

## **4<sup>th</sup> Annual Conference of the EuroXanth COST Action** **Integrating Science on *Xanthomonadaceae*** **for integrated plant disease management in Europe**

Virtual Conference  
28–30 June 2021

Ralf Koebnik, Katarina Gašić, Aleksa Obradović (eds.)

4<sup>th</sup> Annual Conference of the EuroXanth COST Action  
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University of Belgrade – Faculty of Agriculture

4<sup>th</sup> Annual Conference of the EuroXanth COST Action "Integrating Science on Xanthomonadaceae for integrated plant disease management in Europe"

BOOK OF ABSTRACTS

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# Conference Overview

**Opening:** Monday, June 28, 1:00 – 2:00 pm CET  
**Closing:** Wednesday, June 30, 7:15 – 7:30 pm CET

Monday 28 <sup>th</sup> June	Tuesday 29 <sup>th</sup> June	Wednesday 30 <sup>th</sup> June
<b>13:00 – 14:00</b> Opening Ceremony	<b>13:00 – 13:45</b> ePoster Session 2 Diagnostics & Diversity – Population Structure	<b>13:00 – 15:15</b> Session 3 Genetic Resistance – Host Defence
<b>14:00 – 18:10</b> Session 1 Diagnostics & Diversity – Population Structure	<b>13:45 – 17:55</b> Session 2 Pathogen Biology	<b>15:15 – 15:55</b> ePoster Session 4 Disease Management – Vector Control
		<b>16:15 – 18:30</b> Session 4 Disease Management – Vector Control
<b>18:30 – 19:20</b> ePoster Session 1 Diagnostics & Diversity – Population Structure	<b>18:00 – 18:30</b> Celebrating Success	<b>18:30 – 19:10</b> ePoster Session 5 Pathogen Biology Genetic Resistance – Host Defence
	<b>18:30 – 19:15</b> ePoster Session 3 Pathogen Biology Genetic Resistance – Host Defence	
		<b>19:15 – 19:30</b> Closing Ceremony

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# Scientific Program

## Monday 28<sup>th</sup> of June

Program schedule is based on the CET time zone.

13.00 – 13.15	<b>OPENING CEREMONY</b>	
13.15 – 14.00	Julian Smith (United Kingdom)	Opening Talk: How can Plant Health 'Get ahead of the disease curve'

14.00 – 18.10	<b>SESSION 1</b> <b>Diagnostics &amp; Diversity – Population Structure</b> <b>Chairs: Joana Costa (Portugal, WG1 Leader) &amp; Boris Vinatzer (USA)</b>	
14.00 – 14.30	Boris Vinatzer (USA; Key note)	Towards fast, sensitive, and precise plant pathogen detection and identification using metagenomic sequencing and curated databases
14.30 – 14.50	Caroline Bellenot (France)	Distribution of type III secretion systems and effectors in " <i>Xanthomonadales</i> " and <i>Nevskiales</i> orders
14.50 – 15.10	Jamie Harrison (United Kingdom)	Genomics-informed exploration of <i>Xanthomonas</i> threats
15.10 – 15.25	<b>Q&amp;A</b>	
15.25 – 15.40	<b>BREAK</b>	
15.40 – 16.00	Leonor Martins (Portugal)	<i>Xanthomonas arboricola</i> and <i>X. euroxanthea</i> strains isolated from pecan trees: comparative genomics and pathogenicity on walnut
16.00 – 16.20	Enora Dupas (France)	Probable dates and scenario of introduction of <i>Xylella fastidiosa</i> subsp. <i>multiplex</i> in France
16.20 – 16.40	Maria del Pilar Velasco Amo (Spain)	Distribution of pXFAS_5235 plasmid among <i>Xylella fastidiosa</i> infected plant samples in Spain
16.40 – 16.55	<b>Q&amp;A</b>	
16.55 – 17.15	Nay Dia (Switzerland)	Genomics-based loop-mediated isothermal amplification assays for detection of <i>Xanthomonas hortorum</i> complex
17.15 – 17.35	Monika Kałużna (Poland)	Genomics-informed molecular detection systems of <i>Xanthomonas arboricola</i> pv. <i>corylina</i> the causal agent of bacterial blight of hazelnut
17.35 – 17.55	Mafalda Reis Pereira (Portugal)	Tracking changes on host physiological traits promoted by <i>Xanthomonas euvesicatoria</i> : proximal optical sensing as an innovative tool for plant disease detection
17.55 – 18.10	<b>Q&amp;A</b>	



18.10 – 18.30	<b>BREAK</b>
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18.30 – 19.15	<b>ePOSTER SESSION 1</b> <b>Diagnostics &amp; Diversity – Population Structure</b> <b>Chairs: Massimiliano Morelli (Italy, WG1 Vice Leader) &amp; Jamie Harrison (United Kingdom)</b>	
18.30 – 18.32	Manuel Anguita-Maeso (Spain)	Bioinformatic pipelines are determinant in the analysis of microbial communities from different ecological niches in cultivated olive trees
18.32 – 18.34	Ninon Bellanger (France)	Space of spacers: what CRISPR loci can tell us about evolution of xanthomonads
18.34 – 18.36	Pavel Beran (Czech Republic)	Specific DNA markers for detection of <i>Xanthomonas gardneri</i> based on analysis of 2500 genomes within 30 minutes
18.36 – 18.38	Felipe Clavijo (Uruguay)	Genetic and phenotypic characterization of <i>Xanthomonas</i> species pathogenic of wheat in Uruguay
18.38 – 18.40	Mylene Corzo Lopez (Canada)	Assessment of <i>Xanthomonas</i> spp. pathogen variability in Cuban common beans
18.40 – 18.42	Nay Dia (Switzerland)	A new <i>Xanthomonas</i> species pathogenic on <i>Hydrangea</i> ?
18.42 – 18.44	Filip Gazdik (Czech Republic)	Persistence of <i>Xanthomonas campestris</i> pv. <i>campestris</i> in field soil in central Europe
18.44 – 18.46	Irina Ignatyeva (Russian Federation)	An experience of PCR methods implementation for a bacterial blight of bean <i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i> detection in a seed and plant material of legumes
18.46 – 18.48	Monika Kałużna (Poland)	Complete genome sequences and characterization of <i>Xanthomonas arboricola</i> , the causal agent of bacterial leaf blight of blueberry
18.48 – 18.50	Roland Kölliker (Switzerland)	Comparative genomics to understand host range in <i>Xanthomonas translucens</i>
18.50 – 19.15	<b>Poster Discussion</b>	

## Tuesday 29<sup>th</sup> of June

Program schedule is based on the CET time zone.

13.00 – 13.45	<b>ePOSTER SESSION 2</b> <b>Diagnostics &amp; Diversity – Population Structure</b> <b>Chairs: Massimiliano Morelli (Italy, WG1 Vice Leader) &amp; Monika Kałużna (Poland)</b>	
13.00 – 13.02	Amandine Cuntz (France)	An update of the situation of <i>Xylella fastidiosa</i> in plants and vectors in France
13.02 – 13.04	Carla Luís (Portugal)	Dispersion of the bacterium <i>Xylella fastidiosa</i> in Portugal
13.04 – 13.06	Jelena Menković (Serbia)	Etiology of bacterial leaf spot of arugula in Serbia
13.06 – 13.08	Hatice Özaktan (Turkey)	Characterization of <i>Xanthomonas arboricola</i> pv. <i>juglandis</i> strains isolated from brown apical necrosis (BAN) and bacterial blight of walnut by rep PCR
13.08 – 13.10	Jakub Pecenka (Czech Republic)	Integration of virulence factor hpaP in detection of bacterial blight of <i>Apiaceae</i> plants
13.10 – 13.12	Tamara Popović (Montenegro)	<i>Xanthomonas arboricola</i> pv. <i>pruni</i> associated with leaf and fruit spot and twig necrosis of peach, apricot and sweet cherry in Montenegro
13.12 – 13.14	Coline Sciallano (France)	Genetic diversity of <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> strains in Africa
13.14 – 13.16	Dagmar Stehlíková (Czech Republic)	Genome based loop mediated isothermal amplification assays for detection of <i>Xanthomonas arboricola</i> pv. <i>juglandis</i> and <i>X. euroxanthea</i>
13.16 – 13.18	Jan Wohlmuth (Czech Republic)	Symptoms manifestation of the <i>Xanthomonas hortorum</i> pv. <i>carotae</i> on selected representatives of the genus <i>Apiaceae</i>
13.18 – 13.20	Bekri Xhemali (Kosovo)	Phytosanitary quality along the tomato and pepper production chain through an integrated management of bacterial diseases caused by xanthomonads and other bacteria
13.20 – 13.45	<b>Poster Discussion</b>	

13.45 – 17.55	<b>SESSION 2</b> <b>Pathogen Biology</b> <b>Chairs: Joël F. Pothier (Switzerland, WG2 Leader) &amp; Neha Potnis (USA)</b>	
13.45 – 14.15	Neha Potnis (USA; Key note)	Understanding the basis of host adaptation in a stealthy plant pathogenic bacterium, <i>Xanthomonas</i>
14.15 – 14.35	Alice Boulanger (France)	The making of a pathogen: How <i>Xanthomonas</i> adapts to plant environments

14.35 – 14.55	Lucas Morinière (France)	Assessment of the essential genes in vitro and critical metabolic pathways in planta of <i>Xantomonas hortorum</i> pv. <i>vitians</i> using genome-wide Tn-seq screens and computational genomics
14.55 – 15.10	<b>Q&amp;A</b>	
15.10 – 15.30	Miguel Román-Écija (Spain)	Genome comparison of two Spanish strains of <i>Xylella fastidiosa</i> subsp. <i>multiplex</i> ST6 and their potential relationship with phenotypic traits associated to pathogenicity
15.30 – 15.50	Ofir Bahar (Israel)	Distribution dynamics of <i>Xylella fastidiosa</i> within almond tree organs through different physiological stages
15.50 – 16.10	Ralf Koebnik (France)	Complete genome sequences of clade-1 xanthomonads reveal novel genetic traits in the genus <i>Xanthomonas</i>
16.10 – 16.25	<b>Q&amp;A</b>	
16.25 – 16.40	<b>BREAK</b>	
16.40 – 17.00	Mathilde Hutin (France)	Genetic structure of <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> populations and diversity of their TAL effector repertoires in Burkina Faso
17.00 – 17.20	Nicolas W. G. Chen (France)	First description of a TALE target gene in common bean
17.20 – 17.40	Doron Teper (Israel)	Consequences of adaptation of TAL effectors on host susceptibility to <i>Xanthomonas</i>
17.40 – 17.55	<b>Q&amp;A</b>	

18.00 – 18.30	<b>CELEBRATING SUCCESS: What did our COST Action achieve?</b>
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18.30 – 19.15	<b>ePOSTER SESSION 3</b> <b>Pathogen Biology</b> <b>Genetic Resistance – Host Defense</b> <b>Chairs: Eran Bosis (Israel, WG2 Vice Leader) &amp; Roland Kölliker (Switzerland, WG3 Vice Leader)</b>	
18.30 – 18.32	Noemi Casarin (Belgium)	Focus on <i>Salicaceae</i> to investigate potential <i>Xylella fastidiosa</i> -based pathosystems in temperate regions
18.32 – 18.34	Giovane Böerner Hypolito (Brazil)	Characterization of ZapA and ZapB from <i>Xanthomonas citri</i> subsp. <i>citri</i> in cell division
18.34 – 18.36	Aleksandr Ignatov (Russian Federation)	A possible role of EPS structure in climate adaptation of <i>Xanthomonas campestris</i>

18.36 – 18.38	Kristi E. Ledman (USA)	<i>Xanthomonas translucens</i> pv. <i>undulosa</i> identified on non-wheat crops and weedy grasses in Minnesota, United States: describing an expanded host range
18.38 – 18.40	Mateus Terceti (Brazil)	Alterations in essential regulatory two-component system of <i>phoPQ</i> is associated with cell division, cell morphology and Virulence of <i>Xanthomonas citri</i> subsp. <i>citri</i>
18.40 – 18.42	Carlos Andrés Zárate-Chaves (France)	CRISPR interference (CRISPRi), a powerful tool to study the function of gene families in <i>Xanthomonas</i>
18.42 – 18.44	José Gadea (Spain)	PthA4 <sup>AT</sup> , a small TAL effector from <i>Xanthomonas citri</i> subsp. <i>citri</i> induces immunity in <i>Nicotiana benthamiana</i>
18.44 – 18.46	Marlène Lachaux (France)	Functional characterization of the IR64 rice variety resistance towards African <i>Xanthomonas oryzae</i> pv. <i>oryzae</i>
18.46 – 18.48	Lucia Ragasová (Czech Republic)	Visualization and tracking <i>Xanthomonas campestris</i> pv. <i>campestris</i> in cabbage plants by fluorescence in situ hybridization
18.48 – 19.15	Poster Discussion	

## Wednesday 30<sup>th</sup> of June

Program schedule is based on the CET time zone.

13.00 – 15.15	<b>SESSION 3</b> <b>Genetic Resistance – Host Defense</b> <b>Chairs: Jens Boch (Germany, WG3 Leader) &amp; Bing Yang (USA)</b>	
13.00 – 13.30	Bing Yang (USA; Key note)	Exploiting natural and engineered rice resistance to bacterial blight
13.30 – 13.50	Suayib Üstün (Germany)	Self-ubiquitination of a pathogen type-III effector traps and blocks the autophagy machinery to promote disease
13.50 – 14.00	<b>Q&amp;A</b>	
14.00 – 14.20	Shannon Greer (United Kingdom)	Identification, characterisation and mapping of resistance to black rot ( <i>Xanthomonas campestris</i> pv. <i>campestris</i> ) in <i>Brassica</i> spp.
14.20 – 14.40	Florian Goettelmann (Switzerland)	Pooled sequencing identifies candidate genes for resistance to <i>Xanthomonas translucens</i> pv. <i>graminis</i> in <i>Lolium multiflorum</i>
14.40 – 15.00	Joana Vicente (United Kingdom)	Distribution, colonisation and volatile organic compound detection in plants inoculated with <i>Xylella fastidiosa</i>
15.00 – 15.15	<b>Q&amp;A</b>	

15.15 – 15.55	<b>ePOSTER SESSION 4</b> <b>Disease Management – Vector Control</b> <b>Chairs: Tamás Kovács (Hungary, WG4 Vice Leader) &amp; Mária Kocanová (Czech Republic)</b>	
15.15 – 15.17	Irem Altin (Italy)	Synthesis, characterisation and efficacy of chitosan-stabilised silver nanoparticles against <i>Xanthomonas vesicatoria</i> , the causal agent of tomato bacterial spot
15.17 – 15.19	Alice Anzalone (Italy)	Biological control of tomato bacterial diseases by <i>Bacillus</i> sp. and <i>Pseudomonas</i> sp. isolated from tomato endorhizosphere
15.19 – 15.21	Daiva Burokienė (Lithuania)	Influence of natural antimicrobials on <i>Xanthomonas</i> strains growth
15.21 – 15.23	José Juan Cortés Plana (Spain)	Computational model to approach the spread of plant pests and particularly to the propagation of <i>Xylella fastidiosa</i> in the almond trees in Spain
15.23 – 15.25	Guilherme Dilarri (Brazil)	Evaluating alternatives to sodium hypochlorite for the post-harvest sanitization of citrus fruit in packinghouses

15.25 – 15.27	Songul Erken (Turkey)	Sensitivity to copper in <i>Xanthomonas campestris</i> pv. <i>campestris</i> in vitro in Turkey
15.27 – 15.29	Juliano Henrique Ferrarezi (Brazil)	Control of citrus canker symptoms by crude extract from <i>Pseudogymnoascus</i> sp.: a greenhouse assay
15.29 – 15.31	Katarina Gašić (Serbia)	Antagonistic potential of <i>Pseudomonas graminis</i> strains against some economically important xanthomonads
15.31 – 16.55	<b>Poster Discussion</b>	
15.55 – 16.15	<b>BREAK</b>	

