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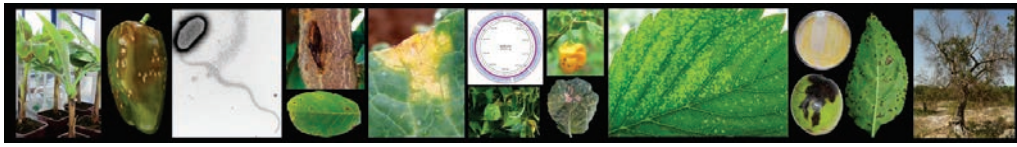
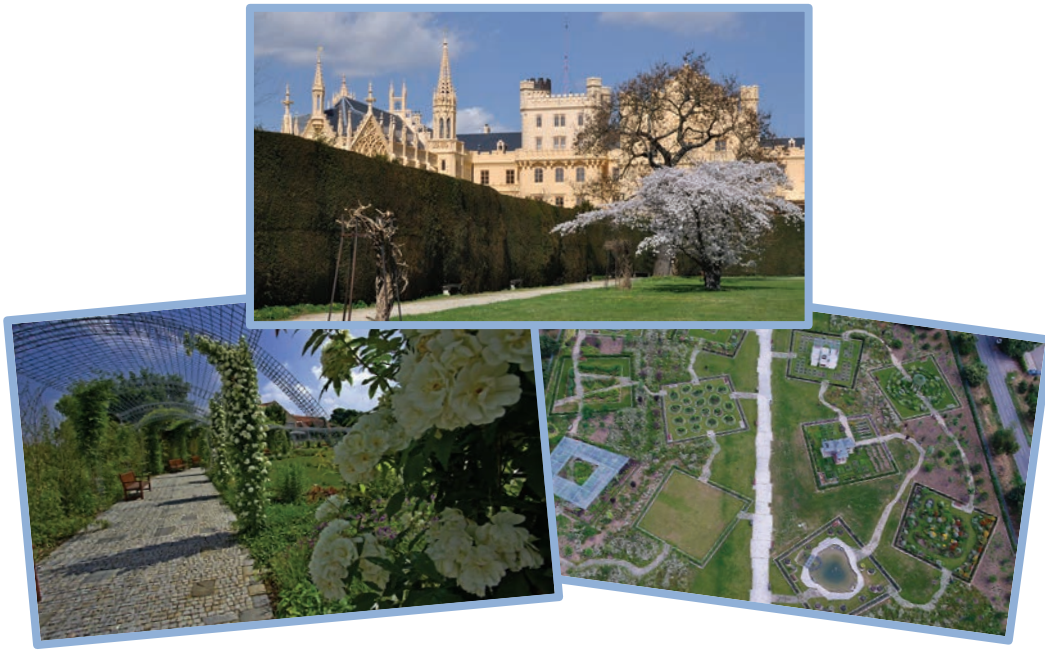


3rd Annual Conference of the EuroXanth COST Action

Faculty of Horticulture, Lednice, Czech Republic

9–11 September 2019



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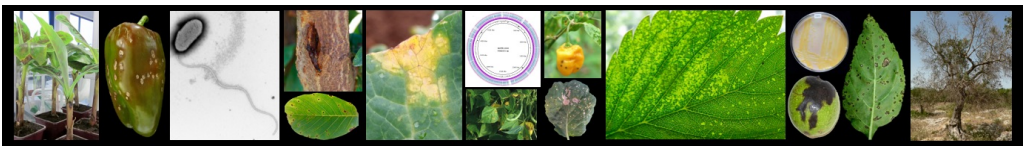

3rd Annual Conference of the EuroXanth COST Action

Faculty of Horticulture, Lednice, Czech Republic

9–11 September 2019

Ralf Koebnik, Miroslav Baránek (eds.)

3rd Annual Conference of the EuroXanth COST Action
Ralf Koebnik, Miroslav Baránek (eds.)

ISBN 978-80-7509-672-2

Conference Overview

Registration: Sunday 19:00 – 21:00 pm with Welcome Drink served at 20:00 p.m.
Monday 8:30 – 9:30 am; Faculty of Horticulture, Lednice, Czech Republic

Monday 9th September	Tuesday 10th September	Wednesday 11th September
8:30 – 9:30 Registration	**8:30 – 10:00** Session 2 (cont'd)	**8:30 – 10:10** Session 4 Disease Management – Vector Control
9:40 – 10:00 Welcome addresses	**Break**	**Break**
10:00 – 12:00 Session 1 Diagnostics & Diversity – Population Structure	**10:30 – 11:00** COST Session	**10:40 – 12:30** Session 4 (cont'd)
	11:00 – 12:30 ECI Session	**12:30 – 12:40** Concluding remarks
12:00 – 13:00 **Lunch**	**12:30 – 13:30** **Lunch**	**12:45 – 13:45** **Lunch**
13:00 – 15:00 Session 2 Pathogen Biology	**13:30 – 15:30** Session 3 Genetic Resistance – Host Defence	
Break	**Break**	
16:00 – 18:00 Social Program Visit of Lednice Castle Guided Park Stroll	**16:00 – 18:00** Poster Session	
19:00 – 24:00 Individual activities	**17:00 – 18:00** Management Committee Meeting	
	19:00 – 24 :00 Gala Dinner	

3rd Annual Conference of the EuroXanth COST Action

Integrating Science on *Xanthomonadaceae* for integrated plant disease management in Europe

Venue:

Faculty of Horticulture (Zahradnická fakulta), Mendel University in Brno
Address: Valtická 337, 691 44 Lednice, Czech Republic

Organisers

Scientific Committee

Miroslav Baránek	Mendel University in Brno, Czech Republic
Pavel Beran	University of South Bohemia, České Budějovice, Czech Republic
Jens Boch	Leibniz University Hannover, Germany
Vittoria Catara	University of Catania, Italy
Joana Costa	University of Coimbra, Portugal
Ralf Koebnik	IRD, Montpellier, France
Tamás Kovács	ENVIROINVEST, Pecs, Hungary
Joël F. Pothier	Zürich University, Switzerland
Emilio Stefani	Università degli Studi di Modena e Reggio Emilia, Modena, Italy

ECI Committee (Early-Career Investigators)

Aitana Ares Yebra	Instituto Pedro Nunes, Coimbra, Portugal
Monika Kałużna	Research Institute of Horticulture, Skierniewice, Poland
Massimiliano Morelli	Instituto per la Protezione Sostenibile delle Piante, Bari, Italy
Eliška Peňázová	Mendel University Brno, Czech Republic
Coline Sciallano	IRD Montpellier, France

Local Organisers and Contact Persons of the EuroXanth COST Action

Miroslav Baranek	Mendel University in Brno, Czech Republic
Aleš Eichmeier	Mendel University in Brno, Czech Republic
Jana Čechová	Mendel University in Brno, Czech Republic
Filip Gazdík	Mendel University in Brno, Czech Republic
Jakub Pečenka	Mendel University in Brno, Czech Republic
Eliška Peňázová	Mendel University in Brno, Czech Republic
Ralf Koebnik	Chair of COST Action, IRD Montpellier, France
Isabelle Sciacco	Grant Holder Administrator, IRD Montpellier, France

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Monday 9th of September

9:40-10:00 **Opening & welcome addresses**
Miroslav BARANEK *(Organiser)*, **Alena SALAŠOVÁ** *(Dean)* & **Ralf KOEBNIK** *(Chair)*

Session 1 Diagnostics & Diversity – Population Structure

Chairs: Joana COSTA & Alič ŠPELA

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10:00-10:40 – **Rodrigo ALMEIDA *(University of California, Berkeley, USA):*** Reconstructing the history of *Xylella fastidiosa* dispersal events

10:40-11:00 – **Lucas MORINIÈRE (*University of Lyon, France*):** Who's who: Clarifying the taxonomy of the causal agent of bacterial leaf spot of lettuce through a polyphasic approach led to combine X. *hortorum* Vauterin et al. 1995 and *X. cynarae* Trebaol et al. 2000 emend. Timilsina et al. 2018

11:00-11:20– **David STUDHOLME *(University of Exeter, UK)*:** Genomics and taxonomy of *Xanthomonas vasicola*: pathogen of banana, enset, maize and sugarcane

11:20-11:40 – **Leonor MARTINS *(University of Porto, Portugal):*** Genomic evidence for a new *Xanthomonas* species isolated from walnut (*Juglans regia* L.)

11:40-12:00– **Joana G. VICENTE *(University of Warwick, UK):*** Recent outbreaks of black rot caused by four races of *Xanthomonas campestris* pv. *campestris* in the UK

12:00-13:00 – LUNCH

Session 2 Pathogen Biology

Chairs: Joël F. POTHIER & Aitana ARES YEBRA

***

13:00-13:40 – **Leonardo DE LA FUENTE *(Auburn University, USA):*** *Xylella fastidiosa* interactions with calcium and copper in the xylem

13:40-14:00 – **Jan VAN DER WOLF *(Wageningen UR, The Netherlands):*** How does Brassica seed become infected with *Xanthomonas campestris* pv. *campestris*?

14:00-14:20 – **Paula Maria MOREIRA MARTINS *(Centro de Citricultura, Brazil):*** Persistence in *Xanthomonas citri* subsp. *citri* and its impact on disease management

14:20-14:40 – **Yael HELMAN *(Hebrew University of Jerusalem, Israel):*** Hitchhiking on swarming bacteria promotes the dispersal of *Xanthomonas* cells

14:40-15:00 – **Alice BOULANGER *(INRA Toulouse, France):*** Genetics of bacterial fitness of *X. campestris* in vitro and in planta

15:00-15:30 – COFFEE BREAK

16:00-18:00 ***– Social Event (Visit of Lednice Castle & Guided Park Stroll)***

Tuesday 10th of September

Session 2 (cont'd) Pathogen Biology

Chairs: Eran BOSIS & Alice BOULANGER

***

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09:00-09:20 – **Aitana ARES *(University of Coimbra, Portugal)*:** Using AnABlast algorithm for the identification of coding genes and fossil regions in a complete genome. A case of study: *Xylella fastidiosa* strain Pr8x

09:20-09:40 – **Jaime CUBERO *(Instituto Nacional de Investigación y Tecnología Agraria, Spain)*:** Comparative analysis of two fruit tree pathogens: *Xanthomonas citri* subsp. *citri* and *Xanthomonas arboricola* pv. *pruni*

09:40-10:00 – **Nay C. DIA *(ZHAW Wädenswil, Switzerland)*:** Is hydrangea a new host for *Xanthomonas hortorum* species level clade members?

10:00-10:30 – COFFEE BREAK

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Chairs: Jens BOCH, Eran BOSIS, Roland KÖLLIKER & Joël F. POTHIER

***

10:30-10:40 – **Ralf Koebnik *(IRD Montpellier, France)*:** Presentation of the EuroXanth COST Action

10:40-11:10 – ***Presentation of the Wiki Project***

ECI Session ECI Poster Talks

Chairs: Massimiliano MORELLI, Eliška PEŇÁZOVÁ & Monika KAŁUŻNA

***

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11:20-11:25 – **Rita P. FERNANDES *(University of Porto, Portugal):*** Genomic analysis and taxonomic inference of *Xanthomonas arboricola* pv. *juglandis* DNA markers using an alignment-free sequence comparison tool

11:25-11:30 – **Katarina GAŠIĆ *(Institute for Plant Protection and Environment, Belgrade, Serbia)*:** Integration of biological and chemical methods in control of pepper bacterial spot

11:30-11:35 – **Marlène LACHAUX *(Institut de Recherche pour le Développement, France):*** Identification of candidate resistance genes in rice induced by TAL effectors of African *Xanthomonas oryzae* pv. *oryzae*

11:35-11:40 – **Jakub PEČENKA *(Mendel University in Brno, Lednice, Czech Republic):*** Graphene oxide nanocomposite as a tool for bacterial spot control

11:40-11:45 – **Julio RETAMALES *(Pontificia Universidad Católica de Valparaíso, Chile):*** Characterization of *Xanthomonas arboricola* pv. *juglandis* strains isolated after field application of phage therapy in Chile and its role in walnut blight disease development

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12:00-12:30 – **Meet the Expert**

Chairs: Massimiliano MORELLI, Eliška PEŇÁZOVÁ & Coline SCIALLANO

***

12:30-13:30 – LUNCH

Session 3 Genetic Resistance – Host Defence

Chairs: Jens BOCH & Florian GOETTELMANN

***

13:30-14:10 – **Helvécio Della COLETTA-FILHO *(Centro de Citricultura Sylvio Moreira, Brazil):*** Host resistance against *Xylella fastidiosa* and strategies for disease control

14:10-14:30 – **Giusy D'ATTOMA *(Institute for Sustainable Plant Protection, Bari, Italy)*:** Unravelling the role of mineral elements in olive response to *Xylella fastidiosa* infections

