

4<sup>th</sup> International Symposium on Biological Control of  
Bacterial Plant Diseases

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## Abstracts



## 4<sup>th</sup> International Symposium on Biological Control of Bacterial Plant Diseases

### Species interactions within the microbiome mediate potential for disease suppression

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Microbes and plants exist within complex networks of interacting plant and microbial species. Our work explores the roles of plant-microbe and microbe-microbe interactions in determining the pathogen-suppressive potential and composition of rhizosphere and endophytic foliar microbiomes, and the potential for managing these interactions to suppress bacterial and fungal plant pathogens. Of particular interest is the role that soil nutrients play in mediating microbial interactions within endophytic and rhizosphere populations. Among foliar endophytes, we found that nitrogen, phosphorous, and potassium (NPK) amendments significantly altered fungal, but not bacterial microbiome composition. Fungi, but not bacteria, had significantly smaller niche widths and were generally poorer competitors for resources in NPK-treated than control leaves. Endophytic fungi that were poor competitors for nutrients were significantly better at inhibiting plant pathogenic bacteria than strong nutrient competitors. Among rhizosphere populations, we found similarly that *Fusarium* populations from high-nutrient soils were more antagonistic than populations from low-nutrient soils. In contrast, *Streptomyces* populations from low-nutrient soils were more pathogen-inhibitory than populations from high-nutrient soils. Moreover, data from both endophytic and rhizosphere populations show that microbial species interactions, including bacterial-bacterial, fungal-fungal, and bacterial-fungal interactions, are critical to determining the pathogen-suppressive capacity of microbiome populations. Our data show that widely-used agricultural inputs significantly alter the composition and functional capacities of soil and endophytic microbiomes, and that these changes are driven by nutrient-related shifts in microbial species interactions. Enhanced understanding of microbial species interaction networks within plant-associated microbiomes will provide novel insights into microbiome management for bacterial (and fungal!) disease suppression.

### Plants attract biocontrol agents and consequently modify their metabolism

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Plants live in close association with a large variety of microbes. The resulting interactions play key roles in important biological processes and affect, for instance, host growth and ability to tolerate stress. Actually, plants are able to change both quantitatively and qualitatively the microbial composition especially of the root zone, thus modulating and adapting to their needs microbiota activity. Studies performed on both rhizobacteria and rhizofungi demonstrate the function of bioactive molecules exuded from roots and acting as chemical signals for beneficial and/or pathogenic microbes. We have recently identified at least two classes of compounds whose secretion by tomato roots is modulated by biotic and/or abiotic stresses and that function as chemoattractants for *Trichoderma harzianum* and *Fusarium oxysporum* f. sp. *lycopersici*. Interestingly, the various patterns of secreted molecules affected the biocontrol and the pathogenic fungi in a different, and sometime opposite, manner. *In vivo* experiments indicate that some of the identified compounds could be used to treat tomato in order to potentiate the effect of applied biocontrol strains. The relevant molecular mechanism could be involved in many plant-microbe chemical cross-talks, including those regulating root-rhizobacteria interactions.

### Sustainable control of Pierce's disease of grapevine and citrus greening with a benign strain of *Xylella fastidiosa*

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Pierce's disease (PD) of grapevine and HLB of citrus, caused by *Xylella fastidiosa* subsp. *fastidiosa* and *Candidatus Liberibacter* spp., respectively, cause large economic losses in their hosts. When injected into the xylem with a pin-pricking technique, a benign strain of *X. fastidiosa* (EB92-1) has provided control of PD in field trials with various *Vitis* spp. In Chardonnay treated at planting in Florida, EB92-1 provided control throughout an 8-year-trial, reducing the incidence of PD from 50% to 17% and the plant loss to PD from 33% to 8%. In a trial established in the UC Riverside vineyard in 2011, EB92-1 provided control of PD for 6 years in Pinot Noir and Cabernet Sauvignon grapevines, reducing the vine loss from 40% in the untreated to 10% in treated vines. In trials established in 2014/2015, a power drill and syringe were used to inject the biocontrol strain into mature grapefruit trees in a Florida grove with a high incidence of citrus greening. Three years after treatment, none of the

non-symptomatic treated trees had developed symptoms, but fifty percent of the untreated had developed symptoms. In 2-year-old sweet orange trees treated with EB92-1, there were moderate symptoms in 18% of the untreated and in 3% of the treated plants 4.5 years after treatment. While a single treatment with EB92-1 at planting sustained PD control in grapevine for up to 8 years, re-treatment every 18-24 months was beneficial for the control of citrus greening.

### Role of cruciferous weeds in the epidemiology and biological control of seedborne *Xanthomonas campestris*

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Weeds serve as an important reservoir of inoculum of seedborne *Xanthomonas campestris* pv. *campestris* (*Xcc*). Surveys have shown black rot (BR) to be common in crucifers in transplant farms in southern Georgia) and in central coastal non-cultivated areas of California. BR was found in *Brassica campestris*, *Lepidium virginicum*, and *Raphanus sativus* in Georgia. In California BR was found along the coast in *B. campestris*, *B. nigra*, *B. geniculata*, *R. sativus*, *Sisymbrium officinale*, *S. irio*, and *Cardara pubescens*. Typical symptoms consisting of yellow v-shaped lesions and atypical lesions consisting of black spots without any yellowing were widespread. The range of symptoms can easily be quite wide making diagnosis somewhat difficult. The symptoms along the coast could easily be confused with *Alternaria* lesions. Field plots in Ga. showed BR to spread up to 12 m from infected *B. campestris* to *B. oleracea*. Using a Anderson particle sampler, counts of *Xcc* in a non-cultivated area with *B. campestris* in Coastal California ranged from 0.84 to 13.68 viable cells of *Xcc* per cubic m of air. These results confirm that *Xcc* can move from cruciferous weeds into adjacent cultivated crucifers. Crucifer weeds are part of the ecosystem along the coast of California and make a good target for biological control.

### Influence of agricultural practices and plant phenological phases on strawberry bacterial microbiome and effects of selected bacteria on strawberry growth and the strawberry-*Xanthomonas fragariae* interaction

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Genomic bacterial DNA was isolated from the rhizosphere of strawberry collected in conventional, organic and biodynamic commercial farms located in Basilicata (southern Italy) in pre-flowering (PFP) and fruit ripening periods (FRS). Their metagenomic profiles were obtained by 454 pyrosequencing of the amplified V1–V3 hypervariable region of the 16S rRNA gene. The weighted UniFrac analyses of the above profiles showed that samples clustered separately on the basis of cultural practices and plant phenological phases. Furthermore, 445 bacterial colonies were isolated from the rhizosphere soil, the outside and the inside (endophytes) of strawberry roots and then evaluated for several characters including the ability to inhibit

*in vitro* the growth of strawberry pathogens such as *Xanthomonas fragariae*, *Pythium* and *Fusarium* spp. Twenty-nine out of 445 isolates showed characters of potential plant growth promoting bacteria as well as bio-control antagonists and for that evaluated either for the ability to promote strawberry plant growth in the field and to protect strawberry plants artificially infected with a virulent strain of *X. fragariae*. Data showed that eight out of 29 isolates resulted highly significant ( $P < 0.05$ ) for the number of buds, leaves, flowers and fruits induced when compared to the control. Conversely, no one of the above bacteria resulted able to antagonize the pathogen reducing the symptom expression and, on the contrary, 23 out of 29 caused an apparent increase of the virulence expression. This unexpected result and quite unknown phenomenon highlights the importance of the microbiome associated to plants in their interaction with microorganisms, including pathogens.