16.15 – 18.30	<b>SESSION 4</b> <b>Disease Management – Vector Control</b> <b>Chairs: Emilio Stefani (Italy, WG4 Leader) &amp; Maria Saponari (Italy)</b>	
16.15 – 16.45	Maria Saponari (Italy; Key note)	Control of <i>Xylella fastidiosa</i> in Europe: from eradication to containment measures
16.45 – 17.05	Conor Horgan (Ireland)	Design and synthesis of potential quorum sensing inhibitors of <i>Xylella fastidiosa</i>
17.05 – 17.15	<b>Q&amp;A</b>	
17.15 – 17.35	Saul Burdman (Israel)	Potential use of antimicrobial random peptide and lipopeptide mixtures for control of <i>Xanthomonas</i> diseases
17.35 – 17.55	Mária Kocanová (Czech Republic)	Identification of novel bacteriophages of <i>Xanthomonas campestris</i> pv. <i>campestris</i> isolated from cabbage
17.55 – 18.15	Irem Altin (Italy)	Encapsulation of phages in liposomes: enhancing their efficacy to control bacterial walnut blight in field
18.15 – 18.30	<b>Q&amp;A</b>	

18.30 – 19.10	<b>ePOSTER SESSION 5</b> <b>Disease Management – Vector Control</b> <b>Chairs: Tamás Kovács (Hungary, WG4 Vice Leader) &amp; Saul Burdman (Israel)</b>	
18.30 – 18.32	Eliška Hakalová (Czech Republic)	Antibacterial effect of thyme essential oil compounds to pathogenic xanthomonads
18.32 – 18.34	Judit Kolozsváriné Nagy (Hungary)	Antibacterial effect of essential oils on the causal agent of bacterial spot of stone fruits and almond
18.34 – 18.36	Julio Retamales (Chile)	<i>Xanthomonas arboricola</i> pv. <i>corylina</i> causing bacterial blight of hazelnut in Chile: genetic analysis of resistance to agrochemicals
18.36 – 18.38	Giulio Flavio Rizzo (Italy)	Efficacy of microbial consortia and natural compounds as seed dressing for the control of tomato bacterial spot

18.38 – 18.40	Vítor Rodrigues Marin (Brazil)	<i>Origanum vulgare</i> essential oil in the control of the citrus canker
18.40 – 18.42	Joanna Świątczak (Poland)	Antibacterial and antibiofilm activity of phenolic compounds against <i>Xanthomonas campestris</i> pv. <i>campestris</i>
18.42 – 18.44	Dorota Tekielska (Czech Republic)	Antibacterial activity of novicidin derived synthetic peptides against <i>Xanthomonas campestris</i> pv. <i>campestris</i>
18.44 – 18.46	Gabrielle Vieira (Brazil)	Penicillic acid isolated from <i>Penicillium</i> sp. from Antarctica effect on citrus canker in greenhouse conditions
18.46 – 19.10	<b>Poster Discussion</b>	

19.15 – 19.30	<b>CLOSING CEREMONY</b>
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# INVITED TALKS



## Opening Talk

### How can Plant Health 'Get ahead of the disease curve'

Julian Smith

Rothamsted Research, Hertfordshire, UK

Keywords: *Xanthomonas vasicola*, Pest Risk Analysis, surveillance, diagnostics, yield loss

In 2001 I isolated and identified, *Xanthomonas vasicola* (then *campestris*) pv. *musacearum* (Xvm), the causal organism for Xanthomonas wilt on banana (BXW). This was a 1st disease report for Xvm in Uganda and outside of Ethiopia. At that time and through the national research institute we raised the concern on how this disease would spread quickly, in planting material and by insect, with devastating consequences to the staple food of the region. Five years on Xvm was widespread across the Great Lakes region of eastern Africa – Burundi, DR Congo, Kenya, Rwanda, Tanzania in addition to Uganda – causing yield losses for millions of smallholders. Twenty years on BXW remains a disease of concern for these countries and a threat to the wider region.

In this talk I will discuss some of the research on Xvm in context to wider questions on how plant pathology can support better the response to new pest outbreaks. In considering Pest Risk Analysis I will question our readiness to future threats, on diagnostics I will question their use and impact, on surveillance I will question how we can do this on a day to day basis, on sequencing I will question how this data can be better valued for accessing future risk and on yield loss I will question if we are capturing the data effectively. In concluding I will ask the question of what is intended by Plant Health National Reference Laboratory status and what may constitute a minimum capability.

# Session 1

## Diagnostics & Diversity – Population Structure

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## Key note

### **Towards fast, sensitive, and precise plant pathogen detection and identification using metagenomic sequencing and curated databases**

Boris A. Vinatzer<sup>1</sup>, Parul Sharma<sup>1</sup>, Marcela Aguilera Flores<sup>1</sup>, Shu Yang<sup>1</sup>, Haijie Liu<sup>1</sup>, Reza Mazloom<sup>2</sup>, Song Li<sup>1</sup>, Lenwood S. Heath<sup>2</sup>

<sup>1</sup> School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA, USA

<sup>2</sup> Computer Science Department, Virginia Tech, Blacksburg, VA, USA

**Keywords:** next generation sequencing, bioinformatics, taxonomy, metagenome-assembled genomes (MAGs)

Outbreaks of emerging diseases require quick interventions to prevent them from turning into epidemics and pandemics. To make this possible, we need fast, sensitive, and precise detection and identification of the pathogens that cause them. Sequencing the entire DNA present in a symptomatic plant sample, *i.e.*, metagenomic sequencing, makes it possible today to quickly obtain sequences of the pathogen. However, the challenge is that the obtained sequences also include sequences of the plant and other microbes. Their presence affects sensitivity of detection and makes it difficult to determine which sequences are of the actual pathogenic agent. An additional challenge with plant pathogens is that the genetic variation that distinguishes pathogens from each other and from non-pathogenic relatives is often within the realm of the same species. Since most genome databases use the species as smallest unit, they are not suitable for plant pathogen identification. To address this problem, we developed a Web service, called [linbase.org](http://linbase.org), which allows within-species identification of bacterial pathogens using genome sequences as query. Here we show how genomes assembled from metagenomes can be successfully used using [linbase.org](http://linbase.org), including *Xanthomonas* and *Xylella*. We then share how we are developing and implementing a future version of [linbase.org](http://linbase.org) that will also accept unassembled metagenomic sequences as query. We finally demonstrate that tools developed for genome-based molecular epidemiology can be used with metagenomic sequences as well. In conclusion, while metagenomics-based pathogen identification is still in its infancy, the potential of this technology in routine plant disease diagnosis is promising.

## Distribution of type III secretion systems and effectors in “*Xanthomonadales*” and *Nevskiales* orders

Caroline Bellenot<sup>1</sup>, Babil Torralba<sup>1</sup>, Sébastien Carrère<sup>1</sup>, Ludovic Cottret<sup>1</sup>, Martial Briand<sup>2</sup>, Marion Fischer-Le-Saux<sup>2</sup>, Erika Sallet<sup>1</sup>, Ralf Koebnik<sup>3</sup>, Marie-Agnès Jacques<sup>2</sup>, Laurent D. Noël<sup>1</sup>, Matthieu Arlat<sup>1</sup>

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Keywords: virulence, evolution

We have conducted comparative genomic and phylogenetic studies on 1468 strains belonging to the orders *Lysobacterales* (earlier synonym of *Xanthomonadales*) and *Nevskiales*, using GTDB-TK (1) and average nucleotide identity calculations. The distribution of type III secretion (T3S) systems and type III effectors (T3E) among these strains including 753 phytopathogenic and commensal *Xanthomonas* strains allowed the characterization of different types of T3S systems and different classes of T3Es. We will also present the first version of a bio-informatic tool designed to mine T3E genes in *Xanthomonas* genomes.

### Reference

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## Genomics-informed exploration of *Xanthomonas* threats

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Several recent genomics-informed taxonomic revisions have transferred pathovars from *Xanthomonas campestris* (Xc) into species that are more closely related phylogenetically. However, phylogenetic analysis of *gyrB* sequences suggests that the species classification Xc still contains several taxa that potentially belong to other species. In an attempt to address this, we sequenced genomes of the pathotype strains of 25 pathovars including *merremiae*, *trichodesmae*, *lantanae*, *zinniae*, *esculenti*, *eucalypti*, *nigromaculans* and *pathenii*. Based on our comprehensive study using a variety of analysis methods including phylogenetic and average nucleotide identity, we propose updated taxonomic positions for these pathovars. This project is part of a larger program of *Xanthomonas* sequencing comprising nearly 1,000 genomes and we will present preliminary data and results emerging from analysis of those to date, including and exploration of the diversity of the recently described *Xanthomonas nasturtii*.

## ***Xanthomonas arboricola* and *X. euroxanthea* strains isolated from pecan trees: comparative genomics and pathogenicity on walnut**

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**Keywords:** *Xanthomonas euroxanthea*, walnut bacterial blight, comparative genomics, pathogenicity

Four *Xanthomonas* spp. strains (CPBF 1494, CPBF 761, CPBF 765 and CPBF 766) have been isolated from two pecan (*Carya illinoensis*) trees showing symptoms similar to walnut bacterial blight, a disease typically associated with *X. arboricola* pv. *juglandis* (*Xaj*) in walnut (*Juglans regia*).

To further characterize these pecan-isolated xanthomonad strains, genomics analysis, plant pathogenicity, and hypersensitivity reaction (HR) assays were carried out. Average nucleotide identity analysis (ANI) revealed that CPBF 761 and CPBF 766 belong to *X. euroxanthea* (ANI > 97 %) and confirmed CPBF 1494 and CPBF 765 as *X. arboricola*. While strains CPBF 1494 and CPBF 761 co-colonize one tree, strains CPBF 765 and CPBF 766, were isolated from another tree of the same orchard. All four strains were HR positive and pathogenic in walnut, but only *Xaj* strains CPBF 765 and CPBF 1494 were shown to be pathogenic in pecan. These results reveal that *X. euroxanthea* isolated from pecan (CPBF 761 and CPBF 766), are pathogenic in walnut but not in the host of isolation, raising the hypothesis that pecan may act as a reservoir for *X. euroxanthea*. Recently, the species *X. euroxanthea* was shown to include both pathogenic (CPBF 424<sup>T</sup>) and non-pathogenic (CPBF 367, CPBF 426) strains on walnut, displaying distinct profiles of type III secretion (T3SS) and effectors (T3E) coding genes. Ongoing genomic characterization of pathogenicity and virulence determinants on CPBF 761 and CPBF 766, adding to the previously described CPBF 424<sup>T</sup>, might contribute to unravel key players for pathogenicity and host specificity in *X. euroxanthea*.

## Probable dates and scenario of introduction of *Xylella fastidiosa* subsp. *multiplex* in France

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**Keywords:** *Xylella fastidiosa* subsp. *multiplex*, bridgehead invasion, MLVA, ABC analysis

*Xylella fastidiosa* was detected for the first time in the French territory in 2015, in Corsica and PACA regions. Most samples were infected by strains from two lineages (ST6 and ST7) of *X. fastidiosa* subsp. *multiplex*. While numerous of foci were detected all over Corsica, in natural, urban and semi-urban environments, in a large range of hosts (39 plant species), a more limited number of mostly urban foci was reported from PACA in a range of 25 plant species, from which 15 were not reported infected in Corsica. Hence, situations look like having different histories. Our aim was to decipher the most probable scenario of introduction of each ST in these two regions. A collection of 82 genome sequences including 56 genome sequences of French strains was used to date the divergence of French *X. fastidiosa* subsp. *multiplex* strains from their American relatives. We developed a 13-VNTR scheme for direct typing of *X. fastidiosa* subsp. *multiplex* in infected plant samples and used Approximated Bayesian Computation analyses to select the best scenario to explain the spread of the bacterium in France. The scenario choice and testing will be presented together with parameters allowing the selection of the best scenario and its interpretation in the context of plant trade.

## Distribution of pXFAS\_5235 plasmid among *Xylella fastidiosa* infected plant samples in Spain

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Keywords: introduction, mobile genetic elements, *traC* gene, Plasmid PCR-based

*Xylella fastidiosa* (*Xf*) is a phytopathogenic bacterium with mobile genetic elements (MGE), including prophages, pathogenicity islands, and plasmids. Genome sequencing of several strains of *Xf* subsp. *fastidiosa* ST1 from Mallorca island, Spain, revealed the presence of a 38kb plasmid (pXFAS\_5235). pXFAS\_5235 has a high sequence similarity to the conjugative plasmid pXFAS01 described in *Xf* subsp. *fastidiosa* strain M23 and to plasmid pXF-RIV5 in *Xf* subsp. *multiplex* strain Riv5. Plasmid comparative sequence analyses and studies of their presence and distribution among different *Xf* strains belonging to same or different subspecies and STs can provide an important information about host adaptation mechanisms with application in future studies of epidemiology, ecology, and evolution of this plant pathogen. In this study we used a PCR-based approach using specific primers targeting the *traC* gene to describe the distribution of the pXFAS\_5235 plasmid on DNA samples obtained from a world-wide collection of more than 60 *Xf* strains from different subspecies and STs isolated from different host plants and regions and from *Xf*-infected plant samples obtained from several hosts from the *Xf* outbreak areas in Balearic Islands and Alicante province in mainland Spain. Our results showed that *traC* gene was amplified in plasmid-bearing strains of *Xf* belonging to subsp. *fastidiosa*, *multiplex*, *pauca* and *sandyi*. Blast analysis of the amplified products with all *Xf* reference genomes revealed more than 10 sequence types; grouping the strains mainly by subspecies or geographical origin. For plant samples, only those infected by *Xf* subsp. *fastidiosa* ST1 contained the plasmid. Moreover, DNA samples obtained from tree rings dated in 1998 previously known to be infected by *Xf* subsp. *fastidiosa* contained the plasmid. Our results confirm previous studies that pointed out to a single introduction of *Xf* subsp. *fastidiosa* ST1 in the Balearic Islands.

Study supported by Project E-RTA2017-00004-C06-02 from AE-INIA Spain, Intramural Project 201840E111 from CSIC, Spanish Olive Oil Interprofessional and Thematic Interdisciplinary Platform on *X. fastidiosa* from CSIC.



# Genomics-based loop-mediated isothermal amplification assays for detection of *Xanthomonas hortorum* complex

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Keywords: diagnostics, in-field detection, genomic-informed, LAMP

The *Xanthomonas hortorum* complex, namely the seven pathovars of *X. hortorum* and newly described *Xanthomonas hydrangeae*, cause disease on a multitude of primary and secondary plants, including crops, ornamental and wild plants. Furthermore, cross-pathogenicity has been proven for some of the strains within this complex. It is thus important to have highly specific and fast diagnostics methods for the *X. hortorum* complex. Comparative genomics was conducted for representative members within the *X. hortorum* complex. Seven LAMP diagnostics assays were developed for the early detection of six *X. hortorum* complex sub-groups, in addition to one assay specific for the entire *X. hortorum* complex. Primer sets were designed for each assay. Primer sets specificity was tested on 88 strains (around 50 % *X. hortorum* complex strains and 50 % outgroup strains). The primer sets amplified their respective targets within 15 minutes. The sensitivity of the assays was tested using ten-fold log dilution series, dilution series spiked with plant material and bead-beated mixtures of fresh bacterial and plant materials. All assays consistently detected, across experimental triplicates, gDNA quantities of 250 fg (45 genome copies). If plant lysate did not delay amplification, the consistent detection limit varied and was either unchanged or decreased to 2.5 pg. For the fresh bacteria and plant lysate mixture, the consistent detection limit, across experimental triplicates and biological replicates, differed between assays and ranged from 10 to 1,000 cells/LAMP reaction. These assays provide thus seven diagnostic efficient tools for the phytosanitary control of an important complex of xanthomonads.

# Genomics-informed molecular detection systems of *Xanthomonas arboricola* pv. *corylina* the causal agent of bacterial blight of hazelnut

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**Keywords:** *Xanthomonas arboricola* pv. *corylina*, hazelnut bacterial blight, real-time PCR, LAMP, molecular detection

Specific primers pairs were designed to detect the causal agent of hazelnut bacterial blight *Xanthomonas arboricola* pv. *corylina* bacterium possessing quarantine status in Europe. A comparative genomic approach was used on the publicly available genomes of *X. arboricola* to select unique targets for designing four specific detection systems relying on: 1) conventional PCR, 2) real-time PCR (SYBRg 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, PCR assays enabled accurate detection of *X. arboricola* pv. *corylina* in pure cultures and in plant material. Validation of the systems and their usefulness for detection of *X. arboricola* pv. *corylina* in infected in plant material with determination of limit of detection is conducted.

