknown bacteria that were targeted by the PCR on the ITS region repeatedly failed. With the help of the QuantPrime, we developed primer pairs for all the recognized ORFs of the genome of *X. ampelinus* strain CECT 7646^T (assembly GCA_003217575.1). By checking the matches of that primer set against a custom database comprising 5878 genomes of the *Burkholderiales*, we devised a subset of primers targeting *X. ampelinus* and its closest relatives. The sequence analysis of the amplicons obtained from the grapevine nucleic acids extracts that were positive only with the diagnostic protocol targeting the ITS region shed light on the diversity of the grapevine microbiota related to the genus *Xylophilus*. Further metagenomic analyses carried out using Oxford Nanopore Technology supported the notions that the endophytic population of grapevine may include members of the *Burkholderiales* taxonomically related to *X. ampelinus,* although they represent a relatively small fraction of the microbial community. Finally, we developed a set of three primer pairs that are able to discriminate the agent of bacterial blight of grapevine, *X. ampelinus,* from taxonomically related strains that share a similar sequence in the ITS region but do not actually belong to the taxon associated with the disease. The developed primer set may prove useful for inclusion in new, improved protocols for the detection of the causative agent of bacterial blight of grapevine for certification purposes.

S6B-KN1

Impact of pollen naturally contaminated by *Pseudomonas syringae pv. actinidiae* (Psa) on disease incidence in a commercial kiwifruit orchard

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Artificial pollination is a common and important feature of kiwifruit production because kiwifruit is a dioecious vine (male and female flowers are carried by different plants). Unfortunately, kiwifruit pollen can harbour the commercially important pathogen *Pseudomonas syringae* pv. *actinidiae* (Psa)[1]. Direct inoculation of kiwifruit flowers with Psa leads to systemic infection[2]. However, the contribution of Psa-contaminated pollen to disease incidence in kiwifruit orchards using artificial pollination is not known. To determine the impact of naturally contaminated pollen on flower infection and fruit set, we compared two years in a row, disease incidence of flowers pollinated with Psa-free pollen with that of flowers pollinated with pollen naturally contaminated with Psa. The percentage of fruit set on vines pollinated with Psa-contaminated pollen was not significantly different from that on vines pollinated with Psa-free pollen. The percentage of fruit set was influenced more by the row in which the vine was located than by whether the pollen used for pollination was contaminated or not by Psa. To determine the effect of naturally contaminated pollen on canopy infection, we compared disease incidence on young susceptible potted kiwifruit plants (trap plants) exposed to Psa-contaminated pollen with that of trap plants not exposed to the pollen. Trap plants exposed to the pollen showed more leaf spots than those not exposed to it, but disease incidence between those two lots of trap plants was not significantly different. Results from this study will help formulate recommendations for the use of kiwifruit pollen in New Zealand.

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S6B-O1

Interference of plant derived phenolics with AHL mediated communication of *Pectobacterium* spp.

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Many Gram-negative plant pathogenic bacteria rely on N-acyl-homoserine lactones (AHLs) to sense the environment and coordinate group behavior, also termed quorum sensing (QS). In *Pectobacterium* a genus responsible for soft-rot and blackleg diseases of potato, vegetables and ornamentals, this signaling mechanism comprises two essential components: a LuxI type AHL synthase (ExpI); and one or more AHL response regulators (ExpR), LuxR homologues. The QS machinery largely regulates virulence by the control of plant cell wall degrading enzymes and secretion systems, production of antibiotics, motility, and biofilm formation[1]. Plants, in order to defend themselves, have evolved an array of low molecular-mass molecules, of which phenolic compounds are probably the most abundant. Here using *P. brasiliense* as a model, we present the interaction of some phenolic compounds, including the plant hormone salicylic acid, with the QS major elements[2]. Our results suggest that some plant derived molecules are potent inhibitors of the QS machinery, and that ExpI (AHL synthase) is their main target[3]. ExpI expression in DH5α, a QS negative *Escherichia coli* strain, supported direct interference of the compounds with AHL synthesis. By combining computational docking simulations and protein ligand thermodynamic approach (Isothermal Titration Calorimetry) we were able to support direct binding of the plant compounds to the bacterial AHL synthase, and suggest a mechanism for the interference of plant molecules with *Pectobacterium* QS machinery.

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S6B-O2

It takes guts to deal with black rot: biofilm degrading enzymes for *Xanthomonas* control

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Xanthomonas campestris pv. *campestris* (*Xcc*) is the causative agent of black rot, an important disease of cruciferous crops worldwide. *Xcc* is primarily a seed-borne vascular pathogen, colonizing the xylem system of infected plants. Biofilm formation plays an important role in the pathogenicity and virulence of *Xcc*, conferring protection against hostile environments and ensuring a long-term bacterial population survival. It requires the synthesis of a complex exopolysaccharide, xanthan gum, that has many industrial applications. Studies have demonstrated that strains lacking or producing truncated xanthan, fail to cause disease in vitro using model plants, such as *Arabidopsis thaliana*[1,2]. Moreover, pre-treatment of plants with xanthan restores the pathogenicity of strains. The aim of this project is to investigate the ability of specific carbohydrate active enzymes (CAZymes) to degrade plant pathogenic biofilms produced by *Xcc* and so decrease its ability to cause disease. Recently, a GH5 enzyme, identified in gut residing bacterium, has demonstrated an endo-acting xanthanase activity[3]. We have confirmed GH5 ability to effectively degrade xanthan produced by various *X. campestris* pathovars and other *Xanthomonas* strains, releasing a mixture of different size oligosaccharides. In addition, ~50% reduction in established *Xcc* biofilms produced in different culture media was observed with GH5 treatment. Experiments to investigate GH5 ability to disrupt *Xcc* biofilms established on seeds and in planta, are in progress. Currently, black rot control measures are limited, thus exploring the capabilities of CAZymes can prove to be a highly effective long-term solution.

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S6B-O3

Sanitation of carrot seeds infected by *Ca.* Liberibacter solanacearum through a thermal treatment and assesment of its efficacy by a viability qPCR protocol

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Candidatus Liberibacter solanacearum is a non culturable bacterium that may affect several important crop species, including host plants belonging to the *Solanaceae* and *Apiaceae* families[1]. In carrot, celery and other *Apiaceae* this pathogen is seed-borne[2], though its transmissibility is currently a matter of debate[3]. In order to ensure an excellent seed quality, we implemented a heat treatment protocol in order to ensure freedom of viable germs possibly present in carrot seeds. Since a qPCR protocol is not able to discriminate viable from dead bacteria infecting any plant matrix, and cultural methods are not feasible either, we implemented a viability qPCR protocol, where seed extracts are treated with monoazides (PMA or EMA). Through the development of a calibration curve, we were able to assess that the naturally infected seed lots used in the experimets harboured bacterial germs in the order of 10^6 cells/g of seed. Our results showed that a thermal treatment at 50°C for 72 hours was able to sanitise carrot seeds: this was confirmed by the viability qPCR detection method that always gave negative results to the analysis. A further confirmation of seed sanitation was obtained by sowing the sanitised seeds under controlled conditions: such seeds were allowed to germinate and produce seedlings. Analysis of seedlings was negative for the presence of *Ca*. Liberibacter solanacearum 30, 60 and 90 days after sowing. Finally, seed quality assays done on thermo-treated seeds confirmed that their viability and their ability to develop strong seedlings did not differ from that of untreated seeds.

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S6B-O4

Bacillus amyloliquefaciens subsp. *plantarum* active against quarantine bacteria causing serious epidemics in North-Central Italy

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In Italy, in the last decades, the continuous spread of bacterial diseases in crops of considerable economic importance, the concomitant climate change and the need of a more environmentally sustainable agriculture have highlighted an increasing attention towards biological control measures integrated with appropriate agronomic techniques and preventive treatments which rely almost exclusively on the use of copper compound sprays on the plant surfaces. Since 2010 and in the following 10 years, we have tested *Bacillus amyloliquefaciens* subsp. *plantarum* (strain D747) to evaluate its efficacy to control some of the most important plant pathogenic quarantine bacteria that caused severe epidemics in North-Central Italy. In particular, the following pathosystems have been studied: *Pseudomonas syringae* pv. *actinidiae* (Psa) – *Actinidia* spp., *Erwinia amylovora* (Ea, from December 2019, RNQP pursuant to Regulation (EU) 2016/2031) – *Pyrus communis*, *Xanthomonas arboricola* pv. *pruni* (Xap) - *Prunus persica*, *Xanthomonas vesicatoria* (Xv) – *Solanum lycopersicum*. The biocontrol efficacy was tested *in vitro* and *in planta*, under greenhouse and field conditions, to test both direct or indirect effects. The strain D747 was highly effective *in vitro* against all four pathogens, it was able to survive on the different hosts and to reduce the pathogen populations and their disease severity. The biocontrol performance of the strain D747 was higher or comparable to the antagonistic strain QST713 of *Bacillus subtilis*, to chemicals as acibenzolar-S-methyl and to copper based compounds in greenhouse and field experiments. Moreover, the strain D747 was able to induce resistance responses against infection of Psa and Xv in their host plants.

S6C-KN1

Biological Control of Citrus Huanglongbing by *Xylella fastidiosa* Strain EB92-1 in Field Trials in Florida

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Huanglongbing (HLB), caused by *Candidatus* Liberibacter asiaticus, is currently the most destructive disease of citrus in Florida. In citrus trials initiated in 2014 and 2015, treatment with *Xylella* fastidiosa strain EB92-1 reduced the incidence and severity of HLB symptoms but did not eliminate the pathogen. In a field trial established in 2-year-old replants in an orange grove that had suffered severe losses from HLB, EB92-1 treated replants had less than 10% of the trees with severe symptoms 8 years after the trial began. Surprisingly, untreated trees also had a very low percentage of severe HLB symptoms. In a trial on mature grapefruit trees, severe symptoms developed in over 50% of the untreated trees in the first year of the trial, but in the next 6 years the incidence of severe symptoms did not increase in either the treated or untreated. This control in untreated trees was due to the transmission of EB92-1 to the untreated. At the end of both the young tree trial and the grapefruit trials, strain EB92-1 was colonizing the treated and untreated tree equally well. In the grapefruit, untreated rows next to treated rows had fewer trees with severe HLB symptoms than untreated rows that were 200 ft or more from the treated rows. Colonization of the roots by EB92-1 appears to result in fewer symptoms and lower populations of HLB in the roots. Treatment of trees younger than 2-years-old was not beneficial. EB92-1 is better able to colonize and protect trees greater than 2-years-old.

S6C-O1

Factors determining the risks of bacterial blotch in *Agaricus bisporus*

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Bacterial blotch is an important diseases of the button mushroom *Agaricus bisporus.* Blotch causing bacteria can be endemically present in casing soils. During a survey of blotched mushroom in Western Europe, we found that not only Pseudomonas species but also representatives of other genera can cause blotch[1]. Studies were done with the two most prevalent pathogens, *P. gingeri* (ginger blotch) and *P. salomonii* (brown blotch). *P. gingeri* was found to be more aggressive than *P. salomonii*[2]. It was shown that the composition of the peat-based casing affected the bacterial blotch prevalence significantly. The ginger blotch, but not the brown blotch prevalence declined from the first to the third harvest cycle. Interestingly the density of *P. gingeri* in the casing soil increased during subsequent flushes. A microbial extract prepared from the casing soil of the blotch-suppressive third flush reduced ginger blotch when added to casing in the first flush. Thus, ginger blotch suppression is transferable. Blotch suppression coincided with specific changes in the microbiome of the casing soil[3]. To investigate the effect of partial replacement of peat by sustainable alternatives on bacterial blotch, peat moss, fibers from agri-residue streams and spent casing soil were used, with or without steam treatment. Steam-treated spent casing had the lowest risk for blotch outbreaks without compromising on yield. We continue to evaluate these alternative casing soils in the EU BIOSCHAMP project, not only for bacterial blotch, but also for three major fungal diseases, i.e. the dry- and wet bubble and the cobweb diseases. (https://cordis.europa.eu/project/id/101000651).

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S6C-O2

Waste valorization for circular protection of tomato by a nanotechnological approach

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Lignocellulosic waste management represent a serious issue in granting agriculture sustainability, which could be solved thank to nanotechnology. Nanomaterials can offer alternative solutions to exploit biomasses components as sources for innotive pesticides[1]. In this work the possibility of extracting cellulose nanocrystals (CNC) from tomato pruning residues was evaluated, as well as their antimicrobial properties against *Pseudomonas syringae* pv. *tomato* (Pst), the causal agent of tomato bacterial speck, in a circular economy context. CNC were obtained through two different protocols: in the first one CNC synthesys was preceded by a 3% sodium chlorite chemical bleaching, while in the latter the cellulosic pulp was treated with a further enzymatic digestion. A mix of cellulases, β-glucosidases and hemicellulases was used. Obtained cellulose resulted purer in terms of amorphous regions, thus it was possible to lower the amount of sulphuric acid from 64% to 62% in the final hydrolisis. Obtained crystals were characterized by TEM, revealing an acicular shape and an average length of 120 nm for both extractions. CNC were tested for their *in vitro* antimicrobial activity on Pst DC3000 strain by several microdilution methods, showing firstly a promising growth inhibition of 60% at 24 hours when used at 1%, then highlighting an ihibition of bacterial biofilm formation of 48% at 24 hours when used at 0,05%[2]. These results indicate the possibility of synthetizing CNC by lowering the amount of used chemicals, while at the same time preserving their antibacterial properties, that could be exploited for the development of new sustainable nanopesticides.

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S6C-O3

Multilocus sequence analysis (MLSA) of *Pseudomonas* spp. causing blotch on mushroom (*Agaricus bisporus*) farms in the U.S.

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Bacterial blotch of white button mushroom (*Agaricus bisporus*) has been reported to cause yield losses in commercial mushrooms for more than 100 years and still to this day it remains a problem[1]. Bacterial blotch is a complex of diseses caused by a variety of bacterial pathogens[1,2,3], including various *Pseudomonas* species. Control of bacterial blotch is complicated by the variety of potentialy beneficial *Pseudomonas* species found on mushroom[4]. The goal of this research is to understand the diversity of *Pseudomonas* spp. which cause blotch in *A. bisporus* in order to apply a more targeted mangement strategy. *Pseudomonas* species were isolated in six survey from mushroom with blotch symptoms using semi-selective media (Kings B medium amended with novobiocin, cycloheximide, and penicillin). Pathogenicity tests were conducted with the isolates and those causing blotch were identified using multilocus sequence analysis (MLSA) by comparing the isolate sequences to 163 type strains of named *Pseudomonas* species and 186 unnamed species. Pathogenic *Pseudomonas* isolates were distribuited among 10 clades hypothesized to represent as many different species. Of the 114 pathogenic bacterial isolates, 56, 13, and 1 were identified as *P. tolaasii*, an invalidly named species [*P. gingeri*], and *P. agarici*, respectively. The remaining 44 strains were shown to be from seven hypothetical species that have not yet been described or named. Interestingly, some of the hypothetical new species were isolated and identified from multiple farms. The analysis of whole genome sequences along with phenotypic testing will be used to determine whether these organisms are distinct and novel species of *Pseudomonas.* The data from the study will allow us to begin to develop management practices targeting only the blotch-causing *Pseudomonas* spp.

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S6C-O4

Light composition modulation of *Actinidia* - *Pseudomonas syringae* pv. *actinidiae* interaction

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Light composition modulates plant growth and development. Chlorophyll absorption peaks correspond to the blue(B) and red (R) spectral regions, and therefore several R:B ratios have been widely tested for yield and quality crop improvement[1]. Moreover, light composition plays an important role in plant resistance against pathogens, by either modulating plant defenses or interfering with pathogen virulence[2,3].
Recently, the use of photoselective hail nets and plastic tunnels has gained interest in kiwifruit production but the consequent physiological changes have not been described yet. Light-emitting diodes(LEDs), used as artificial light source, provide the background to dissect the physiological and molecular mechanisms of plant responses to light conditions. To understand the physiological effects of light composition on kiwifruit, different R:B ratios were tested on *Actinidia chinensis* at a photosynthetic photon flux density (PPFD) of 200µmol.m^{-2}.s^{-1} and photoperiod of 16h.d^{-1} of light. Biometric parameters, chlorophyll a fluorescence, gas exchange and photosynthesis-related gene expression were evaluated in plants subjected to the different light treatments. Furthermore, the influence of light composition was investigated on the infection by *Pseudomonas syringae* pv. *Actinidiae* (Psa). Our study shows that, 50%R-50%B and 25R-75%B lead to the best plant performance but are not effective in controlling Psa endophytic population. Monochromatic red light, on the contrary, severely reduces φPSII, ETR, Pn, TSS and photosynthesis-related gene expression. Conversely, monochromatic blue light significantly decreases disease incidence, though not affecting bacterial endophytic population. These results suggest that monochromatic blue light likely reduces symptom development rather by affecting Psa virulence than by increasing host plant defenses.

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S7-KN1

Identification and use of plant cell-surface immune receptors to improve broad-spectrum disease resistance in crops

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Plants employ a multilayered innate immune system to fight disease. The first layer involves the perception of conserved microbial elicitors known as pathogen-associated molecular patterns (PAMPs) by cell surface-localized pattern recognition receptors (PRRs). PAMPs are often characteristic of whole classes/families of microbes, play an important role for the microbes' life, are generally absent from the host, and therefore represent good targets for recognition of 'infectious non-self'. Plant PRRs can also perceive endogenous signals indicative of a 'stressed' or 'modified self', known as damage-associated molecular patterns (DAMPs), which are thought to amplify immune signaling, locally or systemically. Activation of PRRs results in PAMP-triggered immunity, and acts as an early warning system against danger that is sufficient to restrict growth of most pathogens and pests, and is thus involved in both basal and non-host resistances. Our molecular knowledge on plant PRRs involved in PAMP/DAMP perception has drastically increased in the past decade. Interestingly, it is now clear that while some PRRs are conserved across a wide range of plant species (*e.g.* FLS2 that recognizes the flg22 epitope from bacterial flagellin), many others are family-specific (*e.g.* EFR that recognizes the elf18 epitope from bacterial EF-Tu). This phylogenetic diversification opens ways to engineer broad-spectrum disease resistance in crops through the generation of novel recognition specificities based on the transfer of PRRs across plant species, families, or even classes. We have successfully pioneered this approach with EFR, and I will present recent results illustrating how PRRs can be used to improve resistance of multiple crops against economically-important bacterial diseases, including in field conditions.

S7-O1

Transgenic grapefruit containing a TAL effector trapping promoter provides broad spectrum resistance to *Xanthomonas citri*

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Previously, we created a T-DNA construct that was designated *ProBs3$_{14EBE}$:avrGf1* and which contains 14 *EBE*s corresponding to different *X. citri* TALEs. The construct was designed to confer recognition of different TAL effectors and drive expression of the microbial avirulence gene, *avrGf1*, that in turn elicits a hypersensitive reaction (HR) when expressed in grapefruit leaves. Co-infiltration of Agrobacterium cells containing T-DNA construct when co-infiltrated with *Xanthomonas citri* (Xc) cells that delivered an array of TAL effectors into grapefruit leaves resulted in a hypersensitive reaction (HR) including significant reduction in bacterial populations compared to nontransgenic grapefruit. The construct was transformed into Duncan grapefruit. Several independent transgenic grapefruit trees have been created that contain the intact construct of a variant of *ProBs3$_{14EBE}$:avrGf1* that was designated *ProBs3$_{14EBE}$:avrGf2*. We selected one of the transgenic lines, designated JJ5, for further analysis. Grafted trees containing *ProBs3$_{14EBE}$:avrGf2* were tested extensively in greenhouse and field experiments. In greenhouse experiments, no canker was observed on foliage of JJ5 following artificial inoculation with Xc strains from diverse geographic regions and most of the strains activated an HR. In a field study, JJ5 trees were planted in the field in March 2019. Trees were assessed over a 2-year period beginning in 2019. No lesions were observed on JJ5 trees, but significant disease was observed on control trees at the end of the experiment. This strategy could provide durable resistance to Xc.

S7-O2

Inactivation of tomato *WAT1* results in auxin-dependent tolerance to genetically diverse *Clavibacter michiganensis* strains

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Bacterial canker of tomato caused by the vascular pathogen *Clavibacter michiganensis*, is considered to be one of the most destructive bacterial diseases of cultivated tomato worldwide. To date, no resistance against the pathogen is available[1]. While several molecular studies have identified bacterial factors involved in disease development, the mechanisms associated with susceptibility of tomato to the bacterium remain largely unknown. In our study, we set out to identify host susceptibility (S) genes involved in the interaction[2]. We show that inactivation of the tomato gene *Walls Are Thin1* (*SlWAT1*) through RNAi and CRISPR/Cas9 led to high tolerance to genetically diverse *C. michiganensis* strains, without suppression of bacterial growth. Although a full knock-out of the *SlWAT1* gene led to severe fitness costs, downregulation of the gene by RNAi resulted in high tolerance of transgenic plants to the disease without any pleiotropic effects. Furthermore, our study suggests that the observed tolerance through inactivation of *SlWAT1* is the result of the reduction in biosynthesis of ethylene and auxin. To our knowledge, this is the first report of a S gene against *C. michiganensis*. Moreover, for the first time our study assigns a role to auxin in susceptibility of tomato to *C. michiganensis*.

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S7-O3

Identification, characterisation and mapping of resistance to black rot (Xanthomonas campestris pv. campestris) in Brassica

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Black rot is the most damaging disease of vegetable brassicas (*Brassica oleracea*) and can reduce yields by >50%. The causal agent *Xanthomonas campestris pv. campestris* (*Xcc*) also infects other important brassica crops such as oilseed rape (*Brassica napus*), mustards (*Brassica juncea*) and Chinese cabbage (*Brassica rapa*). This project builds on diverse *Xcc* isolates, including an extensive collection at Warwick University to identify Brassica resistance to the most pathogenic *Xcc* races 1, 4, 5 and 6, with a focus on vegetable brassicas where resistance to these races is rare in extant crop types. We have screened Brassica Diversity Fixed Foundation Sets (DFFSs), for resistance to these four *Xcc* races. The DFFSs have been designed to capture the genetic diversity of ~6000 Brassica accessions in smaller subsets of homozygous lines. The identified resistances will be characterised and mapped to identify resistance-linked markers that can be used to accelerate their introgression into crop types by marker-assisted selection. In parallel, we use chlorophyll imaging and whole plant imaging techniques to visual Xcc infection, as well as the sequencing of over 900 Xcc isolates with the aim to identify effectors that dictate the outcome of Brassica-Xcc interactions.

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S7-O4

Structural restrictions to *Ralstonia solanacearum* colonization in resistant tomato revealed by a novel resistance screening method

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Ralstonia solanacearum is a devastating bacterial vascular pathogen causing bacterial wilt. In the field, resistance against this disease is quantitative and only available for breeders in tomato and eggplant. We have developed reporter strains that allow microscopic location and non-disruptive high-throughput quantification of plant colonisation by *R. solanacearum*. We have applied these strains to evaluate germplasm for resistance to bacterial wilt and to understand the basis of bacterial wilt resistance in tomato. The developed methodology has proven useful to speed up breeding programmes and to identify latent infections on symptomless plants. We have also used this system to investigate the spatio-temporal bacterial colonization dynamics using non-invasive live monitoring and grafting of susceptible and resistant varieties[1]. Our work also reveals four different restrictions to the bacterium in resistant tomato: root colonization, vertical movement from roots to shoots, circular vascular bundle invasion and radial apoplastic spread in the cortex. We demonstrate that structural constraints to bacterial spread are key for tomato resistance to bacterial wilt and that this resistance is expressed both in root and shoot tissues. We also show that *R. solanacearum* is not only a vascular pathogen but spreads "out of the xylem", occupying the plant apoplast niche. Finally, we have investigated the physico-chemical nature of the induced plant barriers as ligno-suberin coatings and tyramine-derived hydroxycinnamic acid amines. In agreement with these findings, overexpression of the ligno-suberin pathway in a susceptible tomato enhanced resistance by restricting R. solanacearum movement inside the plant and delaying disease progression[2,3].
Our findings open new avenues of research to engineer resistance against vascular wilt pathogens, by precisely manipulating the time and location of inducible vascular coatings in the roots of susceptible crops.

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S7-O5

Race-specific genotypes of *Pseudomonas syringae* pv. tomato are defined by mobile DNA elements within the genome

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Pseudomonas syringae pv. tomato is the causal agent of bacterial speck of tomato, an important disease that results in severe crop production losses worldwide. Currently, two races within phylogroup 01A (PG01A) have been described for this pathogen: Race 0 and Race 1. Race 0 strains have avirulence genes for the expression of type III system-associated effectors AvrPto and AvrPtoB, and are recognized and targeted by the effector-triggered immunity by tomato cultivars which have the *Pto* race-specific resistance gene. Race 1 strains instead lack the *avrPto* and *avrPtoB* genes, and are therefore capable of aggressively attack all the tomato cultivars. Here, we have performed the sequencing and the analysis of the whole genome of the strain DAPP-PG 215, which was isolated and described as Race 0 strain in 1996[1]. Our analysis revealed that its genome comprises a 6.2 Mb circular chromosome and two plasmids (107 kb and 81 kb). The results indicate that the strain is phylogenetically closely related to Max13, K40, T1 and NYS-T1 strains. The chromosome encodes Race 1-associated genes like *avrA* and *hopW1* and lacks Race 0-associated genes like *hopN1*[2], giving it a Race 1 genetic background. However, the genome harbors a complete ortholog of *avrPto1*, which allows the strain to display a Race 0 phenotype. It is not the first strain to show intermediate virulence characteristics between Race 0 and Race 1[2]. Comparative genomics with several PG01A genomes revealed that mobile DNA elements are potentially involved in the evolution of the two different races.

[1]Buonaurio *et al*., 1996. J. Phytopathol., 144, 437-440.
[2] Kraus *et al*., 2017. Plant Dis., 101, 1442–1448.

SSA-KN1

The power of nanotechnology for sustainable food and plant protection.