### Exploring rain-isolated bacteria as potential biopesticides to control fire blight

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Fire blight, caused by *Erwinia amylovora*, is a devastating disease affecting apple and pear production worldwide. Disease control includes cultural practices and chemical treatment. However, due to the emergence of antibiotic-resistance, biological control has long been explored as an alternative control option. Unfortunately, the efficacy of biological products heavily depends on environmental conditions. Therefore, we tested the hypothesis that bacteria locally isolated from rain in Virginia, USA, could be particularly well adapted to local conditions and provide effective control. *In vitro* assays with rain-isolated bacteria identified two isolates of the genus *Pantoea* with strong inhibitory effect against *E. amylovora* and other plant pathogenic bacteria. Both isolates were precisely identified as members of *Pantoea agglomerans* based on whole genome sequencing. Moreover, a UV-mutagenesis screen revealed putative genes at the basis of the biocontrol activity of one of the strains. The isolates were then tested for survival on apple branches during the winter and as pre-treatment to protect against fire blight on detached apple blossoms and on whole trees in the field in the same area of Virginia where they had been isolated from rain. Survival was similar to *E. amylovora* and fire blight control was similar to a commercial product but not as effective as a streptomycin control. Our results shows the potential of rain as a source of locally adapted biocontrol strains to control fire blight on apple. However, more field testing will be needed to compare the control provided by the identified isolates with already available commercial products.

### Transcriptional responses of *Xanthomonas oryzae* pv. *oryzae* to type III secretion system inhibitor *ortho*-coumaric acid

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*Ortho*-coumaric acid (OCA), a plant-derived phenolic compound was previously identified as a type III secretion system (T3SS)

inhibitor of the bacterial leaf blight pathogen of rice *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). However, the molecular mechanisms underpinning the T3SS inhibition by OCA and the transcriptional responses to the OCA treatments in *Xoo* remain to be elucidated. RNA-seq-based transcriptomic analysis was conducted in this study to reveal changes in bacterial gene expression in response to 30 min, 1 h, 3 h, and 6 h of OCA treatment. Results showed that OCA significantly inhibited the T3SS gene expression, and that membrane proteins in the functional category of the cellular process became the predominant group after 30 min of OCA treatment. More differentially-expressed genes (DEGs) gathered in the functional category of the biological process over time. Analysis of common DEGs at all time points identified the core elements in *Xoo* during the response to OCA treatment. Notably, a multidrug transporter gene cluster that encodes a MarR-family transcriptional regulator (*mdtX1*), a multidrug RND transporter (*mdtX2*), a multidrug transporter (*mdtX3*), and a MFS transporter (*mdtX4*) was significantly up-regulated by OCA treatment at all time points. Genetic analysis demonstrated that deletion of *mdtX1* in *Xoo* affected the OCA-induced expression of *mdtX2-4*, but not the OCA-inhibited T3SS expression. Therefore, our study revealed the landscape of bacterial responses to OCA at the whole-genome transcription level, and identified an OCA-responsive multidrug transporter cluster, which might be not involved in the T3SS inhibition in *Xoo*.

### Preliminary results of nanopore sequencing for the detection and the identification of *Xylella fastidiosa* subspecies and sequence types from naturally infected plant material

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The recent outbreaks of *Xylella fastidiosa* in Europe raised the need of reliable methods able to detect *X. fastidiosa* and identify its subspecies and sequence type (ST). These latter characterizations, mandatory for new findings, new outbreaks or new plant hosts, are currently performed using Multilocus sequence typing (MLST) analysis, a very time-consuming procedure, based on Sanger sequencing of seven housekeeping genes. The Nanopore MinION platform was assessed for *X. fastidiosa* detection and identification from infected plant material collected in Italian infected areas (Toscana and Apulia regions). The first approach based on direct gDNA sequencing of several infected plant samples showed the possibility to detect *X. fastidiosa* for highly infected samples. The sensitivity was investigated using olive samples artificially inoculated with known amount of *X. fastidiosa* subsp. *pauca*. The result shows that the quality of the gDNA highly affects the performance of Nanopore sequencing. With the purpose to set-up a procedure less influenced by the DNA quality, but highly sensitive, a gDNA PCR amplification step prior sequencing was added, based on two (*cysG*, *malF*) or seven housekeeping genes, for the subspecies and for the ST identification, respectively. A pipeline was developed to generate MLST consensus after Nanopore-sequencing, thus permitting the identification of *X. fastidiosa* in all samples. The overall results showed the possibility to detect and identify *X. fastidiosa* in infected plant material by Nanopore device within few hours.

### Biocontrol of black rot pathogen clonal group predominant in Russia

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*Xanthomonas campestris* pv. *campestris* (*Xcc*) is one of devastating diseases of *Brassica* vegetables. We have collected *Xcc* isolates in the main areas of brassicas cultivation in Russia from 2006 to 2018. Multilocus sequence typing (MLST) based on the partial sequence of loci *dnaK*, *fuyA*, *rpoD*, *gyrB*, *cytP450*, and *avrXcc2109* was applied to over 100 strains representative for 22 collection sites. Comparison of *Xcc* strains collected at the same regions of Russia showed considerable genetic changes occurred after 2012. The phylogenetic reconstruction using a data set of gene *rpoD*, placed the strains into three distinct genetic groups. Group 1 (35 strains collected before 2012 and only 2 - at 2018) was similar to the strain NCPPB5268<sup>1</sup>, LMG8004, and HRI1279a. Small Group 2 (4 strains) was similar to B100 and HRI 3811. Most of strains obtained after 2012 were nearly identical in *rpoD* sequence and similar to *Xcc* 0656 and 0657 from USA. The race structure of *Xcc* strains identified by reaction of differential *Brassica* lines showed that the pathogen strains collected after 2012 showed significant race shift from widespread races 1 and 4 towards to races 5 and 6. No correlation was found between allelic profiles, host of isolation, geographical origin and races. The strains reaction with 25 bacteriophages isolated at 2014–2018 confirmed the distinct grouping of *Xcc* strains obtained since 2012. Application of the bacteriophage cocktails was efficient against black rot caused by homogeneous *Xcc* population on seedlings of brassicas.

### Mechanisms underlying *A. chinensis* var. *deliciosa* defense responses against *Pseudomonas syringae* pv. *actinidiae* after methyl jasmonate and salicylic acid application

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The use of plant elicitors in the control of *Pseudomonas syringae* pv. *actinidiae* (PSA), the causal agent of the kiwifruit bacterial canker (KBC), has been the focus of some research in the past decade. However, information on the mechanisms activated by *Actinidia* spp. plants after elicitation and infection with PSA is still scarce. The aim of this work was to understand the metabolic and genetic mechanisms underlying *Actinidia* sp. defence against PSA after elicitation with two antagonistic compounds: salicylic acid (SA) and methyl jasmonate (MJ). *In vitro* grown *A. chinensis* var. *deliciosa* cv. ‘Hayward’ plants were inoculated with PSA fifteen days after elicitation with SA or with MJ

and colony forming units (CFU), malondialdehyde (MDA), secondary metabolites (total chlorophylls, carotenoids, flavonoids, polyphenols, lignin), proteins, guaiacol peroxidase and the relative expression of defence related genes were analysed fifteen days after inoculation. Plants inoculated with PSA without elicitation showed decreased chlorophyll concentration (by *ca.* 0.4-fold) and increased soluble phenolic compounds (0.4-fold), as well as overexpression of ascorbate peroxidase (*APX*) and superoxide dismutase (*SOD*) genes (2.6- and 4.4-fold, respectively). In plants subjected to exogenous application of SA a 0.54-fold decrease in bacterial density was observed, whereas MJ significantly increased CFU count by 6.6-fold. In general, a decrease in chlorophyll, flavonoid and lignin contents was observed in inoculated plants after both treatments (by at least 0.2-fold), as well as an increase in proteins and guaiacol peroxidase enzyme (by at least 0.5-fold). MJ treatment led to significant overexpression of genes related with antioxidant (*APX* and *SOD*) and ethylene (*ACASI*, *ETR* and *SAM*) pathways, as well as the accumulation of abscisic acid in plant tissues (0.4-fold). It is likely that in *A. chinensis* var. *deliciosa* disease susceptibility increases after MJ treatment due to the activation of pathways related with abscisic acid and ethylene. This study provides evidence on the mechanisms involved in plant defence against PSA after treatment with antagonistic compounds and may be used for the development of novel control methods based on elicitor application.