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# Tracking changes on host physiological traits promoted by *Xanthomonas euvesicatoria*: proximal optical sensing as an innovative tool for plant disease detection

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**Keywords:** Plant disease detection, Plant Pathology, Proximal sensing, Spectroscopy, Precision agriculture

*Xanthomonas euvesicatoria* (Xeu) is a bacterial pathogen known to cause disease in crops of high economic importance worldwide threatening their yield, quality, and economic value. The current methods used to assess this pathogen often depend on the presence of visible signs of the infection, which frequently manifest themselves only in the late stages of this process, compromising the effectiveness of protection measures. Therefore, complementary methods based on proximal optical sensing (POS) have recently been explored. Based on evidence that plant-pathogen interactions promote changes in the biochemical and internal structures of the host, resulting in modifications to their optical properties, this study evaluated the potential use of a POS as an effective technique for the early detection of pathogen infection. A compact, modular sensing system, combining direct UV-Vis spectroscopy with optical fibers, supported by a robust Self-Learning Artificial Intelligence (SLAI), was used to assess the modifications promoted by Xeu in tomato leaves (cv. Cherry). Plant infection was performed by spraying a bacterial suspension ( $1.0 \times 10^8$  cells/mL<sup>-1</sup>) until run-off occurred, and a similar approach was followed for the control group where only water was applied. A total of 270 spectral assessments were performed on leaves, on five different dates, which included pre- and post-inoculation measurements. The spectral signatures were then analyzed by principal components analysis coupled with an innovative SLAI algorithm, which allowed the distinction and differentiation of healthy and infected leaves. These findings indicate that this non-destructive, *in vivo* POS approach may be a promising tool for detecting the changing spectral behavior of diseased plant leaves.

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# Session 2

## Pathogen Biology

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## Key note

### Understanding the basis of host adaptation in a stealthy plant pathogenic bacterium, *Xanthomonas*

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**Keywords:** host range, type VI secretion system, latent infection period, genome-wide association study, apoplast, epiphytic fitness

Genus *Xanthomonas* is a large genus of bacteria that collectively displays broad host range, yet individual species exhibit stringent host and tissue specificity. Bacterial spot xanthomonads present a unique model system to study host specificity because both phenomena of pathological convergence of diverse species on a common host, tomato as well as host range expansion of closely related species on pepper have been observed. Despite convergence on a common host, tomato, the phylogenetically diverse species of bacterial spot xanthomonads differ in the overall aggressiveness, measured by two traits, latent infection period and disease severity. We observed that a mutation in a core gene of type VI secretion system cluster III in *Xanthomonas perforans* leads to a higher disease severity and a shorter latent infection period compared to the wild type. These findings warrant us to consider initial asymptomatic phase while investigating the mechanistic basis of pathogenesis. Other phenomenon observed with closely related *Xanthomonas* species is recent host range expansion of *X. perforans* on pepper. Genome-wide association analyses of two closely related species, *X. perforans* and *X. euvesicatoria*, identified two gene-candidates significantly associated with pepper pathogenic strains. Both candidates are predicted to play a role in the acquisition or transport of nutrients commonly encountered in the apoplast during infection. A minor effect on symptom development and growth was observed with individual mutations in candidate genes. Thus, host specificity is likely a complex and multigenic trait and may have evolved independently within this *Xanthomonas* species complex.

## The making of a pathogen: how *Xanthomonas* adapts to plant environments

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Keywords: *Xanthomonas*, fitness, virulence, plant adaptation

*Xanthomonas campestris* pv. *campestris* (*Xcc*) is a phytopathogenic bacterium which causes black rot disease on cultivated or wild *Brassicaceae*. During its lifecycle, *Xcc* experiences changing environments from leaf surfaces to, distinct endophytic compartments (hydathode, xylem, mesophyll), seeds and plants debris. So far, genetic screens have only identified major determinants important for pathogenicity. Mechanisms of pathogen entry, vascular immunity suppression, metabolic adaptation and microbial fitness in those plant environments are essentially unknown. In order to identify bacterial determinants of microbial plant adaptation at early steps of infection, we have combined *in planta* transcriptomic analyses and high-throughput RB-TnSeq screens during hydathodes colonization.

We will present and discuss these analyses that lead to a better understanding of the physiological state of the bacterium during the homing step of infection and to the characterization of virulence factors.

# **Assessment of the essential genes *in vitro* and critical metabolic pathways *in planta* of *Xanthomonas hortorum* pv. *vitians* using genome-wide Tn-seq screens and computational genomics**

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**Keywords:** *Xanthomonas hortorum*, Tn-seq, essential genome, metabolism, lettuce

*Xanthomonas hortorum* pv. *vitians* is a worldwide-distributed pathogen of lettuce (*Lactuca sativa*) for which no practical and sustainable means of disease control or prevention have been made available yet. These issues are partially related to the virtually complete absence of knowledge about the genetic and molecular functioning of this pathogen, both *in vitro* and *in planta*. Transposon insertion sequencing technics (abbreviated as Tn-seq) have proven to be powerful tools to identify, at a genome-scale, what genes are required for cell fitness in a given condition. Nevertheless, computational genomics must be mobilized to draw biological sense out of the lists of critical genes obtained by Tn-seq screens. In this study, we developed a highly-saturated transposon insertion mutant library of our model strain LM16734 for which a high-quality complete genome sequence was obtained using a combination of short- and long-reads technologies. Then, we explored in-depth the results of the *in vitro* library screen itself, which yielded a set of 370 loci primarily considered indispensable for cell survival in rich medium. We also conducted an *in planta* screen which identified 170 genes that are conditionally essential to the pathogen's fitness during the early steps of interaction with the host. In both cases, a reflexive usage of several computational technics allowed to deepen the biological meaning of these results. We were able to discriminate core essential genes *in vitro* from contextual mobile genetic elements-associated "selfish" genes, and highlight the so far unexamined critical importance of some metabolic pathways and functions during lettuce leaf colonization.

## Genome comparison of two Spanish strains of *Xylella fastidiosa* subsp. *multiplex* ST6 and their potential relationship with phenotypic traits associated to pathogenicity

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The ability of *Xylella fastidiosa* (*Xf*) to acquire new genetic information, via horizontal gene transfer and homologous recombination, has been related with its capacity to colonize new hosts and environments. In this study we have performed a comparative genome analysis of the complete circularized sequences of two *Xf* subsp. *multiplex* ST6 strains (ESVL and IVIA5901) isolated from almond trees showing almond leaf scorch (ALS) symptoms, located at the Guadalest Valley in Alicante province, in mainland Spain. Genome differences detected between both strains and with the reference strain Temecula1 of *Xf* subsp. *fastidiosa* ST1 were then related with phenotypic traits differences associated to infection and disease development observed among those strains. Genome analysis of both strains indicated an average nucleotide identity at the chromosomal level of 99.99%, but main differences were due to the presence of plasmids pXF64-Hb\_ESVL and pUCLA-ESVL only present in the ESVL strain. Our results showed that the ESVL strain had a higher cell motility and a greater surface attachment capability being able to form larger aggregates, while IVIA5901 strain showed a higher planktonic growth. Despite the high genetic similarity between the two strains, we have found important differences in their genomes. The presence of the two plasmids in the ESVL strain provided 48 unique genes to ESVL strain. Also, when comparing the two chromosomes, some unique genes and SNPs differences were found associated to genes potentially related with adhesion, motility, cell wall degradation, toxin-antitoxin system and the development of outer membrane protein, which might have an influence on some of the studied traits associated to pathogenicity.

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## Distribution dynamics of *Xylella fastidiosa* within almond tree organs through different physiological stages

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Keywords: *Xylella fastidiosa*, almond, ALS

*Xylella fastidiosa* was identified in Northern Israel about 5 years ago causing almond leaf scorch (ALS) disease. During field monitoring for ALS, we noticed that when infected almond trees flush in spring they do not show any ALS symptoms and the foliage appear green and healthy. Symptoms are first observed about two month later and become more evident during summer. This phenomenon was observed year after year in the same infected trees. We hypothesized that these changes reflect differences in the annual colonization of *X. fastidiosa* in the tree. To address this hypothesis, we sampled leaf petioles, green and woody stems, roots, green and dry fruit, flowers and buds from infected trees, throughout two complete seasons, and tested for the presence of *X. fastidiosa* using qPCR. *X. fastidiosa* was not detected in leaf petioles of infected trees in spring, but it could be detected before symptoms appear in early summer. Before entering dormancy, *X. fastidiosa* could still be detected in leaf petioles but its titer decreased markedly. Unlike leaf petioles, *X. fastidiosa* could reproducibly be detected in green and woody stems of infected trees throughout the year, maintaining a relatively constant titer, including during dormancy. *X. fastidiosa* was not detected in any of the other tissues tested throughout all sampling points. Our results reveal interesting information regarding the yearly cycle of *X. fastidiosa* in deciduous trees and provide important evidence that *X. fastidiosa* can be detected using molecular methods in infected trees before symptom appearance and even during dormancy.

# Complete genome sequences of clade-1 xanthomonads reveal novel genetic traits in the genus *Xanthomonas*

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**Keywords:** Comparative genomics, flagella, hormones, protein secretion, *Xanthomonas*

Clade-1 xanthomonads, also known as the clade of early-branching species (1), can colonize many crop plants, including banana, hyacinths and other related ornamental plant genera, rice, sugarcane, and tea plants. Using long-read sequencing technology, we have generated complete genome sequences for *Xanthomonas hyacinthi* (2), *Xanthomonas theicola* (3) and three species-level clade strains representing novel, yet unassigned bacterial species within the genus *Xanthomonas* (4). Comparative genomics has identified novel genetic traits in the genus *Xanthomonas* the details of which will be presented.

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## Genetic structure of *Xanthomonas oryzae* pv. *oryzae* populations and diversity of their TAL effector repertoires in Burkina Faso

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**Keywords:** MLVA-10, TAL effectors, *Xanthomonas oryzae* pv. *oryzae*, bacterial leaf blight

Bacterial Leaf Blight of rice (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major threat for food security in many rice growing countries including Burkina Faso where the disease was reported first in the 1980's. In line with the intensification of rice cultivation in West-Africa, BLB has been on the rise along the last 15 years. West-African strains of *Xoo* differ from their Asian counterparts as they (i) are genetically distant, (ii) belong to new races and, (iii) contain reduced repertoires of Transcription Activator Like (TAL) effector genes. In order to investigate the evolutionary dynamics of *Xoo* populations in Burkina Faso, 177 strains were collected from 2003 to 2018 in three regions where BLB is occurring. Multilocus VNTR Analysis (MLVA-10) targeting 10 polymorphic loci enabled to discriminate 24 haplotypes and showed that *Xoo* populations were structured according to their geographical localization and year of collection. Considering their major role in *Xoo* pathogenicity, we next surveyed the TAL effector repertoires of the 177 strains upon RFLP-based profiling. Surprisingly an important diversity was revealed with up to eight different RFLP patterns. Finally, comparing neutral vs. *TAL* effector gene diversity allowed to suggest scenarios underlying the evolutionary dynamics of *Xoo* populations in Burkina Faso, which could be helpful to guide the deployment of BLB resistant varieties in the country.

## First description of a TALE target gene in common bean

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Keywords: Common bean, common bacterial blight, TALE, susceptibility gene

During infection, *Xanthomonas* spp. employ transcription activator-like effectors (TALEs) able to induce susceptibility (*S*) genes to promote infection. Common bacterial blight of bean is due to *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citri* pv. *fuscans* (*Xcf*), two phylogenetically distant groups of strains, yet able to produce the same symptoms on common bean. Pathological convergence of *Xpp* and *Xcf* is associated with the horizontal transfer of dozens of genes, including two *tal* genes located on plasmids: *Xfuta1* and *Xfuta2*. We described six different *Xfuta1* alleles having different internal repeat sequences. We produced a mutant strain devoid of *tal* gene, and complemented it with artificial versions of the six *Xfuta1* alleles. Each allele contributed more or less to symptom development. Using a combination of *in silico* prediction of TALE targets and preliminary RNAseq data, we pointed out a gene potentially targeted by some of the *Xfuta1* alleles. This gene encoded a homologue of the Arabidopsis AINTEGUMENTA-LIKE 1 (AIL1) transcription factor. Voluntary induction of *PvAIL1* using designer TALEs restored the aggressiveness of the mutant strain, thus validating *PvAIL1* as an *S* gene targeted by *Xfuta1* alleles. To our knowledge, *PvAIL1* is the first TALE-induced *S* gene described so far in common bean.

## Consequences of adaptation of TAL effectors on host susceptibility to *Xanthomonas*

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Transcription activator-like effectors (TALEs) are virulence factors of *Xanthomonas* that induce the expression of host susceptibility (S) genes by specifically binding to effector binding elements (EBEs) in their promoter regions. The DNA binding specificity of TALEs is dictated by their tandem repeat regions, which are highly variable between different TALEs. Mutation of the EBEs of S genes is a key strategy to generate resistant crops. However, TALE adaptations through rearrangement of their repeat regions is a potential obstacle for successful implementation of this strategy. We investigated the consequences of TALE adaptations in the citrus pathogen *Xanthomonas citri* subsp. *citri* (*Xcc*), in which PthA4 is the TALE required for pathogenicity, whereas *CsLOB1* is the corresponding susceptibility gene, on host susceptibility. Seven TALEs, containing two-to-nine mismatching-repeats to the EBE<sub>PthA4</sub> that were unable to induce *CsLOB1* expression, were introduced into *Xcc pthA4:Tn5* and adaptation was simulated by repeated inoculations into and isolations from sweet orange. While initially all strains failed to promote disease, symptoms started to appear between 9-28 passages in four TALEs. Sequence analyses of adapted TALEs identified deletions and mutations within the TALE repeat regions, which enhanced affinity to the *CsLOB1* promoter. Analyses suggest that TALEs adaptations result from recombinations between repeats of the TALEs. Reintroduction of these adapted TALEs into *Xcc pthA4:Tn5* restored the ability to induce the expression of *CsLOB1*, promote disease symptoms and colonize host plants. Our study experimentally documented TALE adaptations to incompatible EBE and provided strategic guidance for generation of disease resistant crops against TALE-dependent pathogens.

# Session 3

## Genetic Resistance – Host Defence

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## Key note

### Exploiting natural and engineered rice resistance to bacterial blight

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Keywords: TAL effector, resistance, bacterial blight, *SWEET* gene, *R* gene

Bacterial blight (BB) is a devastating rice disease in Asia and Africa. The causal agent *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) uses TAL effectors (TALEs) to ectopically activate host susceptibility genes (e.g., *SWEET* sucrose transporter genes), conditioning a state of disease susceptibility. Host counteracts TALEs by evolving genetically dominant and recessive resistance (*R*) genes. The *R* genes include the NLR-type *Xa1*, executor-type *R* genes, and loss of susceptibility alleles of *SWEET* genes. *Xoo* uses a limited set of TALEs to target promoters of three *SWEET* genes (*SWEET11*, *13*, and *14*) in rice. Naturally occurring *SWEET* variants, with altered promoter TALE binding elements, act as recessive BB *R* genes by interfering with TALE functioning. We used CRISPR/Cas9 to engineer rice lines that have carried multiple mutations in three *SWEET* gene promoters. The *SWEET* promoter mutations were introduced into different rice varieties, and the disease evaluation showed that editing *SWEET* promoters generated robust, broad-spectrum BB resistance. We also used mapping and functional analysis approaches to identify *Xa7* and multiple functional *Xa1* alleles in various rice cultivars. Our studies reveal the diversity of genetic resistance and provide knowledge for engineering resistance for improvement of rice production.