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Nanotechnology is a promising field of interdisciplinary researches with practical applications into agriculture industry due to the potential benefits that green nanomaterials (NMs) can guarantee respects to plant and food protection. In particular, by nanotechnological approaches, instead of traditional synthesized molecules or chemical-based compounds, the new addresses are respect to both sustainable packaging systems and novel pesticide formulations, representing green strategies for the entire agriculture industry chain, from the field to consumers. Traditional chemical plant protection strategies, esplecially against phytobacteria, aren't sufficient and have negative effects on humans and the environment. Novel greener tools, also valorizing agro-food wastes, could represent efficient alternatives for the management of plant diseases using promising strategies and, the use of nanotechnologies, allows the more efficient assembly and subsequent release of environmentally sustainable active principles, limiting the use of chemicals. Similarly, new sustainable and antimicrobial systems have been rapidly promoted in the food packaging sector to improve the safety and quality of food products and reducing losses caused by different microrganisms. In this scenario, the use of micro- and nano-technology to promote a more efficient assembly and then release of specific and enviromental sustainable active principles seems to offer an incredible power respect to relevant biotic problems the affect food and plant protection strategies. Current research trends and recent advances will be presented while potential future applications will be analysed and discussed.

SSA-O1

Advanced nanomaterials for managing bacterial pathogens affecting vegetable crops and tools for understanding mechanism of action

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Hybrid metallic nanomaterials of Mg, Cu and Zn, and nanoemulsions of plant essential oils are potential options for bacterial disease management in applications that include foliar, soil and seed treatments. Formulations of these materials were developed and tested on *Xanthomonas* spp. affecting tomato and pepper, *Ralstonia solancearum* on tomato and *Pseudomonas syringae* on watermelon[1,2,3,4]. The studies used in vitro analysis of cell viability using epifluorescence microscopy, and PMA-qPCR, structural changes using scanning electron microscopy, biochemical changes using Raman spectroscopy, gene expression using RNAseq, and microbial shifts using high throughput sequencing that provided understanding of the mechanism of action of nanomaterials on bacterial pathogens. Analysis of elemental accumulation in fruits and soil using inductively coupled mass spectrometry provided new information on the fate of particles in the environment. Lab, greenhouse and field studies generated information on disease management, phytotoxicity and yield impact. This presentation will cover a synopsis of these findings, and present a perspective on possibilities for commercialization of advanced nanomaterials against bacterial pathogens.

[1]Carvalho R *et al*. 2019. Scientific Reports 9: 20124.
[2]Fan Q *et al*. 2020. Crop Protection 139:105366
[3]Liao YY *et al*. 2021. Environmental Science & Technology 55:20, 13561-13570
[4]Strayer-Scherer A *et al*. 2022 Microbiology Spectrum (In Press).

SSA-O2

Advanced ultrastructural approaches for the monitoring of nanophytodrugs in plant diseases

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Nanotechnologies are pervading all sciences and thus, the application in agriculture is increasing as well. The most advantageous nanotechnology that is applied to agriculture are related to the targeted/controlled release of agrochemicals in nano-formulated systems, such as nanopesticides or nanofertilizers, that enable more complete biological efficacy without overdosing. The combination of novel antibacterial nanomaterials and the early identification of infectious microorganisms appear to be a promising strategy for the treatment of bacterial infections in plants. However, one of the best advantageous of the use of nanoagrichemicals, lies in the reduction of the major drawbacks that conventional agrichemicals have in relation to efficacy, toxic effects, and environmental impact. On the other hand, the use of nanocompounds has naturally entailed an adaptation of technologies capable of having an adequate resolution to follow morphologically, and not only biochemically, the fate of nanoagrichemicals. A wide choice of ultrastructural technologies allows a precise characterization of nanocompounds, i.e. size, size distribution, shape, dispersion, etc, which is fundamental for safety assessment. Here the data of the analysis of healthy and diseased plants (leaves, stems and roots) treated with metal and organic-based nanoagrichemicals by using transmission electron microscopy (TEM) and X-ray nano-tomography will be discussed, highlighting the pros and cons of the techniques.

SSA-O3

Mechanism of action of a zinc-based nanoparticle with activity against vascular plant pathogenic bacteria

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Vascular-limited phytopathogenic bacteria are difficult to control due to spatial limitations of delivering bactericidal chemicals. Zinkicide® (ZnK) is an antimicrobial formulation containing ~4.0nm coated ZnO nanoparticles[1,2], with the potential to become systemic and control vascular pathogens. ZnK was tested on two vascular phytopathogenic bacteria, the phloem-limited '*Candidatus* Liberibacter spp.', and the xylem-limited *Xylella fastidiosa* (Xf). ZnK efficacy was tested in vitro in comparison to bulk (b-ZnO) on *Liberibacter crescens* (Lcr) and Xf subsp. *fastidiosa* TemeculaL. ZnK displayed a bactericidal effect, while b-ZnO was bacteriostatic at the concentration range tested (0-200 ppm) in microfluidic chambers and batch cultures. ZnK outperformed b-ZnO in bactericidal effect and enhanced antimicrobial mechanisms including Zn solubility, intracellular reactive oxygen species formation, cell membrane disruption, and lipid peroxidation[3]. In planta greenhouse experiments using tobacco and blueberry plants showed efficacy of ZnK to control leaf scorch disease caused by Xf. In tobacco, ZnK three-dose soil drench application with one-week gap intervals of 500/500/1000 ppm, reduced Xf populations by >3 $\log_{10}$ units and disease severity by ≈77% compared to the control. Whereas, in blueberry infected plants treated with ZnK twice at concentrations of 1000 ppm each over a one-week gap interval, Xf population was reduced by ≈ 1-2 log10 units, and disease severity decreased by ≈38% when compared to the control. ZnK enhanced bactericidal activity and translocation makes this formulation a promising product to control vascular pathogens.

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[1]Graham J, *et al*. 2016. Plant Dis. 100:2442-2447.
[2]Santra S, and Berroth M. 2015. US Patent # 9,215,877 (issued on 12/22/2015).
[3]Naranjo, E., *et al*. 2019. Sc. Reports 9:5150, https://doi.org/10.1038/s41598-019-41495-5.

SSA-O4

Advanced copper and Cu alternatives for crop protection

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To meet the increasing demand for global food security and nutrition resulting, current agriculture practices must adapt innovative technologies. Food crop productivity and quality must be improved drastically to feed the future population. Design and development of industrially-viable advanced pesticide and fertilizer formulations associated with low environmental risk factors are expected to significantly contribute to overall crop productivity and nutrition. Excessive use of conventional crop protection chemicals has increased risks of development of pathogen resistance. For example, copper (Cu) bactericides/fungicides are widely used in the agriculture industry in the U.S and worldwide on many crops. There is an increasing concern of Cu accumulation in field soil, Cu leaching potential into the surrounding ecosystem and development of bacterial resistance. Using nanotechnology, it is possible to reduce Cu concentration per application without compromising overall efficacy. Moreover, Zinc (Zn) based nanoparticle can be developed for potential use as an alternative to Cu bactericides/fungicides. This presentation will focus on laboratory, greenhouse and field experiments to demonstrate the efficacy of several nano-based crop protection formulations including Zinkicide® (a Zn based systemic bactericide), along with the challenges associated with developing industrially viable formulations and approaches to minimize regulatory challenges.

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[1]Graham J, *et al*. 2016. Plant Dis. 100:2442-2447.
[2]Huang, Z. *et al.* 2018, J. Agric. Food Chem. 66(33), 8679-8686.
[3]Strayer-Scherer, A. L. *et al.* 2018, *Phytopathology 108(2), 196-205.*
[4]Naranjo, E., *et al*. 2019. Sc. Reports 9:5150, https://doi.org/10.1038/s41598-019-41495-5.

SSA-O5

Nano-Magnesium as an alternative to copper biocide for crop protection

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In agriculture, copper has been an industry standard for controlling a broad spectrum of bacterial and fungal diseases. However, aggressive and prolonged use of copper biocides pose a serious risk of development of Cu tolerance and aggrevate environmental concerns due to their soil accumulation and potential leaching to water bodies. Therefore, there is a strong demand for reducing Cu load in the environment or replacing Cu with a suitable environmentally-friendly alternative. In this presentation, we report antimicrobial nano-magnesium (Nano-Mg) as a potential alternative to Cu biocides. Two non-phytotoxic water-dispersible formulations of Nano-Mg, a hydroxide (MgH) and an oxide (MgO) were developed and characterized [1,2]. *In vitro* antimicrobial studies showed that these formulations are fairly comparable to industry controls when tested against several pathogens including Cu sensitive and Cu tolerant bacterial species. The performance of these formulations were also evaluated in field conditions against citrus canker (MgH) and bacterial spot of tomato (MgH, MgO). Results suggest that Nano-Mg, if formulated correctly, can significantly reduce disease pressure in greenhouse and field conditions. Interestingly, a combination product of MgH-Cu allowed for reducing Cu rate significantly as compared to industry control for citrus canker control. This presentation will primarily focus on the efficacy of several formulations of Nano-Mg formulations (including a combination with Cu) against citrus canker.

Funds: USDA National Institute of Food and Agriculture, Specialty Crops Research Initiative (NIFA-SCRI) grant#s 2019-51181-30010; 2021-67013-33574.

[1]Huang, Z. *et al.* 2018, J. Agric. Food Chem. 66(33), 8679-8686.
[2]Strayer-Scherer, A. L. *et al.* 2018, *Phytopathology 108(2), 196-205.*

SSB-KN1
Mechanism and distribution of natural competence among *Xylella fastidiosa* strains

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Type IV pilus (TIVP) is a multifunctional bacterial structure that is involved in virulence and has roles in motility, adhesion, biofilm formation and natural competence. For this study we dissected the function of 38 genes involved in TIVP biosynthesis and functional regulation in the bacterial pathogen *Xylella fastidiosa*. This naturally competent xylem-limited pathogen causes re-emergent diseases worldwide, notably in Europe and the Americas. Previous studies showed that natural competence is an important mechanism to generate diversity in this pathogen, which potentially influence fitness [1]. Therefore, we were set to understand the molecular function of TIVP as a key component of both virulence and evolution in this pathogen. We identified ten core genes that were essential for natural competence and movement, as well as components that were specific to each trait, or had functions differing from other bacterial species. By combining approaches of molecular microbiology, structural biology, and biochemistry, we determined that the minor pilin FimT3 is the DNA receptor in TIVP of *X. fastidiosa*. This protein is found only in a few plant pathogens of the Xanthomonadaceae family. Additionally, we studied the distribution of natural competence among a collection of *X. fastidiosa* strains isolated from different hosts and geographical locations. We found that this ability is more frequent among strains classified in subspecies *fastidiosa* than others. Our study described the distribution of a critical mechanism required for exogenous DNA acquisition and thus expansion of genetic diversity in this devastating plant pathogen.

Funds: HATCH (Alabama Agricultural Experimental Station, AAES) conferred to L.D and N.P, and Auburn Internal Grant Program (OVPR) .

[1]Potnis N., *et al*. 2019. ISME J. 13:2319-2333.

SSB-O1
***Xylella fastidiosa* epidemics in Europe in a changing climate scenario**

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Introduction events of exotic pathogens are increasing exponentially in recent years, driven largely by altered climatic patterns and increasing rates of travel and trade, modifying their distribution, host range, and impact on crops, forests, and biodiversity. One of the best examples is the quarantine plant pathogenic bacterium *Xylella fastidiosa* (*Xf*) that that is causing several outbreaks in Europe, some of them with devastating effects. *Xf* can develop in a wide range of climate types but is best adapted to areas with mild winters. Temperature is a key factor determining *Xf* current distribution, but climate models project a temperature increase, particularly in winter, which could modify *Xf* ability to expand to regions previously prjected as less suitable. In this work, we have assessed: (i) the effects of temperature on the survival and growth of *Xf* strains representative of the *Xf* subspecies prevalent in the European outbreaks, and (ii) the effects of projected climate on the potential for *Xf* establishment in Europe under different climate change scenarios. Our results indicate that *Xf* strains showed a differential response to temperature. Although no distinctive temperature range was associated with subspecies, the widest range for optimal growth was estimated for subsp. *fastidiosa* (19-33ºC) and *multiplex* (20-31ºC), being lower for subsp. *pauca* (19-27ºC). Extreme temperatures differentially affected cell survival. Thus, temperatures between 4 to 10ºC did not affect cell survival whereas temperatures between 36 and 40ºC resulted lethal. On the other hand, under future climate, the potential distribution of *Xf* would tend to expand to central and northern Europe, although southern regions would still maintain a higher climatic suitability. However, a negative effect on the potential distribution of this pathogen is projected for southern regions in the Mediterranean countries. These results would allow to predict the risk associated with the establishment and spread of *Xf* in Europe under current and future climate scenarios.

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SSB-O2

Xylella fastidiosa situation in France: two new variants detected and a new region contaminated

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Xylella fastidiosa is a xylem-limited bacterium native to America and classified as a priority quarantine pest for EU regulation. Since 2013, *X. fastidiosa* has been identified in European countries with a Mediterranean climate, such as Italy, France, Spain and Portugal, with different subspecies and sequence types (ST) detected. Since 2015 in France, two subspecies has been detected: (i) *X. fastidiosa* subsp. *multiplex* (ST6, ST7) in Corsica and the Provence-Alpes-Côte d'Azur (PACA) regions in almost 70 plant species, and (ii) *X. fastidiosa* subsp. *pauca* (ST53) in only two host plants in a unique area in PACA region. Recently, in two separated contaminated areas of the PACA region, two new variants genetically related to the subspecies *multiplex* have been identified[1] and assigned to: (i) ST88 (Var area) detected on *Polygala myrtifolia*, *Hebe* sp, *Osteospermum ecklonis*, *Lavandula* x *intermedia*, *Coronilla glauca*, *Euryops chrysanthemoides*, and (ii) ST89 (Alpes-Maritimes area) detected on *Myoporum* sp. and *Viburnum tinus*. Both variant strains were isolated and genomic analyses are still in progress. Moreover, in a new region of the South of France, Occitanie (Aude area), *X. fastidiosa* subsp. *multiplex* ST6 was identified in plants from natural and urban settings and from a nursery. A MultiLocus VNTR Analysis was performed on strains isolated from plant samples and also directly on plant samples using an MLVA scheme already adapted to *X. fastidiosa* subsp. *multiplex*[2]. We will present an update of the analyses of the variants and an overview of the genetic diversity of strains present in the Occitanie region.

[1]Cunty A. *et al*., 2022. EJPP, DOI 10.1007/s10658-022-02492-z.
[2]Dupas E. *et al.*, 2022. bioRxiv.

SSB-O3

Genomic and physiological basis of resistance to *Xylella fastidiosa* in olive

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It is almost 10 years since the discovery of *Xylella fastidiosa* in the southern part of the region of Apulia (southern Italy) [1]. The rapid spread of the infections and the large territory interested by the epidemics, urged for the adoption of containment strategies in place of the eradication measures. In the frame of these strategies a breakthrough is represented by the discovery of traits of resistance in the cvs Leccino and FS17. Replanting olive groves with resistant cultivars is of fundamental importance to restore the landscape and the agriculture of the devastated area. Investigations on the mechanisms governing the resistance phenomena in olives, showed that, for example in Leccino, resistance appears to develop from a complex of mechanisms, involving both genomic and physiological basis that keep the bacterium population lower than in the highly susceptible cultivars such as Cellina di Nardò or Ogliarola salentina or other susceptible cultivars. Extensive or targeted gene expression studies indicated that Leccino senses the bacterium by cell wall receptors and manages to contain the induced drought stress by modulating genes involved in the sugar metabolism and water flux across membranes[2,3]. Moreover, the bacterium spread trough the xylem network is likely enhanced in the susceptible Cellina di Nardò because of its facilitated exploitations of pit membranes interconnecting xylem vessels. These studies show that Leccino is more resilient to the infection, whose physiological response to the water stress is not as extreme as in susceptible cultivars. In addition, recent genomic investigations on spontaneous seedlings or cross-bred progenies derived from Leccino, support the evidence that the genetic traits of resistance can be transferred from resistant parentals to the progenies and pave the way to widening the olive germplasm resistant to *Xylella*.

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[2]Giampetruzzi A. *et al*., 2016. BMC Genomics, 17:475
[3]Sabella E. *et al.*, 2019. Scientific Reports, 9:9602

SSB-O4

Progress and difficulties for the management of the "olive quick decline syndrome" in Salento (Apulia, Italy)

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Xylella fastidiosa subsp. *pauca*, the causal agent of "olive quick decline syndrome", has been reported in Salento (Apulia, Italy) on October 2013. To save the valuable olive agro-ecosystem, interdisciplinary studies were undertaken to provide a sustainable strategy to control the pathogen. Confocal laser scanning microscopy and fluorescence quantification showed that a zinc-copper-citric acid biocomplex—Dentamet®—reached the olive xylem tissue either after the spraying of the canopy or injection into the trunk, demonstrating its effective systemicity. The biocomplex showed an *in vitro* bactericidal activity to all *X. fastidiosa* subspecies, and, during a mid-term evaluation, it allowed to reduce the pathogen cell density, twig wilting and tree death in some olive groves of Salento upon spray treatments carried out from spring to early autumn. A ^{1}H-NMR metabolomic approach revealed a general reprogramming of the metabolic activity of the treated trees and a consistent increase in malic acid and γ-aminobutyrate for the treated Ogliarola salentina and Cellina di Nardò cultivars, respectively. Quinic acid, some free fatty acids, and derived oxylipins were found as consistant biomarkers for the disease. Even though the traditional way to manage olive trees in Salento did not include timely treatments and mechanical weed removal or regular pruning, a reasonable number of olive growers currently manage the disease according to the studied strategy. These achievements also promoted the development of a novel endotherapy technique. Epidemiological surveys revealed the occurrence of the aggressive fungal pathogen *Neofusicoccum mediterraneum* in some olive groves of Salento, possibly involved in a similar decline syndrome.

SSB-O5

Using long-read metagenomics to investigate plant disease outbreaks: a highly-resolved phylogenetic reconstruction of Xylella fastidiosa

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To prevent or control plant disease outbreaks, early disease detection and precise identification of pathogen strains responsible for the outbreak are essential. So far, culture-dependent whole-genome sequencing (WGS) has been necessary for the phylogenetic reconstruction of bacterial outbreak strains. However, some plant pathogenic bacteria are slow to grow in culture or are difficult to culture. In this study, Xylella fastidiosa, a high-impact plant pathogen, was used to show that culture-independent metagenomic sequencing using the Oxford Nanopore Technologies MinION long read sequencer can sensitively and precisely detect the causative agent of Pierce's Disease (PD) in grapevine, Xylella fastidiosa subsp. fastidiosa (Xff). Using samples obtained from vineyards across Virginia, USA, we obtained a metagenome-assembled genome (MAG) of sufficient quality for phylogenetic reconstruction with single nucleotide polymorphism resolution. In the phylogenetic tree, the MAG was placed in a clade with isolates from Georgia, USA, suggesting introduction of Xff to Virginia from the Southeastern USA. With this case study, we demonstrated that metagenomic sequencing can be used for reconstructing transmission routes of bacterial plant pathogens and identification of plant pathogens to the strain-level, offering a faster and more precise approach compared to culture-dependent genome sequencing.

POSTER SESSIONS

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S1A-P1

WhpR, an orphan transcriptional regulator of virulence in the pathogen of woody hosts *Pseudomonas savastanoi* pv. *savastanoi*

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The genome of the olive tree pathogen *Pseudomonas savastanoi* pv. *savastanoi* (Psv) NCPPB 3335 encodes a region of about 15 kb named WHOP (from Woody Host and *Pseudomonas*) which is involved in the catabolism of aromatic compounds and is essencial for the virulence of Psv in woody olive plants[1]. This region is shared with other strains of *Pseudomonas syringae* pathovars infecting woody hosts, but it is absent in strains infecting herbaceous plants. The WHOP region is organized into four operons, *antABC* (metabolism of cathecol), *ipoBCA* (conversion of indol to indigo) and *dhoAB* (degradation of fenolic compounds) and three independently transcribed genes, *antR* (positive regulator of the *antABC* operon), *PSA3335_3206* (aerotaxis receptor) and *whpR* (putative AraC family regulator) [2]. In this study we identified two domains in WhpR, a DBD (DNA bingding domain), characterised by a classical HTH (Helix-Turn-Helix) motif and an AraC-like bingding domain. BlastP searches showed that no homologs (≥ 60%) of this protein are found outside the *P. syringae* complex. We also addressed the role of WhpR in virulence by the construction of Δ*whpR* mutants in several *P. savastanoi* strains isolated from olive and oleander (*P. savastanoi* pv. nerii). Moreover, quantitative real-time PCR (RT-qPCR) analysis of Psv NCPPB 3335 and its Δ*whpR* mutant revealed that WhpR is a negative regulator of most of the operons encoded in the WHOP region. Our future aims are to elucidate the mechanism of WhpR-dependent regulation and to determine whether other genes codified outside the WHOP region are also regulated by WhpR.

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[2]Caballo-Ponce *et al*., 2017. Mol. Plant-Microbe Interact., 30, 113-126.

S1A-P2

Identification of new *Dickeya dadantii* virulence factors secreted by the type 2 secretion system

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Dickeya are plant pathogenic bacteria able to provoke disease on a wide range of plants. A type 2 secretion system (T2SS) named Out is necessary for bacterial virulence. Its study in *D. dadantii* showed that it secretes a wide range of plant cell wall degrading enzymes, including pectinases and a cellulase. However, the full repertoire of exoproteins it can secrete has probably not yet been identified. Secreted proteins are first addressed to the periplasm before their secretion by Out. No secretion signal present on the protein allows the identification of substrates of a T2SS. To identify new Out substrates, we analyzed *D. dadantii* transcriptome data obtained in plant infection condition and searched for genes strongly induced encoding a protein with a signal sequence. We identified five new Out-secreted proteins: the iron-binding protein IbpS, the expansin YoaJ, the putative virulence factor VirK and two proteins of the DUF 4879 family, SvfA and SvfB. We showed that IbpS, SvfA and SvfB are required for full virulence of *D. dadantii*. Homologues of IbpS are present in other phytopathogenic microorganisms (fungi and oomycetes) where they also play a role in virulence. *svf* genes are present in a variable number of copies in other *Pectobacteriaceae*, up to three in *D. fanghzongdai*. This work opens the way to the study of the role of non-pectinolytic proteins secreted by the Out pathway in *Pectobacteriaceae*.

S1A-P3

SolR, a LuxR-family solo, modulates cyclic lipopeptide production in *Pseudomonas corrugata*

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LuxR solos are closely related to Quorum Sensing (QS) LuxR-family regulators but devoid of a cognate LuxI-family protein homologue. They possess the typical modular structure with an autoinducer-binding domain at their N-terminus and a DNA-binding helix–turn–helix domain at their C-terminus. LuxR solos may respond to either endogenous or exogenous AHLs or to plant low-molecular-weight compounds[1]. Genome-wide analysis of transcriptional regulators in *Pseudomonas corrugata* revealed the presence of a gene coding for a putative LuxR solo (designated *solR*) in addition to genes for a canonical QS system (*pcoI-pcoR*). According to the adjacent gene context and the protein primary structure, SolR belongs to the subgroup A of *Pseudomonas* LuxR solos with an autoinducer-binding domain likely to bind AHLs[2]. In this study, a *solR*$^-$ mutant was obtained from the *P. corrugata* strain CFBP 5454. Mutational phenotype analysis revealed that the *solR* mutant was more virulent in tomato plants than the parent strain and that *solR* complementation *in trans* restored a wild-type phenotype. Since bacterial colonization and titre was not affected by the mutation, we investigated on the production of cyclic lipopeptides (CLPs) involved in pith necrosis development and for which a role of QS has been demonstrated[3]. Time-course cell-free culture filtrates activity against bioindicator microrganisms showed that CLPs production was greater and anticipated in the *solR* mutant as compared to the parent strain CFBP 5454. In addition, genes involved in the biosynthesis and secretion of CLPs were upregulated in the *solR* mutant. Overproduction strategies for antifungal CLPs *in vitro* could be explored.

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S1A-P4

The *iaaL* gene in the *Pseudomonas syringae* complex: functional characterization and biological activity

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Phytopathogenic bacteria of the *Pseudomonas syringae* complex are causal agents of diseases in a wide variety of woody and herbaceous plants with agronomic and ornamental interest. Indole-3-acetic acid (IAA) is an auxin phytohormone whose production is widely distributed among plant-associated bacteria. Some *P. syringae* strains can further metabolize IAA to the amino acid conjugate 3-indole-acetyl-ε-L-lysine (IAA-Lys), a process involving the enzyme IAA-Lys synthase, encoded by the *iaaL* gene. IAA-Lys is less biologically active than IAA, so it has been speculated that the conjugation of IAA with L-Lysine could allow the bacteria to control the levels of free IAA accumulated in the bacterial cytoplasm and/or secreted to the plant tissues. The *iaaL* gene is widespread in the *P. syringae* complex, and three different alleles (*iaaL*Psv, *iaaL*Psn and *iaaL*Pto) have been described [1]. Recently, we have identified a fourth allele (*iaaL*Psf) specifically encoded in the genome of strains isolated from *Fraxinus excelsior*. However, comparative analyses of the biochemical and biological activities of the different *iaaL* alleles have not been performed. In this work, the genomic context of these four alleles in a collection of *P. syringae* complex strains has been analyzed. In addition, we have constructed strains overexpressing each of these *iaaL* alleles and analysed their biological activities using an elongation assay of *Arabidopsis thaliana* roots. Finally, expression of these alleles in *E. coli* allowed the purification of these four IaaL proteins and the analysis of their specific activities using an *in vitro* enzymatic coupling assay.

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S1A-P5

Soil amendment with crab chitin enhances systemic anti-bacterial resistance via potentiation of pattern-triggered immunity

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Disease outbreaks caused by zoonotic bacteria, such as *Salmonella enterica*, have a heavy toll on people's lives globally. *S. enterica* can survive in plant tissues at low levels, which are nevertheless problematic for food safety. A recent study has shown that soil amendment with crab chitin, which is the second most abundant biopolymer in nature, inhibits the growth of *S. enterica* on the phyllosphere of lettuce[1]. In our study, we found that soil amendment with crab chitin confers resistance against Salmonella and *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000) in both lettuce and Arabidopsis. Notably, this effect is strictly dependent on chitin perception by the plant immune system. Furthermore, using relevant Arabidopsis mutants, we found induced systemic resistance (ISR), receptors/co-receptors involved in pattern-triggered immunity (PTI), but not systemic acquired resistance (SAR), are required for the systemic resistance triggered by crab chitin soil amendment. Our results thus suggest a strong link between chitin perception in the roots and subsequent potentiation of PTI in the leaves, resulting in priming of the plant for enhanced immune responses against bacterial infection. Together, our data provide further evidence that soil amendment with crab chitin can be used as a biocontrol solution to reduce crop diseases, and importantly shed light on the underlying molecular mechanisms involved.