### Specific quantitative viable cell monitoring of the biological control agent *Lactobacillus plantarum* PM411

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*Lactobacillus plantarum* PM411 is a biological control agent (BCA) with broad antagonistic activity against several plant pathogenic bacteria. Strain-specific methods are necessary for field monitoring BCA in order to study its ecological fitness under different environmental conditions and to optimize formulations and delivery strategies to enhance its survival and adaptability in the phyllosphere of plants. In addition, the development of methods capable of discriminating between viable and dead cells is critical since the performance of a BCA depends on its viability. We developed and validated a viability qPCR (v-qPCR) assay for the unambiguous detection and quantification of PM411 in plant material. The v-qPCR method relied on a sample pre-treatment with a nucleic acid-binding dye (e.g. PEMAX) prior to qPCR. First, we coupled the RAPD-PCR technique and whole-genome sequence analysis to find differential DNA sequences. A PM411 strain-specific molecular marker was identified within a region of a predicted prophage with mosaic architecture. Then, three TaqMan qPCR assays with different amplicon lengths (92, 188, and 317 bp) were designed, being the primer set amplifying a 188 bp DNA fragment the most suitable for v-qPCR. The reliability of the v-qPCR together with specific plate counting and qPCR in the monitoring of PM411 cell population on plant surfaces was simultaneously evaluated under greenhouse and field conditions. The three methods contributed to comprehend the behaviour of PM411 in different tissues, plant species and environmental conditions.

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### Biocontrol traits in the *Pseudomonas corrugata* phylogenetic subgroup: a review

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The *Pseudomonas corrugata* phylogenetic subgroup of the *P. fluorescens* group within the *P. fluorescens* lineage includes the species *P. brassicacearum*, *P. corrugata*, *P. kilonensis*, *P. mediterranea*, and *P. thivervalensis* and several other *Pseudomonas* sp. strains whose taxonomic position still needs to be clarified at the species level. Many of them have been isolated from the rhizosphere or from agricultural soils. They have mainly been studied as bio-control strains of soil-borne plant diseases. *P. corrugata* and *P. mediterranea* are the only non-fluorescent species in the group and are also known as plant pathogens. Nevertheless, no T3SS-encoding locus has been detected in any of the examined *P. corrugata* and *P. mediterranea* genomes. Strains within the *P. corrugata* subgroups seem to have very interesting biocontrol traits since they play an antagonistic role against plant pathogenic bacteria and fungi. This role has been demonstrated both *in vivo* and *in vitro*, where the production of diffusible or volatile metabolites are involved. Genome mining however has highlighted some peculiarities as only *P. corrugata* and *P. mediterranea* and some closely-related strains share a canonical LuxI/R N-acyl homoserine lactone-dependent quorum-sensing system (AHL-QS). In these two species AHL-QS regulates the production of antimicrobial diffusible antimicrobial substance via the transcriptional regulator RfiA, which in turn is not present in the other species/strains where the GacS/GacA two-component regulatory system seems to have a pivotal role in the antimicrobial activity. Secondary metabolite production, either proved or predicted *in silico*, and phenotypic traits provide evidence that strains of this phylogenetic subgroup could provide biocontrol agents with a multipurpose activity although that against plant pathogenic bacteria has still to be sufficiently explored.

### Epidemiology and forecasting models in biological control of diseases caused by plant pathogenic bacteria

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Biological disease control is the result of complex relationships between pathogen, host and biological control agent, which interact with the environment across time and space. I will focus my talk in key points for a successful management of disease, and most of the examples will be chosen within the field of biological control of fire blight. Forecasting models of several bacterial diseases have been used for risk mapping or treatment scheduling, as well as to analyse disease dynamics in space and time. However, disease progression and pathogen spread are influenced by pathogen aggressiveness. Among a given plant pathogenic bacteria there is generally a wide range of aggressiveness within the population. The efficiency of biocontrol depends greatly on pathogen aggressiveness. This has been demonstrated by analysing the quantitative relationships between the dose of biological control agent, the dose of pathogen and disease intensity, by means of mathematical models. The environment and host influence the fitness of biological

control agents. This has been the object of several studies, dealing with colonization, survival, spread and decay. Ways to improve fitness of biocontrol agents have been achieved to counteract adverse conditions. The knowledge of the biological cycle of the pathogen and biocontrol agent in relation to the host have provide tools for guided application and timing of biocontrol agents, and several examples have been reported , mainly in fire blight biological control. Finally, it can be speculated about the influence of climate change in biological control of bacterial diseases. The different scenarios suggest a need for new BCA strains because current commercial BCAs may not work due to the arrival of unfavourable environmental conditions. In addition, it will also require to establish new threshold actions and possibly the need for re-evaluation of existing forecasting models.

### Winter population dynamics of *Pseudomonas syringae* pv. *syringae* on cherry buds

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The main disease of cherry tree in Chile is bacterial canker, caused by *Pseudomonas syringae* pv. *syringae* (*Pss*). The control strategy are based on copper and biological control agents (BCA), but there is not a criterion of winter applications, so we considered to establish the winter dynamics of the populations of *Pss* during the winters of 2016 to 2018. For this goal cherry buds were sampled weekly, from leaf fall to bud break, from a Bing/colt orchard located in Talca (Lat 32° S). The buds were subjected to bacteria extraction, both from the surface and inside, accounting bacterial populations of *Pss*. These populations were correlated with climatic information and disease incidence. This was done in plants without treatments and treated with copper and BCA (Nacillus®). The results indicate a high correlation of the internal populations with disease incidence ( $R = 0,68$ ;  $P < 0,05$ ), with a disease incidence threshold of  $1 \times 10^5$  CFU/bud. The second result shows a population dynamic, responding to the combination of temperature ( $R = 0,75$ ;  $P < 0,001$ ), humidity ( $R = 0,69$ ;  $P < 0,05$ ) and rainfall ( $R = 0,55$ ;  $P < 0,05$ ). In cold winters (2017-18), populations were below the threshold until the second week of August, whereas, in 2016, a warm and dry winter, we obtained risk population levels in full winter. There are differences between plants with applications and control. This indicates that winter applications could be adjusted to conditions favorable to the bacterium, such as temperatures, higher than 15 °C, followed by frost or rain.

### The interactions among biocontrol agents, pathogens and the environment: the concept of environmental niches

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The use of biological control agents (BCAs) is supported by Directive 128/2009/EC on the Sustainable Use of Pesticides, which gives priority to non-chemical methods for disease control. Although the intensive research of the last decades, the practical use of BCAs is still challenging. Biocontrol of plant bacterial diseases involves complex interactions among the target pathogen, the host plant, and the BCA population in a

changing environment. The colonization rate and the efficacy of the BCA, as well as the bacterial growth and infectivity, are all influenced by weather conditions, such as temperature and moisture. To achieve an effective integration of BCAs in a disease management program is then relevant to know: i) the life cycle of both, the bacteria and the BCA; ii) the mode of action of the BCA against the target; and iii) how the two are influenced by the environment. The concept of “environmental niches” may help to understand how the environment influence the BCA-bacteria interaction. Environmental niches are defined as the environmental conditions necessary for the presence of a species and the maintenance of its population. This concept is broadly used in ecology, but not in phytopathology. In this work, we use the ecological niches for a case-study bacterial disease and the BCAs used for its control, and we discuss the use of ecological niches for improving the BCA-based control strategies.