## Self-ubiquitination of a pathogen type-III effector traps and blocks the autophagy machinery to promote disease

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**Keywords:** Autophagy, Effectors, Immunity, Ubiquitination, Disease, Xenophagy

Beyond its role in cellular homeostasis, autophagy is considered to play anti- and pro-microbial roles in host-microbe interactions, both in animals and plants. One of the prominent roles of anti-microbial autophagy in animals is to degrade intracellular pathogens or microbial molecules, in a process termed "xenophagy". Consequently, microbes evolved mechanisms to hijack or modulate autophagy to escape elimination. However, the extent to which xenophagy contributes to plant-bacteria interactions remains unknown. Here, we provide evidence that NBR1/Joka2-dependent selective autophagy functions in plant defence by degrading the bacterial type-III effector (T3E) XopL from *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*). We show how XopL associates with the autophagy machinery and undergoes self-ubiquitination, subsequently triggering its own degradation by NBR1/Joka2-mediated selective autophagy. Intriguingly, *Xcv* is also able to suppress autophagy in a T3E-dependent manner by utilizing the same T3E XopL that interacts and degrades the autophagy component SH3P2 via its E3 ligase activity. Thus, XopL is able to escape its own degradation and promote pathogenicity of *Xcv* by inhibiting autophagy through SH3P2 depletion. Together, we reveal a novel phenomenon how NBR1/Joka2 contributes to anti-bacterial autophagy and provide a unique mechanism how a T3E undergoes self-modification to act as a bait to trap host cellular degradation machineries.



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

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**Keywords:** *Xanthomonas campestris* pv. *campestris*, Brassica, black rot, resistance screening, resistance gene mapping

Black rot is the most damaging disease of vegetable brassicas (*Brassica oleracea*) worldwide and can reduce yields by >50 %. The causal agent *Xanthomonas campestris* pv. *campestris* (Xcc) also infects other important brassica crops such as swede, oilseed rape (*Brassica napus*), mustards (*Brassica juncea*), Chinese cabbage and turnips (*Brassica rapa*). This project builds on diverse Xcc isolates, including an extensive collection at Warwick University to identify *Brassica* resistance to the most important Xcc races 1, 4, 5 and 6, with a focus on vegetable brassicas where resistance to these races is rare in existent crop types. We are screening in house *Brassica* Diversity Fixed Foundation Sets (DFFSs) of *B. napus* and *B. oleracea*, 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. Once identified, these resistances will be characterised and mapped using GWAS and QTL mapping techniques, to identify resistance-linked markers that can be used to accelerate their introgression into crop types by marker-assisted selection. This work will be complemented with more fundamental research using chlorophyll and whole plant imaging techniques to visual Xcc infection, as well as the sequencings of approximately 700 *X. campestris* isolates with the aim to identify and functionally characterise effectors that dictate the outcome of *Brassica*-Xc interactions.

## **Pooled sequencing identifies candidate genes for resistance to *Xanthomonas translucens* pv. *graminis* in *Lolium multiflorum***

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**Keywords:** Bacterial wilt, disease resistance, pooled sequencing, *Xanthomonas translucens* pv. *graminis*, *Lolium multiflorum* Lam

*Xanthomonas translucens* pv. *graminis* (*Xtg*) is the causal agent of bacterial wilt, one of the main diseases of Italian ryegrass (*Lolium multiflorum* Lam.), causing considerable losses in yield and quality. Resistant cultivars are currently available, however, since Italian ryegrass is an outbreeding species, cultivars are highly heterozygous, and thus not completely resistant. One major QTL for resistance was previously discovered, but no sequence-specific markers to be used in breeding have yet been identified. In order to fine-map this QTL and characterize the underlying genes responsible for resistance, a mapping population consisting of 7,484 F2 individuals segregating for resistance was established in the greenhouse and inoculated with a highly virulent *Xtg* strain. Two pools of the 750 most resistant and the 761 most susceptible individuals were formed, and genomic DNA from these pools was extracted and sequenced using the Illumina NovaSeq 6000 platform. The frequency of single nucleotide polymorphisms (SNPs) was determined, and SNPs associated with resistance or susceptibility identified. Most of the significant SNPs map to linkage group 4, where the QTL was previously identified. Genes containing these SNPs were determined and constitute candidate resistance genes to be investigated further. Validated genes will allow to better understand the mechanisms of the interaction and to develop DNA markers to breed for resistant cultivars more efficiently.

## Distribution, colonisation and volatile organic compound detection in plants inoculated with *Xylella fastidiosa*

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Keywords: *Xylella fastidiosa*, infection, sampling, diagnostics, detection

Sampling different host plants for diagnostics of *Xylella fastidiosa* should be informed by knowledge on the distribution and colonisation of bacteria in plants in different conditions. The aims of our studies are to understand spread, latency and symptom development in key high-risk plant species for the UK and to develop detection of volatile organic compounds (VOCs) that could assist in targeting sampling.

Three host species (lavender, oleander, coffee) were inoculated with three subspecies of *X. fastidiosa* and kept in two different glasshouse environments (25 °C and ambient temperature) where rates of colonisation and symptom expression were monitored. Symptoms that could be due to *Xylella* infections were not always associated with positive qPCR samples; oleander and coffee plants were successfully infected and, although most positive samples were close to inoculation points, some oleander samples taken up to 10 cm away from the points were also positive showing that the infection had progressed. A follow-on experiment with six host species (including oleander, rosemary, bay laurel, plum, grapevine and blueberry) maintained in four different conditions is underway.

A proof of principle study using coffee plants showed that large amounts of VOCs can be retained on Mono Traps (a portable sorptive solid media), and a number of compounds were different between control and *Xylella* infected plants. We will present preliminary results of new experiments aiming at the detection of VOCs in *Nerium oleander* plants inoculated with *Xylella*.

# Session 4

## Disease Management – Vector Control

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## Key note

### Control of *Xylella fastidiosa* in Europe: from eradication to containment measures

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Keywords: *Xylella fastidiosa*, eradication, containment.

Thought to be restricted to the American continent, it was only recently that *Xylella fastidiosa* emerged as plant pathogen threatening agriculture and landscape in Europe. The discovery in 2013 of *Xylella*-infected olive trees showing a deadly disease in Apulia region (southern Italy), raised major concerns which prompted the implementation of strict EU legislative provisions to protect the European territories from the spread of the pathogen among the Member States and from further introductions from third Countries. The lack of any effective therapeutic approach to cure infected plants imposed the adoption of preventive and eradication legislative measures in all outbreak areas (France, Spain, Germany, Italy, Portugal) where the pathogen has been discovered after its first finding in Apulia. The restrictions imposed by the eradication measures triggered in some area an intense debate and, in southern Italy, their fully implementation encountered several obstacles. On the other hand, European research programs tackling this quarantine pathogen unraveled that several independent and inadvertent introductions have occurred in Europe much earlier than 2013, when presumably an introduction of infected coffee plants from central America has unleashed the epidemic on olives. Genomic studies indicated in fact that the bacterium is likely present in some of the currently known infected areas since the second half of the 1900s (i.e. in Corsica and Balearic Islands), but never emerged as a severe threat or it was confused with other biotic disorders. This means that the bacterium is established already in some areas in Europe and, therefore in such areas, eradication measures are not a practical and feasible approach anymore, with containment measures now being in place. These require the development of tools to reduce the impact of the infections, especially on the highly susceptible plant species. In this regard, in the framework of several European research initiatives main investigations focusses on host plant resistance, sustainable approaches to reduce insect vector populations, antibacterial molecules and microbial antagonists. An appraisal on the state of the art and preliminary results of these research programs will be presented, as well as the advances on the knowledge on the diversity and complexity of the European strains of *X. fastidiosa*.

# Design and synthesis of potential quorum sensing inhibitors of *Xylella fastidiosa*

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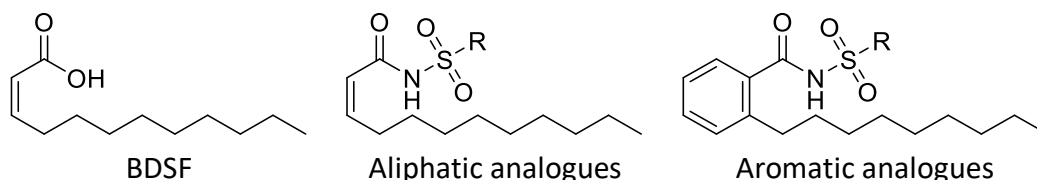
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**Keywords:** Quorum sensing, isosteres, inhibitors

Quorum sensing (QS) is a cell-cell communication process whereby bacteria can activate the transcription of specific genes in response to their environment and bacterial cell density.<sup>1</sup> A family of *cis*-2-unsaturated fatty acid signal molecules, known as the Diffusible Signal Factor (DSF) family, has been found to control biofilm formation and virulence in many bacteria including *Xanthomonas campestris* (DSF), *Burkholderia cepacia* (BDSF) and *Xylella fastidiosa* (XfDSF).<sup>2, 3</sup> It has been shown that interference with DSF signalling may inhibit biofilm formation and virulence in various species of bacteria.<sup>4</sup>

In this work, novel aliphatic and aromatic analogues of BDSF have been synthesised. Acyl sulfonamides are exploited as bioisosteric replacements for the carboxylic acid. Preliminary biological testing of the aliphatic and aromatic analogues assessed their ability to regulate XfDSF dependent phenotypes and biofilm formation in *X. fastidiosa*. Some aromatic analogues displayed an inhibitory effect against biofilm formation, while both agonism and antagonism of DSF signalling has been observed. Biological evaluation of further aromatic analogues is currently ongoing.



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## Potential use of antimicrobial random peptide and lipopeptide mixtures for control of *Xanthomonas* diseases

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**Keywords:** antimicrobial peptides, antimicrobial lipopeptides, *Xanthomonas*, disease control

Management of bacterial plant diseases in agriculture is highly challenging. There are only few means to cope with this type of diseases and they have limited efficacy. Therefore, there is an urgent need for novel alternatives. We have introduced the random antimicrobial peptide mixture (RPM) approach to tackle plant-pathogenic bacteria, and particularly members from the *Xanthomonas* genus. We showed that unique RPMs consisting of random 20-mer combinations of L-phenylalanine and L/D-lysine (FK-20 and FdK-20, respectively) displayed powerful bactericidal activities towards *Xanthomonas perforans* and *Xanthomonas campestris* pv. *campestris*, *in vitro* and *in planta*. Here we report characterization of short lipo-RPMs, which resulted from N-palmitoylation of 5-mer RPMs. Lipo-RPMs containing palmitic acid, L-phenylalanine and D-lysine (p-FdK5) were shown to possess high antibacterial activity against several bacterial strains and reduced disease severity caused by *X. perforans* on tomato. We synthesized and studied all 32 (2<sup>5</sup>) possible lipopeptides that compose the p-FdK5 mixture. Detailed characterization of the individual lipopeptides revealed that their antibacterial activity depends on the peptide hydrophobicity and on the location of the hydrophobic amino acids in the molecule. Remarkably, bactericidal assays with combinations of several lipopeptides revealed the occurrence of synergistic interactions. Microscope observations of tomato leaves treated with fluorescently-labelled p-FdK5 revealed that the lipopeptides do not seem to penetrate the leaf supporting a protectant mode of action for these compounds. Overall our findings support that RPMs and lipo-RPMs have the potential to contribute to management of bacterial plant diseases.

# Identification of novel bacteriophages of *Xanthomonas campestris* pv. *campestris* isolated from cabbage

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Keywords: *Xanthomonas capestris* pv. *campestris*, lytic, bacteriophage, genome sequence, biocontrol

Bacterium *Xanthomonas campestris* pv. *campestris* (Xcc) (Pammel) Dowson is a causal agent of black rot disease, the most destructive disease of the family *Brassicaceae*. Since this pathogen is developing resistance against protective chemical compounds, the study of biocontrol agents like bacteriophages has become attractive again. During the last three years, we monitored the incidence of this disease in more than 30 cabbage fields of the Czech Republic what brought several new natural Xcc strains to our collection. The symptomatic cabbage heads from infected fields were used for isolation of specific lytic bacteriophages which were primary tested against Xcc strains from NCPPB (GB) and against natural isolates collected from the infected fields. Promising bacteriophages were subsequently identified at morphological level by Transmission electron microscopy and by molecular level with whole genome sequencing. Contig annotations, pairwise genome comparison, and phylogenetic analyses allowed us to describe five novel bacteriophages. This experiment was focused on finding and describing novel lytic bacteriophages and shows their relevance in biocontrol in the conditions of Central Europe.



## Encapsulation of phages in liposomes: enhancing their efficacy to control bacterial walnut blight in field

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Keywords: bacteriophage, bacterial walnut blight, liposome, walnut, disease management

The practical use of phages to control bacterial plant diseases requires phages that are more stable and have longer periods of residence in the phyllosphere. Phage encapsulation in liposomes will overcome some of the challenges in developing efficient phage application. Liposomes can serve to protect phages from the harmful effect of UV, thus increasing the span of phage survival in the phyllosphere. Encapsulation of phage and its slow release can help to ensure that the phage concentration in the phyllosphere remains at an effective level over a realistic period. In this experiment, 10 novel phages lysing *Xantomonas arboricola* pv. *juglandis*, the causal agent of the bacterial blight of walnut, were isolated from walnut fruits, leaves, soil and irrigation water in northern Italy. Characterization of phages with restriction endonuclease digestion showed that there are three different phage groups, defined as groups 1, 2, and 3. An analysis of the host range of phages involving 25 different *X. arboricola* pv. *juglandis* strains showed that, despite all isolated phages were specific to *X. arboricola* pv. *juglandis*, the phages can be differentiated into four classes depending on their ability to lyse the 25 strains considered. Four phages were inactivated after 10 minutes of exposure at 68 °C, while six phages were inactivated at 72 °C. All phages survived at a pH range of 5 to 11. When encapsulated and non-encapsulated phages were exposed to UV light at  $\lambda = 254$  nm, 40 % of the encapsulated phages were still detectable in the samples, whereas the non-encapsulated phages were present in only 6 %. Ultimately, our field study showed that encapsulation is an effective technique for improving the bacteriophage stability. To our knowledge, this is the first study that allows bacteriophages to be used in the field by means of liposome technology.

# POSTERS

# ePoster Session 1

## Diagnostics & Diversity – Population Structure

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## Bioinformatic pipelines are determinant in the analysis of microbial communities from different ecological niches in cultivated olive trees

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Olive tree is one of the most important crops in the Mediterranean Basin. However, nowadays its viability is seriously threatened by plant pathogens such as *Verticillium dahliae* and *Xylella fastidiosa* which colonize the xylem vascular bundles and ultimately can cause the death of the olive tree. Recent studies indicated that plant-associated microbial communities play an important role in controlling vascular wilt diseases and could form the basis of sustainable biocontrol strategies for crop production. NGS approaches with advances in bioinformatics and statistical analyses represent valuable tools for characterizing the diversity of these plant-colonizing microorganisms which will lead to a better understanding of their interactions. In this work, we deciphered the bacterial and fungal microbial communities from different olive ecological niches (soil, rhizosphere, root, xylem sap, stem, leaf and fruits) using distinct bioinformatics pipelines based on the identification of operational taxonomic units (OTUs) or amplicon sequence variants (ASVs). Sequence analysis reported a greater number of taxa using ASV-based pipeline (5.891 for bacteria and 3.055 for fungi) in contrast to the OTU-based pipeline (1.269 and 553, for bacteria and fungi, respectively). Rhizosphere was the plant niche with the highest number of ASV for bacteria (5.662), whereas soil was the niche showing highest number when using the OTU pipeline (730). Interestingly, for the xylem, we estimated 689 and 2647 ASV for bacteria and fungi, respectively; whereas a much lower of OTUs was determined (i.e., 205 and 101, for bacteria and fungi, respectively). Our results showed that bioinformatic pipelines may affect significantly the characterization of the plant-associated microbiome and highlight the importance of standardizing the computational methods for downstream analysis of NGS data, especially when searching for potential microbial taxa associated to suppression of vascular plant pathogens.