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S1A-P6

Characterization of the GacS/GacA system in the virulence regulation of *Pseudomonas savastanoi.*

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The two-component regulatory system GacS/GacA is one of the main mechanisms for global regulation in bacteria. GacS/GacA is a highly conserved system that has been studied in many pathogenic bacteria. However, its characterization has been mainly focused on pathogenic bacteria of herbaceous plants. Despite previous works reporting that GacS/GacA regulates the expression of virulence factors, its role in virulence varies among different species and strains. The aim of this work was the identification of virulence factors regulated by the GacS/GacA system in the model bacterial pathogen of woody hosts *Pseudomonas savastanoi* pv. savastanoi (Psv), causal agent of olive knot disease. To this end, we generated a *gacA* deletion mutant in Psv strain NCPPB 3335, whose transcriptomic profile was further analyzed using a massive RNA sequencing (RNA-seq) strategy. The bioinformatic analysis of RNA-seq data showed that the Psv GacS/GacA system regulates a large number of genes, including some virulence factors already described, such as those related to the type III secretion system, the biosynthesis of phytohormones and the catabolism of aromatic compounds, among others. In addition, small Rsm-type RNAs and regulatory proteins (RsmA) were identified in the regulatory cascade of the GacS/GacA system. Finally, the involvement of some of the identified virulence factors of Psv NCPP 3335 were further studied through different phenotypic assays, such as plant virulence assays, induction of hypersensitive response, leaf adhesion tests and translocation of type III effectors. Results obtained in this work indicate that the GacS/GacA system participates in regulation of virulence factors in Psv NCPPB 3335.

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S1A-P8

A new chapter in the old story of indolacetic acid production and transport by *Pseudomonas savastanoi*

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Auxins are key molecules in plant growth and development, as well as in plant defences. Among them, indole-3-acetic acid (IAA) is the most abundant and it is mainly synthesized using tryptophan as a precursor. IAA has been demonstrated to be involved in plant-pathogen interaction, and many phytopathogens can synthesise it, such as *Pseudomonas savastanoi,* which produce IAA through the IAM pathway to induce the development of the typical hyperplastic symptoms. Moreover, many species and pathovars from the *P. syringae* complex can conjugate IAA to Lysine to give a less active form (IAA-Lys). Recent studies have demonstrated that in *P. savastanoi* pv. *nerii* (*Psn*), the *iaaM* and *iaaH* genes for IAA biosynthesis and the *iaaL* gene for IAA conjugation to Lysine are under the control of the Type Three Secretion System (TTSS) [1]. However, IAA transport in *P. savastanoi* is not completely understood. Recent *in silico* and *in vitro* studies have shown the presence of a gene coding for a "Multidrug and toxic compound extrusion" (MATE) transporter upstream of the *iaaL* gene in *Psn*[2]. This bacterial MATE was demonstrated to mediate IAA and IAA-Lys efflux, and to be regulated by the TTSS, due to the presence of a *hrp* box promoter. Homologs of the *Psn* MATE were found in many *P. syringae* pathovars and related species. Further studies are pivotal to confirming the role of this transporter, and ongoing experiments are carried out to solve the structure of the *Psn* MATE and to unveil the specific binding sites for IAA/IAA-Lys.

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S1A-P9

Dynamic changes of the Prf/Pto tomato resistance complex following effector recognition.

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A common plant pathogen virulence strategy is the secretion of effector proteins inside the host cell, with the goal of compromising plant immune responses. In turn, plants have evolved nucleotide-binding leucine-rich repeat (NLR) immune receptors that recognise pathogen-derived effector proteins and initiate effector-triggered immunity (ETI). However, the molecular mechanisms that link NLR mediated effector recognition and downstream signalling are not fully understood. By exploiting the well-characterised tomato Prf/Pto NLR resistance complex, we identified the 14-3-3 proteins TFT1 and TFT3 as interacting partners of both the NLR complex and MAPKKKα. Moreover, we identified the helper NRC (NLR-required for cell death) proteins as integral components of the Prf/Pto NLR recognition complex. Notably our studies revealed that TFTs and NRCs interact with distinct modules of the Prf/Pto complex and, following effector recognition, dissociate to facilitating downstream signalling. Thus, our data provide a mechanistic link between activation of immune receptors and initiation of downstream signalling cascades.

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S1A-P11

Does *Pseudomonas syringae* pv. *actinidiae* differently perceive *Actinidia* species displaying different susceitbility to bacterial canker?

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Bacterial virulence deployment depends on several environmental factors, including nutrient availability and crowding (quorum-sensing), that regulate bacterial behavior[1]. In particular, bacterial phytopathogens express enhanced virulence when cultured in conditions mimicking apoplast environment compared to growth in rich-medium. Moreover, virulence can be further enhanced by the presence of plant signa(s), indicating that the perception of plant-derived factor(s) is required for the full activation of bacterial pathogenic arsenal[2]. *Actinidia* species show different degrees of susceptibility/tolerance toward *Pseudomonas syringae* pv. *actinidiae,* kiwifruit bacterial canker etiological agent . This may be due to the capacity of tolerant species to induce stronger defence responses against the pathogen. Alternatively, plant-derived factors recognized by Psa could be uniquely present, or more abundant, in susceptible species, thus increasing bacterial virulence traits. In this work, we attempted to elucidate this hypothesis by analyzing the capacity of leaf extracts, produced from five *Actinidia* species, to enhance Psa virulence, using reporter strains expressing *gfp* gene under the control of the promoter of several Psa genes involved in pathogenicity. The different extracts were further fractioned by size and polarity in an attempt to characterize the signal(s) responsible for virulence activation. Overall, our study shows that all crude extracts tested enhance Psa virulence, independently of the susceptiblity of the plant. Nevertheless, extract fractionation revealed that the molecule(s) responsible for Psa *hrpA1* promoter induction by the different plant species could differ, suggesting that leaf metabolite composition may play a role in regulating Psa virulence and thus contribute to the outcome of the interaction.

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S1-P12

Identification of aeroreceptor proteins involved in host tobacco infection in *Pseudomonas syringae* pv. *tabaci* 6605

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Pseudomonas syringae pv. *tabaci* 6605 (*Pta*6605) is a causal agent of wildfire disease in host tobacco plants. *Pta*6605 wild-type (WT) strain is highly motile and cause severe disease symptoms on host tobacco leaves. We have investigated virulence factors in *Pta*6605 as model pathogen[1], and we have clarified flagella-mediated motility is important for plant infection. Genes encoding components for chemotaxis signal transduction pathway are mainly localized on two major chemotaxis genes clusters. Our mutation analysis revealed that chemotaxis genes located in cluster II (such as *cheA2* and *cheY2*) are required for optimal chemotaxis and host plant infection and genes in cluster I (such as *cheA1* and *cheY1*) may partially contribute to these phenotypes in *Pta*6605[2]. We found that aerotaxis ability of Δ*cheA2* and Δ*cheY2* was largely and that of Δ*cheA1* and Δ*cheY1* was slightly impaired, indicating aerotaxis requires chemotaxis signal pathway. We found three genes encoding potential aerotaxis transducers from genome information. The *aerA* located in chemotaxis gene cluster I encoded methyl-accepting chemotaxis proteins (Mcp) with a periplasmic 4HB-type ligand binding domain (LBD), whereas *aerB* and *aerC* encoded a cytoplasmic PAS-type LBD containing Mcp. Aerotaxis ability of *aerA* and Δ*aerB* mutants was weaker than that of WT. Virulence of *aerA* and Δ*aerB* mutants was slightly reduced because fewer bacterial populations were recovered from inoculated seedlings and the reduction of the fresh weight of seedlings was slightly smaller in the *aerA*- and Δ*aerB*-inoculation than in WT-inoculation. These results indicated that aerotaxis contributes to host plant infection[3].

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S1-P14

Loss-of-Susceptibility Enables Rice Resistance to Bacterial Leaf Blight and Bacterial Leaf Streak

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Bacterial leaf blight (BLB) and bacterial leaf streak (BLS), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*), respectively, are devastating rice diseases causing significant yield reduction. Transcription activator-like effectors (TALEs), utilized by *Xoo* and *Xoc* as virulence or avirulence factors, bind to effector-binding elements (EBEs) of host target gene promoters and interact with the rice transcription factor *OsTFIIAγ1* or *OsTFIIAγ5* (*Xa5*) to transcriptionally activate expression of target resistance (*R*) and/or susceptibility (*S*) genes[1,2]. Two strategies causing loss-of-susceptibility were applied to confer BLB or BLS resistance in rice; these are editing *OsTFIIAγ* to reduce the expression of *S* genes[2] and disrupting the EBEs of TALE-targeted *S* genes using CRISPR/Cas9 technology[1]. *OsTFIIAγ1* was edited in IRBB5 rice containing *xa5*, and *OsTFIIAγ1*-inactive rice plants are more resistant to BLB[2]. Furthermore, we generated mutations in the EBE of *OsSULTR3;6* promoter that is bound by Tal2g and Tal5d of *Xoc* strain BLS256 and RS105, respectively. And the new germplasm showed resistance to *Xoc* strains containing virulence factors either Tal2g or Tal5d[1]. However, it is worth noting that TALE-triggered and iTALE-suppressed *Xa1*-mediated resistance to BLB and BLS is independent of *OsTFIIAγ* in rice, suggesting that the modifications of *OsTFIIAγ* do not affect the resistance mediated by *Xa1*-like NLR-type genes[3]. In conclusion, loss-of-susceptibility confers BLB and BLS resistance in rice, and the genome-edited mutations in *OsTFIIAγ* and modifications of EBEs of TALE-targeted *S* genes of rice exhibit great potential in breeding with broad-spectrum resistance to BLB and BLS.

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S1-P15
ZAR1-mediated immunity and its link to PTI

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Pattern-triggered immunity (PTI) mediated by surface receptors (PRRs) and effector triggered-immunity (ETI) mediated by nucleotide-binding leucine-rich receptors (NLRs) form a two-tiered surveillance network against diverse pathogenic microbes. Recent advances show that NLRs form different types of resistosomes and activate immune responses via calcium signal. In addition, NLRs and PRRs are found to interact both physically and functionally to potentiate immune outputs. These findings are changing the way we think about the plant immune system. We have been studying mechanisms underlying immunity triggered by the NLR protein ZAR1 and characterizing immune signaling components such as receptor-like cytoplasmic kinases, heterotrimeric G proteins and MAP kinases. I will discuss how signaling from PRRs and NLRs are interconnected at multiple levels. In addition, we will discuss how some components of PTI might have been adopted for ETI through evolution, which supports the zig-zag model.

S2A-P1

Genome-informed design of molecular tests for specific detection of bacterial pathogens for non-bioinformaticians using free web-based programs

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Molecular detection of bacterial plant pathogens is often focused on a few target genes. This limits the design of tests for specific detection of groups or subgroups and design of tests with very specific target sequence requirements, such as isothermal amplification LAMP. On the other hand, more and more genomic data are becoming available and represent an important source of potential new target sequences for specific molecular tests. Therefore, we have designed a protocol that helps non-bioinformaticians to combine bioinformatic analyzes with their nieche knowledge. We tested the feasibility and efficiency of the genome-informed test design using free tools and publicly available genomes. We successfully developed a protocol for target selection and assay design by coupling the web-based program RUCS with additional quality control steps and a primer design program of choice. Using this approach, we identified 11 potential diagnostic markers in phytoplasma genomes and successfully designed 4 experimentaly proficient LAMP test[1,2]. The test with the best performance was validated and showed impressive diagnostic parameters. The approach was also tested for real-time PCR and digital PCR for various bacterial pathogens (e.g. phytoplasmas, bacterial plant pathogens, bacterial human pathogens).

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S2A-P2

Cultural, Physiological, and Biochemical Identification of *Erwinia amylovora* Isolates from the Fire Blight Diseased Fruit Trees in Georgia

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The existence of *Erwinia amylovora* was confirmed by PCR in hundreds of instances on symptomatic pomaceous fruit trees different organs, collected in Eastern Georgia in 2020-2021. For the maximum recovery of the pathogen from the samples three different media: King's B, SNA and CCT agar media were applied. Molecular identification of *Erwinia amylovora* was conducted by PCR using the specific primers set: G1-F and G2-R based on amplification of sequence near termini of the chromosomal pEA71 insert region [1] . In a total of 38 pure isolates were obtained. The Gram-negative *Erwinia amylovora* isolates colonies on King's B appear at 24 h of growth and are creamy white, round shaped; after 48 h, the colonies are non-fluorescent under UV light. Colonies on CCT agar are round, highly convex to domed and mucoid, pale purple in color. The *Erwinia amylovora* Georgian isolates express typical biochemical characteristics of the pathogen but differ from each other according to the nutritional profiling. Almost all isolates produce acid from glucose, fructose, galactose and sucrose; The isolates ability to utilize maltose, xylose, sorbitol and mannitol is variable. *Erwinia amylovora* Georgian isolates differ from each other in Esculin – a coumarin glucoside hydrolysis ability, which was earlier demonstrated by us based on the study of few *E. amylovora* isolates [2]. All strains are characterized by the exopolysaccharide levan production on SNA medium. According to enzymatic profiling *Erwinia amylovora* Georgian isolates are all oxidase test negative and nitrate reductase test negative.

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S2A-P3

Bacterial leaf spot of *Hydrangea*: on a "new old" disease and the importance of getting it right in phytodiagnostics

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The causal organism of bacterial leaf spot of *Hydrangea*, *Xanthomonas hydrangeae*, was the subject of a disease report and a new bacterial species description in 2021 [1,2]. These publications, including the developed *X. hydrangeae*-specific isothermal diagnostics assay, the raising of awareness about this pathogenic bacterium thereof, and the favorable wet weather conditions during the summer of 2021 in Europe uncovered a wider historical and contemporary prevalence of this disease. Global trade of plants appears to play an important role in the dissemination of this pathogen. Furthermore, scouring the literature revealed multiple instances, mainly in the USA, of bacterial leaf spot of *Hydrangea*, that had been attributed to various *Xanthomonas* species (e.g., *Xanthomonas campestris*, *Xanthomonas hortorum*). The first known mention of bacterial leaf spot of *Hydrangea* dates to 1996 in Georgia (USA). However, those 1996 isolates were not available and thus their identity as *X. hydrangeae* cannot be confirmed. This work outlines the challenges encountered when studying *X. hydrangeae*, as related to 1) the development of a diagnostics assay targeting *X. hydrangeae*, especially given its phylogenetic similarity to *X. hortorum* and 2) the information discontinuity regarding the historical and contemporary occurrence of bacterial leaf spot of *Hydrangea*. Suggestions to overcome those challenges, in an ever-increasing global plant trade, are offered and extrapolated to the broader field of bacterial phytodiagnostics.

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S2A-P4

Genetic diversity of *Erwinia amylovora* isolates from fire blight diseased apple, pear and quince trees in Georgia

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Genetic diversity of the 42 *Erwinia amylovora* isolates, recovered from fire-blight diseased pomaceous trees in four different regions of eastern Georgia was studied. Initially, the CRISPR repeat regions (CRR) of four isolates from the years 2016-2018 were sequenced using Sanger Sequencing[1]. The presence of two distinct genotypes was revealed: isolates GE03 and GE04 shown to belong to the genotype (A, a, α) – i.e., identical to that of many historical strains that were among the earliest that colonized Europe[1,2]. On the other hand, isolates GE01 and GE02 belonged to the novel genotype (A, z, α) and displaying the deletion of three spacers next to the leader-proximal region of CRR2[3]. The CRISPR regions of 38 additional *E. amylovora* strains isolated in the years 2020-2021 were analyzed by PCR using primers pairs flanking known spacer deletions or duplications[1,2]. PCR with primers pair C1f04/C1r09 resulted in amplification of a 276-bp fragment in all the studied isolates, indicating that they all have the duplication of spacer 1029 that characterizes archetypal CRR1 genotype A, which was responsible for the first appearance of fire blight in Europe in 1958 and is now widely prevalent in the Mediterranean area, the Caucasus region and Central Asia[2]. PCR with primers pair C2f01/C2r03 resulted in the amplification of 510-bp or 700-bp fragments that are compatible with the CRR2 genotypes z and a, respectively. The vast majority – 81% of these isolates (Shida kartli – main pomaceous fruit growing region) were shown to belong to the CRR2 genotype z, whereas few strains – 19% (Kakheti region) displayed the CRR2 genotype a. These results indicate on the existence of at least two different CRR genotypes in Georgia, although further modifications in the CRR regions could still be revealed by their complete sequencing, which is currently in progress.

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S2A-P5

Application of MALDI-TOF MS technology for fast and accurate identification of phytopathogenic bacteria from different species of *Pectobacteriaceae*

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Soft rot *Pectobacteriaceae* (SRP) are bacterial phytopathogens responsible for soft rot and blackleg diseases on various crops, vegetables and ornamentals. In spite of the economic importance and common occurrence of SRP[1], no efficient methods have been implemented to combat them. Thus, solely preventive approaches relying on early detection of the pathogen are used. Biochemical and molecular diagnostics techniques[2,3] require not only equipped laboratories and trained stuff, but also quite some time to identify the pathogen. Also, in terms of the closely-related species, proper taxonomic classification may be impossible or additional tests, *e.g.* involving sequencing of housekeeping genes, might be necessary. Therefore, we aimed at assessing the applicability of an innovative and fast diagnostic tool, *i.e.* Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS), for to-species identification of SRP. The Bruker's database containing the reference MALDI-TOF MS profiles is not well-represented by proteomic fingerprints from bacterial phytopathogens, therefore our goal was to collect this data for various species of SRP and succeed in building up a library detailed enough for to-species identification of axenic SRP cultures in less than an hour. The MALDI-TOF MS profiles of several SRP strains were utilized for generation of a Bionumerics-computed dendrogram, which reflected the proper taxonomic relations between the evaluated SRP species. We foresee ending up with a library containing the reference MALDI-TOF MS profiles for all SRP species and finally implementing this rapid, robust and cost-efficient technology for reliable to-species identification of pectinolytic isolates during routine monitoring studies [1].

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S2A-P7

Tools for early detection of *Xylella fastidiosa* in almond and olive trees by proximal sensing

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Early detection of infected plants by the quarantine plant pathogenic bacterium *Xylella fastidiosa* (*Xf*) is key for disease management. However, the occurrence of non-specific symptoms, long incubation period and asymptomatic infections make difficult its detection by visual inspection. The objectives of this study were to evaluate the response to *Xf* infection on plant physiological plant traits at leaf level in naturally *Xf* infected almond trees and to which extent these traits could be used to assess the reaction of olive plants artificially inoculated with a set of *Xf* strains representative of the European outbreaks. Leaf measurements were carried out by four proximal sensors: Dualex Scientific leaf clip sensor, Fluor Pen FP100 and two spectrometers (PolyPen RP400 and DLP NIRscan nano) covering the range of the electromagnetic spectrum from 340 and 1700 nm. Firstly, ca. 100 almond trees were selected on the *Xf* outbreak in Mallorca Island (Balearic Islands, Spain) from 12 commercial orchards including rainfed and irrigated management. Measurements were taken on ca. 1,000 leaves from *Xf*-asymptomatic and symptomatic almond trees. To evaluate the response to the infection of the olive plants under controlled conditions, several *Xf*-infected and non-infected leaves were selected at different heights from the inoculation point and *Xf*-specific molecular diagnostic was used to determine the presence of the bacteria in the olive leaves and stem xylem tissues. Hierarchical clustering and stepwise discriminant analysis were performed using a set of 74 pigment content and plant physiological indices calculated from the spectral range measured at leaf level. Our results showed that a set of vegetation indices discriminate between leaves from asymptomatic and *Xf*-symptomatic almond trees. This indices were related to anthocyanin content, carotenoids, blue band of the electromagnetic spectrum region or xanthophyl cycle. Interestingly, *Xf*-infection in olive plants could be discriminated by indices related with carotenoid, xanthophyl and nitrogen content, chlorophyl fluorescence and blue and red bands. These results have allowed the selection of a set of spectral indices with different predictive capacity for *Xf* infection in asymptomatic leaves.

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S2A-P8

The EPPO.Q-Bank Database for accurate identification of regulated phytopathogenic bacteria

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The EPPO.Q-bank Database on Bacteria represents a robust tool to identify and detect regulated plant pathogenic bacteria in support of European legislation. The aim of the Database on Bacteria is to provide phytosanitary organizations with an accurate identification method for regulated bacteria and, in this way to strengthen the plant health infrastructure. The cornerstone of this database is that it comprises only curated sequences of strains that are available in European public collections[1]. Currently, the EPPO.Q-bank Database on Bacteria includes about 300 barcoded strains that represent regulated bacteria and their relatives, belonging to the genera: *Xylella*, *Ralstonia*, *Clavibacter* and *Xanthomonas*. Also detailed protocols for the DNA isolation and barcoding, including PCR amplification and sequencing, are included. A molecular decision scheme showing the workflow for the identification of specific quarantine species, subspecies or pathovars is regularly updated by its curators, in accordance with the EPPO PM7/129 standard [2]. The database is hosted by EPPO since the 1st of May 2019.

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[2]EPPO (2020) EPPO Global Database (available online). https://gd.eppo.int, PM 7/129 (1) DNA barcoding as an identification tool for a number of regulated pests

S2A-P10

Volatile fingerprinting and photoacoustic sensing for innovative and no destructive detection of the quarantine plant pathogen *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

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Food safety and food security certainly represent some of the greatest global challenges. The accidental or deliberate introduction of a quarantine plant pathogen can cause destructive outbreaks, with a dramatic impact on human health and with heavy socio-economic and political effects. Early identification of quarantine plant pathogens is essential to prevent their entry and spread where they are still absent or with a restricted distribution. Here, innovative and complementary strategies have been developed against the EU quarantine plant pathogen *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*), the etiological agent of the bean bacterial wilt and the soybean tan spot. These procedures answer the urgent need of innovative phytodiagnostic methods for the detection of plant pathogens present on asymptomatically vegetable materials and seeds, to overcome the main limits of the current destructive PCR-based methods [1,2]. Analyses of Volatile Organic Compounds (VOCs) have revealed specific diagnostic traits to specifically detect *Cff* also into infected seeds. Moreover, for the first time, the potential feasibility of the photoacoustic (PA) approach as a diagnostic method for *Cff* was here investigated. The PA set-up was successfully developed, and the optical and the non-destructive *in vitro Cff* detection was successfully demonstrated. The methods here developed for *Cff* are easily transferable to other quarantine plant pathogens, and contributed to the further development of economic, rapid, and high-efficiency sensors for many other agri-food applications.

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S2-P11

Characterization of the *Xylella fastidiosa* population in Virginia using metagenomics

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Pierce's disease (PD) of grape caused by *Xylella fastidiosa* (*Xf*) subspecies *fastidiosa* (Xff) is a growing threat to the wine industry in Virginia (VA). PD severity is known to increase after warm winters and under drought conditions. With global warming and the expansion of the wine grape industry in VA, more VA regions, such as northern VA, that were never considered a high-risk area, have now become a high-risk region for PD. To inform management of *Xf* and associated outbreaks, genetic and genomic characterization of *Xf* population present across VA was our main goal in this study. Samples of grapevines, oaks, American sycamore, and hackberries with PD symptoms were collected and sequenced using Oxford Nanopore Technologies sequencing platforms. The obtained *Xf* reads were assembled into genomes for phylogenetic and comparative genomic analysis. Our results identified both Xff and subspecies multiplex (Xfm) to be associated with grapevines with PD symptoms while only Xfm was associated with the deciduous trees showing symptoms of leaf scorch. Since PD is known to be caused by Xff, it was surprising to find Xfm to be associated with symptomatic grapevine. This raises the question if Xfm is one of the causative agents of PD in VA, possibly adapting to grapevine by recombining with Xff. To investigate this possibility, the assembled genomes are being compared with each other and with public Xff and Xfm genomes. We anticipate this data to also allow us to trace the origin of Xfm within VA, which is crucial for preventing further introductions of *X. fastidiosa* into VA.

S2-P12

Characterization of the fire blight pathogen, *Erwinia amylovora,* using short sequence DNA repeats (SSRs) of plasmid pEa29

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Fifteen *Erwinia amylovora* isolates, the causal agent of the fire blight disease, were obtained from five different countries including Egypt, Greece, Italy, Turkey and Germany. All isolates were shown to belong to *E. amylovora* based on cultural, biochemical and pathological characteristics. Identification of these isolates through PCR based on plasmid pEA29 was performed[1]. Genotypic characterization of isolates using the short sequence DNA repeats (SSRs) analysis of a *Pst*1 fragment of plasmid pEA29 was investigated[2]. The number of SSRs motifs represented by the motif GAATTACA was determined for the 15 *E. amylovora* isolates. The motif of eight nucleotides reiterated from three to eight times within the 1 Kb fragment generated by PCR from the pEA29 plasmid. To sequence the part accommodating the variable region, a single primer (seq-ssr) was designed as a universal primer for sequencing the variable region containing the SSRs motifs. The minimum of three SSRs motifs ever found was detected only in isolate EaI23 from Turkey. Three Italian (EaCot1, EaConf1, EaSM1) and two German (Ea385, EaGrossachsen) isolates along with one Turkish isolate (EaI4) appeared similar in possessing the same five SSRs motifs. The majority of the Egyptian and Greek isolates had an SSRs motif number of seven. While the number of SSRs motifs of the German isolate Ea2/79 from *Crataegu*s was six**,** the corresponding number for the Turkish isolate EaI7 derived from Quince was eight. The molecular relationship among isolates was investigated by cluster analysis based on DNA sequence and a phylogenetic tree was established.