### Field evaluation of XAPcast a forecasting system for bacterial spot disease of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*

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Bacterial spot disease of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*, is of high economic importance in the major stone-fruit-producing areas worldwide. XAPcast, a mechanistic forecasting system for this disease based in three components (epiphytic inoculum potential, weather conditions conducive to infections, and disease symptom appearance) was developed by our research group. The forecaster was evaluated for predicting bacterial infections and disease symptoms in peach tree orchards located in Emilia-Romagna Region (Italy). Trials were performed from April to July during 2016 and 2018. Disease incidence and severity were assessed every 7 or 15 days on non-treated trees. Hourly weather data for air temperature, relative humidity, rainfall amount, and wetness were obtained from the orchards (Servizio Fitosanitario Regione Emilia-Romagna). The infection risk was calculated according to XAPcast infection model and daily (S) and cumulated risk (CS) indexes were obtained. S and CS infection risk values were analyzed and compared to the observed disease levels. When the infection model predicted favorable weather conditions for initiating an infection process, the XAPcast symptom model component began to run for prediction of disease symptoms appearance on the basis of daily mean temperature and Cumulative Degree Days. Results indicated that the XAPcast infection and symptoms models accurately predicted the infection periods and the disease progress, and that the threshold values for S and CS should be adjusted to the phenological sensibility of peach trees to the disease.

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### Biocontrol of plant bacterial diseases - recent approaches

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The end point of whole research of plant pathogenic bacteria is to develop strategies for their control. Evolution of agricultural practices, global circulation of plant material, changes in climate, and raised environmental concerns require novel strategies for plant protection from bacterial diseases. Biological control of plant pathogenic bacteria is becoming one of the pillars of integrated disease control strategies, providing acceptable efficacy and sustainability, with no intention to replace chemicals completely. Use of naturally occurring beneficial organisms as control factors of plant pathogenic bacteria, with no toxic side effects on the environment and humans, contributed to an increased interest in the use of biologically based pathogen control. Although the number and diversity of research approaches have increased lately, we must admit that the number and availability of commercial bio-bactericides have remained limited. In most cases, it turns out that application of such compounds and their biocontrol activity are a complex process which requires development of special techniques or combination of more than one biocontrol agent. Biocontrol strategies are mainly based on a reduction of bacterial plant disease incidence either by competition, antibiosis, induced resistance, or interference with pathogens' life cycle. The majority of known biocontrol agents belong to *Bacillus*, *Lactobacillus*, *Erwinia*, *Pantoea*, *Streptomyces*, fluorescent pseudomonads, bacteriophages, protozoa and bdellovibrio. Some include avirulent form of the pathogenic strains. However, recent research approaches indicate that application of biological control agents and expression of their competitiveness and capability in producing antimicrobials or inducing resistance in plants could be improved by better understanding of the nature of their survival and interaction process with plant pathogenic bacteria.

## Evaluation of tannins respect to their biostimulant and antibacterial activity on tomato plants

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The phytopathogenic bacterium *Pseudomonas syringae* pv. *tomato* (*Pst*) is the causal agent of the tomato bacterial speck disease. Its control is mainly related to the balanced agronomic practices and the preventive use of commercial formulations based on cupric salts. The EU restrictions about the copper use and the needs to reduce the chemicals in agriculture requires to find sustainable alternatives that can replace/reduce cupric salts usually employed to control *Pst*. In this study, the *in vitro* and *in vivo* biostimulant and antibacterial activity of different tannins were verified respect to *Pst*. *In vitro* experiments (spot test) were carried out testing different concentration (0.5-5% w/v), measuring the inhibition halo. *In vivo* experiments were conducted by foliar-spraying tannins at 1% before bacterial inoculation. Results underlined that two of the tested tannins (UTUST1, UTUST3) both expressed a remarkable biostimulant and antibacterial activity.

## Biocontrol of *Xanthomonas* spp. by *Bacillus velezensis* cultivated on commercial and raw glycerol

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Production of value-added biocontrol agents intended to replace chemical pesticides by using industrial or agroindustrial waste represents one of the possible ways to reduce final product cost, as well as the way to support sustainable industrial and agricultural development. In this study cultivations of *Bacillus velezensis* in a 3 L laboratory scale bioreactor were performed, using cultivation media based on commercial and raw glycerol from biodiesel production. Antimicrobial activity of the produced biocontrol agent was assessed *in vitro* using the diffusion-disc method against *Xanthomonas campestris* pv. *campestris*, obtained from cabbage with symptoms of black rot, and *X. euvesicatoria*, obtained from pepper leaf with symptoms of bacterial spot. The results of this study indicated very high biomass content (3.65 g/L for commercial glycerol and 1.18 g/L for raw glycerol medium) and the significant antimicrobial activity of *B. velezensis* against the tested bacterial black rot and spot causers. Maximal inhibition zone diameters of 66 mm and 46 mm were achieved after 60 h of cultivation, for media based on commercial and raw glycerol, respectively, pointing out the potential application of this biodiesel industry effluent as promising carbon source for production of microbial biocontrol agents. Further research in this area would implement testing of the produced biocontrol agents *in planta*, as well as the techno-economic analysis of the overall bioprocess aimed at optimization of bioprocess parameters in order to obtain maximal efficacy of the product with minimal costs of its production.

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## Biocontrol of black rot pathogen clonal group predominant in Russia

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*Xanthomonas campestris* pv. *campestris* (*Xcc*) is one of devastating diseases of *Brassica* vegetables. We have collected *Xcc* isolates in the main areas of brassicas cultivation in Russia from 2006 to 2018. Multilocus sequence typing based on the partial sequence of loci *dnaK*, *fuyA*, *rpoD*, *gyrB*, *cytP450*, and *avrXcc2109* was applied to over 100 strains representative for 22 collection sites. Comparison of *Xcc* strains collected at the same regions of Russia showed considerable genetic changes occurred after 2012. The phylogenetic reconstruction using a data set of gene *rpoD*, placed the strains into three distinct genetic groups. Group 1 (35 strains collected before 2012 and only 2 - at 2018) was similar to the strain NCPPB5268<sup>T</sup>, LMG8004, and HRI1279a. Small Group 2 (4 strains) was similar to B100 and HRI 3811. Most of strains obtained after 2012 were nearly identical in *rpoD* sequence and similar to *Xcc* 0656 and 0657 from USA. The race structure of *Xcc* strains showed that the pathogen strains collected after 2012 had significant race shift. The strains reaction with 25 bacteriophages confirmed the distinct grouping of *Xcc* strains obtained since 2012. Application of the bacteriophage cocktails was efficient against black rot caused by homogeneous *Xcc* population on seedlings of brassicas.

## Biocontrol activities of *Bacillus amyloliquefaciens* S20A1 and optimum conditions for secondary metabolites production and control efficacy on bacterial blight disease of rice in Thailand

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Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is an important bacterial disease of rice cultivation in Thailand. Biological control using antagonistic bacteria is an interesting alternative method to control the disease. *Bacillus amyloliquefaciens* S20A1 was isolated from rice rhizosphere. This strain exhibited the antagonistic activities as competition and producing the effective secondary metabolites (SM) to inhibit growth of *Xoo* and various plant pathogenic bacteria and fungi, *X. campestris* pv. *campestris*, *Acidovorax avenae*, *X. axonopodis* pv. *glycines*, *Alternaria solani*, *Fusarium* sp., *Bipolaris oryzae* and *Pyricularia oryzae*. Culture medium and conditions were studied to encourage cell multiplication and SM production. Half YPB medium (half part of yeast extract peptone broth) and incubation on the shaker at 150 rpm under room temperature for 60 h showed the most effective culture conditions for increasing population density and SM production. Cell free filtrate (CFF) of strain S20A1 capable of growth inhibition of *Xoo*, reduced seed born pathogen and enhanced seed germination. The crystallization study of CFF revealed that surfactin tended to be the main bioactive compound. Seed treated with fresh cell and CFF showed strongly effective on plant growth promotion on 30-day-old rice seedling. Two-time foliar spray with 100% CFF of S20A1 on 45- and 48-day-old rice seedling after *Xoo* inoculation showed the highest effect of disease reduction (68.6%) which was better than the treatment with fresh cell and chemical bactericide. The results obtained can be used as culture conditions model for cell multiplication and SM production of *B. amyloliquefaciens* S20A1 in both small scale production and a scale-up to the industrial level.