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## Space of spacers: what CRISPR loci can tell us about evolution of xanthomonads

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Keywords: Database, evolution, molecular typing, spoligotyping, *Xanthomonas*

CRISPR/Cas systems are genetically adaptive defence systems against alien nucleic acids, which have proven extremely valuable for molecular typing of bacteria thanks to the imprint of a historical signal in the evolution of the locus. Two CRISPR/Cas systems have been identified in xanthomonads, the presence of which is conserved at the infraspecific level. Progress on the development of bioinformatic resources for the analysis of CRISPR loci from xanthomonads will be presented and examples giving insight into the evolution of *Xanthomonas citri*, *Xanthomonas vasicola* and *Xanthomonas translucens* will be discussed.

## **Specific DNA markers for detection of *Xanthomonas gardneri* based on analysis of 2500 genomes within 30 minutes**

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Keywords: Genome analysis, primer design, k-mer, KEC, xanthomonads

In this work, we found 47 specific DNA sequences, that can be used as novel markers for development of molecular (PCR, LAMP etc.) based assays for detection of *Xanthomonas gardneri*. The sequences were found using specific tool – KEC (K-mer elimination by cross-reference), which allowed using of all *Xanthomonas* genomic sequences currently available in NCBI (2732) as nontarget and produced potential DNA marker specific for *X. gardneri*. The whole process only required average office laptop computer and took less than 30 minutes to obtain first results. The KEC is freely available for all major operating systems (Windows, Linux, Mac) at <https://github.com/berybox/KEC>.

# Genetic and phenotypic characterization of *Xanthomonas* species pathogenic of wheat in Uruguay

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**Keywords:** multilocus sequence analysis and typing, bacterial leaf streak, wheat, Uruguay

Bacterial diseases affecting wheat production worldwide have been an issue of growing concern in the last decade. Nevertheless, the main bacterial diseases of wheat in Uruguay remain largely uninvestigated. To identify bacterial pathogens associated with diseased wheat fields, 61 fields were surveyed from 2017 to 2019 in western Uruguay, yielding a collection of strains identified as *Xanthomonas* spp. by 16S rDNA sequencing. These strains were further characterized via multilocus sequence analysis (MLSA) and typing (MLST), as well as in planta pathogenicity assays. MLSA grouped 44 strains with reference strains for *Xanthomonas translucens* pv. *undulosa*, the pathovar predominantly associated with bacterial leaf streak of wheat (BLS). To evaluate the genetic diversity among strains, MLST was applied, revealing a low diversity among Uruguayan strains identified as *X. translucens* pv. *undulosa*. In addition, 17 strains in the collection were assigned to a separate clade distant from the *X. translucens* species, grouping together with previously unreported *Xanthomonas* strains isolated from wheat in Minnesota, USA. In planta pathogenicity assays were performed on BLS susceptible wheat seedlings. The 44 *X. translucens* pv. *undulosa* strains caused greasy, dark brown necrosis symptoms typical of BLS, while the 17 non-*translucens* *Xanthomonas* sp. strains caused distinctly different dry tan necrosis symptoms. These results reveal that the main bacterial pathogen affecting wheat crops in Uruguay is *X. translucens* pv. *undulosa*, the causal agent of BLS; and that other pathogenic *Xanthomonas* species are associated with wheat crops in South and North America.

## Assessment of *Xanthomonas* spp. pathogen variability in Cuban common beans

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Keywords: common bacteria blight, phylogenic characterization, *Phaseolus vulgaris* L.

Common bacterial blight (CBB) caused by *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citric* subsp. *fuscans* (*Xcf*), is a serious seed-transmitted disease in both temperate and tropical bean production zones causing crop yield declines of up to 40%. The phylogenic characterization of *Xanthomonas* spp. isolates from different provinces producing common beans in Cuba was the main goal of this work. Samples of leaves and pods from naturally infected plants were collected and processed to isolate and identify pathogenic bacteria. Isolation was performed in culture media (YDCA and XCP1) and the pathogen was characterized by Polymerase Chain Reaction (PCR) amplification and sequencing of housekeeping genes. In addition, the pathogenicity of these isolates was evaluated in the susceptible cultivar *Bat-304*, as well as in resistant and susceptible genotypes from Canada. The DNA fragments obtained by PCR with housekeeping, gene-specific, primers and isolate DNA samples had fragment sizes that corresponded to the sizes reported for these *Xanthomonas* species. The molecular and pathogenic characterizations revealed a high level of variability among the isolates that were isolated and identified. Therefore, the results indicate that there is a high level of diversity among the *Xanthomonas* spp. isolated from common bean production areas in Cuba, but additional work will be required to determine the molecular determinants of pathogenic variability.



## Symptoms manifestation of the *Xanthomonas hortorum* pv. *carotae* on selected representatives of the genus *Apiaceae*

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Keywords: occurrence screening of pathogen; natural isolate; *Xanthomonas hortorum* pv. *carotae*

The bacterial leaf blight of carrot is one of the most serious bacterial diseases in the production of vegetables of the *Apiaceae* family. The causal pathogen, *Xanthomonas hortorum* pv. *carotae*, is able to be transmitted by seeds, but the spread can be increased for example by the subsequent mechanized technology of cultivation. Infection often has an asymptomatic course, but its development is strongly dependent on climatic conditions and the type of host plant. In this study, an epidemiological survey was carried out in the Czech Republic in 2019 and 2020. Dependence of the disease manifestation on climatic conditions and the type of host was confirmed, two natural isolates derived from carrots and parsley were also obtained. Subsequently, phytopathological tests by using of these two newly obtained isolates and registered isolate Xhc NCPPB 4410 were performed on seedlings of carrots, parsley, celery and lovage. Obtained results showed low pathogenicity for plant species that correspond to isolate origin, although live bacteria in seedling plants have been confirmed by both culture and molecular techniques. In contrast, permanent lovage culture showed strong manifestations of symptoms after inoculation by natural isolates and weak manifestation after inoculation by registered Xhc NCPPB 4410 isolate.

## A new *Xanthomonas* species pathogenic on *Hydrangea*?

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**Keywords:** *Xanthomonas hortorum* complex, genomics, pathogenicity, early detection, on-site diagnostics

During the past years, xanthomonads were frequently isolated from brown leaf spots on container-grown hydrangea (*Hydrangea arborescens* and *H. quercifolia*) in commercial nurseries in Flanders, Belgium. The isolates were catalase positive, oxidase negative and produced hypersensitive necrosis in tomato and sedum leaves, but not in leaves of tobacco (Xanthi NN genotype). Pathogenicity was confirmed by spray inoculating young plants with a suspension of 10<sup>8</sup> CFU/ml of phosphate buffer prepared from *Pseudomonas* Agar F cultures. The bacteria were readily reisolated from the inoculated plants and identified as *Xanthomonas hortorum* spp. using Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS). Based on MultiLocus Sequence Analysis (MLSA) of seven housekeeping genes (*gyrB*, *rpoD*, *dnaK*, *lepA*, *efp*, *atpD* and *glnA*; 4,703 bp), these strains form a separate cluster within the *X. hortorum* complex. Phenotypic typing was done using BIOLOG GENIII microplates. Representative strains were genome sequenced using short (MiSeq, Illumina) and long-reads (MinION, Oxford Nanopore) technologies. Average Nucleotide Identity (ANI, fastANI) and *in silico* DNA-DNA hybridization (*is*DDH, GBDP) with known *X. hortorum* strains ranged between 94.35 to 95.19 % and from 55.70 to 59.40 %, respectively. Most of the ANI values and all the *is*DDH values fall below their respective species delineation thresholds (95 % ANI, 70 % *is*DDH), suggesting they form a distinct species within the complex. Furthermore, we have developed a loop-mediated isothermal amplification (LAMP) diagnostics assay based on a singleton approach to best prevent and manage these pathogens in ornamentals and in agricultural crops.

## Persistence of *Xanthomonas campestris* pv. *campestris* in Field Soil in Central Europe

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**Keywords:** brassicas, *Xanthomonas campestris* pv. *campestris*, persistence, soil, detection, PCR, nested real-time PCR

*Xanthomonas campestris* pv. *campestris* (Xcc) is a bacterium that causes black rot of crucifers. The greatest losses of brassica crop production usually result from seed-borne infection, but carry-over of inoculum in field soil may also be possible. The aim of this study was to monitor persistence of Xcc in field soil in central Europe using a conventional PCR assay with hrpF primers and a two-step nested real-time PCR assay using Zur primers. The work has demonstrated that nested real-time PCR can be used to improve the analytical sensitivity for detection of Xcc in soil compared to conventional PCR, and that Xcc may persist in soil for up to two years following an infected brassica crop in central European climatic conditions.

## **An experience of PCR methods implementation for a bacterial blight of *bean Xanthomonas axonopodis* pv. *phaseoli* detection in a seed and plant material of legumes**

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Keywords: bacterial blight of bean, diagnostics, bacteria extraction, PCR

Bacterioses of leguminous crops cause significant damage to the agricultural production in the Russian Federation and its neighboring countries. Today, the growing volume of exports is an urgent issue, while legume yield should meet the phytosanitary requirements of importing countries, including the absence of bacteriosis pathogens. The bacterial blight of bean pathogen *Xanthomonas axonopodis* pv. *phaseoli* is the main phytopathogen of the Common Bean. The causative agent of the bacterial blight of bean is preserved on the surface and inside the seeds, on infected plant residues, on the surface of host plants. Having penetrated the plant, bacteria multiply rapidly in the intercellular space. Causative agent of the bacterial blight of bean affects many plant species of the genus *Phaseolus*. The purpose of this assay is to determine PCR methods for the causative agent of bacterial blight of bean diagnosis using commercial kits officially accepted in the Russian Federation. In the assay, preparing samples and extracting bacteria from plant and seed material methods are proposed, and optimal molecular detecting, identifying and isolating methods are chosen. The plant material was received during the crop examination of the leguminous crops in the Russian Federation. CFBP 2534 reference strain from the CFBP was used as a control sample. Highly sensitive phytopathogen extraction methods were selected. Analytical specificity and analytical sensitivity were obtained for all primer systems. After full testing, the proposed technique can be used in the diagnostic laboratories work for the bacterial blight of bean phytopathogen detection in both plant and seed materials.

# Complete genome sequences and characterization of *Xanthomonas arboricola*, the causal agent of bacterial leaf blight of blueberry

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Keywords: *Xanthomonas arboricola*, MLSA blueberry, genome

In 2013, on the blueberry (*Vaccinium corymbosum*) 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. 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*. Additional analysis to determine if they constitute a new taxon within *X. arboricola* are being conducted.

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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## Comparative genomics to understand host range in *Xanthomonas translucens*

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**Keywords:** comparative genomics, host range, *Xanthomonas translucens*, forage grasses, cereals

The *Xanthomonas translucens* species comprises phytopathogenic bacteria that can cause serious damage small grain cereals and grasses. Based on host range, three major groups can be distinguished: the “*translucens*” group causing blight and/or leaf streak in cereals (pvs. *hordei*, *secalis*, *translucens* and *undulosa*), the “*graminis*” group causing bacterial wilt in forage grasses (pvs. *arrhenateri*, *graminis*, *phlei*, *phleipratensis* and *poae*), and pv. *cerealis* that can infect both groups of plants. In order to gain insight into genomic regions responsible for the different host ranges of the individual pathovars, we obtained complete genome sequences of 18 *X. translucens* strains. In most strains, long-read PacBio sequencing yielded single scaffolds with an average assembly length of ~4.7 Mb and ~4100 genes. Average nucleotide identity (ANI) ranged from 95 to 100 %, with the confirmed *X.t.* pv. *graminis* strains showing nearly 100 % identity. Cluster analysis clearly separated the “*translucens*” and the “*graminis*” groups with further subdivision particularly in the “*graminis*” group. These results are setting the stage for comparative genomic approaches to identify candidate genes for host-range determination.

# ePoster Session 2

## Diagnostics & Diversity – Population Structure

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## **An update of the situation of *Xylella fastidiosa* in plants and vectors in France**

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**Keywords:** *Xylella fastidiosa*, host plant, vector, MLST, ddPCR

*Xylella fastidiosa* (*X. fastidiosa*) is a xylem-limited bacterium native from America and classified as quarantine bacterium for EU regulation. Long-distance bacterial dispersal depends mainly on the human-mediated movement of infected plants and propagating material, while naturally spread on short distances is made by xylem sap-feeding insects. One of the most effective vector of *X. fastidiosa* identified in Europe is *Philaenus spumarius*.

Since 2015, the bacterium has been identified in natural conditions in France in Corsica and Provence-Alpes-Côte d'Azur (PACA) regions, which have a Mediterranean climate. Recently, in 2020, *X. fastidiosa* subsp. *multiplex* was detected in a new region in the south of France. In France, nearly 50 host plants have been identified to date as being infected by *X. fastidiosa* subsp. *multiplex* ST6 and/or ST7 and two host plants by *X. fastidiosa* subsp. *pauca* ST53. In insects, *X. fastidiosa* subsp. *multiplex* ST6 or ST7 were detected in the same location than plants from PACA and Corsica.

The aim of this presentation is to give an update of the situation of *X. fastidiosa*-contamination in France based on the compilation of national field monitoring data, and to present detection and identification of *X. fastidiosa* in plants and vectors using various techniques (real-time PCR, MultiLocus Sequence Typing (MLST), droplet digital PCR (ddPCR)).



## Dispersion of the bacterium *Xylella fastidiosa* in Portugal

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**Keywords:** phytopathogens, forest plant health, xylem-limited bacteria, *Xylella fastidiosa*, *multiplex*

Phytosanitary problems caused by biotic agents, originate physiological imbalances, which lead to loss of vitality and productivity. This increases costs for the control of those agents, impacting the integrated management programs and causing environmental damage due to the frequent use of chemical products. The proclamation of 2020 as the International Year of Plant Health meets precisely the concerns mentioned.

The gram-negative bacterium, *Xylella fastidiosa*, is characterized by its rod shape, with slow growth in xylemic vessels. It has great genetic plasticity, with four subspecies currently recognized and studied. It is considered one of the greatest threats to plant health worldwide.

After the first detection of the bacteria, in Portugal, on January, 2019, was delimited a "Demarcated Area" (comprising the "Infected Area", including all plants that are within a radius of 100m around the contaminated plants, and a "buffer zone" surrounding a 5 km radius) and develop an "Action Plan" to control the pathogen.

The objective is to understand the current dimension of the problem worldwide, in particular, the current study case (Portugal), analysing the state-of-the-art on the knowledge for this bacterium.

This study was developed between January 2019 and June 2020, involved a total of 2261 samples collected.

It was possible to determine that the two initial outbreaks were not an isolated case, as 107 additional outbreaks were detected, revealing a much more worrying panorama of 62000ha of Demarcated Area, which needs further analysis on the real impact of this bacterial strain on the natural environment.

## **Etiology of bacterial leaf spot of arugula in Serbia**

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**Keywords:** *Xanthomonas campestris* pv. *campestris*, arugula, first report, etiology, identification

Brown, necrotic spots, mainly located at the leaf edge, surrounded by weak halo, were noticed on arugula (*Eruca vesicaria* subsp. *sativa* L.) leaves originating from a farm near Belgrade, in early spring of 2019. Creamy yellow, shiny, round, convex colonies, 1 mm in diameter, developed on nutrient agar plates 72 h after isolation. Six strains were further transferred on yeast-extract-dextrose CaCO<sub>3</sub> medium where they formed large, mucoid and shiny yellow colonies. All strains were Gram negative, caused hypersensitive reaction in tobacco leaves, and possessed bacteriological characteristics typical for *Xanthomonas campestris* pv. *campestris*. In order to check pathogenicity of the strains, three-week old arugula plants were inoculated by spraying with bacterial suspension (approx. 10<sup>7</sup> CFU/ml in SDW) with a hand-held sprayer. Inoculated plants were covered with plastic bags to maintain high humidity for 48h, and were kept in a greenhouse. A week later, chlorotic spots, spreading from the leaf edge toward the central vein were observed on leaves of inoculated plants. Blackening of secondary veins appeared within the collapsed leaf tissue. Molecular identification, amplification and sequencing of *gyrB* gene of four representative strains were performed by using primer sets described by Parkinson et al. (1). BLAST analysis showed that DNA sequences of those strains (GenBank acc. nos. MW508894-MW508897) shared 100 % of *gyrB* sequence identity with *X. campestris* pv. *campestris* from different geographical regions. This report indicates that this minor crop could play an important role in the brassica black rot epidemiology in Serbia.