14:30-14:50 – **Guido SESSA *(Tel-Aviv University, Israel)*:** The tomato RLCK BSK830 interacts with transmembrane receptors and plays a role in plant immunity

14:50-15:10 – **Coline SCIALLANO *(Institut de Recherche pour le Développement, France):*** Engineering of loss-of-susceptibility in rice: remove TALE targets to control *Xanthomonas oryzae* pv. *oryzae* in Africa

15:10-15:30 – **Jens BOCH *(Leibniz Universität Hannover, Germany)*:** Strategies for generation of rice plants that are resistant to *Xanthomonas oryzae* infections

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17:00-18:00 – ***COST Management Committee Meeting***

19:00-24:00 – GALA DINNER in the Hotel Galant

Wednesday 11th of September

Session 4 Disease Management – Vector Control

Chairs: Tamás KOVÁCS & Jakub PEČENKA

***

08:30-09:10 – **Jeffrey B. JONES *(University of Florida, Gainesville, USA)*:** Prospects for using bacteriophages for plant disease control

09:10-09:30 – **Massimiliano MORELLI *(Institute for Sustainable Plant Protection, Bari, Italy)*:** Insights on *Paraburkholderia phytofirmans* PsJN behaviour as biocontrol agent of *Xylella fastidiosa* in olive

09:30-09:50 – **Cristina CAMEIRÃO (*Instituto Politécnico de Bragança, Portugal)*:** Olive tree core microbiome: the first learning step towards the development of biological control strategies for *Xylella fastidiosa*

09:50-10:10 – **Saul BURDMAN *(The Hebrew University of Jerusalem, Israel):*** Random antimicrobial peptide mixtures for management of *Xanthomonas* plant diseases

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Session 4 (cont'd) Disease Management – Vector Control

Chairs: Claude BRAGARD & Katarina GAŠIĆ

***

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11:30-11:50 – **Vincenzo CAVALIERI *(Institute for Sustainable Plant Protection, Bari, Italy)*:** First studies on the relationship between *Xylella fastidiosa* subsp. *pauca* ST53 and its insect vector in Apulia

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12:10-12:30 – **Dorota TEKIELSKA *(Mendel University in Brno, Lednice, Czech Republic):*** Antibacterial effect of advanced nanomaterials against *Xanthomonas campestris* pv. *campestris*

12:30-12:40 – ***Concluding remarks by the organisers***

12:45-13:45 – LUNCH

Session 1

Diagnostics & Diversity – Population Structure

***

Reconstructing the history of *Xylella fastidiosa* dispersal events

Rodrigo P.P. Almeida

Dept. of Environmental Science, Policy and Management, University of California, Berkeley, USA

Pathogen introductions are responsible for emerging diseases worldwide. The bacterium *Xylella fastidiosa* is a plant pathogen of economic importance that has been recently detected infecting a range of plants in southern Europe. We have used genomic data from ~350 strains of *X. fastidiosa* to study dispersal pathways associated with the emergence of new diseases, and processes impacting pathogen population structure. A summary of our early findings will be presented.

Who's who: Clarifying the taxonomy of the causal agent of bacterial leaf spot of lettuce through a polyphasic approach led to combine *X. hortorum* Vauterin et al. 1995 and *X. cynarae* Trebaol et al. 2000 emend. Timilsina et al. 2018

Lucas Morinière[1], Alexandre Burlet[2], Emma Rosenthal[3], Xavier Nesme[1], Perrine Portier[4], Carolee T. Bull[3], Céline Lavire[1], Marion Le Saux[4], Franck Bertolla[1]

[1] Ecologie Microbienne Lyon UMR CNRS 5557 INRA 1418, Université Claude Bernard Lyon 1, 69622 Villeurbanne, France

[2] Station d'Expérimentation Rhônes-Alpes Information Légumes, SERAIL, 69126 Brindas, France

[3] Department of Plant Pathology and Environmental Microbiology, Penn State University, University Park, Pennsylvania, USA

[4] IRHS, INRA, Agrocampus-Ouest, Université d'Angers, SFR 4207 QUASAV, 49070 Beaucouzé, France

Keywords: taxonomy, bacterial leaf spot of lettuce, *Xanthomonas hortorum*, *Xanthomonas cynarae*

Assessment of the taxonomy and diversity of the *Xanthomonas* strains causing bacterial leaf spot of lettuce (BLSL), commonly referred as *Xanthomonas campestris* pathovar *vitians*, has been a long-lasting issue which held back the global efforts made to understand this disease and struggle against the damages it causes in the field. In order to restore sane bases essential to the study of this pathogen, we conducted an extensive polyphasic approach on a panel of strains obtained through sampling campaigns or collection strains acquisitions. Results of a multilocus sequence analysis crossed with pathogenicity assays revealed that BLSL-causing strains formed a monophyletic pathovar sparsely diversified in three clonal groups. However, genome-based phylogenies yielded only two major groups of strains. From the bigger picture perspective, phylogenies exhibited the intermediate position of pathovar *vitians* between close species *X. hortorum* and *X. cynarae*, resulting in a phylogenetic continuum regarding the known diversity of the genus. Finally, whole-genome comparisons of all type, pathotype or representative strains of these two species by multiple overall genome relatedness indices (OGRIs) calculation endorsed the hypothesis of one single species. Carbon and nitrogen sources utilization tested using Biolog microplates did not revealed any particular phenotypic differences which would have legitimate the distinction into two species. Therefore, we propose the combination of *X. hortorum*, *X. cynarae* and *X. campestris* pv. *vitians* into a new *X. hortorum* comb. nov. with CFBP 4925^T = LMG 937^T remaining the type strain, and

LMG 938^{neoPT} = NCPB 2248^{neoPT} becoming the pathotype strain of *X. hortorum* pv. *vitians*.

Genomics and taxonomy of *Xanthomonas vasicola*: pathogen of banana, enset, maize and sugarcane

David J. Studholme

Biosciences, University of Exeter, Exeter EX4 4QD, UK

Keywords: sorghum, sugarcane, enset, corn, banana, pathogenicity, sequencing

The bacterial pathogen *X. campestris* pv. *musacearum* (Yirgou and Bradbury) Dye 1978 presents a major threat to cultivation of banana and enset crops in central and eastern Africa, where it causes banana *Xanthomonas* wilt (BXW) and enset *Xanthomonas* wilt (EXW). This pathogen was first isolated from enset and banana in the 1960s and early 1970s, respectively in Ethiopia. Only in the 21st century did the disease establish in the banana-growing areas of Burundi, Democratic Republic of Congo, Kenya, Rwanda, Tanzania and Uganda. In this region around the Great Lakes of eastern and central Africa, BXW disease severely challenges the livelihoods and food security of millions. I present the case for transferring *X. campestris* pv. *musacearum* into the species *X. vasicola* Vauterin and offer an overview of the different evolutionary lineages that constitute the species *X. vasicola*, in the light of our recent genomics analyses. These lineages include strains previously known as "*X. campestris* pv. *zeae*", strains from recent outbreaks of bacterial leaf streak on corn in the USA, *X. campestris* pv. *arecae* (Rao and Mohan) Dye 1978, and strains isolated from *Tripsacum* grass in Sri Lanka. This work includes contributions by a large number of collaborators.

Genomic evidence for a new *Xanthomonas* species isolated from walnut (*Juglans regia* L.)

Leonor Martins[1,2], Camila Fernandes[1,2,3], Jochen Blom[4], Nay C. Dia[5], Joël F. Pothier[5], Fernando Tavares[1,2]

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[2] FCUP- Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Porto, Portugal

[3] Unidade Estratégica de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal, INIAV, Oeiras, Portugal

[4] Bioinformatics and Systems Biology, Justus-Liebig-University Giessen, Giessen, Germany

[5] Environmental Genomics and Systems Biology Research Group, Institute for Natural Resource Sciences, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland

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

Xanthomonas arboricola pv. *juglandis* (*Xaj*), the causal agent of important walnut (*Juglans regia*) diseases leads to major yield losses worldwide. Previous studies stressed the high genetic diversity of *Xaj.* Recently, we observed the frequent occurrence of pathogenic and non-pathogenic xanthomonads lineages co-colonizing the same walnut host, suggesting a sympatric lifestyle which may contribute to unveil genetic trade-offs related to pathogenicity of *Xanthomonas*. Five walnut-associated *Xanthomonas* were isolated from asymptomatic buds and symptomatic leaves of a single walnut host tree (CPBF 367, CPBF 424, CPBF 426, CPBF 427, and CPBF 1521). MLSA based on partial sequences of seven housekeeping genes (*fyuA*, *gyrB, rpoD*, *atpD, dnaK*, *efp* and *glnA*) revealed that CPBF 367, CPBF 424 and CPBF 426 clustered together with non-pv. *juglandis Xanthomonas* strains. The genome of these five strains was determined using Illumina sequencing. Average nucleotide identity (ANI) confirmed CPBF 427 and CPBF 1521 as *Xaj* strains, while CPBF 367, CPBF 424 and CPBF 426, although sharing an ANI>98%, had a low ANI value (<90%) to other *Xanthomonas* species analysed, suggesting that these strains belong to a new *Xanthomonas* species. Pathogenicity assays revealed that while strain CPBF 424 was found to be pathogenic on walnut, CPBF 367 showed a non-pathogenic phenotype. Genomic analysis revealed a functional T3SS and an enriched repertoire of T3E genes for CPBF 424, while CPBF 367 and CPBF426 were deficient for most of T3SS and T3E genes. Ultimately, strain CPBF 424 rises as a valuable strain to address the emergence and evolution of pathogenicity in *Xanthomonas*.

Recent outbreaks of black rot caused by four races of *Xanthomonas campestris* pv. *campestris* in the UK

Joana G. Vicente[1,2], Laura Baxter[1], Vânia H. Passo, Eric B. Holub[1], David J. Studholme[3]

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Keywords: cauliflower, swede, wallflower, pathogenicity, sequencing

Black rot caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*) is one of the most damaging diseases on plants belonging to the *Brassicaceae* family, including economically important crops like cauliflower, kale, cabbage (*Brassica oleracea*) and swede (*B. napus*) and also ornamental plants like wallflower (*Erysimum* spp.). Pure *Xcc* isolates were obtained from samples of *B. oleracea* and *B. napus* leaves showing symptoms of black rot collected in Warwickshire, Lincolnshire and Cornwall from 2014 till 2019.

The isolates were race-typed in a standard range of differential Brassica plants in glasshouse-controlled conditions. Isolates from different races were used as controls. UK isolates from *B. oleracea* crops belonged to three races, 1, 4 and 5. Isolates from swede belonged to race 5 and isolates from wallflower were race 6. This is the first identification of race 5 in the UK. It is possible that swede and wallflower outbreaks can lead to infection of *B. oleracea* crops.

A total of eight isolates were sequenced and were compared with whole genome sequences previously made available. Isolates from different races could be differentiated and some isolates from the same race were distinct indicating that the outbreaks were not caused by only one clonal strain.

Strategies for controlling black rot in the UK should include seed testing to ensure that seeds are not infected and ensuring that planting materials are free from disease. Molecular markers should be developed for different races of *Xcc*. Selection and breeding for disease resistance should consider the different races that can cause the disease.

Session 2

Pathogen Biology

***

Xylella fastidiosa interactions with calcium and copper in the xylem

Leonardo De La Fuente[1], Qing Ge[1], Hongyu Chen[1], Paul Cobine[2]

[1] Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA
[2] Department of Biological Sciences, Auburn University, Auburn, Alabama, USA; lzd0005@auburn.edu

Keywords: sap, ionome, RNA-Seq, microfluidic chambers, natural competence

Xylella fastidiosa (Xf) is limited to live inside xylem vessels, where water and mineral nutrients are distributed throughout the plant. We have studied the role of mineral elements, in particular calcium (Ca) and copper (Cu), in the biology of Xf. RNA-Seq was conducted with Xf cells growing under flow conditions inside microfluidic chambers that mimic Xf natural habitats. Ca transcriptionally regulated the machinery of type IV pili (TFP), and other genes related to pathogenicity and host adaptation. Comparing these results with our previous RNA-Seq study in batch cultures, we concluded that although Ca regulates genes belonging to similar functional categories, the number and tendencies (up-/down-regulation) were different. Recombination-related genes were upregulated by Ca, and we determined experimentally that Xf natural competence is enhanced by Ca. Our results suggest that the regulatory role of Ca in Xf acts differently under flow or batch conditions. Cu was studied as a possible control measure. Low concentrations of CuSO4 increased biofilm formation in vitro, while high concentrations inhibited biofilm formation and growth. Xf -infected and non-infected plants were watered with tap water, or water supplemented with 4 mM or 8 mM $CuSO_4$. Leaf scorch symptoms in Cu-supplemented plants were more severe at later time points. Moreover, $CuSO_4$-amended treatments do not inhibit, but slightly increased Xf growth. Concentrations of Cu in sap were in the range of concentrations that promote Xf biofilm formation. We propose that the plant Cu homeostasis prevents Cu from becoming elevated to a level that would lead to bacterial inhibition.