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S2-P13

Isolation and molecular characterization of some Egyptian isolates of *Agrobacterium tumefaciens*

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Crown gall disease caused by a soil-borne bacterium *Agrobacterium tumefaciens* is a destructive disease of dicotyledonous plants in several areas of the world. Seven isolates of the bacterium were recovered from different infected hosts, showing typical symptoms of the disease that had been collected from guava, apricot, pear and pepper plants. All bacterial isolates were characterized as tumor-inducing on the basis of carrot pathogenisty test. Molecular characterization of these isolates through PCR utilizing specific primers (virD2A/E and tms2A/2B) based on a *virD2* gene of the virulence (*Vir*) region on the Ti-plasmid and the *tms* gene on T-DNA was performed[1,2]. PCR products were positive and produced the expected band 338 bp and 220 bp, respectively. Moreover, one bacterial isolate from each host plant was characterized using 16S rRNA sequence analysis. Partial DNA sequence of the 16S rRNA gene and analysis via BLAST and Genbank data showed that, the bacterial isolates belonging to *A. tumefaciens*.

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S2-P14

Towards a more reliable detection of *Xylophilus ampelinus* using novel diagnostic markers and real-time PCR tests

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Xylophilus ampelinus is a plant pathogen that causes bacterial blight of grapevine. It was first described in Crete (Greece) but has been found in many Mediterranean countries as well as in South Africa and Japan. Because *X. ampelinus* is fastidious, its detection often relies on molecular tests. A specific real-time PCR assay for the detection of *X. ampelinus* in grapevine (leaves, xylem tissue)[1] was one of the first tests to be included in the EPPO standards[2]. *X. ampelinus* is no longer a quarantine organism in the EU, so the focus of testing shifted to international trade. Because the tests are sometimes applied to matrices that were not originally validated (root growth from dormant rootstocks), there can sometimes be discrepant results between different molecular tests. As part of the Euphresco project 2021-A-383 to clarify the status of such results, our goal is to improve the reliability of *X. ampelinus* diagnostics by identifying novel diagnostic markers and designing novel real-time PCR assays. Potential new target sequences were identified using a genome-informed approach. New markers were identified by comparing existing *X. ampelinus* genomes with genomes of closely related bacteria using RUCS[3]. The first set of genomes included genomes of *X. ampelinus* (genome coverage greater than 100×). The second set included sequences from closely related bacteria of the genus *Xylophilus* to which the primers should not bind. One such bacterium is recently described *X. rhododendri* isolated from Royal Azalea (*Rhododendron schlippenbachii*) [4]. In total, more than 60 core sequences unique to *X. ampelinus* have been identified. Sets of primers and probes (TaqMan and MGB) for the selected unique core sequences were designed using the Primer Express 2 program (Applied Biosystems) and are now being evaluated experimentally.

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S2-P15

Detection scheme of the European quarantine pathogen *Pantoea stewartii* subsp. *stewartii* in maize seeds

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Pantoea stewartii subsp *stewartii* (Pss) is a bacterium that causes Stewart's vascular wilt and leaf blight of sweet corn and maize, a disease responsible for serious crop losses throughout the world. Endemic to the USA, Pss has been introduced to other parts of the world with maize seeds. It is now reported in Africa, North, Central and South America, Asia and Europe. In the EU, it has been reported from Italy and Slovenia, with a restricted distribution and under eradication. Pss is regulated according to the Commission Implementing Regulation (EU) 2019/2072 as a quarantine organism and its introduction and spread in the EU is banned on maize seeds. As a National Reference Laboratory, Anses is in charge of Pss tests in France on maize seed imports, applying a detection scheme including a real-time PCR test [1]. However, the subspecies *indologenes* (Psi), which is occasionnally found on maize seed as part of the resident bacterial population, can yield false positive results with this PCR test [1] due to high sequence similarity between Psi and Pss. Psi is avirulent on maize and is not regulated in any country. In this context, we validated a modified real-time PCR test for specific detection of Pss on maize seeds based on the real-time PCR developed by Pal *et al*. (2019) [2]. When the PCR result is positive, isolation of the bacterium is attempted and partial sequences of two housekeeping genes (*recA*, *leuS*) are analyzed to verify and assess its phylogenetic assignation in the *Pantoea* genus.

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S2-P16

Specific and sensitive detection systems for *Xanthomonas arboricola* pv. *corylina* - the causal agent of bacterial blight of hazelnut based on comparative genomics

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Xanthomonas arboricola pv. *corylina* (*Xac*) is responsible for bacterial blight the most important disease on hazelnut (*Corylus avellana* L.)[1]. This pathogen affects mainly nurseries and young orchards by causing significant plant mortality. However, the disease occurs also very often in production crops, especially on susceptible cultivars. Although the disease is devastating and constitutes a major cause of yield losses, causing even 100% death of young trees and planting material, fast, specific and sensitive detecting and diagnosing systems for *Xac* were not yet developed. In the work presented here, sspecific primers pairs designed to detect the causal agent of hazelnut bacterial blight *Xac* are described. Based on a comparative genomic approach used on the publicly available genomes of *X. arboricola*, including *Xac* complete genomes[2], unique targets were selected for designing four specific detection systems relying on: 1) conventional PCR, 2) real-time PCR (SYBR Green and TaqMan) as well as 3) loop-mediated isothermal amplification (LAMP). All assays, done using genomic DNA isolated from all nine pathovars belonging to the *X. arboricola* species, confirmed the specificity of selected primers. Moreover, all PCR assays enabled accurate detection of *X. a.* pv. *corylina* in pure cultures and in plant material. The detection limits depending on the system used were in the order of magnitude ~10^0 to 10^3 cfu per reaction which corresponds to 1 fg to 1 pg of DNA of *Xac*.

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S2-P17

Xylella fastidiosa subspecies and sequence types determination by a third generation sequencing device: the ONT MinION platform

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Currently, *Xylella fastidiosa* (Xf) is detected by using different methods, among these the real time PCR is the most widely used. However, in case of new outbreaks or new host plant the detection of Xf must be followed by the identification of the subspecie and its Sequence Type (ST). This characterization is performed by Multi Locus Sequence Typing (MLST), that requires time and skilled staff. The possibility to use the sequencing platform of Oxford Nanopore Tecnology (MinION) in order to speed up the detection of *X. fastidiosa* and the identification of the subspecie and Sequence Type was investigated. Using the Rapid Barcode kit, we pulled 12 samples in one flow cell, and detected the pathogen and their subspecie in naturally infected olive samples. However, the direct sequencing showed a low sensitivity, that was confirmed by using olive spiked samples with low concentration of *X. fastidiosa* DNA (from 100 fg to 4 fg). To overcome this issue, the amplification of two housekeeping genes (*cysG*, *malF*) for the subspecie identification and of seven MLST genes for the ST determination[1] was performed followed by MinION sequencing on several infected samples collected in Apulia and Toscana regions. This approach permitted to detect *X. fastidiosa* with a similar sensitivity to real-time PCR and simultaneously to correctly identify their subspecie and ST in few hours.

Funding information: Olivicoltura e difesa da Xylella fastidiosa *e da insetti vettori in Italia (Oli.Di.X.I.It (MIPAAF)); Contributi per il controllo delle emergenze fitosanitarie determinate da* Xylosandrus compactus, Xylella fastidiosa, Botrytis cinerea *(COXIBO) (MIPAAF)*

[1]EPPO Standard PM7/24(4) *Xylella fastidiosa* Bulletin OEPP/EPPO Bulletin (2019) 49 (2), 175–227.

S2-P18

FAIR data principles and their applicability to validation of diagnostic tests

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Validated and fit-for-purpose tests are crucial for reliable detection of plant pathogens. There is a wealth of validation data available. Laboratories generate vast amounts of validation data some of which is then made available via dedicated databases namely EPPO List of validation data[1], publications, reports of test performance studies, additional information provided by producers of reagents and other sources. Collecting the data is often a cumbersome and time-consuming process and furthermore, the validation data for a specific test are often not comparable because lack of essential data for comparison. This often applies to the in-house generated data over longer periods of time as well. To address these issues, NIB is introducing in the validation process FAIR data principles. FAIR data is data which meet principles of findability, accessibility, interoperability, and reusability. The approach encompasses development of standardized formats for collecting raw and relevant metadata and standardized ways of summarizing and reporting validation data. An example of summarizing and reporting data in line with FAIR data principle are probability of detecting a pathogen across a range of concentrations as described by non-linear modelling (see e.g. [2]). The approach provides data beyond the specific experimental points tested and thus significantly improves comparability and re-usability of data among laboratories and/or over time.

[1]EPPO (2022) List of validation data (available online). https://dc.eppo.int/validation_data/validationlist
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S2-P19

Phylogeny and identification of pathogenic *Pantoea* species associated with bulb rots and leaf blight of onion crops in Uruguay.

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Onion (*Allium cepa* L.) is among the most consumed vegetables in Uruguay, grown by producers in both the Northern and Southern regions of the country. Onion supply presents significant inter-annual variations associated to high post-harvest losses mainly caused by bacterial rots. Leaf lessions also may be devastating for some varieties under conduce conditions, whereas infections on seed-stalks lead to seed losses. The aim of this work was to identify the causal agents of bulb rots and leaf blight of onion crops in Uruguay. Symptomatic bulbs, leaves and seed-stalks were collected from commercial fields from 2017 to 2019. As a result from this survey, a collection of 54 *Pantoea* spp. strains was generated. The ability to cause disease symptoms was tested by leaf inoculation and red onion scale assays. Multilocus sequence analyisis (MLSA) using four housekeeping genes (*rpoB*, *gyrB*, *leuS* and *fusA*) allowed the assignment of the strains to five *Pantoea* species: *P. ananatis*, *P. agglomerans*, *P. allii*, *P. eucalypti* and *P. vagans*[1]. The last two species have not previously been reported as onion pathogens elsewhere. In addition, five isolates could not be identified to species level by MLSA. Genomic analysis suggest that these strains are related to *P. eucalypti* but may be assigned to a new species (ANI 91.7-91.9%). Overall, *P. ananatis* isolates showed the highest aggressiveness in leaf and bulb inoculation assays. The presence of diverse bacterial pathogens leads to a complex disease management and higlights the need of further knowledge about the biology and epidemiology of these species.

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S2-P20

A TaqMan-based qPCR assay for detection of *Xanthomonas translucens* pv. *undulosa* in infected wheat plants and seeds.

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Bacterial leaf streak, caused by *Xanthomonas translucens* pv. *undulosa* (Xtu), has become in the last decade a major disease in many wheat growing regions around the world [1-3]. Most of the wheat cultivars are highly susceptible and no chemical methods are available for disease control. Seed is considered an important source of primary inoculum, highlighting the importance of using clean seed to reduce bacterial leaf streak incidence. Disease diagnosis in the field is generally difficult mainly because the symptoms resemble those caused by fungal pathogens or environmental disorders. In this context, specific and sensitive diagnostic methods are required to prevent pathogen dissemination through infected seed and to effectively identify new outbreaks in the field. In this study, a TaqMan-based qPCR method was developed for Xtu diagnosis in infected wheats plants and seeds. A specific region of Xtu (*ina* gene) was selected for probe and primer design using a comparative genomics approach. The specificity of the qPCR assay was experimentally validated on 44 Xtu strains and 35 non target strains isolated from wheat plants. The assay was able to detect up to 10 pg of Xtu DNA in sensitivity and spiked assays. In addition, the reliability of the method was verified by detection of the target pathogen in wheat plants naturally and artificially inoculated with Xtu and in comercial seed lots. This TaqMan qPCR provides a valuable tool for pathogen surveillience, routine diagnostics, quantification of Xtu in infected material and for epidemiological studies.

Funding: Grants ANII-FCE_135561 and CSIC Grupos I+D 2000

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S3A-P3

Temperature plays a decisive role in the ability of *Pseudomonas syringae* strains carrying a recognized avirulence gene to induce a hypersensitive response in *Arabidopsis thaliana*

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The phytopathogenic species complex *P. syringae* has a very important economic impact, causing diseases on many important crops and woody plants. The *P. syringae* complex is currently divided into phylogenetic groups, and according to a certain recognized host specificity, they have been divided into more than 50 pathovars- A considerable research effort is invested in understanding how effector repertoires could determine the range of plants that a given strain can infect. Recently, we showed that the incapacity of *P. syringae* pv. *actinidiae* to induce the hypersensitive response (HR) in *A. thaliana* is due to its incapability to inject effectors rather than to the absence of a recognized effector. In this context, we report here results from the comparison of different *P. syringae* strains, belonging to different phylogroups and carrying the same plasmid-borne avirulence gene, for their capacity to induce an HR in *A. thaliana* Col-0, at different temperatures. *Pto* DC3000 and *Pma* M6 consistently trigger a strong hypersensitive cell death, while the other strains induce an HR, at different intensities, significantly dependent on temperature. Suprisingly, differences were also observed among quasi-clonal strains. These results i) highlight the necessity to study bacterial virulence in a broader set of strains and reveal that Pto DC3000 is a reliable model strain but not representative of the *Pseudomonas* complex, ii) support the notion that the presence/absence of effectors is not sufficient to predict the outcome of plant-bacteria interactions, and iii) indicate that temperature may play a crucial role in regulating effector injection.

S3A-P4

Enhancing the UK diagnostic capabilities for *Xylella fastidiosa*

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Reliable and accurate diagnostic tools are critical to enable effective response to *Xylella fastidiosa* in the event of an outbreak. Current testing procedures including different real-time PCR assays are being validated and optimised for use by accredited laboratories in the UK. Informal DNA based proficiency tests for real time PCR assays completed by four institutions gave consistent results. Three host species (*Lavandula dentata*, *Nerium oleander*, *Coffea arabica*) were inoculated with three subspecies of *Xylella fastidiosa* and kept in two different glasshouse environments (25°C and ambient tempreature) where rates of colonisation and symptom expression are being monitored to inform future sampling strategies. Six months after inoculation, there is no evidence of spread in the host plants beyond the area close to the inoculation points. Other approaches, such as detection of volatile organic compounds (VOCs), are being assessed to target sampling. A proof of principle study using coffee plants showed that a large amount of VOCs can be retained on Mono Traps (a portable sorptive solid media), but additional experiments are necessary to determine if the differences detected are due to inoculation with *Xylella*. Methods identifying subspecies and strains are being developed to enable tracing of the infection source. Concatenated MLSA sequences were collected for all publicly available genomes of *X. fastidiosa* and trees built to visualise relationships between clonal complexes. Candidate methodologies for creating cgMLST/wgMLST schemes for *X. fastidiosa* were evaluated and selected. Two prototype cgMLST schemes have been created using publicly available genome data.

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S3A-P5

Identification of phytoplasmas in olive trees infected with *Xylella fastidiosa* in Salento (Italy)

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Sampling was carried out in four olive grove localities in May, July and September 2018 in Lecce province (Italy) to compare the performance in the detection of *Xylella fastidiosa* presence in olive trees among PCR on three genes and qPCR[1]. The *X. fastidiosa* targets were the sigma RNA polymerase (primers RST31/33), the housekeeping gene *lacF* (primers lacF/R) and the hypotethical protein XF1100 (primers 272-1-int/272-2-int). The comparative screening results indicate that the qPCR and the PCR with primers RST31/33 were the most reliable methods for the pathogen detection, irrespectively from the sampling time and the locality. Morevoer considering that in several cases the CT values were very border line (35), the conventional PCR resulted a more suitable method for the confirmation of the presence of the bacterium. Further analyses on the same DNA extracts were performed to verify the possible presence of phytoplasmas and were carried out by nested PCR on 16S ribosomal gene followed by RFLP and/or sequencing analyses[2]. In a number of olive tree samples from one locality that were positive to *X. fastidiosa* (CT values from 32 to 35) or (in one case) negative with all the detection systems employed, the presence of phytoplasmas classified in subgroups 16SrI-B ('*Candidatus* Phytoplasma asteris'-related) and 16SrX-B ('*Ca*. P. prunorum'-related) was detected. The 16SrI-B phytoplasmas were already detected in olive in Italy[3], while the 16SrX-B are detected for the first time. The phytoplasma detection in the olive plants is indicating a possible active role of the *Xylella* insect vectors in the transmission of both bacteria that should deserve further studies for clarification

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S3A-P6

Exploring the diversity within the genus *Dickeya*, new genus and species

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The genus *Dickeya* includes plant pathogenic bacteria attacking a wide range of crops as well as a few environmental isolates from water. It was first defined in 2005 on the basis of six species. Despite the description of several new species in recent years (2014-2019), the diversity of the genus *Dickeya* is not yet fully explored. Many strains have been analyzed for species causing diseases on economically important crops, such as for the emerging potato pathogen *D. solani*. In contrast, only a few strains have been characterized for species of environmental origin or isolated from plants in understudied countries. We have undertaken extensive analyzes of new environmental isolates and poorly characterized strains from old collections. Phylogenomic and phenotypic analyzes led us to propose the new species *D. poaceaphila* for Australian strains isolated from grasses[1], the new species *D. parazeae*, resulting from the subdivision of the species *D. zeae*[2], and the reclassification of *D. paradisiaca* (containing strains from tropical or subtropical regions) in a new genus, *Musicola*[3]. Traits distinguishing each new species were identified from genomic and phenotypic comparisons. Thus the genus *Dickeya* currently includes twelve species with validly accepted names: *D. aquatica, D. chrysanthemi, D. dadantii, D. dianthicola, D. fangzhongdai*, *D. lacustris, D. oryzae, D. parazeae, D. solani*, *D. undicola* and *D. zeae*. In addition, the new genus *Musicola* includes two species *M. paradisiaca* and *M. keenii.*

The remaining heterogeneity observed in some cases, notably for *D. zeae* which appeared to be a species complex, indicates that additional species still need to be defined for certain phylogenomic clades.

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S3A-P7

Comparison of the phenotypic and genomic features of plant pathogenic bacteria from the species *Dickeya solani* and *Pectobacterium parmentieri*

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Dickeya solani and *Pectobacterium parmentieri*, which are classified within the *Pectobacteriaceae* family, are causative agents of soft rot and blackleg diseases of potato. The purpose of this study was to compare the phenotypic and genotypic diversity of both species. We characterised phenotypically and genotypically 20 *D. solani* and 12 *P. parmentieri* strains of various geographical origin and diverse years of isolation (2005-2014). Except for three analysed strains, two from Poland and one from Germany, all others showed high ability to macerate plant tissue and to produce plant cell wall degrading enzymes (pectinases, cellulases, proteases) and other investigated virulence factors [1,2]. The chemical structure of O-antigen of the lipopolysaccharide of four representative *D. solani* strains of different origin was identical. In contrast, in *P. parmentieri* 3 different structures of O-antigen was described [3]. Apart from investigating the differences in the bacterial ability to macerate plant tissue, plant cell wall degrading enzymes activities, siderophores and biofilm production we undertook the analysis of whole genomic sequences. The *de novo*-sequenced and assembled genomes, indicated high genetic homogeneity of *D. solani* in contrast to the heterogeneity revealed for the genomes of *P. parmentieri* [1,2]. The pangenomes constructed on the basis of the studied genomes consisted in 84.7% of core genes in the case of *D. solani* and only in 52.8% regarding *P. parmentieri*. The presented study and especially a genome-wide phylogeny of *D. solani* and *P. parmentieri* species based on core genomes of both species indicated no obvious correlations between their geographical origin and taxonomic relations.

Funding: National Science Centre Poland via grants 2016/21/N/NZ1/02783 and 531-N107-D801-21, 531-N107-D801-22

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S3A-P9

Endophytic distribution of *Pseudomonas syringae* pv. *actinidiae* after a five-year latency into *Actinidia chinensis* var. *chinensis* plants: a real-time-PCR analysis

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The ability of phytopathogenic bacteria to survive for long time within asymptomatic host plants represents one of the main critical factors to control outbreaks. The epidemiological role of the bacterial latency phase is very important since the control strategies are based on preventive chemical treatments by spraying on plant surfaces. In seven-year-old plants of *Actinidia chinensis* var. *chinensis* 'Hort16A' inoculated five years before with a virulent *Pseudomonas syringae* pv. *actinidiae gfp*-expressing/rifampicin-resistant strain (Psa*gfp*-Rif^{res}) at low inoculum dose, the dangerous latency phase of Psa*gfp*-Rif^{res} was studied dissecting the whole plants by cutting, from the apex to the root collar, the shoots/stems in segments of 20 cm (approx. 10-15/plant). In this study, to better clarify the endophyte distribution in asymptomatic plants and the preference of the pathogen for certain portions of the plant stem, the data previously obtained from microbiological (direct isolation on selective media, DI), biological (pathogenicity/HR assay) and PCR (Bio- and Nested) analyses were compared with those from Real-time PCR (RT-PCR) analysis. In the pathosystem Psa–*Actinidia*, the pathogen reached a high degree of pathoadaptation which is highlighted by the pluriannual latency period of Psa. The RT-PCR of the DNA extracted and quantified by the different segments of each entire plant confirmed the results previously obtained from microbiological and Bio/Nested PCR analyses and allowed to detect Psa*gfp*-Rif^{res} in segments of the stem that in Bio/Nested PCR analyses were Psa*gfp*-Rif^{res}-negative. Direct isolation revealed the Psa*gfp*-Rif^{res} presence in accordance with the RT-PCR data. The long time required for DI of Psa*gfp*-Rif^{res} from plant samples five years after the plant inoculation was largely compatible with the low concentrations of Psa*gfp*-Rif^{res} detected by RT-PCR.

S3A-P11

Groundwater, a reservoir for plant pathogenic bacteria : the case of *P. syringae* complex in alluvial aquifer of Avignon

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Groundwater is the main reservoir of freshwater (98 %) on earth excluding glaciers. During last decades the use of groundwater for irrigation have increased following agriculture intensification and climate change, in particular in southern Europe. However, there is a significant lack of information on the presence of plant pathogens in aquifer, the current knowledge being to consider the risk negligible [1]. We decided to evaluate the possibility for groundwater to hosted plant pathogens with the model *Pseudomonas syringae* complex (*Psy*), a bacteria closely linked to the freshwater cycle [2]. We report evidence for the presence of *Psy* population at various places and dates in groundwater of Avignon, an intensive irrigation area in the south-east of France. Their concentration was highly variable and inversely correlated with water conductivity explaining 27% of variability. Their mean abundance were 100 times lower than in the river Durance connected with the aquifer but surprisingly, their genetic structure were more homogeneous i.e. 94 % belonged to *Psy* Phylogroup 02 [3], than in the river (PG02 = 32% of strains). Moreover, most strains (98 %) from groundwater were tested potentially pathogenic on plant, when in river they were only 66%. Determinants of this low diversity and prominence of PG02 in groundwater remains to be identified. In conclusion even if more surveys are needed, aquifers must be considered as potential plant pathogenic reservoirs especially when used for crop irrigation. These results could influence new approaches to disease forecasting and surveillance and could lead to adaptation of agricultural practices.

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S3-P13

Assessing the risk of transmission of '*Candidatus* Liberibacter solanacearum' Apiaceae haplotypes to potato crops

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'Candidatus Liberibacter solanacearum' (Lso) is a phloem-limited, Gram-negative and non culturable bacterium. This emerging pathogen is transmitted to plants by psyllid insect vectors. Lso affects important Solanaceae crops including potato, tomato, pepper, tobacco, and can affect Apiaceae crops such as carrot and celery. It is the causal agent of the disease 'zebra chip' on potato, mainly in the Americas and New-Zealand. The recent detection of Lso on Apiaceae crops in Europe led the potato industry to wonder about the risk of transmission of Apiaceae haplotypes to potato. The objectives of this study are, firstly, to estimate the occurrence of the disease and its vectors in potato fields and, secondly, to assess the ability of psyllids to infect potato plants with Apiaceae haplotypes. Surveys were conducted in France during six summers, in potato fields close to Apiaceae fields. Potato and Apiaceae plants, as well as weeds and volunteers were gathered in 429 fields. A total of 3393 samples were tested for the presence of Lso. Field trials were also carried out for three years beside a carrot field and in a place where the Apiaceae haplotypes of Lso and its vectors occurred. Ten potato cultivars were tested and a total of 543 plants from the different crops were sampled during the growing season. During the same time, a total of 9772 psyllids were collected with yellow traps, and part of them were tested for the presence of Lso. These results suggest that Lso transmission to potato by Apiaceae psyllids is unlikely.

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S3-P14

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[1]. 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[2], 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.

This study was partly financially supported by research grant OS 2016339 project for R. solanacearum species complex of the Ministry of Economic Affairs in the Netherlands.

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S3-P15

The *Pectobacterium punjabense* in Europe: genomic and phenotypic characterization of European strains

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Blackleg causes high economic losses for the seed potato industry worldwide. The disease is caused by bacteria belonging to the genera *Pectobacterium* or *Dickeya*. Since 2016, the number of species belonging to these two genera has increased from 12 to 28 described species. *Pectobacterium punjabense* is one of the recently described species which has been isolated in 2017 from blackleg symptoms in Pakistani potato fields[1]. In order to assess the presence of *P. punjabense* in Europe, we screened bacterial collections by using housekeeping gene sequence analysis[2,3]. The strains identified as *P. punjabense* were further analyzed with genomic and phenotypic characterization methods. Seven *Pectobacterium* strains representative of the closely related species were used as reference. *P.punjabense* was occasionally identified in several collections showing that this species is not restricted to Pakistan and has been already isolated in Europe, sometimes several decades before and always coming from blackleg symptoms. Draft genomes of selected *P. punjabense* strains obtained with Illumina technologies were used for genomic comparisons, together with the reference genomes of the nearest closest species. In addition, based on genomic data, a qPCR TaqMan assay specific for *P. punjabense* was developed and should support further surveys to look for the presence of this pathogen. Finally, potato tuber assays were conducted in order to evaluate the aggressiveness of *P. punjabense* in comparison with others *Pectobacterium* reference strains. Results will be discussed.

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S3-P16

Evaluation of olive (*Olea europaea*) genotypes for resistance to *Pseudomonas savastanoi* pv. *savastanoi* at an International Olive Germoplasm collection

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A 3-year survey (2018-20) was carried out at the International Olive Germoplasm collection of "Villa Zagaria" (Enna, Italy) to assess the incidence and severity of olive knot disease (OKD). This disease, caused by *Pseudomonas savastanoi* pv. *savastanoi* (*Psv*), induces the formation of knots in branches and twigs and seriously affects olive production in the Mediterranean basin[1]. The disease severity was visually rated using a scale that ranged from 0 to 4 based on number, shape and dislocation of knots in the branches[2]. According to visual assessment of 205 Italian olive accessions, the 51.73% showed very severe symptoms (disease severity >3). Over a 3-year period OKD severity was confirmed for all the accessions but 22 which moved one point up the scale expecially in 2020. The most susceptible cultivars with disease severity classified as 4 were: Frantoio, Corsicana da mensa, Cellina di Nardò, Cima di Bitonto, Biancolilla cilentana, Oliva bianca, Giarraffa, Gioconda, Luminario and Riondello. No symtoms were detected on cultivars Negrera, Ascolana semitenera, Favarol and Verdello grosso. Epiphytic *Psv* population sizes in cultivars showing different disease severity did not reveal significant differences, with average values around 4 $\log_{10}$ cfu/mL. The variability of a subset of 32 representative *Psv* isolates collected from different cultivars in different part of the field was investigated. No differences were detected to-date by BOX, REP profiles as well as by High-Resolution Melting (HRM) SNP typing method[3].
Other studies are ongoing to study the microbial endophytic community in genotypes showing different level of resistance.