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## Characterization of *Xanthomonas arboricola* pv. *juglandis* strains isolated from brown apical necrosis (BAN) and bacterial blight of walnut by rep PCR

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**Keywords:** *Xanthomonas arboricola* pv. *juglandis*, brown apical necrosis (BAN), rep-PCR

There are two major disease problems of walnut orchards in Turkey such as walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj), and brown apical necrosis (BAN). Brown apical necrosis (BAN) on walnut fruits caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj) in association with some fungi has started to premature walnut fruit drop. Copper based compounds have been the only means of control for XAJ more than 40 years. Data indicates that copper resistant strains of the walnut blight pathogen are not killed by standard copper applications under field conditions. In this study, XAJ strains were tested in terms of virulence and copper tolerance. According to the results of *in vitro* tests, it was determined that the most tolerant XAJ strains to copper obtained 0.8 mM to 1 mM MIC values and the most sensitive XAJ strains to copper obtained 0.1 mM MIC values. *In vitro* and *In vivo* tests results were confirmed each other when XAJ strains which were selected as tolerant and susceptible to copper depending on *in vitro* test results. However, it has been determined that the virulence of XAJ strains which showed tolerance to copper was low. Rep-PCR method was used for characterization of Xaj isolates from BAN and bacterial blight of walnut. Strains from BAN showed difference from Xaj strains from bacterial blight by rep-PCR. Similarly, copper tolerant strains showed difference from others.

## Integration of virulence factor *hpaP* in detection of bacterial blight of *Apiaceae* plants

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*Xanthomonas hortorum* pv. *carotae* (Xhc) is a serious seedborne pathogen causing bacterial leaf blight on *Apiaceae* plants. In 2019 and 2020 the screening of Xhc occurrence within Czech Republic was performed on 40 localities. Symptomatic and asymptomatic leaves of carrot, parsley, celery and lovage were sampled and processed for detection by dilution plating assay on semi-selective media according to ISTA (2019). Identification of bacterial isolates was carried out by species-specific PCR assay (Meng et al. 2004), however, only few isolates were detected. Sequencing of a partial 16S rRNA (Klindworth et al. 2013), *dnaK* and *rpoD* (Fargier and Manceau 2007) genes confirmed the identity of the obtained isolates as genus *Xanthomonas*. Based on the whole-genome sequence of Xhc strain acc. no. 863365 (GenBank/NCBI), the qPCR (SYBR) assay targeting the *hpaP* virulence factor was designed. This assay positively detected the most of the tested isolates. The obtained partial nucleotide sequences of *hpaP* gene revealed 88-100 % identity sharing with the *hpaP* gene of Xhc strain M081 thus the new approach seems to better reflects the variability of Xhc isolates than the specific marker Xhc3S used mostly as reference. Nevertheless, several of isolates were not detected through *hpaP* approach thus the virulence of bacterial isolates was further evaluated by pathogenicity assay.

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

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**Keywords:** *Xanthomonas arboricola* pv. *pruni*, peach, apricot, sweet cherry, identification

During 2017/20 we surveyed stone fruit orchards for presence of bacterial diseases in Montenegro. Sweet cherry twig cankers were observed on trees near Ulcinj, while peach and apricot leaf and fruit spot and twig necrosis were observed near Podgorica. From the samples, yellow, convex, and mucoid bacterial colonies were isolated on YDC medium. All selected strains were gram negative, HR+, 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 47 strains. Amplification and sequencing of *gyrB* gene of 14 representative strains was performed using primers described by Parkinson et al. (1). Obtained partial DNA sequences showed that 12 strains share 98.97 to 99.71 % of *gyrB* sequence identity with *Xanthomonas arboricola* pv. *pruni* (Xap) pathotype strain ICMP51. The remaining two strains showed 100 % identity with Xap strains originating from peach and apricot in Hungary and peach in Italy. Pathogenicity was tested in host plants by spraying shoots and infiltrating leaves and fruits with bacterial suspension ( $10^7$  CFU/ml in SDW) of all 47 strains and Xap reference strains CFBP3892 and NCPPB 416, 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. These results confirmed presence of *Xanthomonas arboricola* pv. *pruni* in peach, apricot and sweet cherry orchards in Montenegro.

### Reference

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## Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* strains in Africa

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Keywords: bacterial leaf blight, rice, molecular epidemiology, MLVA

Bacterial Leaf Blight (BLB) of rice which is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a major threat to rice production worldwide, leading up to 50% yield losses in Asia and Africa during severe infections. Previous results have shown that African *Xoo* are more closely related genetically to *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) and that there is no common race between African and Asian *Xoo*. While Asian strains of *Xoo*, first described in 1884 in Japan, are widely studied in various countries, the more recent discovery of the pathogen on the African continent (in the early 1980s) and the late introduction of standardized collection campaigns makes these strains less well characterized. Our team has implemented in collaboration with African partners a collection of 420 African *Xoo* strains collected from 1979 to 2018 in nine African countries. In this study, we are aiming to i) describe the genetic diversity of *Xoo* strains in Africa and, ii) discover the pathogen's origin and route(s) of invasion through the continent. Here we present our most recent results upon molecular typing of the 420 strains using Multiple Locus Variable-Number Tandem Repeat (VNTR) Analysis (MLVA). Our 14 *loci* MLVA scheme allows us to characterize the spatiotemporal structure of African *Xoo* population.

## Genome based loop mediated isothermal amplification assays for detection of *Xanthomonas arboricola* pv. *juglandis* and *X. euroxanthea*

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**Keywords:** walnut, bacterial blight, fields detection, phytosanitary controls

The bacterial blight is a major disease of walnut (*Juglans regia*). The disease is present worldwide, wherever walnut is grown and causes huge crop loss. The causal agents of the bacterial blight are *Xanthomonas arboricola* pv. *juglandis* (*Xaj*) and new species *X. euroxanthea* (*Xe*). The disease affects leaves, fruits, branches and trunks. To control these pathogens, tools using rapid and precise in-the-fields detection for these strains are needed. We present genome-based loop mediated isothermal amplification (LAMP) assays for detection and distinction of *Xaj* and *Xe*. Specificity of these assays were tested on the complex of bacterial strains of *X. arboricola* not belonging to the *juglandis* pathovar and other *Xanthomonas* strains. The detection limits were 1 pg of genomic DNA after 30 minutes of amplification. For confirmation purposes DNA from walnut fruits infected by *Xaj* was used. Capability of this assay suggest its use as a standard diagnostic tool during phytosanitary controls.

# Phytosanitary quality along the tomato and pepper production chain through an integrated management of bacterial diseases caused by xanthomonads and other bacteria

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**Keywords:** seed borne, bacterial diseases, *Xanthomonas* spp., *Clavibacter michiganensis* subsp. *michiganensis*

Kosovo has a good potential for agriculture production in terms of range of fruit and vegetable crops that can be cultivated. Pepper and tomato are amongst the main cultivated crops for Kosovo. Many diseases are reported in these crops, but few data are available about bacterial diseases. The aim of this study is to understand the real situation in Kosovo on the phytosanitary quality of pepper and tomato seeds and to propose strategies to ensure production, marketing and use of healthy seeds. This study is carried out by monitoring of vegetable production areas, interviewing public institutions, visiting agricultural pharmacies and investigating domestic production technologies. Seed samples are collected from main vegetable production area in Kosovo and the focus of our study is to check for the presence of seed borne pathogens, particularly referring to *Xanthomonas* spp. and *Clavibacter michiganensis* subsp. *michiganensis*. As an output of our study pepper and tomato production area will be monitored, in order to appropriately investigate and evaluate the impact of bacterial disease on pepper and tomato in Kosovo and possibly develop and implement disinfection method for seed treatment.



# ePoster Session 3

Pathogen Biology

Genetic Resistance – Host Defence

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## Focus on *Salicaceae* to investigate potential *Xylella fastidiosa*-based pathosystems in temperate regions

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Keywords: Salicaceae, *Xylella fastidiosa*, mechanical inoculation

Although the risk posed by *Xylella fastidiosa* to Belgium, and more extensively to northern Europe, is considered limited based on climate-suitability modelling, it should not be undervalued. *X. fastidiosa* occurrence is mostly depending on the presence of efficient host plants and insect vectors combinations. In this view, a screening by inoculation of potential hosts for Belgium, together with the study of potential vector's preferences and dispersion abilities, focused our research on the *Salicaceae*. *Salix alba*, *S. caprea*, *Populus canescens* and *P. tremula* were inoculated mechanically under biosafety conditions with the GFP-labeled strain KLN59.3 (1). Its spread and multiplication in the plants, as well as its distribution in xylem vessels, have been monitored by real-time PCR and by confocal and scanning electron microscopy. Bacteria have also been re-isolated to fulfill Koch's postulate. First results indicate that some species have the potential to act as efficient hosts, asymptotically or symptomatically. Our work shows how *Salicaceae* could serve as stepping stones to facilitate spread of *X. fastidiosa*. Appropriate risk reduction options that can be rapidly deployed in case of detection will be discussed.

### Reference

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## Characterization of ZapA and ZapB from *Xanthomonas citri* subsp. *citri* in cell division

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Keywords: virulence, Asian citrus canker, citriculture, phytopathogen, mutagenesis

Asian citrus canker (ACC) is one of the main diseases that infects citrus and is caused by the bacterium *Xanthomonas citri* subsp. *citri* (*X. citri*). Therefore, it is important to study essential mechanisms, such as cell division, to develop alternative compounds for combating ACC. In this work, we characterize two accessory proteins, ZapA and ZapB, encoded by this pathogen. We investigated its functions by evaluating the *X. citri* mutant phenotype with *zapB* (XAC\_RS17260) and *zapA* (XAC\_RS17265) deletion. Our results showed that the deletion of the *zapB* gene caused the formation of chains and increased cell length, which indicates that this gene participates in the division in *X. citri*. In addition, the deletion of the *zapA* gene did not cause changes in the cell phenotype. Both absence of ZapA and ZapB did not alter cell viability, indicating that these proteins are not essential for the survival of *X. citri*. The expression of the GFP-ZapB fusion in  $\Delta zapA$  cells showed that recruitment of the ZapB protein to the *X. citri*-dividing septum is ZapA-dependent. Regarding virulence, the absence of these proteins did not impair the ability of *X. citri* to infect host plants. This work showed that the proteins ZapA and ZapB in *X. citri* participate in the processes of cell division and contributes for a better understanding of this process in this phytopathogen.

## **A possible role of EPS structure in climate adaptation of *Xanthomonas campestris***

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Keywords: LPS, *Xanthomonas campestris*, temperature adaptation, genome analysis

Black rot of brassicas (caus. agent *Xanthomonas campestris* pv. *campestris* – Xcc) is one of the most devastating diseases of vegetables. Previous comparison of Xcc strains collected before 1998 demonstrated distinct reactions for strains recovered from plants of subtropical (group 1) and temperate climatic zones (group 2) against antibodies reacting with LPS antigens (~77-78 kDa) (Ignatov et al., 1998). The clear difference in LPS antigenic structure was associated with two types of LPS gene clusters represented by Xcc strains B100/3811 (group 1) and ATCC33913/8004 (group 2). The LPS gene cluster of Xcc group 1 is significantly different from the LPS cluster present in group 2, and the last one has evidence of horizontal gene transfer (Patil et al., 2007). LPS locus of B100 was similar in gene organization and content, to that of *X. vesicatoria* strain 85-10, a pathogen of solanaceous plants, normally grown at higher temperature compared to brassicas. Better adaptation and higher virulence of Xcc group 1 at higher temperature was found in a series of artificial inoculation experiments. In contrast, Xcc group 2 strains had better survival rate in infected plants at winter time. A direct mechanism for LPS modifications resulting in better bacterial membrane adaptation and virulence-state adaptation was demonstrated before (Li et al., 2012). It is possible to speculate that the LPS structure of Xcc plays an important role in climate adaptation of Xcc sub-populations.

## ***Xanthomonas translucens* pv. *undulosa* identified on non-wheat crops and weedy grasses in Minnesota, United States: describing an expanded host range**

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**Keywords:** Bacterial leaf streak, *Xanthomonas translucens*, weed hosts, multilocus sequence analysis

Bacterial leaf streak (BLS) of wheat, caused by *Xanthomonas translucens* pv. *undulosa*, has been identified in wheat growing regions worldwide and is now recognized as a major disease of wheat in the Upper Great Plains of the United States. In addition to wheat, other known hosts of *X. translucens* pv. *undulosa* include barley, rye, triticale and the perennial grasses smooth brome (*Bromus inermis*) and quackgrass (*Elymus repens*). In our study, six species of weedy grasses growing near wheat fields and two economically important poaceous crops, intermediate wheatgrass (*Thinopyrum intermedium*) and cultivated wild rice (*Zizania palustris*), were surveyed in Minnesota with symptomatic leaf tissue collected. Multilocus sequence analysis (MLSA) of four housekeeping genes (*rpoD*, *dnaK*, *fyuA*, and *gyrB*) was used, in corroboration with in planta assays, to identify strains isolated from weedy grasses and non-wheat crops. The MLSA phylogeny predicted all strains from five of six weedy grass species, intermediate wheatgrass, and cultivated wild rice to be *X. translucens* pv. *undulosa*. Strains isolated from smooth brome were determined to be *X. translucens* pv. *cerealis*. The in planta character states supported the pathovar identification, with all of these strains having an identical host response profile to previously described *X. translucens* pv. *undulosa* strains, including the pathotype strain LMG 892<sup>PT</sup>. This study identifies seven host species, including both weedy grasses and non-wheat small grain crops, that can harbor *X. translucens* pv. *undulosa*. This work expands our understanding of the host range for this pathogen.

## **Alterations in essential regulatory two-component system of *phoPQ* is associated with cell division, cell morphology and virulence of *Xanthomonas citri* subsp. *citri***

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Keywords: citrus canker, pathogenicity, avirulence

Brazil is the largest producer of sweet oranges in the world, with most of the production destined for juice. Type-A citrus canker is one of the most concerning bacterial diseases for citrus in Brazil and it is caused by the bacterium *Xanthomonas citri* subsp. *citri* (*X. citri*), in which the two-component system PhoPQ represents a typical signal transduction system. In this work, we show that the deletion of the *phoP* gene in *X. citri* 306 caused drastic changes in cell morphology including presence of cell chains and filaments. Regarding subcellular location of the GFP-ZapA protein in the mutant strain  $\Delta phoP$ , we verified that the formation of the Z-ring became anomalous. Nucleoid analysis (DAPI staining) showed absence of genetic material between the septa of the cell chains. These results suggest that the *phoP* gene is directly or indirectly related to cell division processes. In addition, the deletion of the gene made the bacterium less mobile and decreased the production of biofilm leading to a decrease in the pathogenicity of *X. citri*. We conclude that the *phoP* gene could be a target for the control of citrus canker.

## CRISPR interference (CRISPRi), a powerful tool to study the function of gene families in *Xanthomonas*

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**Keywords:** Cassava Bacterial Blight, Bacterial Leaf Blight of rice, silencing, Transcription Activator-Like Effector (TALE), *Xanthomonas phaseoli* pv. *manihotis*, *Xanthomonas oryzae* pv. *oryzae*

Understanding the molecular drivers underlying plant-pathogen interactions is critical for disease resistant crop breeding. This usually requires the manipulation of the host and pathogen genomes. Recently, CRISPR technology has improved genome editing by dramatically increasing efficiency and specificity. However, bacteria are not easily mutated using this technology. A technological variation called CRISPRi employs a Cas9 version that is unable to cut DNA (dead Cas9 – dCas9) but preserves sequence-specific DNA binding. dCas9 interferes with gene transcription when targeted against the promoter or the first nucleotides of the coding sequence, thus resulting in gene knock down. This tool can be extremely useful for the functional analysis of multigene families since a single guide RNA (sgRNA) may knock down several targets at once. As a proof of concept, we aimed at silencing genes of the Transcription Activator-Like Effector (TALE) family, which encode major virulence factors in several *Xanthomonas* species. CRISPRi was implemented through the design of sgRNAs targeting the -10 and -35 promoter elements, the Ribosome Binding Sequence, and a region within the first 50 nucleotides of the coding sequence of *TALEs* of several *Xanthomonas* species. We show that most if not all *TALEs* in a strain are silenced by one individual sgRNA, resulting in decreased bacterial titers in planta and host susceptibility. Furthermore, plasmid-born *TALEs* lacking the sgRNA-targeted sequence can be expressed and used for complementation. Our study provides a useful system to assess the role of *TALEs* during host colonization, and overall, for the functional analysis of multigene families in bacteria.