Acknowledgments
This research was financially supported by the Agriculture and Food Research Initiative competitive grant no. 2015-67014-23085 from the USDA National Institute of Food and Agriculture, the HATCH AAES (Alabama Agricultural Experiment Station) program, and the China Scholarship Council (CSC).

How does Brassica seed become infected with *Xanthomonas campestris* pv. *campestris*?

Jan van der Wolf, Leo Poleij, Pieter Kastelein

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Keywords: plant colonization, microscopy, GFP-tagged strains, black rot, insect transmission

In a Brassica seed crop, seed infections may occur with *Xanthomonas campestris* pv. *campestris* (Xcc), the causative agent of black rot, due to systemic colonization of plants upon leaf infections, or alternatively, after flower infections. Studies from the past in poly-tunnels with cauliflowers showed that spray-inoculation of flowers resulted in a high incidence of seed infections.

To study the translocation of the pathogen in more detail we used Rapid Cycling Brassica (RCB) plants with a GFP-tagged strain of Xcc. Under specific conditions, RCB plants are capable of cycling within a 5-9 weeks period. Multiple spray-inoculations of blooming peduncles in a density 10^7 - 10^8 cells/ml resulted in contamination of flowers, and subsequently in a systemic and symptomatic infection of siliques. Highly infected seed lots were harvested from flower-inoculated plants. Using confocal laser scanning microscopy, Xcc could be observed inside some of the seeds in endosperm and embryo. This indicated that flower inoculations can result in internal seed infections.

Flower infections may occur if contaminated insects visit flowers during pollination (Van der Wolf & Van der Zouwen, 2010). Recently, we showed that bumble bees can transmit Xcc from infected to non-infected plants resulting in symptomatic siliques and contaminated seeds. Xcc could persist for at least 23 days in a colony of bumble bees. We conclude that contamination of flowers is a very efficient way to derive infected seed, but we cannot exclude a role of leaf infections in commercial seed production.

Persistence in *Xanthomonas citri* subsp. *citri* and its impact on disease management

Paula Maria Moreira Martins[1, 2], Alessandra Alves de Souza[1], Thomas Keith Wood[2]

[1] Centro de Citricultura, IAC, Cordeiropolis-SP, Brazil

[2] Chemical Engineering, Pennsylvania State University, State College-PA, United States

Keywords: citrus canker, chemical control, dormancy, growth arrest, stress

In agricultural environments, bacteria are exposed to myriad physical, chemical and biological stresses. High and low temperatures, toxic molecules and UV rays are only a few examples of conditions they face continuously and yet they thrive. Although genetic resistance is the first mechanism that comes to mind when adapting to antimicrobials in the surrounding environment, bacteria can use built-in biochemical pathways in order to survive. The persister phenotype, which can be defined as a "dormant" bacterial state, has been extensively studied in bacteria, but its occurrence in phytopathogenic bacteria is mostly unknown. Here, we present the first study on the impact of different conditions that induce *Xanthomonas citri* persistence phenotype, such as starvation, cupric compounds and high temperatures. We found that after these stressful conditions, cells became more tolerant to killing by antibiotics, in comparison to control cultures. Nevertheless, we could find conditions and chemicals that were able to eradicate persistent cell populations, and these results have the potential to cause in a significant change in the field management of plant diseases.

Acknowledgments
Research grant Fapesp 2018/18550-0

Hitchhiking on swarming bacteria promotes the dispersal of *Xanthomonas* cells

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

Genetics of bacterial fitness of *X. campestris in vitro* and *in planta*

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

Xanthomonas campestris pv. *campestris* (*Xcc*) is the causal agent of black rot disease on cultivated or wild Brassicaceae. So far, genetic screens in *Xcc* have been qualitative and only identified genes essential for pathogenicity or avirulence. Mechanisms of pathogen entry, immunity evasion and microbial fitness in plant environments are not yet elucidated. Through the recent advances in sequencing technologies, numerous bacterial genomes are now available. However, the functions of many genes remain uncharacterized. To bridge the gap between the identification and functional characterization of genes, high-throughput experimental approaches are required. RB-TnSeq (Random barcoding-transposon sequencing) screens have been successfully used over the past few years to identify genes required for the virulence of numerous human bacterial pathogens and more recently of plant bacterial pathogens.

In order to identify the pathways important for bacterial fitness at different steps of plant infection and in different *in vitro* and *in planta* conditions, we have initiated a study using RB-TnSeq, a high-throughput screen of barcoded transposon (Tn) mutants of *Xcc.* The barcoded library of Tn mutants of strain 8004 (ca. 600 000 independent insertions) was produced and characterized. Fitness assays were successfully performed in different *in vitro* conditions (rich and poor media, xylem sap) and at early step of infection during hydathode colonization. Recent results will be presented.

Comparative genomics of the order *Xanthomonadales* with emphasis on type III secretion systems and effectors

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Keywords: virulence, evolution

A comparative genomic study based on 443 strains belonging to orders *Xanthomonadales, Nevskiales* and *Cardiobacteriales* allowed the characterization of more than 5000 *Xanthomonas*-specific genes. Among those genes, we have focused our analyses on genes encoding type 3 secretion systems (T3SS) and their type 3 effectors (T3Es) which are essential determinants of pathogenicity. We studied the distribution of these T3SS and T3Es among 223 pathogenic and non-pathogenic *Xanthomonas* strains. This work allowed the characterization of species-specific T3Es. It also showed that other T3Es are scattered in various *Xanthomonas* strains independently of their phylogeny, suggesting acquisition via horizontal gene transfer. Finally, we will present the first version of a bio-informatic tool designed to mine T3E genes in *Xanthomonas* genomes.

Using AnABlast algorithm for the identification of coding genes and fossil regions in a complete genome. A case of study: *Xylella fastidiosa* strain Pr8x

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Keywords: gene finding, low-score alignments, protein-coding sequences, *Xylella fastidiosa*

The prediction of genesis the first step in studying the functional content of a genome but for genes with no apparent resemblance to others, or genes with no defined start or end their search is difficult and often these genes can keep hidden. AnAblast algorithm allows discern between current protein-coding sequences and "ancestral", surpassing the limitations of the current computational algorithms used in gene prediction. It generates profiles of alignments on query amino acid sequences using a low-score BLAST strategy. It has been successfully tested in the genomes of *Schizosaccharomyces pombe*, and now is being to applied to *Caenorhabditis elegans and Drosophila melanogaster* discovering several marks that could probably be new genes or undescribed exons. Overall our objective was to apply AnAblast algorithm for the search of "lost genes" and "fossil sequences" in bacteria using the complete genome of *Xylella fastidiosa* (Pr8x) for that purpose. We compared the results from AnABlast with gene predictions from Prodigal tool, which only predicts protein-coding sequences. We choose high specificity, even losing sensitivity, because our aim was to analyze former putative false positives which could be coding for new proteins. From the total coding-signals obtained: 1841 were coding sequences, 29 false positives and 667 false negatives; this later corresponding to coding sequences without peaks. So, AnABlast has found 29 candidates representing 0.22% of the total genome. AnABlast seems to be a powerful tool to uncover new coding sequences and these results will be used to locate differential markers by comparing several subspecies of *X. fastidiosa* using traditional gene finders.

Comparative analysis of two fruit tree pathogens: *Xanthomonas citri* subsp. *citri* and *Xanthomonas arboricola* pv. *pruni*

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Keywords: stone fruits, citrus, comparative genomics, bacterial spot disease, citrus

In order to find those elements that contribute to their host divergence despite the convergence in the symptoms that *X. citri* subsp. *citri* (*Xcc*), and *X. arboricola* pv. *pruni* (*Xap*) cause on *Citrus* spp. and *Prunus* spp., respectively, comparative genomic and phenotypic analysis were performed between them. The study reveals genes that could be putatively associated with the adaptation of those pathogens to their hosts, being remarkable those involved in the sensing and the reaction against environmental conditions such as the case of the TonB-dependent transporters, the sensors of the two-component system and the methyl accepting chemotaxis proteins. Other differences were found in processes related to the decomposition of the cell wall as appreciated by their dissimilar set of cell-wall degrading enzymes. Moreover, type three effectors, as one of the most important factors in delineating the host specificity in *Xanthomonas*, also showed a different array when comparing both species. On the other hand, only small variations were found in other features such as the motility appendages and surface adhesion proteins, but these differences were accompanied by a dissimilar capacity to attach in host and non-host leaf surface. The molecular factors found in this work provide the basis to perform a more in-depth functional analyses that unveil those real factors associated with pathogenesis and host specificity in *Xap* and *Xcc*.

Is hydrangea a new host for *Xanthomonas hortorum* species level clade members?

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Keywords: *Xanthomonas hortorum* slc, MLSA, genomics, pathogenicity

Based on partial *gyrB* sequences, the *Xanthomonas hortorum* species level clade (slc) consists of *X. hortorum* and *X. cynarae*, and causes bacterial blight and leaf spot on agricultural crops and ornamentals. Clade members were recently reported in new countries and on new hosts. Two isolates from hydrangea, a host never previously described for *Xanthomonas* spp., originating respectively from Ethiopia (2011) and Belgium (2012), were further characterized. The isolates are closely related to the *X. hortorum* slc based on partial *gyrB* sequencing. They were accurately identified as *X. hortorum* spp. using Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS). ANI (Average Nucleotide Identity) results suggest that the isolates are a distinct species within the clade. MultiLocus Sequence Analysis (MLSA) using partial sequence of seven housekeeping genes was conducted and their genomes were sequenced. Their phenotypic fingerprints were determined using BIOLOG GENIII microplates. The pathogenicity of the isolates was tested on different known hosts of the *X. hortorum* slc clade and on hydrangea plants. The results of the various genomic, taxonomic and phenotypic experiments will be discussed.

Session 3

Genetic Resistance – Host Defence

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Host resistance against *Xylella fastidiosa* and strategies for disease control

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In general, disease resistance in plants is a complex multicomponent system starting by plant receptors that initiate signaling pathways that lead to the expression of defense response genes after the pathogen overcomes the host's mechanical barriers to infection. As is well known, exceptionally within the Gram negative, *Xylella fastidiosa* does not have the Type III Secretion System which is responsible for effector secretion through the host cell membranes. On the other hand, the cell wall released after *Xylella*-encoded cell wall-degrading enzymes, bacterial secreted molecules, and outer membrane vesicle content appears to be associated with molecular patterns that can be recognized by the host plant and trigger immune response. Indeed, among of the economically different *X. fastidiosa* plant hosts, such as citrus, grape and olive there are source of resistance into the species, indicating that these genes are involved in the resistance response. Different approaches have been used to explore the background of genetic resistance against *X. fastidiosa* in order to find key genes and their use in breeding programs or genetic engineering. In addition, despite host susceptibility, selective pressure during co-evolution with the pathogen allows mutations and new sources of resistance to occur. In fact, naturally occurring bud mutations are frequent in most plant species with particular values for vegetative propagation. Thus, mass selection is a potential tool for explore natural resistance already present in cultivars susceptible to *X. fastidiosa*. Here we will focus on the different strategies using information from genetic plant resistance and results obtained so far, aiming at controlling *X. fastidiosa* in citrus species by host resistance.

Unravelling the role of mineral elements in olive response to *Xylella fastidiosa* infections

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Keywords: *Xylella fastidiosa*, olive resistance, ionome remodelling, ICP-OES

The plant vascular system is the route of transport of mineral elements that play key roles in controlling a multitude of metabolic functions. The xylem colonizer *Xylella fastidiosa*, like any other endophyte, has enacted a series of strategies to compete with the host for mineral ions, and ensure its growth. Many of the strategies are linked to the regulation of virulence traits. Previous studies showed that remodelling of leaf ionome occurs during *X. fastidiosa* subsp. *fastidiosa* and *multiplex* infection, uncovering the existence of a mineral element-based response during host-pathogen interaction.

X. fastidiosa subsp. *pauca* is severely affecting olives in Apulia, so to identify possible mechanisms of resistance we investigated whether the mineral elements play a role in OQDS progress and in the differential responses shown by resistant ('Leccino') and susceptible ('Ogliarola salentina') cultivars.