## Antibacterial activity of hydroxytyrosol-enriched extracts obtained from olive mill waste waters by membrane technologies against olive tree pathogens

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Olive Mill Waste Water (OMWW) is one of the wastes generated during olive oil production and, due to the presence of polyphenols, represents a source of valuable compounds to be reused in perspective of a circular economy. Among these compounds, hydroxytyrosol (HTyr) is one of the most important, thanks to its antimicrobial, anti-inflammatory, antioxidant and anticancer properties. Three hydroxytyrosol-enriched extracts (4T, 5T and 6T), from OMWW of different geographical origin (Apulia, Tuscany and Sicily respectively) were obtained by membrane technology, a sustainable separation system. The chemical characterization of the extracts, finely carried out by NMR spectroscopy and HPLC/DAD/MS, confirmed that HTyr was the main polyphenol, and identified minor components. In this study, the antagonistic activity of 4T, 5T and 6T extracts against olive tree bacterial pathogens *Pseudomonas savastanoi* pv. *savastanoi* (*Pss*) and *Agrobacterium tumefaciens* (*At*) was evaluated. Cell viability was verified after adding each extract or pure HTyr, synthesized in our laboratories and used as control, into bacterial growth broths. Notably, 4T and 5T were the

most active extracts which completely inhibited the growth of *Pss* and *At* at 500 µg/ml and 1.0 mg/ml of HTyr respectively, compared to untreated controls. In contrast, HTyr at 1.0 mg/ml was only able to reduce bacterial growth. These results suggest that not only HTyr, but all polyphenols present in the extracts contributed in the antagonistic activity against these bacteria. In conclusion, the antimicrobial activity of the extracts was highlighted, confirming the potentiality of their application for plant disease control.

## Evaluation of biological control of bacterial spot disease (*Xanthomonas euvesicatoria*) in tomato plants stimulated by rhizobacteria

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Bacterial spot has become an increasingly destructive problem on tomato due to the suitability of ecological conditions for bacterial infection in Turkey. The most common bacterial spot agent on tomato and pepper is *Xanthomonas euvesicatoria* (*Xev*) in Turkey. The present study aimed to determine the effects of plant growth promoting rhizobacteria (PGPR) inoculation on bacterial spot disease severity and the induction of defence-related response in tomato plants. Out of 303 epiphytic and endophytic bacterial strains were isolated from internal or superficial tissues of healthy tomato plants which were grown in greenhouse and field in Turkey. These isolates were screened *in vitro* for their plant growth promoting traits and antagonistic activity against the bacterial spot disease of tomato caused by *Xev*. Fifteen bacterial isolates, which were considered as successful by *in vitro* tests were selected for *in vivo* pot tests. Beneficial bacterial inoculation was applied in two different ways: before sowing as seed coating and after transplanting as plant spraying. In pot experiments, 25% of the bacterial isolates inhibited bacterial spot disease development on tomato plants at the rate of 32 to 50% compared to pathogen alone treatment. Especially, PGPR strains 50, 224, and 241 both showed strong PGPR activity and effectively inhibited the bacterial spot development on tomato plants by seed bacterization or spray treatment onto tomato plants compared to non treated control plants. This study continues to investigate some basal defense responses against bacterial spot disease in tomato plants stimulated by rhizobacteria at a molecular level using qPCR.

## The efficacy of bacteriophages for controlling bacterial leaf blight on welsh onion caused by *Xanthomonas axonopodis* pv. *allii*

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This study was conducted to evaluate the efficacy of three *Podoviridae* bacteriophages ΦXan31, ΦXan16 and ΦXan17A for controlling bacterial leaf blight disease on Welsh onion in the greenhouse and field. These bacteriophages were selected from a collection of 10 bacteriophages isolated from the Mekong delta of Vietnam. The three phages had the ability to lyse many strains of *X. axonopodis* pv. *allii* and showed the highest

lytic ability *in vitro*. Evaluation of disease control was assessed individually by applying foliar applications of each of the three phage suspensions alone or a phage mixture ( $10^8$  pfu/ml) to Welsh onion leaves in greenhouse conditions. The results showed that spraying phage  $\Phi$ Xan31 on onion leaves gave higher disease control (>50%) than treatments applying phage  $\Phi$ Xan16,  $\Phi$ Xan17A and phage mixture. However, all these phage treatments had less efficacy in disease reduction compared to bactericide treatment (Starner). In the field conditions, comparison of four treatments, i.e. phage  $\Phi$ Xan31 and phage mixture ( $\Phi$ Xan16 and  $\Phi$ Xan17A and  $\Phi$ Xan31), phage  $\Phi$ Xan31 combined spraying bactericide (Starner) when disease aggressiveness and bactericide treatments. All treatments equally reduced disease severity with the percent infection and AUDPC significantly lower than control treatment through different time of observations. In addition, these phage treatments improved yield equal to the chemical treatment and were significantly higher than the control treatment. Treatment phage  $\Phi$ Xan31 combined with the bactericide gave higher yield than phage mixture. The result indicated that phage therapy can control bacterial leaf blight on onion and when disease aggressive development should apply bactericide as necessary.

### Integrated management of phyto-bacterial diseases through the cultivation of medicinal and aromatic plants and the use of Algerian isolates of *Trichoderma asperellum*

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As a preventive measure against the introduction and spread of quarantine phytopathogenic bacteria and to limit and reduce the damage caused by other bacteria on crops, a new integrated management model is proposed, based on: the establishment of medicinal and aromatic insect repellent plants, the treatment of plants with plant extracts for their bactericidal, insecticidal and insect repellent potentialities in the wood parks, nurseries for the propagation of seedlings and in orchards susceptible to bacterial infections transmitted by insect vectors; the development of large-scale medicinal and aromatic cultures under the effect of biostimulants for the industry in order to increase their biomass as well as the yield and effectiveness of their biomolecules for bactericidal and insecticidal interest; the application of Algerian isolates of *Trichoderma asperellum* and their secondary metabolites in the culture system for their benefic effects as biostimulant, hyperparasitic, bactericidal, insect repellent and nematocidal.

### Biological control of Pierce's disease of grape by an endophytic bacterium

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The potential for *Paraburkholderia phytofirmans* strain PsJN to reduce Pierce's disease of grape caused by *Xylella fastidiosa* was explored. Strain PsJN achieved population sizes as large as  $10^6$  cells/g and moved 1 meter or more within 4 weeks after inoculation into vines. While *X. fastidiosa* grew and moved extensively in grape when inoculated alone, few viable cells were recovered when it was co-inoculated with strain PsJN and disease severity was always greatly reduced. Large populations of strain

PsJN could be established in both leaf lamina and petioles by topical application of cell suspensions in 0.2% of an organo-silicon surfactant conferring low surface tension; such treatments were as effective as direct puncture inoculations of this biocontrol strain in reducing disease severity. Inoculation of strain PsJN into plants at the same time as or 4 weeks after the pathogen resulted in large reductions in disease severity; much less disease control was conferred by inoculation 4 weeks prior to that of the pathogen. The expression of grapevine PR1 and ETR1 was substantially higher in plants inoculated with both *X. fastidiosa* and strain PsJN compared to that in plants inoculated only with the pathogen or strain PsJN, suggesting that this biological control agent primes innate disease resistance pathways in plants that otherwise would have exhibited minimal responses to the pathogen. Disease control in extensive field trials using Cabernet Sauvignon grape were similarly high as in greenhouse studies with topical applications of PsJN made up to 4 weeks after inoculation with the pathogen most efficacious.