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S3-P17

Leafhopper *Bothrogonia sinica* is a natural vector of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit orchards

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Kiwifruit bacterial canker (KBC), caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), is the most disaster disease destroyed kiwifruit plants throughout the world. Psa spreads quickly in orchards via abiotic elements, such as rain, wind, as much as by human activity. In this study, we isolated Psa from the interior and exterior bodies of a commercial kiwifruit pest, the leafhopper *Bothrogonia sinica*. Moreover, we detected Psa symptoms on the punctured leaves of the kiwifruit cultivar 'Jinfeng' (*Actinidia chinensis* var. *chinensis*). 60% of *B. sinica* adults isolated from Psa-infected kiwifruit orchards were positive for the Psa presence after PCR detection. Interestingly, the peak of dispersal of *B. sinica* onto kiwifruit (March-April) fits with the highest seasonal Psa infectivity. Using a green fluorescent protein-labeled strain of Psa JF8, microscopic observations showed that the pathogen could colonize mandibular and maxillary stylets. The maxillary stylets are interlocked to food canal and salivary canal. Under controlled conditions, the leafhoppers collected from infected orchards were able to transmit Psa to healthy kiwifruit plantlets. The present research work is the first finding that *B. sinica* is a vector for Psa, which could potentially accelerate Psa transmission over large distances.

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S3-P18

Presence of *Pseudomonas syringae* pv. *actinidiae* in buds of asymptomatic *Actinidia chinensis* var. *deliciosa* plants in late autumn and winter

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In bacterial diseases, the latency period, during which there are no visible disease symptoms, can be prolonged depending on the conditions unfavorable for the growth of pathogens. As for *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of the bacterial canker of kiwifruit (BCK), the ability to survive several years in asymptomatic plants was recently highlighted. The Psa pluriannual latency in susceptible plants raises serious problems to control BCK for which it is necessary to consider defense strategies other than those based on spray treatments. Phytopatogenic bacteria can be hosted in buds of asymptomatic plants and their survival is regarded as a factor facilitating the overwintering of certain diseases caused by *P. syringae*. To clarify the bud epidemiological role, the Psa early detection in dormant buds (≈300) was carried out. In late autumn, 10 asymptomatic *A. chinensis* var. *deliciosa* plants were selected in an orchard located at the edge of a safety area that in the current year did not show BCK symptoms. At the beginning of dormancy (mid-December) and of "bleeding" (end of February), the buds were removed from a one-year old shoot. The Psa presence was determined by direct isolation and PCR analysis. At the end of the two surveys, the plant phytosanitary state was monitored to relate the orchard situation with the effective Psa absence/presence as pointed out by the early detection on bud samples. Psa was present in buds from 1 out of 10 and in 4 out of 10 plants in the first and in the second survey, respectively.

S3-P19

The phylogenetic position of *Agrobacterium* strains isolated from blueberries in Poland, *Agrobacterium vaccinii* – new species isolated from galls on shoots.

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Although crown and cane gall are one of the most important bacterial diseases in blueberry (*Vaccinium corymbosum*) production, little is known about the causal agent of these diseases. In Poland, three types of symptoms were recorded on blueberries: i/ galls on the stem at the soil line; ii/ galls on the cane surface that completely girdled affected cane sections, and iii/ small galls on branches. Bacteria isolated from these tumor tissue on selective media were pre-identified as *Agrobacterium*/*Rhizobium* spp. using 23S rDNA-based multiplex PCR[1]. Additionally, for selected isolates, 16S rDNA, *recA, atpD* and *rpoB* gene regions were sequenced and phylogenetically compared to the sequences of reference strains. Among isolates obtained from galls on the stem at the soil line, majority of isolates were classified as *Rhizobium rhizogenes* and "*Agrobacterium tumefaciens* species complex" (biovar 1). From cane galls, *Agrobacterium rubi* was frequently isolated and, as from small galls on shoots, bacteria belonging to different new phylogenetic lineages in *A. rubi* clade. However, none of the isolates was pathogenic nor positive in PCRs with primers amplifying tumor-inducing (Ti) plasmid genes. Four strains isolated from the galls on blueberry shoots were characterized additionally by whole-genome-based phylogeny which indicated that they form a novel *Agrobacterium* species. Average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) comparisons between genome sequences of representative strains B7.6^T and B19.1.4, and their closest relatives, confirmed that the strains studied represent a novel species of the genus *Agrobacterium*, for which the name *Agrobacterium vaccinii* sp. nov. was proposed[2].

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S3-P20

***Pseudomonas syringae* pv. *actinidiae* targets kiwifruit ethylene metabolism and volatile organic compound emission to faciliate host colonisation and insect-mediated dispersal**

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The bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), is the most significant limiting factor in kiwifruit production worldwide[1]. This bacterium is able to infect host plants via natural openings, such as stomata and nectarthodes, or wounds, such as those caused by sucking insects. Moreover, the planthopper *Metcalfa pruinosa* Say (1830) has been demonstrated to directly transmit Psa from infected plants to healthy ones[2]. The role of volatile organic compounds (VOCs) in insects attraction and behavior is well known. Plants emit a wide range of VOCs in response to abiotic stresses, herbivory or pathogen infection. Some of those VOCs are key infochemicals playing a role in plant defenses, in the recruitment of functional microbiome or in the attraction of parasitoids to protect the plant against herbivorous insects. Psa emits specific VOCs in cell-free plant medium and induces substantial changes in plant VOCs upon infection [3]. Furthermore, Psa targets ethylene signaling which directly regulates plant VOCs emission. Our study indicates that VOCs changes may influence insect mediated transmission of Psa. In fact, in Y olfactometer test, 37% of *M. pruinosa* adults were attracted toward infected plants, while only 5% preferred the healthy ones. Our results suggest that Psa evolved a complex strategy based on manipulation of ethylene metabolism and VOCs signaling to both suppress host defenses and facilitate insect-mediated dispersal.

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S3-P21

Comparative genomic analysis and phenotypic characterization of *Pectobacterium brasiliense* from different origins

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The *Pectobacterium brasiliense* strains were first isolated in 1999 from potato tubers in Brazil[1]. Since then, this species was detected on all continents in different climate zones. These bacteria are highly aggressive and cause disease in many ornamental and horticultural plants species. A wide spectrum of infected plants, and heterogeneous environments occupied by these bacteria. correlates with the wide variation of the *P. brasiliense* strains. At least two clades could be described on the basis of phenotypic and genotypic features of the strains[2,3]. In this study, the genomes of two *P. brasiliense* strains isolated from groundwater and insects were compared with thirty five genomic sequences of other strains from this species, which were isolated from five plant species orignating from six continents. Overall genome relatedness methods revealed that two separate phylogroups corresponding to the species level could be discriminated. Genomic comparison of *P. brasiliense* with 150 strains of other 17 *Pectobacterium* species, for which genomes are available in Genbank, did not allow the selection of genes that were characteristic and unique for this species. The forty *P. brasiliense* strains isolated from different origins were subsequently submitted to phenotypic characterization. Tested strains exhibited different ability to cause plant tissue maceration. Moreover, observed variability in biochemical properties, protein profile and fatty acid content correspond with genetic diversity based of fingerprinting profiles, as well as MLSA analysis. *P. brasiliense* strains originating from insect and water samples did not exhibit significant differences from those isolated from plants.

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S3-P22

Glandular and non-glandular trichomes are colonization sites and host entry points of the fire blight pathogen on apple leaves

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Unlike fungal pathogens, bacterial plant pathogens do not have penetration structures thus rely on natural openings or wounds to enter host and cause disease. Characterized natural openings as host entry points by bacterial pathogens include stomata (*Pseudomonas syringae*) and hydathodes (*Xanthomonas campestris*). Fire blight, caused by a bacterial pathogen *Erwinia amylovora*, is a devastating disease of apple and pears. On leaves and shoots, *E. amylovora* has been long thought to enter host through injuries caused by insects, wind, and hailstorm, however, the level of infection observed in the field suggests there could be additional host entry points other than artificial wounds. In this study, we demonstrated that *E. amylovora* can infect apple leaves without artifiicial injuries (grown in a plant growth chamber). Epiphytic colonization of *E. amylovora* was observed on glandular and non-glandular trichomes. *E. amylovora* was later found in interceluar space of leaf tissue adacent to the glandular trichomes and the veins. Additionally, we observed the glandular and non-glandular trichomes gradulally rupture and fall off during leaf development, which provide naturally occurred wounds for *E. amylovora* to enter. Although the type III secretion system is not required for colonization of *E. amylovora* on the glandular trichomes, it is however essential for *E. amylovora* to establish initial colonziation in messophil tissue adjacent to the glandular trichomes. Finally the host entry and infection of shoots is heavily impacted by the shoot water content. When shoot water potential is below -18 bar, pathogen entry and shoot blight infection would not occur.

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S4-P1

Combating onion bacterial diseases with pathogenomics tools and enhanced management strategies: Research objectives and progress towards reducing crop losses

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The 'Stop the Rot' project (https://alliumnet.com/stop-the-rot/) is funded by the USDA National Institute of Food & Agriculture Specialty Crops Research Initiative (2019-2023), with scientists from diverse disciplines in the United States and South Africa researching the host, pathogens, and environmental factors influencing bacterial diseases of onion. The objectives are to: characterize onion bacterial pathogens across the USA through field surveys, pathogenomics, and microbiome studies; develop robust molecular diagnostic tools for timely detection and identification of bacterial pathogens; establish a National Onion Bacterial Strain Collection to support onion research; complete field trials to optimize disease management strategies and identify risk-based management options; and employ economic evaluations to ensure recommendations are practical, viable, and financially sustainable. Although five bacterial genera are associated most commonly with onion diseases (*Pantoea, Burkholderia*, *Enterobacter, Xanthomonas*, and *Pseudomonas*), surveys to date have revealed 95 genera of bacteria associated with symptomatic onions. The distribution and pathogenicity of strains varied across onion production regions, although strains of very few genera caused symptoms in onion pathogenicity tests. Microbiome analyses revealed different complex bacterial communities in asymptomatic vs. symptomatic onion bulbs. Species-specific diagnostic tests are being developed for pathogenic *Pantoea agglomerans,* a ubiquitous bacterium of national concern in the USA. Irrigation, fertility, cultural, and post-harvest practices as well as bactericide applications are being evaluated in field trials in multiple regions across the USA. Results to date indicate that irrigation timing and method of application, and other cultural practices can be optimized to reduce onion crop losses caused by bacterial diseases.

S4-P2

How does *Arabidopsis thaliana* fight off its ever-present opportunistic pathogen *Pseudomonas viridiflava?*

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During evolution of host-pathogen interactions, pathogens improve their virulence tools while hosts advance their defenses. *Pseudomonas viridiflava,* an opportunistic pathogen, is the most common *Pseudomonas* clade in the phyllosphere of European *Arabidopsis thaliana* populations. Belonging to the *P. syringae* complex, it is genetically and phenotypically distinct from well-characterized *P. syringae sensu stricto*. Despite its broad range we lack knowledge of how *A. thaliana* responds to diverse co-occurring *P. viridiflava* strains and how they colonize *A. thaliana*. Here, we characterized the host response in a *A. thaliana* - *P. viridiflava* pathosystem. We measured host and pathogen growth in infections to ascertain genotype x genotype interactions and used immune mutants and transcriptomics to determine defense pathways influencing infection. We found a large effect of host genotype on infection outcome and evidence of host x pathogen genotype interactions. Immune mutant and transcriptome analyses showed that *A. thaliana* uses jasmonic acid (JA)/ethylene (ET) signaling to defend itself against *P. viridiflava,* more so than salicylic acid. Wounding of the plant increased resistance to infection. Our results suggest JA/ET is important for suppression of *P. viridiflava*, yet suppression capacity varies between host accessions due to still unknown mechanisms. Our results shed light on how *A. thaliana* suppress the ever-present *P. viridiflava*, but further studies are needed to understand the mechanism of this interaction and how it contributes to persistence of *P. viridiflava* in *A. thaliana* populations.

S4-P3

Investigating the targets of conserved essential bacterial effectors

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Crop losses caused by pathogens and pests threaten food security. Using chemicals to control the ensuing diseases imposes significant monetary and/or environmental costs. A more sustainable approach is to use genetics to increase disease resistance against important pathogens in crops. This however requires a more detailed understanding of the molecular mechanisms controlling the interaction between pathogens and their host plants. This project is based on the characterization of conserved, essential virulence effector proteins that are injected within plant cells by pathogenic bacteria. A phylogeny-based approach using the model plant *Arabidopsis thaliana*, the crop maize and the fruit crop apple identified four clusters of receptor kinases as putative targets of a broadly conserved and pathologically important bacterial effector family. These candidates were screened for their interaction with these effectors by yeast two-hybrid and split-luciferase assays. Positive interactions are currently being confirmed *in planta* using co-immunoprecipitation. In parallel, knock-out mutants in Arabidopsis and apple are currently being generated using CRISPR/Cas9, and will be investigated for disease resistance against phytopathogenic bacteria. This comparative study between different plant species will provide scientific insights and potential practical applications for crop protection.

This project is supported by the Zürich-Basel Plant Science Center (PSC)- Syngenta Fellowship

S4-P4

Different Lifestyles, Different Polymorhpic-Toxin Repertoires: Abundance, Diversity, and Function of Polymorhpic-Toxins in Phytopathogenic Bacteria

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Prior to infection, most phytobacteria exist as epiphytes and must successfully compete against other microbes for both resources and space. This competition drives microbial ecology and evolution with potentially significant impacts on the phytobiome. A fundamental understanding of the molecular mechanisms bacteria employ to compete for resources is essential to the development of environmentally sound, targeted, and effective disease management tactics. Polymorphic-toxins, also known as Rearrangement hot-spot (Rhs) toxins, are secreted by many bacteria and play an important role in intra- and interspecies competition. To this end, we investigated the abundance, diversity, and function of Rhs-toxins in gram-negative bacteria in reference to a pathogen's lifestyle. A Hidden Markov Model was used to identify a 36 Rhs aminoacidic motif in the proteomes of 1,356 publicly available genomes in the genera: *Xanthomonas, Pseudomonas, Pectobacterium, Dickeya*, and *Ralstonia.* Strains of soilborne *Pectobacterium spp.* had the highest number of Rhs-toxins encoded in their genomes. In contrast, the foliar pathogens *X. vesicatoria* and *X. perforans* show a reduced number or lack of Rhs-toxins. Our data indicate that soilborne bacteria encode more predicted Rhs-toxins than their foliar pathogen counterparts. These data will be fed into bacteria-bacteria and bacteria-microbiome models that may inform the viability and efficacy of Rhs-toxins as a biological control against phytopathogenic bacteria.

S4-P6

The root of the problem, potential origins of the bacteria associated with Acute Oak Decline.

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Acute Oak Decline (AOD) is a complex decline disease affecting the British oaks *Quercus petraea* and *Quercus robur*. The disease is fatal in 3-5 years and has rapidly progressed throughout the UK [1]. Similar cases are emerging across the globe and on other broadleaf hosts, marking a worrying turn in forest health.*Brenneria goodwinii* and *Gibbsiella quercinecans* have been identified as key agents of disease, while *Rahnella victoriana* and *Lonsdalea britannica* play secondary roles in disease development. Rapid molecular identification methods have been designed to monitor the development and spread of the disease. However, the source of these pathogens is still unknown [2]. To assess whether the rhizosphere soil could be a reservoir for the AOD bacteria, soil samples from healthy and symptomatic oak trees were collected from Hatchlands National Trust site, UK. Using molecular and traditional culturing methods, *G. quercinecans* and *R. victoriana* have been identified in healthy and diseased rhizosphere soil samples. At the same time, while assessing oak material both *G. quercinecans* and *R. victoriana* were also identified from acorns and leaves. This provides evidence that soil could act as a reservoir for pathogens contributing to the development of AOD symptoms, but most probable is that these bacteria are endophytes that are inherited through seeds.
These methods also yielded several novel species of *Scandianvium* which have been classified using a polyphasic approach. These bacteria could be pathogenic in nature, having been isolated from both the roots of oaks suffering from AOD as well as weeping lesions on *Tilia* species, though further testing is required to confirm this.

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S4-P7

Methylases and methylation in gene regulation of *Pseudomonas syringae.*

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DNA methylation carried out by methyltransferases not associated with restriction enzymes, known as orphan methyltransferases, is an epigenetic mechanism widely spread in bacteria and archea. These orphan methylases show motif specificities and methylation patterns that support their function in gene regulation and DNA replication[1]. In *E. coli* and *Salmonella*, this mechanism has been related to the regulation of the initiation of replication, DNA mismatch repair and transcription. DNA methylation is also involved in generating phenotypic heterogeneity within clonal populations in these species, with strong implications for virulence[2]. However, little is known about the role of methylation in *Pseudomonas syringae*. *P. syringae* is a relevant phytopathogenic bacteria, responsible for a great variety of plant diseases and with a huge impact in crop production worlwide. This bacteria is also used as a model for the study of plant-pathogen interactions[3]. In order to unveil the importance of methylation in *P. syringae,* we decided to address the characterization of this epigenetic process in this pathogenic bacteria. For that, we have studied the methylases identified in *P. syringae,* and established its methylome. Regarding the methylome, we have identified several methylation motifs.

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S4-P8

Immune Recognition of the Secreted Serine Protease ChpG Restricts the Host Range of *Clavibacter michiganensis* from eggplant varieties

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Bacterial wilt and canker caused by *Clavibacter michiganensis* (*Cm*) inflicts considerable damage in tomato-growing regions around the world. *Cm* harbors a narrow host range and can cause disease in tomato but not in most eggplant varieties. The pathogenicity of *Cm* is dependent on secreted serine proteases of the Pat-1 family, that are encoded on the *chp*/*tomA* pathogenicity island (PI), and the pCM2 plasmid. Screening combinations of PI deletion mutants and plasmid-cured strains found that *Cm*-mediated hypersensitive response (HR) in eggplant is dependent on the *chp*/*tomA* PI. Singular reintroduction of the four PI encoded Pat-1 family proteases into *Cm*ΔPI identified that the HR is elicited by the Pat-1 family protease ChpG. Eggplant leaves infiltrated with *chpG* marker exchange mutant (*Cm*Ω*chpG*) did not display HR, and infiltration of purified ChpG protein elicited immune responses in eggplant but not in *Cm*-susceptible tomato. Virulence assays found that while wild-type *Cm* and *Cm*Ω*chpG* complemented strains were nonpathogenic on eggplant, *Cm*Ω*chpG* caused wilt and canker symptoms. Additionally, bacterial populations in *Cm*Ω*chpG*-inoculated eggplant stem tissues was ~1000 folds higher than wild-type and *Cm*Ω*chpG* complemented *Cm* strains. Alanine substitution of Serin231 of the putative serine protease catalytic triad eliminated the ability of purified ChpG protein to elicit HR on eggplant and introduction of $ChpG_{S231A}$ into *Cm*Ω*chpG* did not affect its ability to cause disease, indicating that protease activity is required for immune recognition of ChpG. Our study identified ChpG as a novel avirulence protein that is recognized in eggplant and restricts the host range of *Cm*.

S4-P9

In vitro screening of antimicrobial activity of epiphytes and endophytes against the phytopathogen *Xylella fastidiosa*

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Xylella fastidiosa represents a global threat to crops of great economic importance, such as citrus, olives, grapes, and almonds. At present, effective control of this dangerous bacterium is still lacking. In this context, the screening for antimicrobial activity of endophytic and epiphytic bacteria isolated from different host plant species might be a promising control strategy for xylem colonizing pathogens such as *Xylella fastidiosa.* Indeed, the antimicrobial metabolites produced by antagonistic microorganisms used against plant diseases are of great importance. Natural antagonists have long been used to control plant diseases. Comparatively, the use of microbial metabolites has many advantages over whole microorganisms. In the present study, *in-vitro* well diffusion and broth dilution assays were carried out to test antimicrobial metabolites secretion by endophytes and epiphytes bacteria isolated from different host plants of *Xylella fastidiosa* ST53, using their culture-free supernatants. Our results revealed the antimicrobial activity of five antagonistic bacteria: *Paenibacillus rigui, Bacillus subtilus, Bacillus pumilus, Microbacterium oxydans*, and *Stenotrophomonas rhizophila* were able to produce in liquid culture substances with inhibitory effect and reduce *X. fastidiosa* growth with an appearance of clear zones between 10 and 32 mm. Therefore, the potential role of these bacteria to control *X. fastidiosa* should be further investigated and can pave the way to develop a sustainable strategy for the control using their secreted metabolites.

S4-P10

Investigations on plant-associated bacteria with inactive crown galls of grapevine in Lebanon

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In February 2019, widespread symptoms of old and dry crown galls on trunks near the soil surface were observed in vineyards of Bekaa Valley, Lebanon. Twelve samples from inactive crown galls found in 6 vineyards were collected from the trunks close to the graft unions and used for bacterial isolation on LB Broth (Sigma, USA) agar plates. Isolated colonies were screened by PCR using specific primers for *Rhizobium* spp. and then the DNA region encoding 16S ribosomal RNA from selected isolates was sequenced. DNA sequencing results showed the absence of *Rhizobium* spp. while revealed the presence of important bacterial communities, including the following beneficial microorganisms known as antagonists able to counteract plant pathogens: i) *Pseudomonas frederiksbergensis* known to produce diketopiperazines (important bioactive compounds); ii) *Rahnella aquatilis*, a bacterial endophyte able to efficently reduce plant root colonization by fungal pathogens[1], and reported also as antagonist of *Rhizobium vitis*[2]; iii) *Pantoea agglomerans*, an ubiquitous bacterium, known as a biocontrol agent, inhabiting various environments (plants, water, soil, humans and animals), but also causing gall-forming diseases in certain plant hosts[3]. Our results showed that *Rhizobium* spp. was absent in old and dry crow galls, suggesting either that these tumors had a different origin or that the microbial communities occuring in the Lebanese diseased grapevines were effective in couteracting *Rhizobium* spp. infections. Perhaps, further investigations to clarify the aetiology of the crow galls disease and the role of the isolated bacteria on grapevine in Lebanon are necessary.

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S4-P11

Exploring the bacterial community associated with cane gall tumors on thornless blackberry

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Tumorigenic strains of the family *Rhizobiaceae* are responsible for crown gall and cane gall diseases of various agricultural crops. In our previous study, we identified a novel tumorigenic species, *Rhizobium tumorigenes*, associated with cane gall disease of thornless blackberry[1]. However, microbiota inhabiting cane gall tumors on thornless blackberry has not been studied before. Therefore, the aim of this study was to investigate the composition of bacterial community associated with this ecological niche. In 2021, nine tumor samples were collected at one of the localities (Lučani, Serbia), where we initially identified *R. tumorigenes* five years earlier[1]. Total bacterial community of crown gall tumors was analyzed by amplicon sequencing targeting 16S rRNA gene region V3-V4. Amplicon sequence variants (ASVs) obtained were taxonomically annotated using the blastn program. *R. tumorigenes* was identified in all samples with a relative abundance ranging from ~14% to ~95%. This was a dominant taxa in five samples tested (~42-95%). However, in the remaining four samples, *Rachnella* was the most dominant genus detected (~24-63%), although it was also detected in other five samples (~0.4-9%). In several samples, *Bacillus* sp. (~6 and ~25%), *Rhizobium* spp. (~8 and ~20%), *Pectobacteriaceae* (~13%), *Pantoea* sp. (~11%), *Novosphingobium* sp. (~7%) and/or *Burkholderiaceae* (~5%) were also enriched. All other taxa that are detected in some samples had relative abundance <5%. Apart from *R. tumorigenes* which was reported as tumorigenic bacterium, the remaining taxa detected in this study are also known as plant-associated bacteria, among which some were described as biocontrol and/or plant growth promoting agents.

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S4-P13

First report of *Pectobacterium odoriferum* causing bacterial soft rot of cabbage in Serbia

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In August 2021, symptoms of soft rot appeared on cabbage in Futog (Bačka, Vojvodina) locality known for traditional cabbage cultivation in Serbia. Symptoms appeared in the form of sunken and soft lesions on outer head leaves, while interior tissue was macerated, with cream to black discoloration. A strong, specific odor followed the breakdown of the affected tissue. From the 1 ha field, a total of 5 heads were collected for the isolation of causing pathogen. Margins of healthy and dead tissues were macerated and plated on Crystal Violet Pectate medium. Five creamy-white colonies forming pits in the medium were purified. These bacterial isolates were positive for pectinolytic activity on cabbage heads and potato slices. They were all facultative anaerobic, Gram-negative, catalase-positive, and oxidase-negative; unable to produce diffusible fluorescent pigments. Multilocus sequence typing of cabbage isolates was performed by sequencing of four housekeeping genes (*proA*, *dnaX*, *icdA*, *mdh*). Based on the nucleotide BLAST (National Center for Biotechnology Information, NCBI) analysis of the sequences of genes *icdA* and *mdh*, Serbian cabbage isolates were 100% identical with *Pectobacterium carotovorum* subsp. *odoriferum* strain BC S7. Based on the *dnaX* gene, identity with the same strain was 99.79% and 99.57% based on the *proA* gene. Serbian cabbage isolates also showed 100% identity with other NCBI deposited *P. odoriferum* strains CFBP1878 and ATCC 25272 based on the *icdA* gene, as well as ICMP 11533 based on *mdh* gene. Therefore, this first report of *P. odoriferum* indicates a wider range of *Pectobacterium* spp. recently described in Serbia.