Leaf ionome of symptomatic and asymptomatic samples collected in two fields in the infected area was determined by Inductively coupled plasma-optical emission spectrometry. Data showed that 'Leccino' maintains consistently higher Mn concentrations than 'Ogliarola salentina', independent of disease status. Given the prominent role of Mn in plant enzymes, we hypothesized that the increased availability of this element may provide a basis for 'Leccino' resistance by activating antioxidant enzymes and counterbalancing the uptake of transition metals, triggered by the pathogen. 'Leccino' also showed a significant increase in Ca levels as reported before for other *X. fastidiosa* hosts, in response to the progress of symptoms. This Ca increase was not detected in 'Ogliarola salentina', probably due to the advanced stage of symptomatology evidenced in the field. Previous data suggested that the levels of increased Ca were correlated to severity of disease, therefore,

understanding how 'Leccino' is able to withstand the activation of a *X. fastidiosa*-induced cascade driven by Ca increase, may help further our knowledge of the disease progression in other hosts.

Acknowledgements
This work was done in the framework of an STM granted by the EU COST Action CA16107 and with the financial and scientific support of the EU Horizon 2020 research and innovation programme under Grant Agreement N. 635646 "Pest Organisms Threatening Europe POnTE" and N. 727987 "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

The tomato RLCK BSK830 interacts with transmembrane receptors and plays a role in plant immunity

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Members of the *Arabidopsis* brassinosteroid signaling kinase (BSK) family of receptor-like cytoplasmic kinases have been implicated in brassinosteroid signaling and immunity. To investigate a possible role of tomato BSK830 in pattern-triggered immunity (PTI), we analyzed the physical interaction of BSK830 with various components of PTI signaling. BSK830 interacted in yeast with the pattern recognition receptors FLS2 and FLS3, which perceive the bacterial flagellin peptides flg22 and flgII-28, respectively, with the LysM receptor-like kinase Bti9 and the co-receptor SERK3A. Interaction of BSK830 with FLS3, FLS2 and Bti9 was confirmed *in planta* and plant treatment with flgII-28 reduced the BSK830-FLS3 interaction. Consistent with a role in PTI signaling, preliminary analysis of CRISPR/Cas9-generated *bsk830* mutant plants revealed a reduced accumulation of reactive oxygen species upon treatment with the flg22 and flgII-28 peptides. However, MAPK activation by flg22 and flgII-28 remained unaltered in these plants. In addition, *bsk830* mutant plants displayed enhanced susceptibility to *Botrytis cinerea*. Finally, we started to investigate whether type III effectors of the bacterial pathogen *Xanthomonas euvesicatoria* may target BSK830. Split luciferase complementation assay performed in *Nicotiana benthamiana* plants revealed that seven *Xanthomonas* effectors, out of the 35 tested, interacted with BSK830 *in planta*. Together, these results support a role for BSK830 in PTI signaling and as a target of bacterial effectors.

Engineering of loss-of-susceptibility in rice: remove TALE targets to control *Xanthomonas oryzae* pv. *oryzae* in Africa

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Keywords: *Xanthomonas oryzae* pv. *oryzae*, BLB, TAL effectors, durable resistance

Bacterial Leaf Blight (BLB) 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. *Xoo* pathogenicity relies on the injection into the host cell of Transcription-Activator Like Effectors (TALEs) which are effective transcription factors specialized to hijack the plant transcriptional machinery. TALEs bind to Effector Binding Elements (EBE) in the promoter of susceptibility (*S*) genes and activate transcription, which is essential for disease development. Clade-III members of the family of *SWEET* sugar transporters are major BLB susceptibility genes. Naturally occurring polymorphism in the EBEs of *SWEET* promoters prevent TALE binding, resulting in loss of *SWEET* gene induction. These "unresponsive" alleles act as recessive resistance genes, preventing symptoms formation and reducing bacterial colonization *in planta*. African *Xoo* are more genetically related to *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) and differ from Asian *Xoo* by their TAL effectors's content and targets. African *Xoo* has fewer TALEs and specifically targets *SWEET14* of Clade III as a major susceptibility gene. Besides, African *Xoo* use two distinct TAL effectors to induce *SWEET14*, highlighting the importance of this gene for bacterial proliferation. Genetic engineering to gain resistance is an effective, economical and ecological way to guarantee food and economic safety of rice producing countries. Here, we investigate the robustness of a resistance based on loss-of-susceptibility upon multiple editing of *SWEET* genes towards broad-spectrum resistance of rice against African *Xoo*. Our most recent results will be presented.

Strategies for generation of rice plants that are resistant to *Xanthomonas oryzae* infections

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Keywords: TALE, genome editing, rice, *Xoo*, resistance

Rice-pathogenic *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) cause severe harvest loss. The pathogen virulence strongly relies on bacterial TALEs (transcription activator-like effectors) which function as transcriptional activators for plant genes. We determined the full genome sequences of *Xoo* strains and compared their TALE repertoires. Genome-wide TALE targets in rice were then identified by combining computational TALE target predictions and the induction of rice gene expression during a bacterial infection. The induction of specific rice promoters by individual TALEs was experimentally validated. In summary, a comprehensive view was gained on the different plant processes that are manipulated by the pathogen. This knowledge can now be used as a basis to develop plants that are resistant to an *Xoo* infection. The rice target promoters can be modified via genome editing to generate allelic variants that are no longer recognized by TALEs, effectively inactivating key virulence factors of the pathogen. Alternatively, TALE target sequences can be placed in front of a resistance gene which triggers cell death to generate pathogen-inducible plant resistances. We will present recent progress on these approaches.

Session 4

Disease Management – Vector Control

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Prospects for using bacteriophages for plant disease control

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Keywords: Biocontrol, phage therapy, phage ecology, phytobacteria

Significant challenges exist when using bacteriophages for controlling bacterial plant diseases associated with the phyllosphere. Effective disease control requires that phage populations are at high enough concentrations to come into contact with the pathogen on the leaf surface. However, maintaining high populations on leaf surfaces is the major challenge given adverse environmental conditions. Bacteriophages were shown not to persist on plant surfaces for long periods of time mainly as a result of exposure to UV light where phage populations plummet rapidly. Therefore, several strategies were undertaken to enhance phage efficacy. The application timing was altered to increase the period of time phage persisted on leaf surface. Phages were applied late in the day to avoid UV exposure for a longer period of time and resulted in a longer period of phage survival on leaf surfaces. Protective formulations were developed to prolong phage viability on the leaf surface. Various formulations were identified that extended phage survival in the field. Finally, we have learned that identifying the correct phages is critical. We demonstrated that selecting the phages based on in vivo assays is important to consider when developing use for field application. Although phages have potential in biological control, there are major obstacles to consider.

Insights on *Paraburkholderia phytofirmans* PsJN behaviour as biocontrol agent of *Xylella fastidiosa* in olive

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Keywords: *Paraburkholderia phytofirmans* PsJN, *Xylella fastidiosa* 'De Donno', biological control, OQDS

Numerous research efforts are currently underway to identify novel biocontrol agentsfor the management of diseases associated with *Xylella fastidiosa*. Despite the encouraging premises, most of these studies proved unable to provide long-term effects or failed once they were examined at the field-scale. An intriguing exception was that of the rhizosphere-colonizing bacterium *Paraburkholderia phytofirmans* PsJN, which appears efficacious to suppress Pierce's Disease development when inoculated in grapevine infected by *Xf* 'Temecula'.

Having verified that PsJN did not exhibit any antagonistic *in vitro* effect towards *Xf* 'De Donno', we wanted to determine if it might colonize and protect olive from OQDS progress. We tested different inoculation approaches, before concluding that pin-prick method ensures the highest success rate. Motile populations of PsJN could be consistently detected distant from the inoculation point, with a newly developed SYBR® Green qPCR assay, and their long-term viability was demonstrated by successful isolation on TSA medium.

We set field trials in the Apulian area affected by OQDS to investigate prophylactic and therapeutic effects of PsJN inoculation in plants naturally exposed to *Xf* infection. Current observations, limited to a single season, did not evidence any statistical correlation between the population dynamics of Xf and PsJN, but still, require validation on a larger time-frame. Although we collected evidence that PsJN could multiply and move in olive xylem vessels, we also observed that its concentration decreased significantly over time, suggestive of a plant response that limits PsJN expansion. We attempted a WGS metagenomic study to further investigate if PsJN

might prime a host response in turn leading to a knock-on effect on the population indices of *Xf* and the residential microbial communities.

Acknowledgements
This work has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N. 635646 "Pest Organisms Threatening Europe POnTE".

Olive tree core microbiome: the first learning step towards the development of biological control strategies for *Xylella fastidiosa*

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Keywords: plant-associated microbiota, plant susceptibility, *Xylella* infection, core microbiome

The core microbiome has been increasingly recognized to be central to plant health. However, the extent to which host plant may play a role in shaping its core microbiome and their implications for host susceptibility/resistance to a particular disease, remains poorly understood. Such knowledge, besides providing insight on the potential role of core microbiome in plant resistance, could additionally contribute to the identification of microbial strains that can be used as inoculant to drive the plant microbiome to a pathogen-resistant composition. *Xylella fastidiosa* is now recognized as one of the major threats to the olive tree culture. In this study, the xylem tissue microbiome of infected and non-infected olive tree cultivars, with contrasting susceptibilities to *X. fastidiosa*, was analyzed through Illumina amplicon sequencing and further compared. This analysis was performed in samples collected in spring and in autumn. Overall, the core microbiome was dominated by members of the *Proteobacteria* (81% of the total bacteria reads) and Ascomycota (98% of the total fungal reads). In non-infected trees, the core microbiome was found to differ between susceptible and resistant cultivars, in particular within fungal communities. Indeed, the interaction between the host cultivar and the presence of *X. fastidiosa* was found to shape the core fungal community in xylem vessels. Specific fungal/bacterial signatures were detected to either the presence or the absence of *X. fastidiosa* in the xylem vessels, suggesting an important role of these microorganisms in pathogen establishment/development.

Acknowledgments
This work was supported by European funds in the framework of the project H2020-SFS-2016-3 "XF-ACTORS - *Xylella fastidiosa* active containment through a multidisciplinary-oriented research strategy" (Grant Number 727987).

Random antimicrobial peptide mixtures for management of *Xanthomonas* plant diseases

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

Management of bacterial plant diseases vastly relies on copper bactericides. According to the European Food Safety Authority, the use of copper is of concern to public health and the environment, and copper compounds are due to be gradually phased out. Considering the lack of alternatives, there is an urgent need to develop novel technologies to manage bacterial plant diseases. We have recently introduced the random antimicrobial peptide mixture (RPM) approach to tackle pathogenic bacteria. 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 several phytopathogenic bacteria. Importantly, these compounds significantly reduced disease severity of tomato and kohlrabi plants infected with *Xanthomonas perforans* and *Xanthomonas campestris* pv. *campestris*, respectively. Recently, we generated lipo-RPMs by conjugating short RPMs with fatty acids. While short 5-mer RPMs FK-5 and FdK-5 did not show significant antimicrobial activity, their conjugation with palmitic acid (FK-5-p and FdK-5-p) led to antimicrobial activities that were similar or higher than those of the 20-mer RPMs. We synthesized the 32 individual molecules that compose the FdK-5-p mixture, and studied the effects on antimicrobial activity of the ratio between hydrophobic and cationic amino acids, as well as of their proximity to the fatty acid. Both traits were shown to be important determinants of antimicrobial activity. Moreover, combinations of lipo-peptides were shown to possess enhanced antimicrobial activity than individual ones. Our study demonstrates the potential of RPMs and lipo-RPMs as novel tools for management of bacterial plant diseases.

Nanoparticles as new weapons in the war against antibiotic resistant bacterial strains

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Keywords: antimicrobial nanomaterials, metals, bacteria, toxicity

Research and development of antibiotics has been very intense since their discovery in 1928. However, in the last decade, there has been a dramatic shift in their effectiveness. More and more bacterial strains have developed resistance to antibiotics and these resistant microorganisms are able to withstand the activity of antimicrobial drugs in a way that standard treatment becomes ineffective and infections persist, which increases the risk of their spread. It is not therefore surprising that numerous researchers have been looking for new way to kill bacteria. We aim our research at development, characterization and modification of nanomaterials based on Selenium and other metals and semi-metals having wide-spectrum antimicrobial effects on the one hand, but no or only minor cytotoxic effect on eukaryotic cells on the other. In this study, we tested nearly one hundred types of nanoparticles composed of Selenium, Zinc or Copper and their couples with graphene/graphene oxide. The prepared nanoparticles were further modified to increase the effects or to prevent negative effects by targeting ligands that are tailored to the particular application. Their antimicrobial effects were studied on both non-resistant (e.g. *Staphylococcus aureus*, *Escherichia coli*) and resistant strains (e.g. methicillin resistant *S. aureus*). The most promising nanoparticles designed in this way were tested on *Xanthomonas campestris* pv. *campestris* (Xcc).