### Successful control of fire blight: can bacteriophages do the job?

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Development of antibiotic resistance, popularity of organic fruit production and the consumers' desire for pesticide free food has reinvigorated interest in biological control of *Erwinia amylovora*. While commercial products containing epiphytic bacteria or yeast are available for the control of the fire blight pathogen, the use of bacteriophages as biologicals exploits the ability of lytic phages to kill the pathogen. In this novel system, an epiphytic bacterium, a carrier, is infected by phages and is used to deliver the phages to the blossom surface. The carrier acts as a propagation system for the phage thus allowing an increase in phage population prior to the arrival of the pathogen. The carrier additionally serves as a biocontrol agent on its own. This presentation will examine the multiple step process from phage isolation and characterization, bioassays, the impact of bacterial host resistance mechanisms, formulation and field trials. Past field trials with non-formulated carrier-phage preparations were conducted in order to demonstrate proof of concept under field conditions. The results were variable from failed trials to 50-65% efficacy. Current focus is on developing a stable formulated carrier-phage preparation and conducting field-based trials.

### Evaluation of different strains of *Bacillus* for their activity against *Erwinia amylovora*

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The effectiveness of antagonistic bacteria to control *Erwinia amylovora* was evaluated under *in vitro* and *in vivo* conditions. Among thousands of sporulating *Bacillus* strains, ten isolates showed antibacterial activity against *E. amylovora*. For these strains, PCR analyses were performed for

the detection of genes involved in the biosynthesis of lipopeptides, polyketides and the dipeptide bacilysin. These include the *srfA*, *ituC*, *bmyB*, *fenD*, *dfnA*, *mlnA*, *baeA* and *bacA* genes involved in the biosynthesis of synthetases of surfactin, iturin, bacillomycin, fengycin, difficidin, macrolactin, bacillaene and bacilysin, respectively. Differential detection of genes between the strains was observed. There was a high association of the antibacterial activity of strains against *E. amylovora* and the presence of the polyketides biosynthetic genes and the *fenD* gene ( $p < 0.05$ ). All strains produced at least four bioactive compounds against *E. amylovora* as detected using bioautography. Study of the genetic variability of the antagonistic *Bacillus* strains using Rep-PCR showed clusters according to their antibacterial activity against *E. amylovora*. Further investigation was performed to assess the antibacterial activity *in vivo* of the studied strains. The obtained results indicated that a strain of *Bacillus amyloliquefaciens* showed the highest biocontrol efficacy of the disease that was comparable to that obtained with streptomycin, used as a reference pesticide against *E. amylovora*. Thus, this strain could be a promising biopesticide for the biological control of fire blight disease.

### Light quality modulates infection and defence response to fire blight in pear trees

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*Erwinia amylovora* causes fire blight disease. Pathogenesis-related (PR) proteins are part of the systemic signaling network that perceives pathogens and activates defenses in plant. Eukaryotic and bacterial species have a 24 hour 'body-clock' known as circadian rhythm. This rhythm regulates organisms' lives, as the activity and/or mRNA accumulation of phytochromes (*phys*) and cryptochromes (*crys*) genes, which in turn synchronize the internal clock, working as zeitgeber or time-keeper molecules. Salicylic acid accumulation is under light control, and upregulates the PR gene expression, and from *Arabidopsis thaliana phys* nil mutants clearly appears that PR1 gene expression is photoreceptors regulated. Moreover, overexpressing *phyA* in cherry plants increases resistance to *Pseudomonas syringae*. In this work, five bacterial transcripts (*erw1-5*), expressed in asymptomatic *E. amylovora*-infected plants, have been isolated. The research aims to understand how the circadian clock, light quality and related photoreceptors regulate PR and *erw* genes expression, *in vitro*-cultured plantlet system of pear of three lines of cv. DarGazi: DarGazi-wt, DarGazi-overexpressing *phyB* and DarGazi-overexpressing *cryI*. Plantlets were exposed to different circadian conditions, and continuous Blue-, Red- and Far-Red- light. Results showed that PR10 is under circadian control, PR1 is expressed without clear evidence of circadian regulation, whereas *erw* genes are regulated by circadian rhythms. In this regulation framework the active form of phytochrome enhances the expression of PR1 five to 15 times more. An ultra-dian rhythm has been observed according with the zeitgeber role played by CRY1. Results will be discussed in relation to the regulative role of photoreceptors during photoperiod and pathogen attack.

### The influence of physical mutagenesis on the antagonistic activity of *Bacillus amyloliquefaciens* against fire blight

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Induced mutations allow obtaining a biological object with high useful productive properties. Chemical and physical mutagens are used for this purpose. One of the most common methods is physical mutagenesis. Based on this, the aim of our research was to find out how different doses of ultraviolet (UV) radiation and radiation multiplicity affect the antagonistic activity of the bacterium *Bacillus amyloliquefaciens* against the causative agent of bacterial blight of fruit crops *Erwinia amylovora*. The study materials allowed to obtain data on the action of UV radiation on the inhibitory activity of the bacterium-antagonist *Bacillus amyloliquefaciens*. UV radiation was used as a mutagenic factor in the range of 254 nm with 30 W using a bactericidal lamp "TUV TL-D 30W SLV". Different time variants of irradiation were estimated for 15, 30, 35, 40 and 45 min. It was found that the most effective exposure to UV radiation to increase the antagonistic activity of *B. amyloliquefaciens* is the exposure of 45 min. As a result of UV irradiation a mutant strain *B. amyloliquefaciens* MB40 was obtained exceeding the antagonistic activity of the original parent strain 2.8 times. At the same time, the zone of suppression of the pathogen of bacterial burn *E. amylovora* by the mutant strain of *B. amyloliquefaciens* MB40 was  $60 \pm 0.7$  mm, whereas before the mutation it was  $21.6 \pm 0.5$  mm. Repeated irradiation of the mutant strain MB40 UVI led to the emergence of new types of mutant colonies, which differed significantly from the original form of the antagonist in their morphological and cultural characteristics. Testing them for inhibitory activity by diffusion into agar showed that there is a decrease in the suppressive effect of the pathogen of the bacterial burn. It is established that secondary irradiation stimulates the active growth of the antagonist itself. Component composition of active substances produced by the antagonistic bacterium was analysed using GC-MS methods.

### Phage-mediated biocontrol of *Erwinia amylovora*: screening for the ideal carrier

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The use of bacteriophages as a potential replacement, or supplement, to antibiotics for the control of bacterial pathogens is a continually expanding area of research. *Pantoea agglomerans*, an orchard epiphyte with antagonistic properties against *Erwinia amylovora*, is able to act as a carrier to phages which target the pathogen. Infection of *P. agglomerans* allows for the delivery of protected phages onto the blossom, where they can then populate the stigma surface. The strain of *P. agglomerans* chosen as a carrier is critical to the efficacy of the biological. Wild type isolates of *P. agglomerans* were collected from the Niagara region of southern Ontario, and 24 isolates were screened against four species of

*E. amylovora* phages. The use of the classical soft agar overlay technique remains highly prevalent in the literature to study phage-host interactions but producing quantitative data from this technique can be highly variable and time-consuming. To study phage proliferation on a specific host we developed a small-scale broth-based protocol. This method uses plasmid standardized quantitative PCR to quantify phage genomes produced after 8 h. Using this methodology carriers capable of producing all four species of phages were easily identified. This allowed us to choose an ideal carrier for a phage-mediated biocontrol treatment and also facilitated further study into phage-host population dynamics and comparative genomic studies to model their interactions.