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S4-P14

Comparative genomic analysis of the endophytic bacterium *Pantoea agglomerans* DAPP-PG 734 and its synergistic interaction with *Pseudomonas savastanoi* pv. savastanoi DAPP-PG 722 in olive knots

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Pantoea agglomerans DAPP-PG734 was isolated as endophyte from olive knots caused by *Pseudomonas savastanoi* pv. savastanoi DAPP-PG722 in olive trees. To understand the plant-pathogen-endophyte interaction on a genomic level, the complete genome of *P. agglomerans* DAPP-PG734 was sequenced and annotated. The genome had a total size of 5'396'424 bp, containing one circular chromosome and four large circular plasmids. Unlike most other *P. agglomerans* strains, in *P. agglomerans* DAPP-PG734, a gene cluster for the synthesis of the Hrp-1 type III secretion system (T3SS) was identified. To assess its role in the interaction with *P. savastanoi* DAPP-PG722, we generated independent knockout mutants of *hrpJ*, *hrpN*, and *hrpY* genes in *P. agglomerans* DAPP-PG734. In contrast to *P. savastanoi* DAPP-PG722, the wildtype strain *P. agglomerans* DAPP-PG734 and its three Hrp T3SS mutants did not cause olive knot disease in 1-year-old olive plants. Coinoculation of *P. savastanoi* DAPP-PG722 with the *P. agglomerans* wildtype strain did not significantly change the knot size, while the *P. agglomerans hrpY* mutant induced a significant decrease in knot size, which could be complemented by providing *hrpY* on a plasmid[1]. By epifluorescence microscopy and confocal laser scanning microscopy, we found that the localization patterns in knots of fluorescently labeled strains were nonoverlapping for *P. savastanoi* DAPP-PG722 and *P. agglomerans* DAPP-PG734 when coinoculated. Comparative genomic analysis of the *P. agglomerans* DAPP-PG734 genome and related *Pantoea* spp. indicated, that the potential biosynthesis of two antibiotics by DAPP-PG734 may be responsible for the distinct localization in the olive knots[2].

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S4-P15

Pseudomonas savastanoi pv. *glycinea* affecting a vegetable soybean for commercial edamame production in Serbia

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The vegetable soybean "edamame" (*Glicine max* L.) is a nutritious legume having pods and seeds that can be harvested and consumed while they are still fresh and premature. Edamame is rich in micronutrients and vitamins and is therefore used as food and for medicinal purposes. In Serbia, edamame production started for the first time in 2019 on a 10 ha field in Bečej locality (Bačka, Vojvodina). Later, in 2021, its production was expanded to 30 ha, but in summer was followed with symptoms of bacterial leaf spot. The symptoms appeared on leaves in the form of water-soaked spots surrounded by a chlorotic halo that enlarged and coalesced into necrotic lesions. Disease incidence was from 15-20%. Isolation of the causal pathogen was performed by sowing of suspension of the macerated margins of spots/lesions from ten collected symptomatic leaves on nutrient agar supplemented with 5% sucrose. Ten representative, purified isolates were whitish, circular, smooth, shiny, levan-positive, strictly aerobic, gram-negative; positive for green-fluorescent pigment and tobacco hypersensitive response, and negative for oxidase, arginine dihydrolase, and potato soft rot (LOPAT group Ia). Pathogenicity of the isolates was confirmed on soybean cotyledons by under pressure atomizing a bacterial suspension (10^{7-8} CFU mL^{-1}). Sequencing of genes *gapA*, *gyrB*, and *rpoD* showed 100% homology of the obtained isolates with *Pseudomonas savastanoi* pv. *glycinea* pathotype strain LMG 5066 and strains BR1, KN166, KN28, KN44, LN10, MOC601, R4a (*gapA* and *gyrB*), and M301765 (*gapA* and *rpoD*) originated from soybean, all from the Plant Associated and Environmental Microbes Database (PAMDB).

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S4-P18

Dissecting strawberry microbiota for the selection of improved biological control agents againts *Xantomonas fragariae*

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Niche-specific fungal and bacterial microbiomes of three strawberry genotypes were taxonomically and functionally characterized to investigate their influence on plant health, productivity and fruit quality[1]. The core microbiome included 24 bacterial and 15 fungal operative taxonomic units which were present in all compartments and plant genotypes. However, both plant organ and genotype had a significant role in assembling the microbial communities. The microbial community assemblage across different soil and plant compartments significantly correlated with disease resistance. In fact, only the genotype 'Monterey', which is tolerant to angular leaf spot (*Xanthomonas fragariae*) was able to recruit the potential biological control agents *Pseudomonas fluorescens* in all plant organs and to establish symbiosis with the arbuscular mycorrhiza *Rhizophagus irregularis*. The cultural-dependent characterization of microbiota led to the selection of 43 bacterial strains belonging to 20 genera. Among them, 10 strains had a direct antagonism against *X. fragariae* and 21 presented a plant growth and resistance promotion activity. In greenhouse experiments, *P. fluorescens* and *Rhizobium soli* resulted in the highest reduction of angular leaf spots and in a significant increase of fruit productivity. Isolates of *Stenotrophomonas rhizophila*, *Bacillus pumilus* and *Rhodococcus spp.* had an efficacy comparable to streptomycin application in reducing disease incidence. Our results indicates that the selection of organ- and plant specific biological control agents is a promising strategy for achieving both a reduction of pesticide use and an increase of horticultural production.

[1]Sangiorgio D. *et al*., 2022. Journal of Advanced Research, https://doi.org/10.1016/j.jare.2022.02.009.

S4-P19

Mobilization of the virulence plasmids from *Pseudomonas syringae* pv. savastanoi NCPPB 3335

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P. syringae pv. savastanoi NCPPB 3335 is the causal agent of olive knot disease and contains three completely sequenced native plasmids —pPsv48A (pA), 80 kb; pPsv48B (pB), 45 kb, and pPsv48C (pC), 42 kb—. Here we show that pB contains a complete type IVA secretion system (comprising genes *virB1-virB11* and the coupling protein gene *virD4*) and a functional origin of conjugational transfer (*oriT*, 107 nt) adjacent to a MOB_P relaxase; pPsv48C also contains a functional *oriT* (146 nt)-MOB_P array, whereas pA contains an incomplete type IVB, but not a recognizable *oriT*. pB is a conjugative plasmid with a high frequency of transfer ($9.7 \pm 1.1 \times 10^{-3}$ transconjugants per recipient) and that pC is mobilizable ($6.2 \pm 1.1 \times 10^{-5}$), with plasmid transfer occurring on solid and in liquid media, and on bean (*Phaseolus vulgaris*) leaf surfaces. A mutant in gene *virB4* of pB was not conjugative and could not mobilize pC, indicating that this gene is essential. The MOB_P relaxase genes of pB and pC were functionally interchangeable for conjugal transfer, although with differing efficiencies. We also identified a functional MOB_Q mobilization region in pC, which could mobilize pC using the type IVA system of pB. Plasmid pB could be efficiently transferred to strains of six phylogroups of *P. syringae* but not to other species of pseudomonads, whereas pC could only be mobilized to two strains of phylogroup 3, also encompassing *P. syringae* pv. savastanoi.

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S5A-P2

Characterisation of phage that lyse *Gibbsiella quercinecans,* a causative agent of Acute Oak Decline

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Acute oak decline (AOD) is an emerging disease of oak trees (*Quercus* spp.), which causes dark weeping lesions on bark and potential tree mortality within 3 to 5 years. AOD is caused by a polymicrobial complex of three bacterial species, one of which is *Gibbsiella quercinecans*. No treatments are yet available against AOD or other diseases associated with *G. quercinecans*, such as bacterial canker of Russian olive (*Elaeagnus angustifolia*) and walnut (*Juglans regia*) [1,2]. Additionally, bacterial resistance and negative environmental impacts restrain the use of antibiotic treatments and copper compounds. However, a potential avenue for biocontrol of *G. quercinecans* is the use of bacteriophage (phage). Phage have been proven effective in treating several other bacterial tree diseases [3]. We isolated multiple phages from diseased lesions of oak trees that could lyse *G.quercinecans* in England. The genomes of phages that produce unique plaque sizes and morphologies are being sequenced. This will enable us to differentiate between individual phages, assign phage families, and perform genetic analysis. To determine the specificity and safety of the phages, host range assays are being performed against several *G. quercinecans* strains and other pathogenic, beneficial and commensal species. To determine the efficacy of the phages against their host, killing curve assays are being performed, via the application of phages both individually and in combinations. The results of this study will help identify phages or phage cocktails to treat *G. quercinecans*. It will also form the basis of research on phage identification and dynamics within the AOD pathosystem.

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S5A-P3

Dickeya solani D s0432-1 produces an arsenal of secondary metabolites with anti-prokaryotic and anti-eukaryotic activities against a wide rande of organisms.

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The necrotrophic plant pathogenic bacterium *Dickeya solani* is an invader of potato agrosystem in Europe. All isolated strains of *D. solani* contain several large polyketide/fatty acid/non-ribosomal peptide synthetase clusters. Analogy with genes described in other bacteria, suggests that two clusters are involved in the production of secondary metabolites of the oocydin and zeamine family. In this study, we constructed by an approach of reverse genetics mutants affected in the three secondary metabolite clusters *ssm*, *ooc* and *zms* in order to compare the phenotype of the *D. solani* strain D s0432-1 with its derived mutants. We demonstrated that the zeamine cluster *zms* inhibits growth of gram-positive and gram-negative bacteria. The oocydin cluster *ooc* inhibits growth of fungi of the phylum *Ascomycota*. Finally, we unveiled the function of a new secondary metabolite cluster *ssm* (for *solani* secondary metabolite), only conserved in some *Dickeya* species. This cluster produces a secondary metabolite inhibiting yeasts. *D. solani* therefore produces several molecules that are toxic to a wide range of living and potentially interacting organisms.

S5A-P4

Complete genome sequences and characterization of _Xanthomonas arboricola_, the novel causal agent of bacterial leaf blight of blueberry

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The cultivation of blueberry (*Vaccinium corymbosum* L.) is becoming increasingly important due to high content of beneficial nutrients in the fruit, its attractiveness, and the high profitability. For many years this plant species remained rarely infected by bacterial pathogens. Hitherto described, were tumorigenic *Agrobacterium* spp., *Burkholderia andropogonis, Xylella fastidiosa* and *Pseudomonas* spp. Recently, new pathogenic bacteria – the subject of this study[1] – were discovered. In 2013, on the blueberry cv. Toro and Duke growing in a nursery located in Central Poland russet brown, irregular spots on leaves were observed. From these leaf spots, fluorescent and yellow bacteria were isolated. Two yellow isolates, named 1311a and 1314c, were positive in a PCR assay using primers X1 and X2 specific for bacteria belonging to the genus *Xanthomonas*. Based on partial sequences analysis of *gyrB*, *fuyA* and *rpoD* (totalizing 1,635 bp), the strains were not closely related to each other, however, both were placed within the strains of *Xanthomonas arboricola*. Their complete genomes were determined using short (MiSeq, Illumina) and long-read technologies (MinION, Oxford Nanopore). The genomes size of the strains 1311a and 1314c are 4,889,189 bp and 4,891,143 bp, respectively, with a G+C content of 65.7%. Whole genome-based taxonomic analysis using the Type (Strain) Genome Server (TYGS; https://tygs.dsmz.de) confirmed the affinity of these two strains to *X. arboricola*. The genomes will be used for further analysis of evolution within the species *X. arboricola* for determining if the strains constitute a new pathovar within the species and improving the molecular diagnostics of this relevant pathogen of blueberry. It is the first report on the occurrence of bacterial leaf blight on blueberry caused by a *Xanthomonas* species. The further inspections confirmed the presence of *Xanthomonas* on blueberry in other geographic localizations.

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Based upon work from COST Action CA16107 EuroXanth supported by COST (European Cooperation in Science and Technology).

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S5A-P5

Phylogenomic status of two *P. syringae* strains P16 and P21 with different pathogenicity isolated from sugar beet in Serbia

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Members of the *Pseudomonas syringae* species complex are heterogeneous bacteria with strong abilities to exist on and infect different plant hosts and survive beyond agro-ecosystems[1]. The *P. syringae* strains previously collected from sugar beat commercial fields in Serbia were characterized as a genetically and pathogenically diverse strains collection[2]. This study provides comparative genomics and phylogenomic status assessment of two *P. syringae* strains from this collection, P16 and P21, with distinctive pathogenicity. The whole-genome data obtained in this study were combined with the publicly available genomes of 34 strains from the *P. syringae* species complex, including strains from the most ubiquitous and pathogenic phylogroups and strains isolated from non-agricultural environments. The core SNP and ANI analysis revealed four distinctive clusters among compared genomes, where P16 and P21 were clustered on the same branch, mostly with strains from PG02 and two strains from PG01 (*P. syringae* isolated from kiwi fruit). The pangenome analysis revealed 6.03% of the core genome and 93,97% of accessory genes. Based on the presence/absence of individual genes, both strains are grouped in the same branch of the phylogenomic tree together with other *P. syringae* strains from PG02b. We observed slightly different virulence factor gene composition between P16 and P21 strains, especially the T3SS effector and T4SS *virB* genes repertoire. The extensive accessory genome revealed a high degree of variability among *P. syringae* complex, which could be associated with their ability to survive in diverse ecological niches and the extensive horizontal gene transfer[3].

[1]Xin *et al.*, 2018. Nat. Rev. Microbiol., 16, 316–328
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[3]Dillon *et al.*, 2019. Genome Biol., 20: 1-28.

S5A-P6

Killing effect of *Bacillus velezensis* FZB42 on a *Xanthomonas campestris* pv. *campestris* strain newly isolated from cabbage: a metabolomic study

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Potential of *Bacillus velezensis* for biological control of various phytopathogens has been documented over the past few years but its antagonistic interactions with xanthomonads have not been studied in detail. The findings documented a strong killing effect on *Xanthomonas campestris* pv. *campestris* (Xcc) cells in a co-culture with *B. velezensis*. Lipopeptides and the siderophore bacillibactin involved in the killing process were quantified. A new robust Xcc-SU isolate tolerating high concentrations of ferric ions was used. In a co-culture with the antagonist, the population of Xcc-SU was annihilated within 24–48 h, depending on the number of antagonist cells used for inoculation. No inhibitory effect of Xcc-SU on *B. velezensis* was observed. Bacillibactin and lipopeptides (surfactin, fengycin, bacillomycin) were present in both the co-culture and the monoculture of *B. velezensis*. Except for bacillibactin, the maximum contents of lipopeptides were higher in the antagonist monoculture compared with the co-culture. Scanning electron microscopy showed that the death of Xcc-SU bacteria in the co-culture was caused by cell lysis. The analysis by mass spectrometry showed four major compounds, bacillibactin, surfactin, fengycin, and bacillomycin D. Different forms of surfactin and fengycin with variations in their side-chain length were detected. The results demonstrated the ability of *B. velezensis* FZB42 to act as a powerful antagonistic strain against Xcc.

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S5A-P8

Comparative genomics of native plasmids from phytopathogenic bacteria

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Plasmids are widely distributed in bacteria, often carrying genes participating in pathogenicity, virulence, or survival in hostile environments. Completely sequenced plasmids deposited in the NCBI from 15 genera of phytopathogenic bacteria showed high variability in size (1.3 kb-2.2 Mb) and G+C content (24.7 % -70.2 %). The characteristics of around 300 plasmids from six genera separated in three families: Enterobacteriaceae (*Erwinia*, *Pantoea* and *Pectobacterium*), Pseudomonadaceae (*Pseudomonas*) and Xanthomonadaceae (*Xanthomonas* and *Xylella*) were examined. We found replication initiation protein (Rep) genes in 69% of them, with a large percentage containing two or three replicons (14%). All three bacterial families contained homologues of one of the Rep genes whereas two of the families contained homologues of three Rep genes, indicating the possibility of plasmid exchange among these bacteria. Most plasmids within Pseudomonadaceae appear mobilizable, because 63% contained sequences homologous to *oriT*. About 68% of all native plasmids analysed contain transposable elements. In addition, we found annotated coding sequences with importance in virulence and resistance to antibiotics and heavy metals in about 15% of the plasmids analysed. Our results stress the plasticity and dynamism of the phytobacterial native plasmids, which could provide advantages in adapting to the environment and in the evolution of the bacterial populations through horizontal genetic exchange.

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S5A-P9

Uncommon *Curtobacterium flaccumfaciens* pathovars revive in other plant hosts: pv. *betae* in sugar beet and pv. *oortii* in garden lily

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Silvering disease appeared mainly in red beet from the 1950's till the 1970's in the UK[1]. The bacterial pathogen, *Corynebacterium betae*, was later renamed *Curtobacterium flaccumfaciens* pv. *betae*[2]. Tulip yellow pock disease was originally described in the Netherlands[3]. The bacterial pathogen, *Corynebacterium oortii*, was later renamed *Curtobacterium flaccumfaciens* pv. *oortii*[2]. Both vascular diseases cause silvery discoloration spreading through the leaves with cracks in the mesophyll. Severe infections result in stunting of the beet tap-root or form yellowish soft patches on the tulip bulb scales. Both pathovars have a limited geographical distribution. With records of silvering disease unknown for almost 40 years, it was surprisingly spotted in the 2011 Official Variety Test (OVT) of sugar beet in Belgium. Symptoms increased from 2016 onward and *Curtobacterium* isolates were recovered from 50 different sugar beet cultivars/selections in the 2018 OVT. Conversely, tulip yellow pock disease lingered on in bulb cultivation, although not at an economic damaging level. Remarkably, during 2019, *Curtobacterium* isolates were recovered from garden lily bulbs showing yellow softening of the basal plate.
Identification was done with a partial sequence of the *gyr*B and *egl* gene. Concatenated sequences of all isolates from sugar beet were identical and exactly matched the pathotype strain of *C.f.* pv. *betae*. The isolates from lily bulbs displayed sequence diversity, conforming with strains of *C.f.* pv. *oortii*. The genetic affiliation investigated in ANI analysis showed that none of the *C. flaccumfaciens* pathovars formed a monophyletic cluster. Pathogenicity was assessed on sugar beet, red beet, common bean, tulip, garden lily and poinsettia, confirming the pathovar designation.

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S5A-P10

Could the degradation of antibiotics using cold atmospheric pressure plasma brushes have an impact on the multidrug resistance of various pathogens?

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Nowadays, antibiotics are widely used in agriculture to prevent and treat crop diseases and increase harvest yield. At least 30 different antibiotics are commonly used in agriculture and veterinary. The increasing prevalence of antibiotics in the natural environments and the ability to acquire resistance by virulent pathogens from environmental bacteria directly trait to human health. Taking into consideration the lack of effective methods for degradation of antibiotics, we present a new method for inactivation of antibiotics from liquid disposals that relies on cold atmospheric pressure plasmas (CAPPs), *i.e.* pulse-modulated radio-frequency atmospheric pressure glow discharge (pm-rf-APGD) or dielectric barrier discharge (DBD) generated in the continous flow pm-rf-APGD or DBD-type plasma brushes (P.440185, UPRP). The effectiveness of antibiotics degradation from single solutions or mixtures involving fluoroquinolones, tetracyclines, trimethoprim, chloramphenicols and β-lactams, followed by om-rf-APGD- and DBD-type plasma brush was examined. The conducted research revealed that application of pm-rf-PAGD-type plasma brush lead to change the the values of Total Organic Carbon and Total Nitrogen Contents, which indicated antibiotics decomposition. High performance liquid chromatography showed that application of the pm-rf-APGD-type plasma brush led to 91.4%-100% removal efficiency for trimpetoprim and doxycycline, respectively. In turn, utilisation of the DBD-type plasma brush resulted in degradation of 4 antibiotics from a mixture in rates from 49.8% for chloramphenicol to 65.7% for doxycycline. Finally, disc-diffusion assays conducted on the members of *Enterobacterales* confirmed the reduction in the antibacterial properties of the DBD- and pm-rf-APGD-treated antibiotics solutions, which might lead to diminshment in the multidrug resistance among pathogens.

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S5-P11

Genetic diversity of *Xanthomonas arboricola* strains isolated from symptomatic hazelnuts in Chile

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The bacterial blight of *Corylus avellanae*, caused by *Xanthomonas arboricola* pv. *corylina* (Xac), induces severe yield losses in all the regions of its intensive cultivation around the world. The aim of this study was to assess the genetic diversity among 60 *Xanthomonas*-like strains isolated from symptomatic tissues of hazelnut cvs. Barcelona and Tonda di Giffoni in six Chilean regions during the period 2016-2018. These isolates were identified through molecular analyses. The evaluation of genetic diversity was then carried out by Rep-PCR (BOX-, ERIC- and REP-PCR) analysis. The genetic profiles were transformed into a binary matrix in order to perform the phylogenetic analysis and the dendrogram construction (by using UPGMA cluster analysis and Jaccard similarity index). In addition, the same profiles were analyzed by the BioNumerics v.7.6. software using the Xac strain NCPPB935 as reference and the *X. axonopodis* pv. *vitians* DISTAL9081 as out-group strain. Selected isolates were then subjected to the pathogenicity test on highly susceptible hazelnut cultivars. Among the 60 isolates, 19 were identified as *X. arboricola* (Xa) and 20 as Xac strains, whereas 21 isolates resulted Xa- and Xac-negative. The 39 Xac strains were differentiated in 5-6 genetic groups at approx. 50-60% similarity level by the BioNumerics elaboration, whereas the elaboration of the binary matrix of concatenated profiles allowed their separation in 7 genetic groups at approx. 60-70% similarity level. No relationships were found between amplification pattern, the plant organ of isolation, or the geographical origin of the isolates with the similarity groups delineated by the bioinformatics analyses.

S5-P14

Xanthomonas arboricola pv. *pruni* associated with leaf spot and twig necrosis of peach and sweet cherry in Montenegro

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In Montenegro we surveyed stone fruit orchards for presence of *Xanthomonas arboricola* pv. *pruni* during 2017/18. Twig cankers were observed on sweet cherry near Ulcinj, while leaf and fruit spot and twig necrosis were observed on peach near Podgorica. From the samples, yellow, convex, and mucoid bacterial strains were isolated on YDC medium. All selected strains were HR+, gram negative, strictly aerobic, oxidase negative, catalase positive, hydrolyzed esculin, and did not grow at 37°C. Three strains hydrolyzed starch and two strains did not hydrolyze gelatin. PCR analysis, with primer pair XapY17-F/XapY17-R, produced single band of 943 bp in all 37 strains [1]. Amplification and sequencing of *gyrB* gene of ten selected strains was performed using primers described by Parkinson et al. [2]. Obtained partial DNA sequences showed that eight strains share 98.97 to 99.71% of *gyrB* sequence identity with Xap pathotype strain ICMP51. The sequences of two strains showed 100% identity to *gyrB* gene of Xap strains isolated from peach and apricot in Hungary and peach in Italy. Pathogenicity was tested by spraying shoots and by infiltration of leaves and fruits with the bacterial suspension (10^7 CFU/ml in SDW) of all 37 strains and Xap reference strain 69VR (CFBP3892) respectively. Lesions appeared on all inoculated shoots, leaves and fruits within a week after inoculation. The pathogen was reisolated from the symptomatic tissues, and identity was checked by the PCR. According to their characteristics, the strains isolated from peach and sweet cherry in Montenegro were identified as Xap.

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S5-P16

Exploring *Acidovorax* species causing leaf spot on ornamentals and vegetables

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The bacterial genus *Acidovorax* was established in 1990, initially without phytopathogenic species. Shortly thereafter, several phytopathogenic (commonly called) non-fluorescent *Pseudomonas* species were transferred therein as subspecies of *Acidovorax avenae*[1], which were later elevated to species status[2]. They were rather unknown for significant economic damage until the early 1990s when *Acidovorax citrulli*, the cause of bacterial fruit blotch of cucurbits, was spread globally and became a major threat. Furthermore, bacterial blight and leaf stripe diseases caused by *Acidovorax avenae* in domesticated cereal crops such as corn, wheat, rice, barley and millet increased in importance in recent years. Somewhat underrated are *Acidovorax* species causing bacterial black spot in vegetables and on ornamentals. Isolates from *Phalaenopsis*, *Calathea*, *Pelargonium*, *Valerianella locusta* (lamb's lettuce) and *Cichorium endivia* (frisée endive) were analyzed on the 16S rDNA gene and by conventional PCR and in-house developed qPCR. They were identified by DNA barcoding using a partial sequence of the *rpo*B and *rpo*D gene[3]. The isolates from lamb's lettuce displayed identical barcode sequences which matched exactly with the type strain of *A. valerianellae*. The isolates from *Phalaenopsis* clustered in a dichotomous *A. cattleyae* species clade. The isolates from *Pelargonium* clustered with *A. konjaci*. The isolates from *Calathea* and *Marantha* formed a species-like clade and the isolate from frisée endive was assigned to an orphan cluster with *A. valerianella* as closest relative. The investigated genetic affiliations showed that the isolates from *Calathea* can be considered as a previously undescribed species, additional to the seven phytopathogenic *Acidovorax* species already defined.

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S5-P17

Isolation and identification of *Pantoea ananatis* as potential causal agents for fire blight-like disease in strawberry

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Recently, symptoms resembling fire blight such as brownish lesions, necrosis, and production of exudates were observed on a new cold-resistant strawberry variety (Ania®). Thus, the aim of this study was to isolate and identify the causative agents. Bacterial isolation was carried out from symptomatic tissues (leaves and stems) collected from four strawberry plants. Tissues were surface-sterilized and then homogenized in 0.85% (w/v) NaCl. Dilution plating was carried out using Nutrient Agar (NA) as growth medium. Representative bacterial isolates (120) were tested for the induction of the hypersensitive response (HR) in tobacco leaves according to [1]. Bacterial isolates able to induce HR were identified by 16S rDNA gene sequencing, an results revealed the presence of bacterial isolates belonging to *Pantoea ananatis*. To validate these results, further PCRs using the primer pair 61F/1009R specific for *P. ananatis* [2] were carried out. Results confirmed that the tested bacterial isolates belonged to *P. ananatis*. Pathogenicity tests were carried out by wounding detached ripe pseudo-fruits from strawberry plants (Elsanta®) and inoculating five µL of bacterial cell suspension (~1×10^8 CFU/mL) of the identified bacterial strains. Strawberries were incubated in the dark at 27°C and 90% RH. Appearance of symptoms was monitored daily and symptoms appeared after 5 days. Bacteria belonging to *P. ananatis* were re-isolated in purity according to the procedure reported above. To the best of our knowledge, this is the first report of *P. ananatis* on strawberry in Italy and shows that *P. ananatis* may represent a threat to strawberry cultivation in Italy.