Potential vectors of *Xylella fastidiosa* in olive groves, almond orchards and vineyards in Trás-os-Montes region, Portugal

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Keywords: emerging plant diseases, *Cicadomorpha*, sweepings, *Philaenus*, *Neophilaenus*

Xylella fastidiosa, was recently detected in Portugal (January 2019), this phytopathogenic bacterium is a threat to important crops of agricultural interest. It is transmitted by xylem-feeding insects that belong to the suborder Auchenorrhyncha. However, the knowledge about these insects in Portugal is scarce. In this context, the goal of this work was to identify the frequency and biodiversity of *Auchenorrhyncha* in Trás-os-Montes region, Portugal. For that, in 2018, in Trás-os-Montes region, five olive groves, five almond orchard and five vineyards with ground cover were sampled for adults of *Auchenorrhyncha*, during three distinct periods (beginning of July, mid-September and mid-October). Sampling was performed in the ground and in the aerial part of the plants. In each sampling date, 10 samples of 10 sweepings were collected on the ground in each orchard and vineyard. On the aerial part of the orchards 10 samples of 4 sweeping in 6 trees were collected and in the vineyard 10 samples of 50 sweepings were collected. A total of 3741 adults of *Auchenorrhyncha* were recovered on the three sampling dates. From these, 949 were *Fulgoromorpha* and 2792 *Cicadomorpha*, being the highest abundance observed in the beginning of July. In general, the vineyards presented the higher number of individuals of the *Cicadomorpha* and the olive orchards presented the smaller number of individuals of this infraorder. However, almond orchards presented the higher abundance of confirmed vectors of *X. fastidiosa,* 16 individuals belonging to the genus *Neophilaenus* and seven individuals of the genus *Philaenus* in the three periods. The higher number *Philaenus* individuals was observed in mid-October on the vegetation cover.

Acknowledgments
This work has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement N. 727987 "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

First studies on the relationship between *Xylella fastidiosa* subsp. *pauca* ST53 and its insect vector in Apulia

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Keywords: *Philaenus spumarius*, spittlebug, OQDS, environmental factors, kinetics

To elucidate the relationships between *Xylella fastidiosa* and its main European insect vector, the spittlebug *Philaenus spumarius*, experiments were conducted for two consecutive years to assess the kinetics, multiplication and persistence of the bacterium in the spittlebug. To this end, insects were first caged on infected source plants and then isolated, in groups of five, on olive or periwinkle plants at different times post-acquisition (3,7,14, 28, 56, and 72 days). Insects and recipient plants have been individually tested for *X. fastidiosa* by quantitative PCR. The bacterial load estimated in the infected insects at different times post-acquisition varied from few thousands to tens of thousands cells per insect's head. The second experiment was carried out to assess the influence of environmental factors (temperature, season) and insect age on simulated epidemics progression on olive plants. Adults of *P. spumarius,* fed on infected olive branches, were then released in cages containing 16 olive plants and collected after 3-7-14-21 days. Although most of the diagnostic tests will be carried out only in the upcoming months, preliminary results indicate a higher acquisition efficiency in September as compared to July, and a lower acquisition efficiency from periwinkle compared to olive as source plants, but higher transmission efficiency to periwinkle compared to olive as recipient plants.

Rapid spread of black rot (*Xanthomonas campestris* pv. *campestris*) in brassica transplants

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Keywords: *Xanthomonas*, spread, epidemiology, modelling

Xanthomonas campestris pv. *campestris* (Xcc) is well known as an important seed-borne pathogen of brassicas. Most vegetable brassicas are grown from transplants. These transplants are generally produced under glass by specialist plant-raisers. We conducted a series of experiments that examined the rate of spread of Xcc during during transplant production under conditions that mimicked commercial production systems. Primary inoculum was introduced as infested seeds in one or more module cells. Disease symptoms were mapped, and the asymptomatic presence of the pathogen was determined by leaf washings from samples at different distances from the primary infector at intervals after sowing. These experiments showed that the rate of spread during transplant production could be very high: spreading from one primary infector (infested seed) to 98% contaminated within six weeks in a block of 4500 transplants. The data were used to develop models to predict potential contamination in commercial scale systems. Such rapid spread explains why, despite relatively low levels of seed infestation, disease symptoms can 'suddenly' appear simultaneously on large numbers of plants in the field.

Antibacterial effect of advanced nanomaterials against *Xanthomonas campestris* pv. *campestris*

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Keywords: nanomaterials, black bacterial vein, antibacterial effect

Xanthomonas campestris pv. campestris (Xcc) is a very destructive biotic agent for cruciferous plants worldwide. A control of black bacterial vein caused by Xcc is difficult due to a deficiency of effective remedies. Therefore, a development of new treatments against Xcc is substantial in order to reduce the negative economic impact of this pathogen. In the present study, we tested several types of advanced nanomaterials based on metals and semi-metals against Xcc strain. In order to determine bactericidal properties of tested nanomaterials, we performed various *in vitro* trials. The nanomaterials were incubated with Xcc culture and bacterial growth inhibition was evaluated by method involving colony forming units enumeration. Subsequently, minimum inhibitory concentration (MIC) was determined for nanomaterials with the highest antibacterial activity. For this purpose, bacterial growth curves were established by spectrophotometric measurement of absorbance, as well as quantification of viable bacteria was performed. The results obtained from *in vitro* trials give a strong basis for selection of advanced nanomaterials for further tests *in vivo*.

POSTERS

Improved diagnostics of common bacterial blight of beans using DNA barcoding and MALDI-TOF analysis

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Keywords: *Xanthomonas axonopodis* pv. *phaseoli*, DNA barcoding, MALDI-TOF, identification

A seed- borne pathogen *Xanthomonas axonopodis* pv. *phaseoli* causes common bacterial blight on beans. It is regulated on seeds (EPPO A2 list and EU Annex II/A2). Although even low levels of seed contamination can lead to symptoms development in the field and consequently to yield losses. Infected seeds are usually symptomless. Therefore, laboratory testing is necessary to manage the disease together with good sanitation practices during seed production. The laboratory testing relies on isolation of bacteria on general and selective media, followed by their identification and final confirmation of bacterial pathogenicity by artificial inoculation of bean plants (*Phaseolus vulgaris*). The aim of our study was to test faster methods than classical microbiology for identification of *Xanthomonas* spp. and compare them to currently established methods described by the ISTA protocol (Grimault et al., 2014). We focused on generic methods of identification, MALDI-TOF *(*MALDI Biotyper, Bruker Daltonik) which was found to be a useful screening test to select isolates for further identification and DNA barcoding (partial sequencing of *gyrB* and *avrBs2* gene) which allows for accurate identification and differentiation of the target bacteria from similar isolates occurring in bean seeds. Between the years 2010 and 2018 our diagnostic laboratory received altogether 34 bean seeds samples. From these, 62 isolates of genera *Xanthomonas* were isolated from 22 samples (65 % of samples). Using generic methods of identification we were able to differentiate *Xanthomonas axonopodis* pv. *phaseoli* from morphologically similar bacteria that are frequently occurring on plates and resulted in a faster and more reliable of diagnosis.

Impact of different chemicals with presumed epigenetic effect on virulence of *X. campestris* strain

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Keywords: epigenetics, *Xanthomonas campestris*, virulence, cabbage

Epigenetics study heritable changes in phenotype that are reasoned by stable alterations in the gene expression manner. In the present study, a selected strain of *X. campestris* pv. *campetris* (*Xcc*) was treated by different chemical substances with more or less described epigenetic effect. They were subsequently used for inoculation of sensitive variety of cabbage. The speed and vigour of symptoms typical for *Xcc* infection was evaluated in details for a long period, until majority of variants died. Obtained results prove that some of the treated strains showed significant differences in their virulence compared to inoculation with untreated *Xcc* strain. These differences were both - towards higher virulence, while some treated strains showed also reduced virulence. In some cases, noticed changes in virulence were even confirmed within second round of inoculations by using re-isolated strains from first circle of inoculations.

Presented results are the basis for further planned research, where will be evaluated the influence of individual substances on other molecular genetics properties (RNASeq of treated strains) or on biological properties (versatility of promising treatment in terms of their re-inoculation to other Brassica species or if applied to other bacterial pathogens).

Acknowledgments

This work was supported by the project LTC18009, program INTER-COST provided by the Ministry of Education, Youth and Sports of the Czech Republic.

Realtime colorimetric loop mediated isothermal amplification for point of care detection of *Xanthomonas gardneri* causal agent of bacterial spot of tomato and pepper

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Keywords: *atpD* gene, primer design, field-deployable, portable instrument

Xanthomonas gardneri is one of three causal agents of bacterial spot of tomato and pepper (BS). DNA sequence of *atpD gene* was chosen for primer design to develop a loop-mediated isothermal amplification (LAMP) based assay. Significant advantages in terms of rapid and sensitive detection can be achieved by using field-deployable portable LAMP-based instruments. In order to rapidly and accurately identify and differentiate *X. gardneri* from other BS causing *Xanthomonas* ssp. using real-time monitoring LAMP-based method has been optimized on Smart-DART™ (Diagenetix, USA) platform instrument using colorimetric assay. Specificity and sensitivity were tested on the complex of bacterial strains pathogenic to tomato, pepper and related crops. The assay detection limit was 1 pg of genomic DNA with result visible in only 30 minutes. The use of various portable and handled heating instruments allows a fast analysis reducing the diagnosis time and may have implications for the disease management and for the control of *X. gardneri*. The high efficiency of this method suggests its use as a standard diagnostic tool during phytosanitary controls.

Computational analysis of type VI secretion systems in *Xanthomonadaceae*

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Keywords: secretion, effector, antibacterial competition, virulence, comparative genomics

Xanthomonadaceae is a widespread family of the Gammaproteobacteria class. More than hundred different species were sequenced to date, and a total of two thousand genome sequences are available. Many *Xanthomonadaceae* contain a type VI secretion system (T6SS), a multi-protein machine used to deliver effector proteins into neighboring bacteria and host cells. The genes coding for the T6SS are usually located within large 'gene clusters'. In most cases, the genes coding for the effector proteins are located within these clusters, although, sometimes, they are located outside these clusters. We employ computational approaches to systematically identify and characterize T6SSs in *Xanthomonadaceae*. Furthermore, we use comparative genomics and advanced data mining to identify new effector proteins with focus on polymorphic toxins. We discuss possible roles of T6SSs in antibacterial competition and virulence.

Molecular characterization of *Xanthomonas* spp. isolates detected in *Fabaceae* plants

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Keywords: *Xanthomonas*, PCR MP, rep-PCR, MLSA, T3E, *Fabaceae*

The genus *Xanthomonas* represents a widely spread group of phytopathogenic bacteria that can cause serious economic and ecological losses. However, there have been no detailed investigations about bacterial diseases caused by *Xanthomonas* in Lithuania so far.

The aim of this study was to detect and to characterize xanthomonads isolated from different species of *Fabaceae* plants. Nucleic acid-based techniques as PCR (using *Xanthomonas* genus specific primers X1 and X2; Maes M., 1993), PCR melting profile (PCR MP), repetitive PCR (rep-PCR), multilocous sequence analysis (MLSA) and detection of type three effector (T3E) genes were used for identification and characterization.

Isolates that belong to the *Xanthomonas* genus were found in this study. PCR MP with *NcoI*, *PstI* and *ApaI* was performed. However, PCRs failed with all our *Xanthomonas* spp. isolates where endonuclease *ApaI* was used. ERIC and BOX PCR allowed to assess the genetic diversity of *Xanthomonas* strains, but REP PCR didn't discriminate our strains as only few products were obtained. Two (*gyrB* and *rpoD*) out of three housekeeping genes were present in all *Xanthomonas* strains, and *fyuA* gene was detected only in 4 strains. 15 primers for detection of T3E genes were used, but only *xopA*, *xopG*, *avrBs2* and *xopR* genes were found in some isolates.

After a molecular study, it is predicted that a new pathovar could be exist, and further studies are needed to confirm this.

Investigations on Belgian flora and on xylem-feeding insects to evaluate the risk of introduction, establishment and spread of *Xylella fastidiosa* in Belgium

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Following the EFSA opinion (2015) on the risks to plant health posed by *Xylella fastidiosa* in the EU territory and later on, its report in 2018 in a nursery of West Flanders, the threat of this pathogen for the Belgian flora needs to be assessed.
Based on a first screening of the *Xylella* host plants, four model tree species were selected to be subject to susceptibility investigations: *Prunus domestica* cv. Opal, *Quercus petraea*, *Salix alba* and *Populus tremula*. The susceptibility to the bacterium of these plant species is investigated in different ways. Firstly, it is assessed through "Sentinel plantation", which consists in the establishment of these plant species in areas contaminated by the bacteria to evaluate their susceptibility under natural conditions. The second complementary way consists in mechanically inoculating different strains of *X. fastidiosa* into the plant xylem in biosafety quarantine-controlled glasshouses. The presence and the progression of the bacterium into the plants is monitored by PCR as well as by confocal microscopy with the use of a GFP-tagged strain kindly provided by Steven Lindow.
On the other hand, three potential insect vectors for Belgium were selected, the Hemiptera *Cicadella viridis*, *Philaenus spumarius*, and *Aphrophora salicina*. Their potential for bacterial acquisition through artificial diets and artificially infected plants is also studied using a set of *X. fastidiosa* strains. Furthermore, the bulk sampling of these potential vectors through Belgium is undertaken, with the aim of using an "insect spy" strategy for an early detection of the bacteria in the country.