### Microbial groups and biocontrol agents associated with different crop succession in relation to potato brown rot suppression

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Four reasonable naturally infested areas in Egypt (0.12 ha each) were selected to follow three years of crop succession. Two areas were sandy (Wardan, Giza governorate and Ganoub El-Tahrir, Behera governorate) and two areas were silty clay soils (Talia, Minufya governorate and Sids, Bani Suef governorate). The applied cropping succession system included corn, potato intercropped with cabbage and onion (first year), cowpea, and wheat (second year), corn again and ended by potato (third year). The pathogen was undetectable after onion, wheat and second corn in the four fields. The pathogen was undetectable at the end of the crop succession program in all fields under investigations except in Sids. The failure of the eradication of the pathogen in this field correlated to the high ratio of NO<sub>3</sub><sup>-</sup> and Na<sup>+</sup> in this area as compared to the other three fields. Disease suppression at the end of crop rotation associated with clear shift in cultural soil biodiversity as indicated by enhancing oligotrophism, increased ratio of fluorescent pseudomonads, endospores bacteria and actinomycetes ratios. Also, the suppressed potato soil and rhizosphere supported high ratio of a diverse of *R. solanacearum* antagonists, similar to *Pseudomonads* spp., *S. maltophilia*, *Citrobacter freundii*, *Acinetobacter* sp., *Delftia* sp. and *Serratia marcescens* as identified by 16S rRNA gene sequencing.

### Control of tomato bacterial wilt (*Ralstonia solanacearum*) by grafting and bacteriophage application

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Tomato bacterial wilt disease caused by *Ralstonia solanacearum* is difficult to control. Grafting with H-7996 and local rootstocks suppressed bacterial wilt into some degree of protection. This paper reported the use of grafted plant and application of bacteriophage to control tomato bacterial wilt. Local rootstocks (Amelia, Mawar) were sown two days before scion (Servo) while H-7996 was sown the same day with scion. The plants were grafted by tube grafting method at three weeks after

sowing the scion. Bacteriophages were isolated from several region of tomato fields and tested *in vitro* for their infection activity against *R. solanacearum*. The phage that had widest spectra against several isolates of *R. solanacearum* was then chosen for the next experiment. Soil in polybags were infested with 20 ml of water suspension of *R. solanacearum* at 10<sup>8</sup> cfu/ml then added with 10 ml bacteriophage solution. A bacteriophage from a region in Central Java showed the widest host range compare with the others. Clear phages were produced in the lawn of *R. solanacearum* in CPG medium. Combination of grafting and bacteriophage application enhanced protection against bacterial wilt of tomato caused by *R. solanacearum*.

### Principles of bacteriophage use to control soft-rot bacteriosis of potato

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Pectolytic enterobacteria (*Pectobacterium* spp. and *Dickeya* spp.), causing soft rot and black leg (Soft Rot *Pectobacteriaceae*, SRP) result in substantial losses of potatoes during vegetation and post-harvest storage. The use of bacteriophages (bacterial viruses) is considered as a prospective method to control bacterial phytopathogens. Due to high specificity of most bacteriophages, their application requires precise diagnostics and differentiation of target pathogens. Based on RAPD, BOX-PCR, MLST profiling, as well as complete genome sequencing, we were able to divide the *Pectobacterium* and *Dickeya* strains abundant in Russia, into 17 strain groups. The representatives of each strain group usually have the same phage susceptibility. This systematization enabled us to rationalize the selection of specific lytic bacteriophages. In the course of the present project we have designed enrichment cultures composed of strains representing each genetic group, that allows us to isolate target bacteriophages from pathogenic plant tissues and sewage water. The current collection of characterized specific bacteriophages includes above 60 phage units, and their combined infection range covers all abundant SRP. Analysis of phage genomes reveals the gene encoding structural proteins with predicted polysaccharide-depolymerizing and polysaccharide-deacetylating activity. We propose that primary interaction of such phages occurs in surface polysaccharide of bacterial cells. On the basis of the above observations we have designed experimental phage cocktails demonstrating high activity against circulating *Pectobacterium* and *Dickeya* pathogens *in vitro* and in biological models.

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### Recent advances on the control of *Xylella fastidiosa* and its vectors in olive groves: state of the art from the ongoing Europe's Horizon 2020 research program

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Search for therapeutic solutions to suppress the development of diseases caused by strains of *Xylella fastidiosa* in infected plants has been, since

the last century, one of the most challenging objective in several applied research programs developed in the Americas, and more recently this topic is of high priority in the European research programs. Likewise other vector-borne diseases, tools for control are directed to both the pathogen in the host plant(s) and the insect vector(s). A wide range of sustainable approaches are included in the multidisciplinary research workplan funded by the European Commission to tackle, in particular, the severe disease associated with the infections of *X. fastidiosa* subsp. *pauca*, ST53, in olives. Search for olive varietal resistance or tolerance is currently one of the main focus, with a large panel of olive cultivars under screening in greenhouse and open field. Bacterial population size, phenotypic response and expression level of candidate genes involved in the host response are the main experimental parameters evaluated. Applications of antimicrobial formulations and use of antagonists are also at an advanced stage (i.e. *in planta* and under field conditions), that while confirming the difficulties to counteract the impact of *X. fastidiosa* in susceptible olives, provide some encouraging evidence to be further implemented and investigated (i.e. attenuation of symptoms upon N-acetylcysteine endotherapeutic applications; colonization of olives by *Paraburkholderia phytofirmans*, previously shown to be effective in grapes). Control of the vector populations is a complementary research task, aiming on one side to elucidate the feeding behavior toward setting strategies for disrupting the transmission events, on the other side by testing organic or inert compound to suppress juveniles and adults. The implications of having strategies for mitigating *Xylella*-disease are particularly relevant for those European territories (demarcated areas) where the bacterium has established and containment strategies are in place.

### How early detection of *Xylella fastidiosa* can contribute to strategies of control of the bacterium? Status of France

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In France, diverse subspecies of *Xylella fastidiosa* (*Xf*) were first intercepted in 2012 on *Coffea* plants, the subspecies *multiplex* was detected in natural condition in 2015, in Corsica and French Riviera (PACA) region. Since then nearly 40,000 samples have been tested at the national level by official laboratories. French National Reference laboratory (Anses) is submitting methods of detection to optimization and evaluation process to verify whether the performance criteria reach the required levels. Up to now, after the various work carried out in intralaboratory at Anses and by partners of H2020 POnTE project or EPPO members, within interlaboratory comparisons, the Real-Time PCR has shown the best analytical sensitivity (detection threshold) on the great majority of *Xf* hosts. However, depending of host plant and the inhibitors it contains, DNA extraction method makes difference in terms of sensitivity. CTAB DNA extraction is the most adapted to the complex matrices such olive tree for high efficient extraction when the use of commercial kits give reliable detection with the other hosts. Within Anses/INRA collaboration, an improvement of target extraction has been obtained by inclusion of a sonication step prior to the extraction of the DNA. This accurate detection method fully adopted by EPPO diagnostic protocol and used in official surveys represents a precious and accurate tool for control of the presence and the spread of the disease in symptomatic and asymptomatic samples. It can help to determine the relevant period for biocontrol measures. In addition, the Real-Time PCR combined with QuickPick™ SML Plant DNA (Bio-

Nobile) kit validated for *Xf* detection in vectors as *Philaenus spumarius* could be valuable for determination of vectors status in a specific zone.