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S5-P18

Ralstonia pseudosolanacearum (phylotype I) in waterways and bittersweet (*Solanum dulcamara*) in the Netherlands

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Ralstonia solanacearum (phylotype II), the causal agent of the brown rot bacterium of potato, is a major threat to the potato industry. As it has the potential to survive in surface water, annual surveys in waterways are performed to monitor the spread of *R. solanacearum* in the Netherlands. In autumn 2020, the outcome of a diagnostic investigation on water samples originating from surface water in two provinces in the Netherlands confirmed the presence of *R. pseudosolanacearum* (phylotype I). *R. pseudosolanacearum* (phylotype I and III) is one of the three described bacterial species inside the *Ralstonia solanacearum* species complex (RSSC), next to *Ralstonia solanacearum* (phylotype II) and *Ralstonia syzygii* (phylotype IV) [1]. Additional surface water samples taken in 2021 within a 5 km radius of the initial findings in 2020 confirmed the presence of *R. pseudosolanacearum* (phylotype I). Further, analysis of lower stem and root parts of bittersweet (*Solanum dulcamara*) collected from the same areas demonstrated the systemic presence of *R. pseudosolanacearum* in this plant. This suggests that *R. pseudosolanacearum* (phylotype I) overwinters in water or infected S. *dulcamara* in the Netherlands**.** Sampling and isolation of *Ralstonia pseudosolanacearum* was performed according to protocols described in the annexes of commission directive 2006/63/EC. Isolates were identified to species level based on matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)[2] and Sanger sequence analysis of the *egl* locus[3]. The results and implications of these finding will be presented and discussed during the conference.

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S5-P20
Fire Blight and *Erwinia amylovora* in Georgia

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The fire blight disease affecting pomaceous fruit trees appeared in Georgia in 2016. Since that it has spread to several other regions of the country during the years 2017-2018, where the most affected plant species in eastern Georgia was apple, while in western Georgia the disease was limited to few pear and quince trees [1]. A 99 samples of different organs of the disease suspicious plants have been collected in Kakheti Region and the main pomaceous fruit growing region Shida Kartli, Eastern Georgia in the years of 2020-2021. In a total of 42 *Erwinia amylovora* isolates were recovered from samples, where the pathogen was confirmed by PCR. Isolates displayed the typical biochemical and physiological properties of the species, differing from each other according to maltose, xylose, sorbitol and mannitol utilization abilities, as well as to esculin hydrolysis. Sequencing of the CRISPR regions of four *E. amylovora* isolates revealed the presence of two distinct genotypes (A, a, α) – i.e., identical to the earliest strains that colonized Europe and to the novel genotype (A, z, α), displaying the deletion of three spacers next to the leader-proximal region of CRR2 [2]. The CRISPR regions of 38 additional *E. amylovora* strains isolated in the years 2020-2021 were analyzed by PCR using primers pairs flanking known spacer deletions or duplications [3]. The vast majority – 81% of these isolates (Shida kartli) were shown to belong to the CRR2 genotype z, whereas few strains – 19% (Kakheti region) displayed the CRR2 genotype a.

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S6A-P2

Antimicrobial composites for management of bacterial plant diseases

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Pathogenic bacteria are among the most important causal agents of plant diseases with almost all major crops being severely affected by one or more important bacterial diseases. While a large diversity of fungicides are available for management of plant diseases caused by fungi and oomycetes, the chemical control options to cope with bacterial diseases in agriculture are limited. To date, chemical control of bacterial plant diseases in agriculture predominantly relies on copper (Cu)-based bactericides. These compounds, however, possess limited efficacy. Therefore, there is an urgent need to develop novel technologies to manage bacterial plant diseases and reduce food loss. In the present study, we report the development of a new antimicrobial agent, based on a doping method, which enables the entrapment of bioactive organic molecules within metals. Since Cu bactericides are the most commonly used compounds to manage bacterial plant diseases, Cu was selected to serve as the metal matrix. The food preservative, lauroyl arginate ethyl ester (ethyl lauroyl arginate; LAE) was chosen as the doped organic compound. We studied the effects of LAE-Cu composites on *Acidovorax citrulli*, the causal agent of bacterial fruit blotch of cucurbits, as a model species for plant-pathogenic bacteria. We found that the new composites had strong bactericidal activity against *A. citrulli* in both *in vitro* and *in planta* conditions. Moreover, the two components of the composites, LAE and Cu, interacted in a synergistic manner in terms of antimicrobial activity. Overall, our findings demonstrate the potential of a new family of bactericides as crop protection agents.

S6A-P3

The high potential of essential oil emulsions in restricting *Pseudomonas syringae* pv. *actinidiae* virulence

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Crop pathogen management is a priority for sustainable agriculture development. Indeed, the large use of pesticides is threatening both human and environmental health, while simultaneously pushing antimicrobial resistance occurrence. Although it is not trivial to develop solutions concurrently durable, effective and environmentally neutral, exploiting defence arsenal already present in natural ecosystems represents a promising approach to meet sustainability criteria. Essential oils (EOs) are naturally occurring compounds displaying a potent antimicrobial activity [1]. Indeed, they can have multiple targets among molecular and structural components of microbial cells. However, their high extraction cost, low stability in the environment and poor water solubility slow down their potential application in crop protection. Modern approaches like nanotechnologies can provide solutions to the previous issues and, at the same time, can outperform the antimicrobial activity of essential oils [2]. The nanoformulation of EOs improves their solubility in water, hence their availability, and simultaneously can prevent their degradation in a natural environment like open fields.
Here, we investigate i) the antimicrobial activity of 4 different EOs, single or combined, against *Pseudomonas syringae* pv. *actinidiae*, the causal agent of the kiwifruit bacterial canker, evaluating bacterial growth inhibition and biofilm formation prevention *in vitro*, and ii) their encapsulation using different formulations. Consistently, our purpose is the development of an innovative and environmentally safe alternative to traditional pesticides based on the nanoformulation of single and/or multiple EOs combining different antimicrobial activities to provide a durable efficacy of the product.

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S6A-P5

Paenibacillus dendritiformis and *Bacillus mycoides* as biocontrol agents against bacterial plant diseases

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The use of beneficial microorganisms as biocontrol agents is considered a sustainable alternative to the use of chemicals to promote growth of healthy crops. In this study, we examined the potential use of two bacterial strains, *Paenibacillus dendritiformis* T and *Bacillus mycoides* YH1, as biocontrol agents against bacterial fruit blotch disease of cucurbits caused by *Acidovorax citrulli*. *P. dendritiformis* T was chosen for this project because its genome is rich in genes and pathways required for competition over resources and for producing offensive as well as defensive compounds[1]. *B. mycoides* strain YH1 was chosen based on its ability to secrete enzymes that inactivate quorum sensing molecules[2]. Here we show that pretreatment of melon plants with the two strains, each one alone or together, efficiently reduces disease severity caused by *A. citrulli* M6 in leaves. We tested *in vitro* interactions between the biocontrol agents and two plant-pathogenic bacterial strains, *A. citrulli* M6 and *Pseudomonas syringae* pv. *tomato* (Pst) DC3000. *In vitro* assays indicated that neither *P. dendritiformis* T nor *B. mycoides* YH1 inhibited the growth of both pathogens. However, when glucose was added to the medium, *P. dendritiformis* T colonies exhibited specific directional swarming movement towards *A. citrulli* colonies while *B. mycoides* YH1 spreaded towards *A. citrulli* M6 and *Pst* DC3000 colonies. We are now aiming at improving our understanding of the mode of action of the two biocontrol agents with the overall goal being developing practical means for management of a wide range of bacterial diseases in agricultural crops.

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S6A-P6

Assessment of transcriptional reprogramming of lettuce in response to chitin soil amendment in relation to its effect on plant growth and disease resistance

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Chitin soil amendment is known to improve soil quality, plant growth and plant resilience. The first goal of this work was to study the effect of chitin soil amendment on the lettuce physiology. Secondly, we analysed the transcriptional reprogramming of lettuce roots in response to chitin treatment. Lettuce grown in chitin-amended soil had a higher flavonoid content two weeks after treatment. After four weeks of chitin treatment, lettuce exhibited a higher chlorophyll content, developed more leaves, and had a higher fresh weight at harvest. Over 300 lettuce genes were found to be significantly differentially expressed upon chitin soil treatment. Consistent with the phytiological changes observed, a GO-term enrichment analysis revealed statistical overrepresentation of GO terms linked to pigment metabolic processes, photosynthesis, and detoxification. Further analysis of the differentially expressed genes showed that the flavonoid pathway is mostly upregulated whereas the upstream phenylpropanoid pathway is mostly downregulated. Our data, in light to what is known in the litterature, suggest that the downregulation of the lignin biosynthetic pathway might lead to a change of flux to the flavonoid pathway – a hypothesis that we aim to test further. Since flavonoids play an important role in plant defense, our results are concurrent with available data describing higher resistance to diverse pathogens upon chitin soil amendment. Together, the work from this project will contribute to a better underdstanding of the modes-of-action of chitin soil amendment that could be used as a biostimulant for a more sustainable agriculture.

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S6A-P7

Target oriented functional peptides for control diseases caused by plant-pathogenic bacteria

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Antimicrobial peptides have been identified as potential candidates for plant disease control, and have a strong potential to manage diseases caused by plant pathogenic bacteria, fungi and phytoplasms. We have developed synthetic peptides, either as analogues from natural compounds or completely *de novo* synthetized, with minimal length and optimized for activity, toxicity and biodegradability. A library of linear undecapeptides (CECMEL11) derived from sequences of cecropin and melittin have shown strong activity, both *in vitro* and *in planta*, at low micromolar concentrations. Specifically, derivatives of BP100 successful control fungal and bacterial plant pathogens in apple, pear, *Prunus,* tomato, and pepper. *Xylella fastidiosa* is one of the most harmful bacterial plant pathogen, causing a variety of diseases limiting fruit crop productivity worldwide, and emerging diseases in Europe. Although *X. fastidiosa* has been extensively studied, there are no methods to supress disease in infected plants due to the lack of effective bactericides and to the difficulty to access to the xylem vessels, where the pathogen establishes. The kinetics of cell inactivation and the susceptibility of *X. fastidiosa* subspecies *fastidiosa*, *multiplex* and *pauca* to antimicrobial peptide derivatives belonging to CECMEL11 and CYCLO10 libraries have been studied. BP178, a 29 amino-acid peptide derived from BP100 was the most bactericidal peptide at micromolar concentrations, resulting in rapid pore formation in cell membranes, abundant production of outer membrane vesicles and cell lysis. Interestingly, there was a strong differential susceptibility to the peptides among strains, STs and subspecies of *X. fastidiosa*. To set-up a plant pathosystem platform for testing the peptides, we first studied the aggressiveness of strains in different almond cultivars, under greenhouse conditions. We confirmed that the growth rate observed in young plants and the parameters such as maximum pathogen levels, median and minimal effective dose varied depending on the strain. Finally, using the most adequate strain and cultivar we assessed the efficacy of the bifunctional peptide BP178 by endotherapy treatment. The treatment was efficient in controlling infections and induced defence responses on the plant.

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S6A-P10

Exploring amino acids for controlling bacterial blight on cabbage

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Bacterial blight disease of Brassicaceae caused by *Pseudomonas cannabina* pv. *alisalensis* (*Pcal*) is an economically important disease worldwide [1]. Management of bacterial blight based on copper fungicides and antibiotics resulted in the occurrence of a *Pcal* resistant strain. Therefore, there is an increasing demand for new sustainable disease control strategies. Previous studies demonstrated that several amino acids protect plants from pathogens [2;3]. We here focused on amino acids protective effects for bacterial blight on cabbage. To investigate the effect of proteinogenic amino acids on disease resistance, we treated cabbage with amino aicds, and then spray-inoculated with *Pcal* (5×10^7 CFU/ml) 0 hour and 24 hours after treatment. As a result, disease development and bacterial multiplications were suppressed by several amino acids, including glutamate and cystein. We also conducted syringe infiltration with *Pcal* (5×10^5 CFU/ml) in cabbage. Bacterial populations showed no significant differences after any amino acids treatments we tested. Therefore, we assumed that amino acids, by which showed reduced bacterial populations, have a protective effect before *Pcal* entering plants. To investigate the protective mechanisms, we conducted stomatal assay and, gene expression analysis of plant defense-related genes and *Pcal* virulecne-related genes. In this presentations, we present the latest findings on these analyses.

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S6A-P11

Antibacterial properties of nanocrystals based on chitosan and Fosetyl-Al against *Xylella fastidiosa*

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The olive quick decline syndrome (OQDS) is a serious phytosanitary emergency that is compromising Apulian olive groves. Currently, one of the greatest difficulties is the containment of the spread of the associated bacterium *Xylella fastidiosa* (Xf) for which, research is targeting from the individuation of resistant varieties to the control of the insect vectors. The control of Xf, given its endophytic nature, is affected by the inability of some agrochemicals to reach the xylematic vessels. Natural and synthetic products for the control of bacterial and fungal diseases include chitosan and Fosetyl-Al. These substances migrate systemically and/or stimulate the plant defense, important aspects for the potential control of Xf. In this study the potential antibacterial activity of chitosan-based fosetyl-Al nanocrystals (CH-nanoFos) was compared with naked Fosetyl-Al nanocrystals (nano-Fos) and Fosetyl-Al. All compounds were tested *in vitro* for the ability to control the planctonic and the biofilm growth of Xf, and *in vivo* for their ability to reduce the bacterial concentration in artifically inoculated plants of *Nicotiana tabacum* (Petite Havana SR1) assessed by real-time PCR. The *in vitro* results showed a capacity of CH-nanoFos to both reduce the planctonic and the biofilm growth of Xf with respect Fosetyl-Al and nano-Fos. The *in vivo* experiments on tobacco plants inoculated with three different Xf subspecies (Xf *pauca*, *fastidiosa* and *multiplex*) showed a statistically significative bacterial growth reduction and a partial simptoms regression. On the other hand no effect was observed on tobacco plants inoculated with XF pauca and treated with Fos, nano-Fos and nano-chitosan. The bacteriostatic efficacy of CH-nanoFos could depend on the synergy of Fosetyl molecule and chitosan shell.

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S6-P13

Are the waterways important paths of spreading plant pathogenic bacteria from the genera *Dickeya* and *Pectobacterium*?

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Pectinolytic bacteria are causative agents of blackleg and soft rot diseases of potato and other crops and vegetables. Thus far, *Pectobacterium* and *Dickeya* spp. have been isolated from distinct environments such as rotten or asymptomatic plants, soil, and waterways. Due to the ongoing climate change, it is anticipated that the number of fields subjected to irrigation will constantly rise. The aim of this project was to monitor the presence of *Dickeya* and *Pectobacterium* spp. at different depths in the Pomeranian lakes. Water samples were collected at every 5 m depth from 9 lakes in Northern Poland by a qualified scuba-diver. Samples of 100 µl of each collected suspension was plated on Crystal Violet Pectate medium and incubated at 28°C for 48h. With the use of multiplex PCR and/or species-specific PCR we identified 15 strains of pectinolytic bacteria from 2 lakes. One of them was classified to *Pectobacterium aquaticum* IFB5637 (Paq), notably being the first reported Paq strain in the Polish waterways. Characterisation of the isolated Paq strain was based on phenotypic features and phylogenetic analysis. In addition the analytical profile index (API 20E) was determinate. Paq IFB5637 possesses a smooth form of a lipopolysaccharide with O-polysaccharide consisting of mannose, glucose, and abequose. It this study, *Pectobacterium* and *Dickeya* spp. isolates were solely isolated from 0 m nearby the shore. Therefore, the usage of irrigation water originating from depths instead of the surface water, might contribute to the limitation of spread of the soft rot *Pectobacteriaceae* in the natural environment.

S6-P14

Evaluation of copper resistance of *Pseudomonas syringae* pv. *actinidiae* populations in Italy

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Bacterial canker of kiwifruit still represents one of the biggest threat in kiwifruit cultivation all around the world. This vascular disease is caused by the bacterium *Pseudomonas syringae* pv. *actinidiae* (PSA), for which no effective chemical control methods are reported, except for copper. Cupric salts are able to prevent and diminish the risks of infections and reinfections if applied with the correct dose and timing. Due to the emergence of copper resistance in PSA populations in different kiwifruit countries and the economic importance of kiwifruit cultivation in Italian agro-food chain, the aim of this work was to perform a wide kiwifruit sampling to detect the possible presence of copper resistant PSA strains in the most important Italian kiwifruit districts[1]. A total amount of 58 strains were collected from symptomatic and asymptomatic kiwi plants in 2018-2019; 27 strains (ten strains came from the Latium region, six from Emilia Romagna, one from Piedmont, one from Lombardia and nine from Campania) were selected and submittted to known molecular techniques to confirm to be PSA. To evaluate possible copper resistance among the collected PSA strains a known *in vitro* test was developed[2]. No copper resistant PSA strains have been recorded. For italian farmers the results highlight the possibility to continue to use cupric salts for an effective strategy to contrast PSA.

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S6-P15

New tools for the biological control of *Xanthomonas fragariae* in orchard

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The angular leaf spot (ALS) disease caused by *Xanthomonas fragariae* (Xf) in strawberry is present in all growing areas seriously damaging the fruit quality. The Xf control mainly relies on preventive treatments with chemicals, such as copper-based products. Biological agents could represent an effective alternative within integrated control strategies. The antibacterial activity of two essential oil-based products (OEBPs) - VitiBioSap Plus® 1R45 and VitiBioSap Plus® 458 - was evaluated in orchard, by treatment with OEBPs against ALS, comparing it to the standard chemical treatments (control, SCT). In an orchard severely contaminated by Xf, in Emilia Romagna region (North-Central Italy), from August to November 2019, the OEBPs- or SCT-treatments were performed 13 times on plants belonging to five different cultivars (Asia, Malga, Roxana, Olympia and Tea). From October to November, three phytopathometric assessments were carried out to determine the leaf spot number to evaluate the disease severity (DS) using disease classes. At the first assessment, the DS resulted low in all OEBPs-treated cultivars. In particular, on cv. Asia, the OEBPs-treated plants showed a reduced DS compared to those SCT-treated, whereas on cvs. Malga and Roxana, the DS was higher with respect to that detected on SCT-treated plants. At the second and third assessments, the DS greatly increased in the orchard, but the OEBPs-treated plants of cvs. Asia, Malga, Roxana and Olympia showed a significantly reduced ALS severity compared to that of SCT-treated plants. During all the assessments, on cv. Tea the DS was low compared to the other cvs. regardless of treatment.

S6-P16

Screening of microorganisms for antagonistic activity against pathogenic bacteria *Xanthomonas* spp.

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Plant pathogenic bacteria belonging to the genus *Xanthomonas* are spreading rapidly around the world and causing significant crop losses, leading to intensified research in this area worldwide. However, information about these pathogens and their control methods is still lacking in Lithuania. The aim of this study was to identify the endophytic bacteria with the highest antagonistic activity against phytopathogenic *Xanthomonas*. During 2017-2018, plant material of various *Fabaceae* species growing in Lithuania was collected. In this study, 295 bacterial isolates were obtained and investigated, where endophytic bacteria were isolated from legume roots and nodules. All strains were tested by Gram staining and hypersensitive reaction (HR) on tobacco and tomato plants, also the ability to cause rot on potato and pectolytic activity was investigated[1]. All bacteria used in this study were identified by sequenced 16S rDNA (accession numbers in GenBank: OL505113-OL505122). Yellowish *Xanthomonas*-like bacteria were selected for further molecular studies: screened by PCR using genus-specific primers X1 and X2, genetic diversity and phylogenetic analysis of *Xanthomonas* were performed using PCR melting Profile (PCR MP), repetitive PCR (rep-PCR), multilocus sequence analysis (MLSA)[2] and type three effector (T3E)[3] genes identification. The antimicrobial activity of endophytic bacteria was determined by agar diffusion method. Thus, 10 out of 73 endophytic isolates belonging to *Bacillus*, *Paenibacillus*, and *Pseudomonas* genera were selected as the most effective isolates against pathogenic *Xanthomonas* strains.

This work was based on a collaboration established by the COST Action CA16107 "EuroXanth: Integrating science on Xanthomonadaceae for integrated plant disease management in Europe", supported by European Cooperation in Science and Technology (COST).

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S6-P17

Synthesis and application of artificial lipid nanoparticles to protect plants from bacterial infection

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Despite the numerous examples of nanotechnology solutions in life science, the potential benefit of its application in agriculture have received less attention. In this study, we explore the use of synthetic lipid nanoparticles (LNPs) as delivery vehicle of biomolecules to induce disease resistance in plants. LNPs with different lipid composition and fluorescently labelled with Cy3-siRNA (small interfering RNA) were synthesized and evaluated for their performance in terms of uptake, distribution and persistent in plants. Plant uptake was more effective when LNPs were applied through the root system compared to drop placement on leaves or leaf infiltration. Once LNPs reached the root surface, they are transported to the stem and upper leaves preferentially *via* the vascular system. LNPs were detectable in the plant tissue at least 25 days post-application. To examine whether synthetic nanoparticles can activate the plant immune response, we rationally designed LNPs coated with a known plant immune elicitor, flg22 (LNPs-flg), and used them to treat *Arabidopsis* plants. LNPs-flg induced a robust upregulation of immune gene markers in seedlings and elicited a reactive oxygen species burst in disc leaves. We further demonstrated that plants pretreated with LNPs-flg promote a significant reduction in bacterial growth in infected leaves and this priming effect was evident up to a week after LNPs application. Finally, we showed that LNPs treatment induce systemic immunization in distal leaves. In summary, results show that our immunogenic-designed LNPs represent a promising alternative to mitigate plant diseases.

S6-P18

Antibacterial activity of new bioactive compounds against *Xylella fastidiosa* subsp. *pauca*

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Following the first discover of an outbreak of *Xylella fastidiosa subsp. pauca* (*Xfp*) on olive trees (*Olea europaea*) in the Salento Peninsula (southern Italy) [1], a rapid spread of the pathogen has been observed, determining the current severe epidemic. Quarantine and control measures contributed to reduce the initial high rate of spread of the infections, however the lack of effective therapeutic tools to cure infected plants represents a major constrain to cope with the bacterium in area where management strategies to cope with the bacterium are needed to mitigate the impact of the infections. In this scenario, the interest on the development of new active compounds for controlling phytopathogenic bacteria is a big challenge toward the current trends and the new directions in crop protection. In this work new sustainable bioactive substances were selected and evaluated *in vitro* and *in vivo* for their antimicrobial activity against the *Xfp* isolates responsible for the Olive Quick Decline Syndrome (OQDS) in Apulia. Results attested the large antimicrobial spectrum of some of the tested compounds and their bactericidal activity against *Xfp in vitro*. Furthermore, *in planta* experiments in green-house conditions were carried out to evaluate the capability of the selected compounds to control the disease caused by *Xfp* on potted olive plants grown under controlled conditions. The results showed that some foliar treatments significantly reduced, in the short period, *Xfp* symptoms on treated plants and at the end of the experiments, some treatments also induced a significant reduction of the bacterial population. Further investigations are in progress to optimize activity an use of the most promising products under field conditions.

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OLIDIXIIT, "Olivicoltura e difesa da Xylella fastidiosa e da insetti vettori in italia" – Mipaaf - DM n.23773/2017;
XF-ACTORS "Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy" H2020 GA 727987.

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S6-P19

Evaluating the antimicrobial activity of natural organic products for their potential use against *Xylella fastidiosa*

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Xylella fastidiosa (*Xf*), including various subspecies, is one of the 20 regulated quarantine pests in Europe, regarded as one of the "priority pest" according to the new plant health regulation. Because several outbreaks were discovered in the last decade in southern Europe, relevant research programs are ongoing to tackle, in particular, the severe epidemics occuring in southern Italy, where *X. fastidiosa* subsp. *pauca* (*Xfp)* causes a devastating disease on olives. So far, due to the unavailability of effective agrochemicals to cure infected plants, prevention is the only measure to limit the spread and impact of the pathogen. Therefore, an intensive screening program is underway to find new environmentally-safe products against *Xf*. In this scenario, we are evaluating new natural compounds against *Xf* subspecies and strains. Results of *in vitro* experiments showed a dose-dependent and broad-spectrum antibacterial activity of some tested products. Field testing with most promising products on naturally-infected *Xfp* olive trees significantly reduced plant desiccation and disease severity. To further study the activity of the most effective products, clarify their mechanisms of action and optimize their application, experiments are ongoing under controlled condition on potted olive plants. Treated and untreated plants were inoculated by grafting infected scions from susceptbile and resistant olive cultivars. Periodic diagnostic tests are performed to monitor bacterial colonization within the host-plant. The results are discussed in relation to the possible mechanisms of action of the tested products and their potential use for a sustainable management of *Xf*.

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BIOLIX, "Valutazione di strategie di difesa biologica su olivo per limitare la diffusione di Xylella fastidiosa pauca e del suo vettore Philaenus spumarius" – Puglia Region, Proj. Cod. C - CUP B36J16002340009;
OLIDIXIIT, "Olivicoltura e difesa da Xylella fastidiosa e da insetti vettori in italia" – Mipaaf - DM n.23773/2017;
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S6-P20

Prunus persica* plant endogenous peptides PpPep1 and PpPep2 cause PTI-like transcriptome reprogramming in peach and enhance resistance to *Xanthomonas arboricola* pv. *pruni

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Rosaceae species are economically highly relevant crops. Their cultivation systems are constrained by phytopathogens causing severe losses. Plants respond to invading pathogens through signaling mechanisms, a component of which are plant elicitor peptides (Peps). Exogenous application of Peps from the same species activates defense mechanisms and reduces the symptoms of pathogen infection in a few model pathosystems [1,2]. Here we demonstrate the effectiveness of *Prunus persica* peptides PpPep1 and PpPep2 in protecting peach plants from the bacterial pathogen *Xanthomonas arboricola* pv. *pruni* (Xap), *in vivo.* They are active upon topical application of nanomolar doses, conferring 40% reduction of the symptoms following Xap massive infection. We used deep sequencing to characterize the transcriptomic response of peach plants to preventive treatment with PpPep1 and PpPep2. The two peptides induced highly similar massive transcriptomic reprogramming in the plant. One hour, 1 day and 2 days after peptide application there were changes in the expression of 8% peach genes. The transcriptomics dynamics was visualized in a custom background knowledge network, which made comparison of our data and previously published immune response datasets possible. *P. persica* Peps mode of action is induction of the PTI natural response. Thus, *P. persica* Peps are good candidates for deployment of natural, targeted and environmental friendly strategies to enhance resistance in *Prunus* species and prevent important biotic diseases.