## **PthA4<sup>AT</sup>, a small TAL effector from *Xanthomonas citri* subsp. *citri* induces immunity in *Nicotiana benthamiana***

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Transcription-activator-like effectors (TALE) are secreted by *Xanthomonas*, acting as eukaryotic transcription factors that can target both susceptibility or resistant genes in the plant cells. They recognize specific DNA sequences in the host promoters through a domain consisting of a variable number of ~34 amino acid repeats following a well characterised code. The main virulence factor of *Xanthomonas citri* subsp. *citri* (*X. citri*), the bacteria causing citrus canker, is PthA4, a 17.5-repeats TAL effector activating susceptibility genes in *Citrus*. Recently, a natural variant of *X. citri* triggering a hypersensitive response (HR) in *C. limon* and *C. sinensis* was isolated, and the effector triggering this HR was shown to be a 7.5-repeat PthA4-derivative TALE (PthA4<sup>AT</sup>), likely activating a resistance gene on these citrus hosts.

Interestingly, PthA4<sup>AT</sup> also causes HR in the non-host plant *Nicotiana benthamiana*. In this study, we have characterised the defense response leading to the HR deployed by *Nicotiana* when challenged with this effector. Moreover, we show how this defense response prevents *Nicotiana* from further infections by virus and bacteria, raising the possibility to use it as a biological control. Finally, artificial TALE effector technology combined with transcriptomic data has allowed us to refine PthA4<sup>AT</sup> targets in the plant cell as an initial step towards the identification of the *Nicotiana* resistance gene responsible for this HR.



## Functional characterization of the IR64 rice variety resistance towards African *Xanthomonas oryzae* pv. *oryzae*

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Keywords: *Xanthomonas oryzae* pv. *oryzae*, Recombinant Inbred Lines, RNAseq, TAL effectors, African strains

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the causal agent of Bacterial Leaf Blight (BLB), a devastating disease of rice. Among the cocktail of effectors secreted by *Xoo*, the Transcription Activator-Like Effector (TALE) family plays a critical role in the interaction. Some of these TALEs are major virulence effectors that target susceptibility (*S*) genes, the overexpression of which contributes to disease development. In some incompatible interactions, TALEs can induce the expression of so-called executor (*E*) genes leading to a resistance reaction that blocks disease development. To date four *E* genes were cloned in rice but none of them are adapted to control African *Xoo*. With respect to the importance of TALEs in the *Xoo*-Rice pathosystem, we aimed at identifying resistance sources mediated by African TALEs. To this end, a population of 180 Recombinant Inbred Lines (RIL) of F11 generation derived from a cross between the resistant rice variety IR64 and the susceptible one Azucena, was screened with the two reference African *Xoo* strains, BAI3 and MAI1, in order to identify quantitative trait loci (QTL) responding to African strains of *Xoo*. Several QTLs were identified in IR64 and preliminary results show that one of them responds specifically to a TALE present in both African strains. To go further on this lead, a transcriptome analysis was carried out with a strain mutated for the candidate avirulent TALE in order to sort out differentially expressed genes in comparison to the wild type strain and identify candidates that may act as *E* genes.

## Visualization and tracking *Xanthomonas campestris* pv. *campestris* in cabbage plants by fluorescence *in situ* hybridization

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**Keywords:** fluorescence *in situ* hybridization, *Xanthomonas campestris* pv. *campestris*, confocal laser scanning microscopy

Methods based on fluorescence *in situ* hybridization (FISH) are widely used for visualization and cultivation-free detection methods of microorganisms in plant tissues, seeds or insect vector bodies. The aim of this study was to visualize the black rot disease development in time by the estimation of the colonization of cabbage plant tissue by *Xanthomonas campestris* pv. *campestris* (Xcc) bacterium after artificial inoculation. Three cabbage cultivars with different susceptibility to black rot; very susceptible mediate tolerant and tolerant; were used in the experiment. Plant tissues were sampled at 3, 6, 9 and 14 days post-infection from several parts of plant: i) stalk under the inoculated leaf, ii) stem of inoculated leaf, iii) inoculated leaf and iv) leaf younger than the inoculated one. After the fixation and dehydration, plant tissues were hybridized using double-labeled EUB338 I-III probes, *Xanthomonas* specific probe and negative control probe. The presence of bacteria in samples were confirmed by confocal laser scanning microscopy (CLSM). The observation implies higher Xcc colonization of stalk in susceptible cultivar. To confirm the presence or absence of Xcc in plant tissues, the real-time PCR approach was used. According to results of both FISH and real-time PCR, differences in disease development within three cultivars and various sampling points were defined.

# ePoster Session 4

## Disease Management – Vector Control

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# Synthesis, characterisation and efficacy of chitosan-stabilised silver nanoparticles against *Xanthomonas vesicatoria*, the causal agent of tomato bacterial spot

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Keywords: nanoparticle, silver nanoparticle, disease management, *Xanthomonas vesicatoria*, tomato bacterial spot

Silver nanoparticles (AgNPs) gained increased interest, since silver may penetrate into bacterial cells inducing cell death. Several factors during their production (pH, temperature, reaction time and the method used for synthesis) greatly influence the quality of nanoparticles, thus limiting or enhancing their applications. We aimed to: 1) understand the effect on AgNPs synthesis of two different methods (heating or injecting the precursor solution) during two different exposure times (12 or 15 h); 2) characterise synthesised nanoparticles and 3) assess the *in vitro* efficacy of nanoparticles against *Xanthomonas vesicatoria*. In our study, AgNPs were synthesised by chemical reductions, using chitosan as a capping agent that stabilises the synthesised nanoparticles. As a precursor of Ag, silver nitrate was added. The size and shape of silver nanoparticles were characterised by Transmission Electron Microscopy (TEM). Confirmation of the atomic species was done using the Energy Dispersive X-ray Spectroscopy (EDS). TEM micrographs demonstrated that we synthesised monodispersed, cubic shaped AgNPs, ranging from 5 to 80 nm. *In vitro* experiments showed a marked antibacterial activity of AgNPs against *X. vesicatoria*, better than copper sulphate. The observed minimum inhibitory concentration (MIC) of nanoparticles was 15 and 20 µg/ml, when they were obtained by the injection method, with 12 and 15 h reduction time respectively. Alternatively, when the precursor solution was heated and exposed to 15 h reduction time, nanoparticles agglomerated, thus reducing their activity. Our study showed the potential of silver nanoparticles to control *X. vesicatoria*.

## Biological control of tomato bacterial diseases by *Bacillus* sp. and *Pseudomonas* sp. isolated from tomato endorhizosphere

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Keywords: Biocontrol, Tomato, Bacterial diseases, *Xanthomonas*, *Clavibacter*

Tomato is subject to several bacterial diseases affecting both field- and greenhouse-grown plants. To select cost-effective potential biocontrol agents, we used a laboratory throughput screening to identify bacterial strains with versatile characteristics suitable for multipurpose uses in tomato cultivation. Once set up a collection of bacteria isolated from tomato endorhizosphere was genotyped by partial sequencing of 16S rRNA gene and characterized phenotypically for plant growth promotion and biocontrol traits. Ten endophytes belonging to the genera *Pseudomonas* and *Bacillus* were further evaluated for their *in vivo* biocontrol activity against *Clavibacter michiganensis* pv. *michiganensis* (Cmm), causal agent of bacterial canker, and *Xanthomonas euvesicatoria* pv. *perforans* (Xep), one of the causal agents of bacterial spot disease. Two isolates, *Pseudomonas* sp. f1 and *Bacillus* sp. 265, significantly reduced bacterial canker by delaying disease progress and reducing the number of dead plants compared to the control. All the ten bacterial strains were able to reduce the symptoms of bacterial spot. Since the phytopathogenic bacterium in this case was leaf-inoculated, a mechanism of induction of systemic resistance is suggested. Two *Pseudomonas* strains with promising biocontrol activity were selected for genome sequencing. Results suggested that strain f1 belongs to the species *P. citronellolis*, within the *P. aeruginosa* genomic group. Strain 172 belongs to the *P. putida* genomic group, although ANIb values suggest that this strain belongs to a new species. The microbial collection generated could provide the basis for the future development of bio-inoculants using single strains or synthetic microbial communities with potential root colonization properties.

## Influence of natural antimicrobials on *Xanthomonas* strains growth

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**Keywords:** *Xanthomonas*, antimicrobial substances, inhibition

The aim of this work was to investigate the effect of natural antimicrobials on the growth of *Xanthomonas* strains detected in plants growing in Lithuania. The research objects were some strains of *Xanthomonas* spp. isolated from plant tubers and stems: *Xanthomonas translucens* NRCIB X6, *X. arboricola* NRCIB X7, *X. arboricola* NRCIB X8, *X. arboricola* NRCIB X9, *X. arboricola* NRCIB X10. Natural antimicrobials were used in the study: the supernatants of 12 lactic acid bacteria strains (*Lactococcus lactis*, three strains; *Lactobacillus helveticus*, four strains; *Lactobacillus reuteri*, two strains; *Streptococcus thermophilus*, one strain; *Enterococcus faecium*, two strains) and emulsions of lavender (*Lavandula angustifolia*), grapefruit (*Citrus paradisi*), pine (*Pinus alpinus*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), peppermint (*Mentha piperita*), lemon (*Citrus limetta*) 1.0 % (v/v) and 2.0 % (v/v) essential oils, aqueous extracts of blueberries (*Vaccinium myrtillus*). The antibacterial activity of tested substances was determined by agar diffusion method. Supernatants of strains *L. reuteri* 7, *L. helveticus* 14, *L. helveticus* R, *L. helveticus* 3, *L. helveticus* 148/3 were found to have a strong antibacterial activity against *Xanthomonas* spp. strains when compared to control solution (1.0 % copper sulfate, the mean inhibition zones 28.8±0.7 mm). The diameter of inhibition zones of supernatants ranged from 24.5±0.6 mm to 32.0±0.8 mm. Emulsions of thyme and lavender essential oils 2.0 % (v/v) inhibited the growth of *Xanthomonas* spp. strains (the mean inhibition zone 19.0±0.1 mm) slightly lower. Aqueous extracts of blueberries showed a very weak antibacterial activity (the mean inhibition zones 11.0±0.1 mm).

# Computational model to approach the spread of plant pests and particularly to the propagation of *Xylella fastidiosa* in the almond trees in Spain

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Keywords: *Xylella fastidiosa*, insect vector, disease expansion, computational modelling, spacetime framework, neighbourhood, update rules

Biological invasions are a process with four stages: arrival, establishment, spread and concentration. All the processes involved can model on a system of ordinary differential equations (ODE) that describes the evolution of populations confined in compartments according to their states.

In addition, differential equations assume that individuals are homogeneously distributed, and all connected to each other, therefore, it is difficult to access the individual dynamics that can occur depending on the climate conditions, as well, temperature, humidity, etc. These difficulties can be overcome by discrete models (based on agents or cellular automata). Our compartmental model bases on a time-space representation in the form of a grid in which each cell represents a tree that can be infected under the influence of the whole ecosystem. The state of the grid is updated over time. As many grids as factors in the entire ecosystem will be considered. The update rules and the definition of the probabilities of infection will be described by Boolean rules and neighbourhood environments in the grids.

Our proposal provides suitable modelling to the spread of plant pest and particularly to the propagation of *Xylella fastidiosa* in the almond trees. This approach allows the study of the response of the individual trees according to both the combination of variables of an ecosystem and their intensity. Another advantage of the model is that it allows easy scaling when the number of characteristics of the ecosystem increases. These encouraging results can guide the modelling of tools to advise the appropriate control.

## Evaluating alternatives to sodium hypochlorite for the post-harvest sanitization of citrus fruit in packinghouses

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Keywords: citrus canker, orange, peracetic acid, phytopathogen, *Xanthomonas citri* subsp. *citri*

*Xanthomonas citri* subsp. *citri* (*X. citri*) is a quarentenary plant pathogen and the causal agent of citrus canker. During infection, the bacterium forms biofilms on the surface of the host plant, therefore, plant parts and fruit have to be sanitized before they can be commercialized. In Brazil, citrus fruits have to be sanitized in packinghouses using a solution of NaClO at 200 ppm. However, NaClO reacts with the organic matter to form carcinogenic and toxic compounds. Because of this negative side effect, the European Union no longer accepts fruits that have been sanitized with NaClO. Thus, the aim of this study was to evaluate potassium bicarbonate ( $\text{KHCO}_3$ ), calcium hydroxide ( $\text{Ca(OH)}_2$ ), calcium hypochlorite ( $\text{Ca(OCl)}_2$ ) and peracetic acid ( $\text{CH}_3\text{CO}_3\text{H}$ ) as alternatives to NaClO for the sanitization of citrus fruits. By monitoring cell respiration and bacterial growth, we showed that  $\text{CH}_3\text{CO}_3\text{H}$  and  $\text{Ca(OCl)}_2$  are bactericides against *X. citri* when applied at 25 ppm and 100 ppm, respectively. Time-response growth curves and membrane integrity analyses showed that  $\text{CH}_3\text{CO}_3\text{H}$  and  $\text{Ca(OCl)}_2$  targeted the bacterial cytoplasmatic membrane in the first minutes of contact.  $\text{CH}_3\text{CO}_3\text{H}$  at 25 ppm displayed a reduction of 1.79 log CFU mL<sup>-1</sup> of *X. citri* when compared to the negative control (0.86% NaCl). The performance of  $\text{CH}_3\text{CO}_3\text{H}$  in experiments that simulate the disinfection of citrus fruit in packinghouses was statistically equal to the activity observed for NaClO. Among the tested compounds,  $\text{CH}_3\text{CO}_3\text{H}$  was the only one that exhibited satisfactory results, and constitutes an alternative to NaClO for citrus fruit sanitization.



## Sensitivity to copper in *Xanthomonas campestris* pv. *campestris* in vitro in Turkey

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Keywords: black rot, *Xanthomonas campestris* pv. *campestris*, copper tolerance

Black rot caused by *Xanthomonas campestris* pv. *campestris* is the main destructive and widespread disease on cruciferous crops worldwide. Copper derivatives have been used and shown various efficacy in controlling this disease. The objective of this work was to determine the sensitivity to copper of isolates of *X. campestris* pv. *campestris* collected in different locations cabbage growing regions of Turkey and over a period of years, from 2018 to 2020. All isolates were evaluated for sensitivity to copper in nutrient agar plates amended with copper sulfate at the following concentrations: 10 to 150 ppm. The minimum inhibitory concentration (MIC) was varied from 50 to 110 ppm among 304 isolates tested. Although all pathogenic native strains were grown at 50 ppm concentration of copper sulfate, the majority (221 of 304) were not grown at 100 ppm showing high sensitivity. Only six isolates were grown at 110 ppm concentration. *X. campestris* pv. *campestris* strains collected in the cabbage-growing regions are highly sensitive to copper indicating copper sprays still could be used for effective control of black rot in Turkey.