EFSA PLH Panel (EFSA Panel on Plant Health), 2015. Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. EFSA Journal 13 (1): 3989. DOI: 10.2903/j.efsa.2015.3989.

IBER-XYFAS – Ibero-American network for the surveillance of *Xylella fastidiosa*

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Xylella fastidiosa is the causal agent of various plant diseases that continuously challenge agroforestry production causing significant losses to European and American countries. IBER-XYFAS is an international network of research groups, agrofood companies and regional governments, financed by CYTED that aims gather all available data on the bacterium, on its vectors, on the crops affected in Ibero-American countries and on the prevention and control activities that are being carried out. The specific objectives of the network will focus on: P1) Information on the bacterium; P2) Information on transmission vectors; P3) Information on the interaction of the bacteria with the plant; P4) Information on therapies; P5) Information on remote sensing methodologies; P6) Information on the environmental, social and economic impact of diseases and control measures. The purpose of the information exchange is to generate knowledge that will contribute to the development of a technological alert and surveillance system that will enable local or national governments to take the necessary measures to contain and ultimately eradicate the disease. The main outputs of IBER-XYFAS are: promote the scientific and technological integration of the Ibero-American region and the effective transfer of knowledge and technologies; encourage the participation of researchers from the Ibero-American region in other programs and the active participation of different actors in the control of the disease; promote the development of effective transfer activities to small producers and vulnerable sectors; contribute to the improvement of the social perception of R+D+I and its results; promote the improvement of production through the integration of technological and economic aspects; design technological protocols for the control of the disease.

Monitoring plant and microbial volatile organic compounds for disease detection

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Keywords: volatile organic compounds, sensors, disease monitoring, microbial interactions

Can microbial ecology inform pathogen detection and monitoring? Plants and microorganisms emit volatile organic compounds (VOCs) which are indicators of microbial and plant-microbe interactions, potentially allowing early detection of infections. However, important bottlenecks remain. For the detection of VOCs to be useful to farmers, it is important that plant and/or soil VOC profiles can be determined in-situ. However, VOC profile determination requires expensive sampling equipment, such as VOC-sorbent tubes, and complex laboratory analyses, e.g. thermal-desorption gas-chromatography mass-spectrometry (TD-GC-MS). Furthermore, existing research suggests that VOC emissions from plants and soil can be considerably variable depending on environmental conditions and the low VOC concentrations hinder passive detection strategies. In addition, the linkages between specific VOCs and plant pathogens are generally not well established. However, recent developments in low-cost sensor technology, microfluidics, machine learning algorithms, environmental wireless sensor networks and data analytics are enabling substantial developments in pathogen monitoring approaches. In this study, a semi-continuous monitoring strategy for sampling VOC emissions from plants and soil is being developed. Our aim is to design a VOC sensor prototype that will enable future in-situ detection of soil and plant health status and ultimately a low-cost sensor system that can be used for early-warning detection of infections by specific plant pathogens. The sensor concept is designed not only to provide an early-warning system but also to inform and educate farmers, government bodies and other stakeholders for increased uptake of evidence-based plant-protection measures.

Comparative genomics highlight putative pathoadaptations of a new *Xanthomonas* sp. isolated from walnut and *Xanthomonas arboricola* pv. *juglandis*

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Keywords: bacterial walnut disease, *Xanthomonas*, comparative genomics, virulence-related genes

The occurrence of multiple *Xanthomonas* lineages within the same walnut host has been reported by different studies. More recently, it was shown that these consortia of walnut associated *Xanthomonas* include both pathogenic and non-pathogenic strains and non-*arboricola Xanthomonas* species, as assessed by Average Nucleotide Identity (ANI) analysis. The ecological, evolutionary and pathogenicity implications of this co-colonization is still poorly understood. In order to unveil niche-specific adaptations, the genome of five *Xanthomonas* strains isolated from a single walnut tree in Loures (Portugal) were sequenced. Core genome phylogeny allowed separating these isolates in two distinct clusters, one grouping CPBF 427 and CPBF 1521 with *Xanthomonas arboricola* pv. *juglandis* (*Xaj*) strains, and another clustering together CPBF 367, CPBF 424 and CPBF 426, constituting a likely new species according to the low ANI value (<90%) to other *Xanthomonas* species. Beyond the distinct profile of T3SS and T3E genes, detailed in another work, comparative genomics revealed relevant genomic differences between the typical *Xaj* isolates (CPBF 427 and CPBF 1521) and the *Xanthomonas* sp. (CPBF 367, CPBF 424, and CPBF 426), which could translate into distinct pathogenicity and virulence features. In fact, when compared with *Xaj*, these strains hold an exclusive set of gene homologs encoding for chemotaxis related proteins, pectate lyase E, pectinesterase, and XspN

from T2SS, suggesting distinct pathoadaptations. Most interestingly, is the presence of genes encoding for a putatively functional type IV pilus in strain CPBF 424 which pathogenicity in walnut has been demonstrated. Altogether, the distinct genomic repertoire of these *Xanthomonas* sp. isolates raise the hypothesis of their importance as walnut opportunistic pathogens.

Genomic analysis and taxonomic inference of *Xanthomonas arboricola* pv. *juglandis* DNA markers using an alignment-free sequence comparison tool

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Keywords: *Xanthomonas*, alignment-free methods, genomics, phytopathogens detection

The current availability of thousands of complete microbial genome sequences provides a wealth of new data and new bioinformatics solutions are being developed to operate at this level. Among these methods, alignment-free sequence algorithms are particularly promising since they require low computational processing and memory and are not dependent on sequence identity nor on homology, making these approaches faster than alignment-based algorithms for sequence analysis.

In this work we used Chaos Game Representation (CGR), an iterative mapping method for representing sequences, to assign taxonomically DNA markers regardless their size, by applying Genomic Signal Processing (GSP) tools capable to retrieve their similarity and dissimilarity. Whole and partial genomic sequences from distinct species of *Xanthomonadaceae* were compared with four *Xanthomonas arboricola* pv. *juglandis* (*Xaj*) DNA signatures (XAJ1, XAJ4, XAJ6 and XAJ8). In order to solve inconsistencies when comparing sequences with different sizes, a new algorithm is proposed to efficiently compare short DNA sequences (<1 kb) with whole chromosomal sequences.

The algorithm, implemented in MATLAB, showed that *Xaj*-specific DNA sequences clustered together with *Xanthomonas arboricola* pv. *juglandis* as hypothesized, contrary to other *Xa* pathovars or other *Xanthomonas* species. These results suggested that this method might be particularly useful to rapidly infer the taxonomic identity of short DNA sequences to accurately detect *Xanthomonas* phytopathogens and contribute to improve the assembly of genomic and metagenomic reads.

Currently, we are assessing the genotyping resolution of this approach using larger genomic regions capable to discriminate different *Xaj* strains, which will be valuable for epidemiologic surveys.

Role of cuticle thickness and wax composition in the resistance to *Xanthomonas citri* ssp. *citri* during mandarin leaf-development

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Satsuma (*Citrus unshiu*) 'Okitsu' is a mandarin presenting substantial resistance to *X. citri* ssp. *citri* (*X. c*itri), causal agent of citrus canker disease. We have previously shown that 'Okitsu' is more resistant to *X. citri* than mandarin 'Clemenules' (*C. clementine*), particularly during early stages of leaf development and exclusively when the leaves are inoculated by a noninvasive spraying method, suggesting that the leaf surface could be contributing to this resistance. In this work, we study the structural-chemical properties of leaf surface barriers of both mandarin cultivars. Ultrastructural analysis showed a thicker cuticle covering epidermal surface and guard cells in young 'Okitsu' leaves that was associated with a smaller stomatal aperture, reduced cuticle permeability and increased stomatal defense to *X. citri* infection. These findings were correlated with an early accumulation of cuticular wax components, including primary alcohols, alkanes and fatty acids. Expression of the major wax-associated genes confirmed these results. None of these differences described for young leaves were observed in mature leaves, where both cultivars are equally resistant to the bacterium. Remarkably, mechanical alteration of cuticular thickness of young 'Okitsu' leaves allows *X. citri* colonization and canker development. Taken together, these data suggest that a faster cuticular wax development in 'Okitsu' leaves play a central role in its resistance to *X. citri*.

Integration of biological and chemical methods in control of pepper bacterial spot

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Keywords: *Xanthomonas euvesicatoria*, copper, antibiotics, resistance inducers, bacteriophages

Bacterial spot caused by *Xanthomonas euvesicatoria* is one of the widespread and economically most important pepper diseases in Serbia. The disease management practices were either omitted or failed to provide satisfactory control, especially when weather conditions favored spread of the pathogen. In order to develop an efficient integrated disease management program for pepper bacterial spot control, we studied efficacy of biocontrol agents: bacteriophages (strain KΦ1) and two strains of *Bacillus subtilis* (AAac and QST 713), systemic acquired resistance (SAR) inducer (acibenzolar-S-methyl - ASM), a commercial microbial fertilizer (Slavol), copper based compounds (copper hydroxide and copper oxychloride) in combination with or without mancozeb, and antibiotics (streptomycin sulfate and kasugamycin). Based on the single treatment efficacy, various combinations of the treatments were integrated for further testing in three separate field experiments. Additionally, we evaluated potential negative effect of ASM on pepper growth and yield in the growth chamber experiments and field. Spraying of ASM in concentration of 0.0015% effectively controlled the disease intensity and caused minimal negative effect on pepper growth and yield. All the tested single treatments significantly reduced disease severity compared to the untreated inoculated control (UTC), except microbiological fertilizer and *B. subtilis* strain AAac. All integrated treatments of biocontrol agents, ASM and copper hydroxide, significantly reduced disease severity as compared to the UTC. Integration of bacteriophages, ASM and copper hydroxide was the most efficient treatment combination, reducing the disease intensity by 96-98%, indicating that this may be an adequate alternative program for control of pepper bacterial spot.

Identifying resistance genes in the *Lolium multiflorum* – *Xanthomonas transluscens* pv. *graminis* pathosystem

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

Xanthomonas transluscens 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. Since its discovery, resistant cultivars have been bred, however, since *L. multiflorum* is an outbreeding species, cultivars are highly heterozygous, and susceptibility still occurs. One major QTL for resistance was previously identified, however no sequence-specific marker to be used in breeding has yet been identified. A mapping population consisting of 7530 F_2 individuals segregating for resistance was established in the greenhouse, and inoculated with a highly virulent *Xtg* strain. Two pools of the 1000 most resistant and the 1000 most susceptible individuals, respectively, were formed and sequenced using the Illumina HiSeq2500 platform. SNP frequencies will be determined, and SNPs associated with resistance or susceptibility will be identified. This will help to identify candidate resistance genes for marker assisted breeding and to better understand the mechanisms of this complex interaction.

Detection of *Xanthomonas campestris* pv. *campestris* and *Xanthomonas campestris* pv. *raphani* in seeds of cabbage crops with Seed-Extract PCR followed by dilution plating

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Keywords: TaqMan PCR, seed extract, dilution plating, black rot

Cabbage crop seeds should be free of living *Xanthomonas campestris* pv. *campestris* (Xcc) and *Xanthomonas campestris* pv. *raphani* (Xcr), the causal agent of black rot of cabbage crops, when sold to our customers. To assure this, all seed lots of cabbage are tested for the presence of Xcc/Xcr. The International Seed Health Initiative (ISHI) developed a method to detect Xcc/Xcr by PCR with Seed Extract-PCR (SE-PCR) which has become an ISTA (International Seed Testing Association) rule, meaning that this method has been validated and internationally recognized.

At Bejo Zaden B.V., this method is adopted and used as a prescreening method for routine testing of untreated cabbage seed lots. The first step is to use the extract of soaked seeds for Xcc/Xcr detection by TaqMan PCR. If Xcc/Xcr DNA is found, the presence of living Xcc/Xcr colonies is confirmed by dilution plating of the seed extract and colony identity confirmation by a second PCR.

A schedule was created in which a PCR results should be obtained within a certain amount of time, followed, if necessary, by dilution plating of the same extract. It assures a timely and reliable result.

After testing of several hundreds of seed lots, it was possible to correlate PCR to dilution plating results and refine the results.

CRISPR elements provide a new framework for the genealogy of the citrus canker pathogen *Xanthomonas citri* pv. *citri*

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Keywords: molecular typing, genetic diversity, Clustered Regularly Interspaced Short Palindromic Repeats, spoligotyping, epidemiology, phylogeny, *Xanthomonas citri* pv. *citri*

Background: Xanthomonads are an important clade of Gram-negative bacteria infecting a plethora of economically important host plants, including citrus. Knowledge about the pathogen's diversity and population structure are prerequisite for epidemiological surveillance and efficient disease management. Rapidly evolving genetic loci, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), are of special interest to develop new molecular typing tools.