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S6-P21

In vitro antibacterial activity of a novel naotechnology-based green agrochemical to control three _Pseudomonas_ spp. bacterial plant pathogens

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Pseudomonas syringae pv. *actinidiae* (Psa), *P. syringae* pv. *tomato* (Pst) and *P. savastanoi* pv. *savastanoi* (Psav), are the causal agents of bacterial canker of kiwifruit, tomato bacterial speck, and olive knot, and are managed by the preventive application of cupric salts. Such compounds accumulate in soil and water while favouring the selection of resistant strains. Thus, there is an urgent need to find efficient and bio-based solutions to mitigate bacterial diseases. Nanotechnology-based agrochemicals are promising, since cellulose nano-crystals (CNCs) can be obtained by plant wastes and employed as green nano-carriers to deliver active molecules. High-amylose starch (HAS) consisting of a high content of amylose, can act as excipient by increasing the solubility of active compounds. We extracted CNCs and HAS from wastes of the bread wheat cultivar Cadenza high-amylose while chitosan and gallic acid were assayed as active molecules. *In vitro* experiments individuated the optimal active concentration in order to formulate a bio-based composite of CNCs, HAS, chitosan (25% w/w), and gallic acid (2.5% w/w). The bio-based composite was tested *in vitro* at 2% against Psa, Pst and Psav. Antibacterial assays demonstrated that the composite inhibited the cell multiplication from 50% to 80%. In-broth assays demonstrated that the composite drastically reduced the ability of the *Pseudomonas* spp. to produce biofilms and its adheration to plastic surfaces. Such experiments demonstrated that the composite acts directly as antibacterial, but also it physically interacts with bacterial cells to inhibit the biofilm formation as much as the adhesion to surfaces.

S6-P22

Biocontrol of black rot on autochthonous cabbage cultivar 'Futoški'

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The potential of two biocontrol strains, *Bacillus velezensis* X5-2 and *Pseudomonas orientalis* X2-1P to control black rot caused by *Xanthomonas campestris* pv. *campestris* was evaluated *in vivo* on autochthonous cabbage cultivar 'Futoški', under the condition of natural infection. Liquid formulations of biocontrol strains were prepared in fermenters (*B. velezensis* - 10^{10} CFU mL^{-1}, *P. orientalis* - 10^{9} CFU mL^{-1}) and diluted with water in a 1:5 ratio, before use. Treatments were performed with each strain and their mixture (1:1). The experiment included the following: (i) seed treatments (15, 30, 60 min), (ii) foliar treatments (three treatments: when the first symptoms appeared and remaining every two weeks), and (iii) combined seed and foliar treatments. Experiments were conducted in four replicates, with 10 plants per replicate. Two controls were used, untreated and conventionally treated cabbage (pesticides). Assessment of disease intensity was rated one month after the last foliar treatment, using the Horsfall-Barratt scale (1-12). During ripening, cabbage heads' weights were measured and data were statistically processed (Minitab). All performed treatments were effective in the control of black rot (85.7-98.9%). The highest efficacy was obtained in combined seed (60 min) and foliar treatments with *P. orientalis*, as well as its mixture with *B. velezensis* (98.9% both). All treatments influenced the increase of cabbage heads' weight (up to 1.3 times), with the highest found in combined treatments (60 min seed + foliar) using a mixture of *B. velezensis* and *P. orientalis*. A negative correlation ($P \leq 0.05$) was found between disease intensity and cabbage heads' weight.

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S6-P23

Enzymatic disruption of *Erwinia amylovora* biofilms to reduce pathogenicity

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Fire blight, caused by the plant pathogen, *Erwinia amylovora*, is of outmost agricultural and thus economic importance. This disease affects a wide range of plant species, but it is most destructive to the plants of family Rosaceae, especially the commercial production of apples and pears. The production of exopolysaccharides, amylovoran and levan by *E. amylovora*, is necessary for biofilm formation, which contributes considerably to pathogenesis and xylem vessel colonization. Studies have demonstrated that a lack of both exopolysaccharides results not only in delayed development of symptoms, but also in reduced or no virulence to susceptible plants[1]. Currently, there are no known treatments capable of complete eradication, once infection has established. Better understanding of pathogen biology and the collective of pathogenicity factors is crucial for developing novel and sustainable strategies that offer sufficient disease control. The aim of this project is to investigate the ability of specific carbohydrate active enzymes to degrade plant pathogenic biofilms produced by *E. amylovora* and so decrease its ability to cause disease. Successful enzymatic degradation of purified levan and levan portion of the established *E. amylovora* biofilms was achieved by GH32-CBM66 from *Bacillus subtilis* and BT1760 from *Bacteroides thetaiotaomicron*, β-(2, 6) specific levanases with an exo- and endo-activity, respectively[2, 3]. Furthermore, the ability of EPS depolymerase from *E. amylovora* bacteriophage ø-Ea1h, an endoglycosidase, to break down both, purified amylovoran and its component of the biofilm matrix was also demonstrated. Further experiments to explore the capabilities of these enzymes to disrupt *E. amylovora* biofilms in planta are pending.

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S6-P24

The endophytic fitness of five *Salmonella* serovars in butterhead lettuce with chitin soil amendment

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The human pathogen *Salmonella enterica* is one of the major causes of outbreaks related to the consumption of fresh produce. Previous studies revealed that *Salmonella* serovars differ in their endophytic fitness in certain plants [1], and some serovars could cause chlorosis and wilting in *Arabidopsis* [2]. Morover, chitin soil amendment has potential for suppressing *Salmonella* in the lettuce phyllosphere [3]. However, the endophytic fitness of different *Salmonella* serovars as well as, whether chitin could suppress their endophytic colonization in lettuce, and the mechanisme behind it, are largely unknown. Here, we tested the endophytic survival of five *Salmonella* serovars (*S*. Enteritidis ATCC BAA 1045, *S*. Montevideo ATCC BAA 710, *S*. Senftenberg ATCC 43845, *S*. Thompson RM 1987N and *S*. Typhimurium SL 1344) in butterhead lettuce *Lactuca sativa* L. var. *capitata* "Alexandria" with or without chitin soil amendment. Lettuce were grown in potting soil with or without chitin for 4 days, subsequently, leaves were infiltrated with *Salmonella* inoculum. Fourteen days after inoculation, the population of *S.* Enteritidis, *S.* Senftenberg and *S.* Typhimurium all decreased significantly compared to the initial inoculum, while the population of *S*. Montevideo and *S*. Thompson remained more or less at the same level. The population of *S*. Enteritidis in chitin-treated plants started decreasing 1 day after inoculation, and was significantly lower than that in non-chitin treated plants 7 days after inoculation. Our preliminary results suggest that *S*. Montevideo and *S.* Thompson have better endophytic fitness in the tested lettuce cultivar; *S*. Enteritidis is more sensitive to chitin treatment.

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S6-P25

Criticity on the molecular detection of *Xanthomans citri* pv. *citri* and *Xanthomans citri* pv. *aurantifolii*: outputs from the EURL Experience

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EU reference laboratory for pests of plants on bacteria [1] aims to assess the proficiency of the EU national reference laboratories (NRLs) and to provide advice on the available diagnostics. *Xanthomonas citri* pv. *citri* (Xcc) and *Xanthomonas citri* pv. *aurantifolii* (Xca), are not known to occur in the Union territory. An intralaboratory study was performed to give guidelines for NRLs on the best DNA extraction methods (DEM) to be used on different citrus matrices (lemon fruit/leaves, orange/lime fruit). The analysis compared the standard curves of spiked samples of DNA extracts (1ng/ul to 10 fg/ul) and bacterial culture (10^7-10 cfu/mL). The results showed that at lower target concentration the DEM can affect the reliability of the detection tests; QuickPicK and DNeasy-Plant-Mini Kit showed the most reliable results. Late Cq values occurred by real-time PCR on healthy matrices, particularly for samples extracted by CTAB and by DNeasy-Mericon-Food Kit. A proficiency test (PT) was organized to assess the proficiency of 25 NLRs. Twelve test-items were prepared by mixing DNA of plant matrices with DNA of Xcc (A, A*, A^W) and Xca (B and C). The participants reported 1351 results of which 98,7% were conforming, 1% false-negative and 0,3% false-positive. Xcc was detected with overall conformity of 100% whereas Xca showed a detection conformity of 90-92% for pathotype C and B respectively. Overall results highlight that the criticalities are mainly related to the diagnosis of Xca, due to the lack of specific tests targeting this pathovar and consequent difficulties in the results interpretation.

The EU Reference Laboratory for pests of plants on bacteria is a consortium led by the the Netherlands Food and Consumer Product Safety Authority, National Reference Centre Plant Health (NVWA-NRC, The Netherlands) and is also composed of the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO, Belgium), the National Institute of Biology (NIB, Slovenia) and the Research Centre for Plant Protection and Certification (CREA-DC, Italy), funded by the European Commission (grant S12. 809508).

S6-P26

New approaches to citrus canker management using nanotechnology

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In recent years, challenges for Brazilian agriculture have increased. Climate change, high costs fertilizers and diseases are problems that significantly impact agricultural production. Among the diseases bacterioses are phytosanitary problems without efficient products for control. While new fungicides, acaricides and insecticides are continually being launched, the control of bacterial diseases still lacks continuous innovation. With the use of antibiotics banned in the field for safety reasons, the producer is forced to use one of the only tools available for them: copper compounds. Used since its discovery in the 19th century, it has some effect, but not without causing damage such as phytotoxicity and accumulation in soils or groundwater. Recently, an alternative from Brazilian research came to change this scenario: the use of the molecule N-acetylcysteine (NAC) proved to be useful in the control of many bacterial diseases such as HLB, CVC and citrus canker, with no known adverse effects on plants or the environment. We tried to push forward this achievement, adding NAC to an especially formulated nanoemulsion, composed of biodegradable and safe components for both plants and its surrounding environment. It contained NAC, zinc and/or copper, in different amounts and reservoirs in the nanoemulsion. We tested 14 different compositions, in early-stage seedlings and found that two of these nanoemulsions could reduce the appearance of symptoms up to 80% compared do the control treatment, which contained no active ingredients in the nanoemulsion. Besides, this symptom reduction is comparable to other control treatment, using NAC at higher concentrations without the nanoemulsion. This shows the potential of cost-reduction and efficiency nanotechnology can provide, and the potential of its effective use in the field.

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S6-P27

Peptides with multifunctional activities to protect plants against *Xylella fastidiosa* infections

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Xylella fastidiosa is one of the most harmful bacterial plant pathogens worldwide, causing a variety of diseases in different host plants such as grapevine (*Vitis vinifera*), olive (*Olea europaea)* and almond (*Prunus dulcis*), and resulting in a huge economic impact on agriculture and the environment [1]. *X. fastidiosa* invades the host xylem, multiplies and forms biofilm occluding the vessels and killing its host. Despite great research efforts, there is no method to effectively prevent or cure hosts from infections [2]. Peptides can be considered promising candidates against *X. fastidiosa* due to their broad spectrum of activity and low environmental impact [3]. They can affect the pathogen directly (e.g. bactericidal activity) or indirectly by acting as plant defense elicitors which may induce a priming state on treated plants. Priming allows a faster and stronger defense response upon infection which could prevent the development of diseases. In this work, 20 peptides, previously reported in the literature to be active against other pathogens, and 11 newly designed analogues were synthesized and tested for their bactericidal and antibiofilm activity against *X. fastidiosa* subsp. *fastidiosa* IVIA 5387.2. Moreover, their hemolytic activity and effect on tobacco leaves were determined. This study allowed the identification of peptides, such as **1036** and **RIJK2**, with both *in vitro* bactericidal and antibiofilm activities against *X. fastidiosa* [4]. Additionally, a screening platform to identify defense elicitor peptides was developed on *P. dulcis* using RNA-seq and RT-qPCR technologies and the defense elicitor peptide **flg22.** This platform was employed to test different peptides.

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S6-P28

Identification and characterization of tomato bacterial endophytes and preliminary evaluation for consortia-based biocontrol products

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The design of microbial consortia for improving the reliability and versatility of current biological control practices is a major trend in biotechnology [1]. A collection of 94 bacterial isolates obtained from the endosphere of tomato seeds and roots of plants grown on different substrates (soil, peat, coconut fiber) were characterised. Isolates based on 16S rRNA gene sequencing belonged to genera of seven orders: the Gram-positive Bacillales, and Micrococcales, and the Gram-negative Pseudomonadales, Enterobacteriales, Flavobacteriales, Burkholderiales, and Xanthomonadales. A high percentage of strains showed PGP-related traits (ability to grow on NaCl, siderophores, aminocyclopropane-1-carboxylate deaminase production, ability to solubilize phosphate). In addition, approximately the 32% of the isolates showed antagonistic activity against tomato phytopathogenic bacteria *Clavibacter michiganesis* pv. *michiganensis*, *Pseudomonas syringae* pv. *tomato*, and *Xanthomonas euvesicatoria* pv. *perforans* when tested in vitro according to Anzalone *et al.* [2]. Based on previously collected metagenomics data, we selected isolates that were either highly (mainly *Pseudomonas* and *Bacillus*) or lowly represented in taxa, to evaluate their application in bacterial consortia. *In vitro* tests highlighted incompatibility groups between bacterial isolates. For further studies three bacterial consortia with 4 to 10 isolates were assembled and tested for synergistic antagonistic action. Preliminary results obtained using consortia-bacterised seeds showed that plants treated with the bacterial consortia had a higher average root length and a more abundant production of root hairs as compared to untreated plants. In addition, all consortia were successful in reducing the incidence and severity of bacterial spot disease.

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S6-P29

Highly efficacy of silver ultra nanoclusters against phytopathogenic bacteria

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The effective management of plant bacterial diseases is extermely difficult for the few and often partially efficacy of control measures available. Copper compounds are the most commonly used mean for controlling these diseases. However, the frequent occurrence of copper resistant bacterial strains in copper-treated crops and the restricted use of copper compounds imposed by European Union for the enviromental and public health problems renders inadeguate this control mean. The use of nanoparticles is an emerging and promising measure for controlling plant bacterial disease as an alternative to copper compounds. Electrochemically synthesized Silver Ultra Nano Clusters (Argirium-SUNc®) with strong antimicrobial activity (ED_{50} < 1 ppm) against mammalian pathogenic bacteria have been recently reported [1,2,3,4]. We here demonstrated *in vitro* and *in vivo* efficacy of Argirium-SUNc against a number of Gram-negative and Gram-positive phytopathogenic bacteria. *In vitro* analyses demonstrated that ED_{50} of Argirium-SUNc for *Pseudomonas syringae* pv. tomato, *Xanthomonas vesicatoria*, *Xylella fastidiosa* subsp. *pauca* and *Clavibacter michiganensis* were 0.33, 0.12, 0.05 and 0.3 ppm, respectively. In addition, Argirium-SUNc is able to reduce biofilm formation and to provoke biofilm eradication. Tomato plants treated with Argirium SUNc (root uptake) at a nonphytotoxic concentration of 10 ppm were significantly protected from *P. syringae* pv. tomato attacks. Through a trascriptomic approach we are verifying the toxicity mechanisms of Argirium-SUNc in *P. syringae* pv. tomato.

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S6-P31

A novel Zinc Borate nanoformulation for crop protection againts foliar pathogens

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Exhausted soils and pathogens are the common factors leading growers towards the use of multiple agrochemicals to increase yield and crop protection. Continuous use of these chemicals can lead to salt build up in the soil, fertilizer run off and pathogen resistance. Therefore, to minimize these negative effects, it is necessary to develop multifunctional formulations capable of controlling plant disease while at the same time satisfaying the plant nutrational needs. Recently, nanotechnology has been proposed as a way to formulate the new generation of agrochemicals. In this work, a novel zinc borate nanomaterial (Bz) was developed to target specific parts of the leaf and protect tomato plants against foliar pathogens, while also providing valuable micronutrients. This nanomaterial was synthesized from EPA approved chemical by the coprecipitation method. In colloidal state, the nanomaterial has an average diameter of 9.8 nm, but as it dries the particle size increases to 200 – 400 nm. Bz demonstrated great affinity for colloidal cellulose, which enabled it to preferentially deposited on areas commonly colonized by bacteria[1]. Moreover, the nanoformulation ameliorated zinc phytotoxicity without affecting its antimicrobial properties on Pseudomonas syringae and two other different strains of Xanthomonas perforans. These findings show the multifunctionality of nano zinc borate and how it can be utilized as a targeted pesticide for protection against tomato foliar pathogens.

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S6-P32

Effective citrus canker control using the biofilm-disruptive antioxidant NAC

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Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (XCC), is one of the most destructive diseases that occur in orange orchards worldwide. It affects all citrus varieties, and no cure is known so far. Regular management approaches rely on the elimination of infected trees and the application of metallic compounds to prevent bacterial infection. Regular and continuous application of these products are causing side-effects, such as accumulation in soils, and even phytotoxicity to trees, in some cases. New approaches to control this bacterial infection, that are less harmful to both plants and environment are needed. Although new molecules are continuously being discovered to fight bacterial diseases, effective field trials are scarce. Here, we present a case of successful technology transfer from academia to productive sector, with the use of the molecule N-acetylcysteine (NAC). This compound breaks the biofilm cells, limiting XCC entry into the plant mesophyll and preventing disease. We prepared a formulation (called "ÍKONE") containing NAC as its main active ingredient and applied in a productive orange farm in Sao Paulo state (Brazil). Its application was intercalated with the regular copper compounds used, and compared with the control, where only cupric compound was applied. We observed an increase of 20% in total production (Kg/tree) and 36% increase in the number of fruits in the parcels that received the ÍKONE/copper treatment. These results were obtained after a 3-year experiment and were cost-effective for the grower.

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S6-P33

Defense response induced by essential oils in tomato plants against *Xanthomonas vesicatoria*

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The control of *Xanthomonas vesicatoria* (Xv), one of the aetiological agents of tomato bacterial leafspot, is limited to copper compounds, few resistance inducers and biocontrol agents (BCA). Moreover, the main prophylactic strategy still consists of diagnostic analyses of seeds. Among BCA, natural compounds as essential oils (EO) and plant extracts were studied to find out eco-friendly tools to counteract phytopatogenic bacteria. An essential oil from *Origanum compactum* (OR; 48.13% carvacrol) and an hydrolate (HyAA; 47.7% linalool) obtained from EO of *Citrus aurantium* var. amara were assayed for their ability to induce resistance against Xv. The root apparatuses of *Solanum lycopersicum* cv. VF10 plants were treated with OR (0.03%) or HyAA (4.5%) by 10 minutes soaking and, after 48h, were inoculated with the Xv strain DISTAL 2684 (approx. 10^7 CFU/ml) by leaf spraying. Acibenzolar-S-methyl (75 ppm) and sterile distilled water (SDW) were used as controls. Neither the treatment with OR nor that with HyAA were phytotoxic.The disease severity (DS) was evaluated by counting the number of leaf spots 14 days after Xv inoculation. In the plants treated with both OR and HyAA (approx. 57 spots/leaf), the DS resulted lower compared to the SDW-treated control samples (approx. 98 spots/leaf). It is likely that the root treatment with the OR or HyAA might have stimulated the host defense responses against Xv since a relative protection of about 42% was detected. Further transcriptomic analyses will be undertaken to support the hypothesis of the activation of the defense responses of the plant.

S6-P34

Screening *in vitro* of eco-friendly control tools against *Xylella fastidiosa* subspecies

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The control of *Xylella fastidiosa* (Xf), causing Olive Quick Decline Disease (OQDS), represents a challenge in the management of olive groves. In this study the *in vitro* antibacterial effect of natural molecules and resistance inductors (*Trametes versicolor* -extract, BABA®, *Punica granatum* extract, Atholio Bio®, free-fatty acids, diacylglycerides and oxylipins) and synthetic compounds with low environmental impact (Biozon®, Dentamet®, Propionic acid) was investigated against Xf subspecies. Since Xf is a fastidious bacterium to cultivate, a preliminary screening of different methodologies was carried out to evaluate the best one. The methods evaluated were: i) broth microdilution [1]; ii) broth macrodilution [2]; iii) agar dilution assays; [3]; iv) agar disk diffusion [3]. For each substance and/or for the different methodology we evaluated both the ability to limit the planktonic growth (reported as minimum inhibitory concentration, MIC) and the ability to inhibit the biofilm formation. Moreover MBC (minimum bactericidal concentration) was evaluated as the lowest product concentration able to completely inhibit the growth of the pathogen on PD2 plates. The results demonstrate that the broth macrodilution method was the most appropriate and reproducible method. The results showed a good efficacy for most of the products used against Xf subspecies highlighting antimicrobial or anti-biofilm activity. This study contributes to the choice of new and promising products eco-compatible for open field application for OQDS managing.

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S6-P36

Novel CNC and thyme extract-based nanocapsules to control the olive knot causal agent

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Nanopesticides represent one of the most innovative tools to control plant diseases. Nanotechnology's application in crop protection can provide effective compounds to counteract bacterial pathogens and at the same time reduce the amount of traditional synthethic agrochemicals[1]. In this work cellulose nanocrystals (CNC) and thyme extract were functionalized to develop an innovative nanopesticide, tested against *Pseudomonas savastanoi* pv. *savastanoi* (Psav), the causal agent of olive knot disease. CNC were obtained from grapewine pruning redidues by a 5% sodium chlorite bleaching followed by an acid hydrolysis, while thyme extract was produced through a supercritical CO_2 extraction. Both CNC and thyme extract were investigated for their biocompatibility on olive seedlings by measuring leaf area, chlorophyll and flavonol contents after application on canopy. Nanocapsules were synthetized by using CNC as nanocarrier adding calcium choride as cross-linker to encapsule the extract. Obtained nanomaterials were morphologically characterized by electron microscopy revealing an average dimension of 180 nm. Nanocapsules were then investigated by an *in vitro* micrudilution test and an agar disk diffusion test to assay their antimicrobial properties against Psav (strain PvBa206)[2]. Obtained results showed a almost total inhibition on the bacterial growth when the nanocompound was used at the concentration of 0,5%, comparable to the one showed by copper sulphate at the field dose. These preliminary results highlight the possibility of using innovative technological compounds obtained from agricultural wastes as a sustainable tool for the management of bacterial crop diseases without any negative effects on the biological development of plants.

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S6-P38

Xylella fastidiosa infections reveal different physiological response in resistant and susceptible olive cultivars

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Recently, a strain of *Xylella fastidiosa* subsp. *pauca* (*Xfp*) was introduced into Southern Italy, where it caused the Olive Quick Decline Syndrome [1], a disease that induces a severe dieback in susceptible olive cultivars. Interestingly, *Xfp*-infected olives of resistant cultivars do not develop severe dessication and branch dieback. Mechanisms of resistance to *Xfp* are not fully understood although evidences suggest that resistant olive genotypes actively limit *Xfp* replication by molecular and physiological responses [2]. In this study we compared the stem water potential (Ψstem) and stomatal conductance (g_s) of healthy and *Xfp*-infected olives of Leccino, FS17 (resistant) and Cellina di Nardò (susceptible) in greenhouse controlled conditions. Time-course measurements allowed to calculate the differential Ψstem (ΔΨstem) and the differential g_s (Δg_s) from both healthy and *Xfp*-infected plants within each tested cultivar. Higher Δg_s and ΔΨstem were found in Cellina di Nardò, compared to Leccino and FS17. These preliminary results, while supporting previous molecular data differentiating resistant and susceptible cultivars [3], indicate that *Xfp* infection limits the water supply to leaves and suggest a differential physiological response in resistant and susceptible olive cultivars. Thus, recording physiological parameters can help the pre-selection of olive genotypes with resistant traits to *Xfp* in the framework of breeding programs.

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S6-P39

Harmonization of laboratory diagnosis for *Candidatus* Liberibacter spp. on Citrus among National Reference Laboratories in Member States

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Huanglongbing (HLB) is a lethal disease of *Citrus* and associated with three obligate biotrophic bacteria; '*Candidatus* Liberibacter africanus' (CaLaf), '*Candidatus* Liberibacter americanus' (CaLam) and '*Candidatus* Liberibacter asiaticus' (CaLas). Although not yet present in the European Union, these quarantine bacteria are phytosanitary categorized as priority pests for Member States and they have, therefore, been included in the activities of the EU Reference Laboratory (EURL) for pests of plants on bacteria. Various detection tests have been developed for this pathogen in host tissues. An international test performance study (TPS) was organized in the framework of the EURL by our laboratory in 2020 to evaluate three promising molecular detection tests. The participants had to perform an adapted real-time PCR test (HLB TaqMan) by Li *et al*. (2006) [1], a recently developed real-time PCR test (CaLib TaqMan) by Quintana-González *et al*. (*in preparation*) and a combination of two conventional PCRs (HLB-PCR) described by Hocquellet *et al*. (1999) [2] and Teixeira *et al*. (2005) [3]. Results showed that these tests can be regarded as fit for purpose and are recommended for routine testing of *Citrus sinensis* and *Citrus reticulata* leaves for the detection of *Candidatus* Liberibacter spp. Following the TPS, a Proficiency Test (PT) on the molecular detection of *Candidatus* Liberibacter spp. in *Citrus* was organized in the framework of the EURL by our laboratory in 2021, in order to assess the diagnostic competence of European NRL's. Results showed that an overall accuracy of 97,8 % was achieved among the NRL's.

The EU Reference Laboratory for pests of plants on bacteria is a consortium led by the Netherlands Food and Consumer Product Safety Authority, National Reference Centre Plant Health (NVWA-NRC, The Netherlands) and is also composed of the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO, Belgium), the Research Centre for Plant Protection and Certification (CREA-DC, Italy) and the National Institute of Biology (NIB, Slovenia), funded by the European Commission (grant S12. 809508, co-funded by the Ministry of Agriculture, Nature and Food Quality in the Netherlands).

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