## Control of citrus canker symptoms by crude extract from *Pseudogymnoascus* sp.: a greenhouse assay

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**Keywords:** citrus canker, management, crude extract, Antarctica, greenhouse

Citrus canker is responsible for large losses in the worldwide production of citrus. In this regard, natural products can be a great alternative in the control and management of this disease. The search for alternative compounds that are less harmful to the environment is necessary to replace the commercial pesticides currently used. In this study, we evaluated a crude extract from *Pseudogymnoascus* sp. as a preventive treatment against *Xanthomonas citri* subsp. *citri* 306. The fungus isolated from Deception Island, Antarctica, was grown in 2% malt broth for 21 days (15°C). After incubation, supernatant was collected and submitted to liquid-liquid extraction with ethyl acetate (C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>). The crude extract obtained was applied on leaves of *Citrus sinensis* (L.) Osbeck nursery trees (rootstock *Citrus x limonia* Osbeck) in greenhouse conditions. After 40 days of the experiment, lesions on the abaxial epidermal surface of the leaves were counted. Plants treated with commercial pesticide (positive control) presented 0.27 lesions per cm<sup>2</sup>, an 82.5% decrease of symptoms compared to the negative control (1% dimethyl sulfoxide). Plants treated with crude extract (2.1 mg/mL) presented 0.04 lesions per cm<sup>2</sup>, a 97.4% decrease of symptoms compared to the negative control. Both results were statically different ( $p < 0.05$ ). Thus, we concluded that crude extract produced by *Pseudogymnoascus* sp. has potential phytosanitary use and it was better than negative and positive controls.

## Antagonistic potential of *Pseudomonas graminis* strains against some economically important xanthomonads

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Keywords: *Pseudomonas graminis*, epiphytes, biological control, antagonism, *Xanthomonas* spp.

The plant phyllosphere is colonized by various microorganisms including bacteria that could act as natural antagonists of plant pathogens. In order to find an alternative to chemical pesticides in plant protection, our study was focused on epiphytic bacteria as potential biocontrol agents. Among 104 bacterial strains collected from apple phyllosphere in Serbia, four strains showed potential to inhibit growth of *Xanthomonas euvesicatoria* strains in preliminary *in vitro* screening. According to physiological and biochemical characteristics, as well as 16S rRNA and *rpoD* genes sequences, all four strains were identified as *Pseudomonas graminis*. Its antagonistic activity was further tested against some of the economically most important xanthomonads *in vitro*. Plates of KB medium were spot inoculated with antagonistic strains, and after 48 h of incubation, cultures were scraped from the medium afterwards plates were exposed to chloroform vapor. Plates were subsequently flooded with suspension of each pathogenic strain in 0.7 % water agar (conc.  $10^7$  CFU/ml). Antagonistic activity was evaluated by measuring the widths of the inhibition zones after 48 h of incubation at 27 °C. Results of our research revealed that all tested strains showed antagonistic activity against *X. euvesicatoria*, *X. vesicatoria*, *X. gardneri*, *X. perforans*, *X. arboricola* pv. *juglandis* and *X. hortorum* pv. *pelargoni* by producing an inhibition zone of 5-27 mm. Our study showed that strains of *P. graminis* are able to inhibit *Xanthomonas* spp. growth *in vitro*, suggesting their potential use as a biocontrol agent in plant disease control.

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# ePoster Session 5

## Disease Management – Vector Control

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## Antibacterial effect of thyme essential oil compounds to pathogenic xanthomonads

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Keywords: *Thymus*, antibacterial activity, essential oils, *Xanthomonas*

Bacteria from the genus *Xanthomonas* represent serious pathogens of economically important crops of *Apiaceae*, *Brassicaceae* and *Solanaceae* families. As none chemical substance is registered for use against xanthomonads in the Czech Republic, the antibacterial potential of biological compounds was evaluated. Essential oils (EOs) derived from species of genera *Lavandula*, *Mentha*, *Origanum*, *Salvia* and *Thymus* were tested for the elimination of three pathogenic xanthomonads – *X. campestris* pv. *campestris*, *X. euvesicatoria* and *X. hortorum* pv. *carotae*. The primary screening of antibacterial properties of concentrated EOs reveals the thyme EO as effective as the positive control (streptomycin) when showing equal values of inhibition zones through agar diffusion method. Based on the variable composition of EOs caused by environmental parameters, the minimal inhibition concentrations and minimal bactericidal concentrations for each examined bacterium were determined for five major constituents reported for thyme EO (namely thymol, p-cymene, carvacrol, eugenol and linalool) using microdilution methods. Subsequently, the fractional inhibitory concentration index showing the synergistic effects of the most effective constituents, thymol and eugenol, were evaluated.

## Antibacterial effect of essential oils on the causal agent of bacterial spot of stone fruits and almond

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Keywords: bioactive components, essential oil, *Xanthomonas arboricola* pv. *pruni*, direct bioautography, microplate assay

*Xanthomonas arboricola* pv. *pruni* (Smith 1903) Vauterin *et al.* 1995 (Xap), the causal agent of bacterial spot of stone fruits and almond, is one of the most important diseases of several *Prunus* species. Hungarian occurrence of the pathogen was first reported in 2004, when its spread was prevented, however, since 2016 Xap was repeatedly identified. Using bioactive compounds produced by many plants is a promising alternative control measure. In this study antibacterial activities of several essential oils were investigated against two Hungarian Xap strains (isolated from *Prunus salicina* Lindl., 2004 and *P. armeniaca* L. cv. Bergecot, 2016) by means of microplate assay and high-performance thin-layer chromatography (HPTLC) coupled with bioassay (direct bioautography, DB). In the course of direct bioautography, the dried HPTLC chromatogram was dipped into the bacterial cell suspension and after appropriate incubation the bioautogram was visualized by MTT staining (the bright zones indicated the antibacterial activity). By the microplate assay the efficacy of the crude essential oils was tested in liquid shaken culture, while the HPTLC-DB newly developed to Xap pointed to the active separated components of the essential oils. The two strains showed similar sensitivity. In microplate assay the growth of both Xap strains was inhibited by the essential oils such as of cinnamon (*Cinnamomum ceylanicum* Nees.), clove (*Syzygium aromaticum* L.) and thyme (*Thymus vulgaris* L.). HPTLC-DB analysis of these essential oils confirmed their antibacterial effect on the Xap strains. Moreover, the bioautograms indicated the bioactive components that were identified as trans-cinnamic aldehyde, eugenol and thymol, respectively.

## ***Xanthomonas arboricola* pv. *corylina* causing bacterial blight of hazelnut in Chile: genetic analysis of resistance to agrochemicals**

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**Keywords:** Bacterial blight of hazelnut, *Xanthomonas arboricola* pv. *corylina*, agrochemicals resistance

*Xanthomonas arboricola* pv. *corylina* (Xac) is the etiological agent of bacterial blight of hazelnut. This pathogen was detected for the first time in Chile in 1987 and it is considered a persistent pathogen in our country, generating losses of around 30% of production. For its control in the field, cupric compounds are preferably used, in spite of their negative effects on the environment and human health. In this work, we analyzed the genetic basis of resistance to copper and antibiotic of an isolate of Xac obtained from productive hazelnut fields located in VII region of Chile. Tissue samples from hazelnut trees (leaves and twigs) were macerated and seeded on YPG agar. These samples were incubated at 28°C until typical colonies of *Xanthomonas* were obtained (yellow, shiny and rounded bacterial colonies). The identity of these colonies was confirmed by using PCR reactions employing genus/species-specific primers. All identified isolates were tested for resistance to copper (copper sulfate) on CYEG agar plates. Subsequently, a biochemical profile and antibiogram of one Xac isolate considered resistant (Xac J401) was performed and subjected to whole genome sequencing. Preliminary analyses confirm the presence of copper resistance genes and a high number of antibiotic resistance genes, which correlate with the phenotypes observed in in vitro assays. This is the first report showing genetic evidence of resistance to conventional agrochemicals in Chilean isolates of Xac.

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## Efficacy of microbial consortia and natural compounds as seed dressing for the control of tomato bacterial spot

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Keywords: PGPR, biocontrol agents, *Xanthomonas*, seed dressing, organic seeds

Different microbial consortia (combinations of different species of *Azotobacter*, *Bacillus*, *Pseudomonas*, *Streptomyces*, *Glomus*, *Trichoderma*) and natural compounds (chitosan and glucosinolates) have been evaluated as seed dressing for the containment of tomato bacterial spot. Organic tomato seed (San Marzano nano) treatments were performed by soaking in microbial consortium suspensions and natural compound dilutions as recommended by the company who provided them. Seeds soaked in sterile distilled water were used as control. All trials were performed by artificial inoculations with suspensions of *Xanthomonas euvesicatoria* pv. *perforans* (Xep) strain NCPPB 4321 ( $10^8$  cfu ml<sup>-1</sup>). A grow-out test in Petri dishes was performed and seedlings germinated from seeds inoculated with Xep bacterial suspension before dressing were evaluated observing lesions in the cotyledons at the stereomicroscope. A significant reduction of disease severity was observed, in seedling treated both by microbial consortia and natural compounds. A second trial was performed by leaf spray inoculating Xep bacterial suspensions on 30 days old tomato plantlets obtained from treated seeds that had been placed to germinate in nursery trays. Ten days post-inoculation 10 leaves were randomly collected from each replicate plant and the numbers of lesion and leaf area calculated to obtain lesions per cm<sup>2</sup> of leaf area. A significant reduction in the number of lesions was observed in plants obtained from seeds treated with 2 of the 3 microbial consortia and with chitosan.

*These trials were performed in the framework of the European project H2020 BRESOV Breeding for Resilient, Efficient and Sustainable Organic Vegetable production.*



## ***Origanum vulgare* essential oil in the control of the citrus canker**

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**Keywords:** essential oils, citrus canker, biocontrol, natural products

The search for natural products capable of inhibiting bacterial phytopathogens, and reducing the number of regular pesticide applications, are an ever-present need. Essential oils are potential sources for antimicrobials agents and are widely accepted as GRAS (generally recognized as safe), becoming valuable study objects. In this light, we evaluated the antibacterial capabilities of the oregano essential oil (EO) against the known causer of the citrus canker, the bacteria *Xanthomonas citri* subsp. *citri*. Initially, a sensitivity assay was performed, which revealed complete bacterial growth inhibition in all EO concentrations tested (0,0312%; 0,0625%; 0,125%; 0,25%; 0,5%; v:v) after a 4-hour incubation period. To further investigated these results, the EO was utilized in a preventive treatment against the bacteria in order to assess its capabilities to inhibit the emergence of symptoms in citrus plants. A greenhouse experiment was conducted for 40 days in *Citrus sinensis* (L.) Osbeck nursery trees. Results revealed that plants treated with oregano oil (0,0312%) presented 0,741 lesions per cm<sup>2</sup> while the positive control (chemical pesticide) presented 0,25 lesions per cm<sup>2</sup>, being both statically different ( $p < 0.05$ ) than the negative control plants (ethanol 1% v:v) which presented 2.289 lesions per cm<sup>2</sup>. While the oregano EO did not perform similarly as the chemical control applied, these results prompt further investigations around the antibacterial capabilities of this natural product.

## **Antibacterial and antibiofilm activity of phenolic compounds against *Xanthomonas campestris* pv. *campestris***

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**Keywords:** phenolic compounds, antimicrobial activity, biofilm formation, *Xanthomonas campestris*

Synthetic chemicals have been used to control plants diseases for a long time. However, most of these chemicals cause a number of undesirable effects on the environment as well as human health. Therefore, there has been an increased interest in natural and plant-based products which are considered as better alternative to synthetic chemicals. Plants synthesize a variety of groups of compounds as secondary metabolites. They show antimicrobial activity and they can be easily extracted from plant tissue. Therefore, the aim of this study was to evaluate the antibacterial and antibiofilm activity of several types of phenolic compounds e.g. carvacrol, eugenol, geraniol, citral, thymol, guaiacol and cinnamaldehyde against *Xanthomonas campestris* pv. *campestris* (Xcc). To test antibacterial effect, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Antibiofilm activity was evaluated by quantifying total biomass using crystal violet assay. The obtained *in vitro* results allowed to select the best phenolic compounds for further analysis *in vivo*.

## Antibacterial activity of novicidin derived synthetic peptides against *Xanthomonas campestris* pv. *campestris*

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**Keywords:** antimicrobial peptides, antibacterial activity, *Xanthomonas*, disease control

Crop plants are constantly exposed to various biotic and abiotic stresses that significantly affect their growth and productivity. Plant protection involves mostly the application of chemical pesticides, which are under strong restrictions and regulatory conditions. Control of bacterial diseases in plants is based mainly on copper compounds and other synthetic chemicals, which contribute to pollution of the environment. In the consequence much attention has been paid to development of less toxic and less polluting treatments. In the present study we have tested three novicidin (NVC) derived synthetic peptides (NVC-F>A, NVC-AA, NVC-G) in order to evaluate their antibacterial activity against *Xanthomonas campestris* pv. *campestris*. NVC sequence modification can lead to design of nontoxic peptide with maintained cell membrane penetrating capabilities. NVC-F>A peptide has phenylalanine residues substituted with alanine, NVC-AA peptide has both phenylalanine and tyrosine substituted with alanine, while NVC-G peptide has deleted central glycine. For determination of minimum inhibitory concentrations (MICs) of tested peptides growth curve establishment by spectrophotometric measurements and colony forming units (CFU) enumeration was performed. Two peptides (NVC-F>A and NVC-G) showed antibacterial activity at concentration 1 µg/ml, while MIC of NVC-AA was at 2 µg/ml. Conducted *in vitro* trials gave the basis for test on cabbage plants in isolated cultivation room, which included two approaches: preventive treatment of seeds and preventive spray treatment of plants.

## Penicillic acid isolated from *Penicillium* sp. from Antarctica effect on citrus canker in greenhouse conditions

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Keywords: management, fungal metabolites, penicillic acid, Antarctica, citrus canker

Alternative compounds for agricultural use are an important topic in the control and management of citrus canker. The discovery of novel molecules or new applications of known metabolites may assist in the elaboration of new efficient chemicals even more so considering the emergence of copper tolerant and resistant strains of *Xanthomonas*. We applied penicillic acid ( $25 \mu\text{g} \cdot \text{mL}^{-1}$ ) isolated from a *Penicillium* strain from marine sediments from Antarctica as a preventive treatment against *Xanthomonas citri* subsp. *citri* strain 306 in leaves of *Citrus sinensis* (L.) Osbeck nursery trees (rootstock *Citrus x limonia* Osbeck). The experiment was conducted in greenhouse conditions for 40 days. Penicillic acid ( $0.437$  lesion per  $\text{cm}^2$ ) decreased symptoms by 75.31%, close to the positive control ( $0.270$  lesions per  $\text{cm}^2$ , reduction of 84.74%) and was statistically different ( $p < 0.05$ ) than the untreated control ( $1.772$  lesions per  $\text{cm}^2$ ). Evaluation of lesions per  $\text{cm}^2$  (mean  $\pm$  standard error) on the leaves surface revealed the potential use of the compound in chemical formulations against citrus canker.

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## Integrating science on *Xanthomonadaceae* for integrated plant disease management in Europe

**COST Action CA16107 EuroXanth  
2017 | 2021**

### Participating Countries

AL, BA, BE, BG, CH, CZ, DE, DK, EE, ES, FR, GB,  
GR, HR, HU, IE, IL, IT, JO, LT, LV, ME, MK, NL,  
NO, PL, PT, RS, SE, SI, SK, TN, TR, US, XK, ZA

### Challenge

Present, emerging or re-emerging plant diseases due to infection by of the genera *Xanthomonas* and *Xylella* are continually challenging food security and cause significant losses to the EU economy each year, thus demanding for concerted R&D actions at the international level, which will be supported by the COST Action networking instruments.

### Working Groups

**WG1 – Diagnostics & Diversity**

**WG2 – Pathogen Biology**

**WG3 – Resistance & Defence**

**WG4 – Disease Management**



Olive trees cut down because of *Xylella*.

Photo credit: PanareoFotografia via Thinkstock.

### Objectives

- ✓ Develop, implement, compare and standardize methods of pathogen detection
- ✓ Estimate the risk of epidemics and outbreaks
- ✓ Develop, distribute and valorize bioinformatics tools for data analysis
- ✓ Identify key bacterial factors in the microbe-eukaryote interaction at different steps of the infection/dissemination cycle
- ✓ Identify elicitors of plant defense responses as targets for resistance breeding
- ✓ Discover novel resistance traits
- ✓ Generate durably resistant crop cultivars
- ✓ Evaluate and establish disease control measures
- ✓ Evaluate and compare approaches to eliminate or reduce vector populations

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