Results: We analyzed CRISPR loci of 56 *Xanthomonas citri* pv. *citri* strains of world-wide origin, a regulated pathogen causing Asiatic citrus canker in several regions of the world. With one exception, 23 unique sequences built up the repertoire of spacers, suggesting that this set of strains originated from a common ancestor that already harbored these 23 spacers. One isolate originating from Pakistan contained a string of 14 additional, probably more recently acquired spacers indicating that this genetic lineage has or had until recently the capacity to acquire new spacers. Comparison of CRISPR arrays with previously obtained molecular typing data, such as amplified fragment length polymorphisms (AFLP), variable-number of tandem-repeats (VNTR) and genome-wide single-nucleotide polymorphisms (SNP), demonstrated that these methods reveal similar evolutionary trajectories. Notably, genome analyses allowed to generate a model for CRISPR array evolution in *X. citri* pv. *citri*, which provides a new framework for the genealogy of the citrus cancer pathogen.

Conclusions: CRISPR-based typing will further improve the accuracy of the genetic identification of *X. citri* pv. *citri* outbreak strains in molecular epidemiology analyses, especially when used concomitantly with another genotyping method.

Identification of candidate resistance genes in rice induced by TAL effectors of African *Xanthomonas oryzae* pv. *oryzae*

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Keywords: *Xanthomonas oryzae* pv. *oryzae*, rice, executor *R* genes, TAL effectors, Africa

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 three *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 *E* genes induced by African TALEs. A hundred rice accessions were screened to select accessions that were resistant and susceptible to African and Asian *Xoo* strains, respectively. By a gain-of-function approach consisting in the introduction of each of the 9 *tales* of the Malian African strain MAI1 into the Asian virulent strain PXO99^{A}, three African *tales* were found to trigger resistance on specific rice accessions. To confirm these results further, we generated and characterized a collection of *Xoo*Δ*tale* mutants in the African strain BAI3 which TALome is close to that of MAI1. Preliminary results indicate that the resistant phenotype observed in some of the identified accessions may result of a combination of executor *E* gene and *Xa1*-like *R* gene activities.

Monitoring of *Xanthomonas* spp. bacteria in plant raw materials and foods of plant origin

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Keywords: *Xanthomonas*, identification, plant raw materials, foods of plant origin

The aim of the research was to determine the prevalence of *Xanthomonas* spp. bacteria in plant raw materials and foods of plant origin. The objects of the research were plant raw materials: vegetables (avocado, white radish, cauliflower, broccoli, cabbage, tomatoes, cucumbers, potatoes, carrots, leaf lettuce); fruits (grapes, mandarins, banana, pears, oranges, lemons, apples); seeds (pumpkin seeds, hemp seeds); crops (wheat, barley, buckwheat, long grain rice); hazelnuts and foods of plant origin (wheat grits, pearl barley, barley grains, oatmeal, wheat bran). Total number of samples was 120. Number of samples of vegetables, fruits, seeds, crops, hazelnuts and foods of local origin consisted 25%; 0%; 56%; 87.5%; 0% and 100%, respectively.

According to morphological, physiological and biochemical tests, 80 cultures of presumptive *Xanthomonas* spp. were isolated on yeast dextrose calcium carbonate agar after incubation for 48-72 hours at 27±1 °C Identification of all 80 isolates was done by PCR method with specific *Xanthomonas* spp. primers X1 and X2. Though all isolates were suspected as presumptive *Xanthomonas* spp., molecular method did not confirm them as *Xanthomonas* spp.

Factors influencing community composition of bacterial endophytes in the leaves of the evergreen shrub cherry laurel (*Prunus laurocerasus* L.)

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In this work we examined the community composition of bacterial endophytes residing in leaves of the evergreen shrub *Prunus laurocerasus* L. growing in Mlynany Arboretum using a meta-genomic approach. We focused on two factors that could influence the composition of bacterial communities, namely the proximity of leaves to the soil and the presence of fungal disease. Significant differences have been shown for community composition of 50 most abundant endophytic bacterial genera between leaves originating from upper and lower plant parts. However, the community composition differences between diseased and non-diseased leaves were not apparent. Great portion of residual variance points on additional factors that could influence the composition of endophytic bacterial assemblages within the leaves of this evergreen shrub which is also discussed.

Evaluation of xylem vascular occlusions in olive cultivars infected by *Xylella fastidiosa*

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Keywords: vascular occlusions, *Xylella fastidiosa,* light microscopy

Vascular occlusions of the secondary xylem vessels were studied in olive cultivars infected by the *Xylella fastidiosa* strain *De Donno.* The presence and distribution of occlusions were investigated in stem sections recovered from infected and non-infected plants of Cellina di Nardò (susceptible cultivar), Leccino and Fs17 (resistant cultivars) artificially inoculated and grown under controlled conditions. Light microscopy observations were carried out on toluidine blue stained cross-sections from 1-year old olive twigs collected from mock inoculated plants and from systemically infected plants (18-24 months after the inoculation). A total of 79813 vessels were observed (ranging from 4487 to 7774 vessels for each twig). Negligible values of occlusions were found for Cellina (0.15%), Leccino (0.02%) and FS17 (0.13%) mock-inoculated control plants. While these were significantly higher in twigs of the same cultivars but collected from infected plants: 9.65% in Cellina, 6.81% in Leccino and 1.33% in FS17. Thus, suggesting that infections by *X. fastidiosa* are able to induce the formation of occluded vessels and that their number is significantly higher in the susceptible cultivar. Distribution of occluded vessels was not uniform among individual twigs of the same cultivars, ranging from 1% to 34% in Cellina, 0.044% to 14% in Leccino and 1.09% to 1.53% in FS17, most probably reflecting the erratic distribution of the bacterium in the infected tissues. However, within each cultivar it was not possible to establish a correlation between the percentage of occluded vessels and the presence or absence of symptoms.

Status of bacterial plant diseases on woody hosts caused by *Xanthomonadaceae* and their vectors in Latvia

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Keywords: *Xanthomonas*, *Xylella fasidiosa*, hosts, vectors

The plant pathogenic bacteria of *Xanthomonadaceae* family are one of the most devastating plant pathogens causing significant losses in the food production chain. Several *Xanthomonas* species and *Xylella fastidiosa* are recognised as emerging threats to food production globally and are listed as quarantine pathogens in European countries. In Latvia, the purposeful research on *Xanthomonadaceae* and diseases they cause is not carried out. So far, the outbreaks or damages caused by this group of bacteria have not been noted and detected although the susceptible hosts are widely cultivated (e.g. cereals, forage crops, vegetables, strawberry, stone fruits), they are natively present in wild habitats, and introduction of susceptible crops occurs widely from other countries. In the previous researches on diseases caused by bacteria on fruit trees, only *Pseudomonas* have been detected and indications about the presence of *Xanthomonas* were not noted. During the yearly monitoring carried out by State Plant Protection Service suspicions samples of tomato, wheat, plums and beans probably infected with ***Xanthomonas*** have been noted, but laboratory tests were negative. Since 2015, State Plant Protection Service performs regular monitoring and laboratory diagnostics for presence of *X. fastidiosa*, which has not been yet detected. However, the risk for its spread exists due to the climate change, plant introduction, and since the susceptible hosts are widely present in cultivated and wild landscapes and several of the known vectors are prevalent. The details of hosts and vectors are presented and discussed.

The *xopJ6* gene encodes a PopP2-like acetyl transferase from *Xanthomonas campestris* pv. *campestris* recognized by *RRS1/RPS4 R* genes in Arabidopsis

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Xanthomonas campestris pv. *campestris* (*Xcc*) is the causal agent of black rot disease of *Brassicaceae* and an Arabidopsis pathogen. *Xcc* relies on a cocktail of type III effectors for its pathogenicity. We identified the *xopJ6* type III effector gene encoding a close homologue of the *Ralstonia solanacearum* acetyltransferase PopP2. *xopJ6* is located on a transposable element found in 1 to 4 copies on chromosome or plasmids of *Xcc.* XopJ6 recognition causes hypersensitive response in specific *Brassica olearacea* cultivars and avirulence on given Arabidopsis ecotypes. Similar to PopP2, XopJ6 acetyltransferase activity triggers activation of the *RPS4/RRS1-R* immune receptor complex, through manipulation of RRS1-R WRKY DNA-binding domain. Importantly, a natural single amino-acid polymorphism in XopJ6 allows *Xcc* to evade *RRS1/RPS4* recognition in Arabidopsis. This study identifies a cognate *RRS1/RPS4* avirulence determinant in a bioagressor of *Brassicaceae*. Latest results will be presented.

Confrontation of *Xanthomonas campestris* pv. *campestris* strains with various bacterial and fungal antagonists

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Keywords: confrontation tests, Xcc sensitivity, *Bacillus amyloliquefaciens*, pseudomonads, *Trichoderma harzianum*

Use of antagonistic organisms in biocontrol is a powerful tool decreasing the crop infections. The purpose of the study was a comparison of the sensitivity of various *Xanthomonas campestris* pv. *campestris* (Xcc) strains to selected antagonistic bacteria and fungi. Growth inhibition was measured as a confrontation in dual, agar-medium cultures at the Fe^{3+} concentrations of 8 and 200 µM. Grown in liquid Bushnel-Haas medium at 200 µM Fe^{3+}, the doubling times of the collection strains Xcc HRIW 3811, HRIW 1279A and HRIW 3871A were 1.1-1.3-fold compared to the strain Xcc SU that was isolated from a cabbage field. Growth of all Xcc strains was sensitive to inhibition by *B. amyloliquefaciens*. The sensitivity of Xcc to *Pseudomonas aeruginosa* PAO1, *P. putida* IBU, *P. fluorescens* Flu, and *Pseudomonas* sp. DF1 strains was in the range of a medium- to no-inhibition; the concentration of Fe^{3+} affected the inhibition. With *Trichoderma harzianum* the inhibition could not be evaluated as the growth of the bacterium was much faster compared to the fungus. The fungus colonized the agar surface even after robust Xcc biofilms developed on the agar surface. The analyses of the metabolomic profile of the dual culture *B. amyloliquefaciens*/Xcc SU are ongoing.

Acknowledgments
This work was supported by the project LTC18009, program INTER-COST provided by the Ministry of Education, Youth and Sports of the Czech Republic.

2015-2019: four years of *Xylella fastidiosa* surveillance in France

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Keywords: *Xylella fastidiosa*, detection, identification, survey, host, vector

In France, *Xylella fastidiosa* (Xf) has been detected in natural condition since 2015, in Corsica and French Riviera (PACA region) on a large range of ornamentals, fruit crops and wild hosts. During the last four years, more than 30,000 samples have been tested at the national level by official laboratories.

French National Reference laboratory (Anses - plant health laboratory) develops, optimizes diagnostic methods and evaluates the performance criteria in order to verify that they reach the required levels on plants.

Up to now, after the various works carried out in intralaboratory at Anses and within interlaboratory comparisons by partners of H2020 POnTE project or EPPO members, the Real-Time PCR (Harper *et al*., 2010) has shown the best detection threshold on the great majority of hosts, combined with the more efficient as possible DNA extraction step. Direct MLST on DNA extracts obtained from samples positive to Xf is the best way to identify the subspecies and sequence-type (ST) present in samples. In addition, these methods were adapted and efficient to detect Xf in the vector *Philaenus spumarius*.

These accurate detection methods fully adopted by EPPO for diagnostic protocol are now used in official surveys and represent precious and accurate tools for control of the presence and the spread of the disease. These four years of experience have allowed getting data on foci locations of positive plants and insects, range of hosts and strains found in French area. These results allow gathering knowledge on Xf and its epidemiology.

A study for identification and pathogenicity of *Xanthanomas campestris* pv. *campestris* on some *Cruciferaceae* species

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Keywords: *Xanthomonas campestris* pv. *campestris*, *Cruciferaceae*, diagnosis, pathogenicity

Xanthomonas campestris pv. *campestris* (*Xcc*) is considered to be one of the most destructive causal agent of black rot disease of Crucifer plants. The aim of this study was to identify of *Xcc*-suspected isolate, which was obtained from cabbage plants and kept in Bacteriology laboratory stocks of Ege University, with biochemical and molecular diagnostic methods. In addition, the susceptibility of some crucifer species to *Xcc* infection was evaluated under *in vivo* conditions. In this study, suspected isolate was initially identified by biochemical tests including, Gram reaction, Fluorescent pigmentation, Levan production, Oxidase activity, Pectolytic activity, Arginine dihydrolase, Tobbaco hypersensitive response, Gelatin hydrolysis, Aesculin hydrolysis, Starch hydrolysis, Aerob/Anaerob respiratory test and Utilization of some carbon sources. Afterwards, it was sequenced with 16S rRNA primers. The sequences were browsed on BLAST application in NCBI and constituted to phylogenetic timeline trees and compared other *Xcc* isolates, which existing NCBI database. Pathogenicity test was applied to crucifer species including broccoli, cabbage, cauliflower, garden rocket, and radish. Two inoculation methods were applied for evaluation of species susceptibility to the black rot disease of crucifers: seed inoculation and spraying onto leaves of tested crucifer plants. As a result of this study, the isolate was precisely identified as *Xcc* via both biochemical and molecular diagnostic methods. Two inoculation methods produced typical black vein necrosis symptoms. Among the *Cruciferaceae* species, garden rocket was the most susceptible crucifer species while broccoli was the least in both inoculation methods.

Graphene oxide nanocomposite as a tool for bacterial spot control

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Keywords: nanocomposite, graphene oxide, bacterial spot, antibacterial effect

Nanomaterials are used for many applications including phytopathology and crop protection. Development of a new nanomaterials is in rapid progress worldwide. Substantial effort to find out the nanomaterials with efficient antibacterial effect and low toxicity for eukaryotic cells can help in the process of crop production generally. In this study based on a known bacteriostatic effect of some metals we prepared a nanocomposite of silver and copper nanoparticles (NPs) bound on graphene oxide (GO). Graphene oxide (GO) was prepared by chemical oxidation according to the simplified Hummer's method. GO was used as a starting material for synthesis of nanocomposite consisting of GO, silver and copper NPs. Characterization of this nanocomposite was performed using a scanning electron microscope (SEM) with energy dispersive X-ray spectroscopy (EDS) and Fourier transform infrared spectroscopy (FT-IR). Antibacterial effect of this nanocomposite was tested by *in vitro* trials on bacterial spot-causing xanthomonads (BSX). Minimum inhibitory concentration was determined using colony counting method. Positive bacteriostatic effect after spraying of this nanocomposite on artificially BSX infected peppers and tomatoes was observed.

A metabolomics study of the antimicrobial compound N-Acetyl-Cysteine on the growth of the plant-pathogen *Xanthomonas citri* subsp. *citri*

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Keywords: N-Acetyl-Cysteine, metabolites, *Xanthomonas citri*, growth curve, GC-MS

N-Acetyl-Cysteine (NAC) is known as antioxidant, anti-adhesive and antimicrobial compound. In this study we investigated the metabolic changes induced by NAC and how it interferes in bacterial growth. For this, we performed a *Xanthomonas citri* subsp. *citri* growth curve analysis at 0, 1, 2, 4, 6, 12 and 24 hours in presence or absence of NAC. Subsequently the primary metabolites were identified using GC-MS. The growth curve showed cell death occurring after 6 hours in presence of NAC. The Principal Component Analysis and Hierarchical Cluster Analysis, supervised method by Partial Least-Squares to latent structures as well as pathway analysis were performed using MetaboAnalyst 4.0 in which two main clusters were identified, one group of control samples and other with bacterial cells incubated with NAC. A total of 54 metabolites were identified and monitored in the samples. The pathways impact analysis showed that arginine and proline, alanine, aspartate and glutamate, D-glutamine and D-glutamate, purine, pyrimidine as well as nitrogen metabolisms are deregulated in comparison with the control group. Since the metabolic activity of the cells is deregulated, there is less energy available due to the reduction in energy generated by the central carbon metabolism, which therefore leads to cell death.

Acknowledgments
Financial Support: FAPESP

Etiology of peach leaf and fruit spot and twig cancer in Montenegro

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Keywords: bacterial spot, peach, biochemical tests, PCR analysis, *Xanthomonas arboricola* pv. *pruni*

Bacterial spot of stone fruits and almond, caused by *Xanthomonas arboricola* pv. *pruni* is one of the most important bacterial diseases of *Prunus* spp. worldwide. The bacterium is listed as a quarantine organism in the EU as well as in Montenegro. In Montenegro, the disease was first described on almond trees in 1994. During 2017-2018, leaf and fruit spot and twig necrosis were observed in peach orchards near Podgorica. The leaf lesions were initially small, angular, water-soaked, surrounded by a halo. As the disease progressed, the necrotic areas dropped out, leaving a 'shot-hole' leaf appearance. Eventually, infected leaves turned yellow and dropped off. On fruits, small, circular, water-soaked or dark brown spots were observed. Lesions on twigs were dark brown, elongated and sunken. From the symptomatic tissue, yellow, convex and mucoid bacterial colonies were isolated on YDC medium. Thirty-seven strains were selected based on the tobacco hypersensitivity. All strains were gram negative, strictly aerobic, oxidase negative and catalase positive, hydrolyzed esculin and didn't grow at 37 °C. Out of all, two strains hydrolyzed starch but not gelatin. PCR analysis of 36 strains, with a pair of primers XapY17-F/ XapY17-R produced a single characteristic band of 943 bp. Pathogenicity tests were conducted by spraying young peach shoots (cv. Royal Time) with a suspension of tested strains (10^7 CFU/ml in SDW) respectively. The shoots were maintained at about 25 °C and high humidity in a glasshouse. Lesions appeared on all inoculated shoots 11 days after inoculation. The symptomatology and bacteriological characteristics indicated that the strains are closely related to *X. arboricola* pv. *pruni*, which supposed to be confirmed by further studying.

Comparative genomics of *Xanthomonas* sp. bacteriophages

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Keywords: *Xanthomonas* sp., bacteriophages, comparative genomics, genome arrangements, phylogenetics

Numerous complete genomes of bacteriophages providing possible biological solutions against the plant pathogenic bacteria belonging to the *Xanthomonas* genus have been submitted to GenBank. In addition to these data, we determined the complete genome sequence of 10 novel *Xanthomonas oryzae* pv. *oryzae* (XOO) bacteriophages. Phylogenetic trees were constructed based on the predicted protein sequences of portal proteins and terminase large subunits. Novel bacteriophage phylogenetic groups have been proposed based on these results. Possible genome region rearrangements were analysed within each phylogenetic groups using progressive Mauve algorithm. We have exploited the opportunity that we were able to sequence genomes of ten closely related bacteriophages. We mapped the reads originating from the phage genomes to two selected XOO genome sequences and analysed the alteration frequencies along the genomes. Alteration hotspot regions valid for the investigated genomes could be observed during these investigations. Our results could contribute to a better understanding of the phylogenetic relationships of *Xanthomonas* sp. bacteriophages.

Characterization of *Xanthomonas arboricola* pv. juglandis strains isolated after field application of phage therapy in Chile and its role in walnut blight disease development

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Keywords: walnut blight, *Xanthomonas arboricola* pv. *juglandis*, phage therapy, phage-resistant

Xanthomonas arboricola pv. *juglandis* (Xaj) is the etiological agent of walnut blight, a disease characterized by generating necrotic damage in fruits and other young tissues plant. Chilean strains of Xaj presents high level of resistance to cupric derivatives and antibiotics. As an alternative to the use of conventional agrochemicals, we have evaluated the use of bacteriophages in field conditions. Own results show a reduction of the bacterial load of Xaj and significative decrease of incidence/severity index in comparison to conventional management of copper sulphate and absolute controls (without treatment). However, post-treatment of bacteriophages we detect the presence of phage-resistant Xaj isolates and their participation in the development of the disease is unknown. Our research aims to characterize phage-resistant strains of Xaj and determine their role in the walnut blight disease. Two colonies morphotypes of phage-resistant Xaj have been obtained, yellow phage-resistant colonies like wild type strain and yellow less small colonies inducible only by the presence of bacteriophages. both types of colonies have been characterized according to biochemical test, outer membrane proteins (OMPs) and LPS profile, detection of exoenzymes secretion and siderophores production in addition to motility test under *in vitro* assays. To determine the role of these phage-resistant strains in the development of walnut blight disease compared to wild type strain of Xaj, trials on walnut plant tissues are being developed. These results are being carried out to optimize the use of bacteriophages as a real alternative against walnut blight disease in field conditions.

Acknowledgments
Funding: FONDECYT-CONICYT Postdoctoral Grants nº 3180660

Comparison of the effect of *Stenotrophomonas rhizophila* strains isolated from different plant rhizospheres on plant growth promotion

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Keywords: *Stenotrophomonas rhizophila*, PGPR, rhizosphere, plant pathogens

Stenotrophomonas rhizophila are known as plant growth promoting, Gram-negative bacteria. The aim of the research was to compare the effect of *S. rhizophila* isolated from the maize and canola rhizosphere on plant growth promotion. The strains were identified by MALDI-TOF MS. Our results indicated that the application of *S. rhizophila* from canola rhizosphere showed a significantly high effect on plant growth promotion compared to *S. rhizophila* strain from maize rhizosphere. A significant increase in canola's chlorophyll content, root and shoot length was observed over the un-inoculated control. Both isolates were able to produce salicylic acid, auxins and could solubilize phosphate. Moreover, *S. rhizophila* from canola rhizosphere synthesized ACC deaminase, decreasing plant ethylene levels which in high concentrations can lead to plant growth inhibition. Also, this strain was capable of producing many secondary metabolites like siderophores, ammonia and fungal cell wall lysing enzyme - chitinase with antagonistic effect on mycelial growth of diverse plant pathogens. The study indicates the potential of this PGPR strain for inoculums production for enhancing the growth of canola under field condition.

Actinomycetes as means of increasing yield and yield components and decreasing the disease incidence of faba bean plants

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Keywords: faba bean, actinomycetes, yield, spores

Actinomycetes is being involved in plant defense response and, plant-microbe interactions, flavonoid production by plants may be increased when the plant recognizes certain molecules or structures that characterize pathogen. Actinomycetes produce a wide variety of antibiotics and of extracellular enzymes. The objective of the present study was to determine the effects, under field conditions, of foliar applications of actinomycetes spores on the yield and yield components of mature faba bean, and also on the disease incidence. Actinomycetes spores were suspended insterile 10% glycerol solution and concentration was adjusted prior to use to 2.5×10^5 and 2.5×10^9 spores per mL using sterile distilled water. Actinomycetes were applied at 4 mL per plant using a hand-held spray bottle. Actinomycetes applied at two faba bean stages of development [vegetative (V4), and early podding (R3)]. Untreated controls were also included. Results indicate that actinomycetes hold promise as a way of increasing yield and yield component of faba bean plants. Also, our results indicated that, the application of actinomycetes reduced the disease incidence pronouncedly comparing with the untreated control.

A GWAs in *Arabidopsis thaliana* identified novel resistance traits against *Xanthomonas campestris* pv. *campestris* in a hydathode-based entry assay

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The bacterial plant pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*) exclusively colonizes members of the *Brassicaceae* family through organs on the leaf margins, called hydathodes, which stand in direct contact with the leaf vasculature. Recent studies showed that *Xcc* first proliferates in hydathodes and then colonizes the vasculature in a type III secretion-dependent manner, indicating that the epithem tissue layer in the hydathodes can mount an immune response after recognition of bacterial effectors. Taking this natural hydathodal entry into account, we screened 332 Arabidopsis accessions for their susceptibility to *Xcc*. Our screen was conducted in the absence of the ZAR1/RKS1 resistance response that mediates recognition of the *Xanthomonas* effector XopAC, which otherwise would have occurred in roughly 50% of the accessions screened. In the absence of this major resistance trait, we still found a large number of accessions to show (partial) resistance to *Xcc* (strain 8004 Δ*xopAC*). Using a Genome Wide Association (GWA) analysis the underlying genetic loci were revealed using the imputed full sequence of all accessions. We identified several novel candidate genes with a possible role in (partial) resistance to *Xcc*. Validation of these candidate genes will provide opportunities for plant breeders to enhance resistance against black rot in crop plants.

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

EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

COST Action CA16107 EuroXanth
2017 I 2021

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

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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Funded by the Horizon 2020 Framework Programme of the European Union

The organisers kindly acknowledge the support from the following sponsors, either as in-kind support or via financial contributions:

MENDELU
Faculty
of Horticulture

Mendel
University
in Brno

This brochure is based upon work from COST Action CA16107 EuroXanth, supported by COST (European Cooperation in Science and Technology).

COST (European Cooperation in Science and Technology) is a pan-European intergovernmental framework. Its mission is to enable break-through scientific and technological developments leading to new concepts and products and thereby contribute to strengthening Europe's research and innovation capacities. www.cost.eu

Funded by the Horizon 2020 Framework Programme of the European Union

Ralf Koebnik, Miroslav Baránek (eds.)

3rd Annual Conference of the EuroXanth COST Action

Published by Mendel University in Brno, Zemědělská 1, 61300 Brno

First printing edition 2019

120 copies

Printed in Mendel University Press

ISBN 978-80-7509-672-2