### Development and prospects of nanostructured smart systems for sustainable strategies control of *Xylella fastidiosa*

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The success of nanomedicine has paved the way for the development of agri-nanotechnology. For that reason, the as-synthesized CaCO<sub>3</sub> nanocrystals (nanoCaCO<sub>3</sub>) were tested as innovative smart nanomaterial against the pathogen *Xylella fastidiosa*. NanoCaCO<sub>3</sub> interaction with bacteria was studied through cells ultrastructural analysis by transmission microscopy. CaCO<sub>3</sub> nanocrystals affect *X. fastidiosa* causing bacteria wall alteration up to cell destruction. The action of this nanomaterial will be thorough with *in planta* experiments. We are analyzing the absorption of nanocrystals into rooted olive cuttings. Nanocrystals were adsorbed from roots and they translocated in plants tissues. NanoCaCO<sub>3</sub> carriers were able to encapsulate efficiently two types of antimicrobial substances and the potential efficacy was tested in experiment under greenhouse conditions. A good alternative to inorganic systems is represented by natural organic polymers. Chitosan is a promising material thanks to its antibacterial properties, its bioadhesion and ability to stimulate defense responses in plant tissues. We have investigated nano-phytodrugs based on chitosan and Fosetyl-Al to synergize the activities of these two components acting on the adhesion of the bacterium and / or its ability to form the biofilm. We have developed chitosan coated nanocolloids of Fosetyl-Al and their activity was studied through *in planta* and *in vitro* experiments.

### Understanding the olive microbiome of susceptible and resistant cultivars for sustainable biocontrol

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Olive Quick Decline Disease (OQDS) is a devastating olive disease emerged few years ago in the region of Apulia (southern Italy) as a result of the bacterial infections caused by *Xylella fastidiosa* subsp. *pauca*. Bacterial infections were consistently associated to severe desiccations

on the local cvs Cellina di Nardò and Ogliarola salentina, whereas traits of resistance, i.e. mild symptoms of OQDS were found in the cvs Leccino and FS17. Investigations on the olive microbiome of OQDS-resistant and susceptible cultivars were undertaken to identify potential protecting endophytes for a strategy of biocontrol. The xylem bacterial and fungal communities of infected trees of the cvs FS17 and Kalamata, respectively symptomless and highly symptomatic, were analysed by barcode (16S rRNA V4 and ITS1-spanning amplicons) and whole shotgun sequencing (WSS). The core microbiome was dominated by fungi, accounting 99.8% and 88.4% of the total reads by barcode and WSS sequencing, respectively, while Proteobacteria and Ascomycota are the most represented phyla with both techniques. This fungi/bacteria ratio was maintained in all FS17 olives while it was inverted in the susceptible cv. Kalamata, where *Xylella* colonized the majority of the ecological niche in the heavily affected plants. Moreover, bacterial communities of trees of the cvs FS17 and Leccino were purified and co-inoculated with *X. fastidiosa* into potted olives of the cv. Cellina di Nardò for evaluating potential effects on the progression of *Xylella*-infections in this susceptible cultivar. Data on the progress of *Xylella* infections and/or symptoms appearance in these microbiome-*Xf* co-inoculated plants will be presented.

### Sustainable pesticides to contrast the bacterium *Xylella fastidiosa*: current situation and reasonable perspectives

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Since 2013, the Olive Quick Decline Syndrome caused by *Xylella fastidiosa* subsp. *pauca* strain De Donno (*Xf*), determined a dramatic situation for the whole olive/oil production in Apulia region (Italy). Different research projects are in progress and, in the same time, a containment plan (early detection, monitoring, eradication and sanitation of *Xf*-infected plants, strategies against its vector, selection of olive tolerant *Xf*-cv.) is recommended. Moreover, changes in cropping systems (e.g. pruning, fertilization and irrigation), could have some impacts on the disease development but this is not enough to protect the plants. Until now, no new pesticides to contrast *Xf* have been registered. In addition, according to the recent report of EFSA Plant Health Panel, there is currently no method available to cure a diseased plants in the field. The main activities in progress to develop new sustainable pesticides to contrast *Xf* are related to: induce resistant compounds, biocontrol agents, peptides, bacteriophages, natural active ingredients, nanoparticles. As for all systemic phyto-bacteria, also respect to *Xf* it is complex to develop an effective pesticide able to contrast/kill its bacterial cells outside the plants, and particularly when they are inside xylematic vases of the host plants. The different approaches against *Xf* are analyzed and future perspectives are discussed.

### Effects of agronomic practices for contrasting the spread of *Xylella fastidiosa*

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Following the emergency caused by the presence of *Xylella fastidiosa* subsp. *pauca* ceppo CoDiRO in the Salento area, several professionals were called to use their expertise to fight its diffusion. Among these, there

are the local agronomists, working as consultants for the farmers; their task is to recommend to the farmers the most suitable practices to adopt. The objective of the presentation is to explain such strategies. Given the bacterial nature of the disease, the agronomists aimed at opposing its diffusion through indirect measures, which were structured according to the most recent scientific advancements concerning bacterial diseases in general and *Xylella fastidiosa* in particular. The approach which was considered more suitable to adopt in the area is intended to: decrease the insect vectors; decrease the inoculum; improve the environment where the trees grow, with special regard to the microbial processes of the soil; rapidly take action to reduce stress conditions in the olive orchards at risk. To ensure the environmental and economic sustainability of these measures, we proposed the integration of different methods (Integrated Pest Management), more specifically: spraying of pesticides with low environmental impact; soil tillage; tree pruning. Moreover, the type and intensity of the recommended actions are different in the infected area, in the buffer area and in the containment area. The efficacy of these methods has been acknowledged by the policy makers and thus integrated in the laws regulating the actions to take against *Xylella fastidiosa*.

### EPPO activities in efficacy testing and safety assessment in biological control

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Requirements for the approval of biological control agents (BCAs), such as micro-organisms and invertebrates vary widely between EPPO countries. It differs whether the BCA is in a formulation (i.e. a product), or just released as such. The approval process would differ for a BCA native to the area compared to a non-native species. Within the remit of plant protection products, EPPO's work focuses on efficacy of plant protection products including biopesticides. EPPO Standard PP 1/296 *Principles of efficacy evaluation for low-risk plant protection products* describes the requirements for an efficacy evaluation of low-risk plant protection products in a registration procedure. More specifically, PP1/276 *Principles of efficacy evaluation for microbial plant protection products* describes the principles for determining requirements for an efficacy evaluation of plant protection product containing micro-organisms. The work of the Joint Panel on Biological Control Agents focuses on safety. For a non-native BCA being released in a new area, the comparative assessment of ecological benefits and safety is an important aspect in the approval procedure. For this assessment, the EPPO Standard PM 6/4 *Decision-support scheme for import and release of biological control agents of plant pests* provides guidance on impact assessment for a BCA. It supports decision-making on introduction and aims to harmonize the assessment procedure within the region. It addresses the probability of establishment of a BCA and potential environmental consequences. Using expert judgement, an overall conclusion on the benefit and associated consequences of the introduction of a BCA is drawn.

### A holistic model can be used to explain the symbiotic mitigation of the olive quick decline syndrome

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The introduction of a bio-fertilizer (BF) into the soil, based on symbiotic micro-organisms as agents to promote the yield and health of crops, is aimed at inducing modifications in the rhizosphere, as well as in the plant phenotype. In *Olea europaea* cv. Ogliarola di Lecce groves affected by Olive Quick Decline Syndrome (OQDS, involving *Xylella fastidiosa* subsp. *pauca*) we report that: i) the vegetative responses to the disease appeared highly variable, but the symptoms were significantly mitigated in two groves out of six and aggravated in only one; ii) the NIR-tomography of hay-litter-bags from non-inoculated soils can be used to forecast the outcome of BF inoculation; iii) a holistic model that gathers differential and compositional analyses of the leaf (pH, crude protein, water) and of the soil (respiration) can explain over 95% of the average mitigation response to BF inoculation. The two keys for a successful inoculation have been identified as a high degree of variability of the soil conditions, which is favourable for welcoming the guest BF (lowering the fingerprint of the control litter-bags) and for an enhancement of the homogeneity of the leaves (with increases in the fingerprint of the leaves treated with BF). However, inoculation of BF consortia is far from being the ultimate remedy to mitigate OQDS. Further field experiments are needed to clarify the soil hosting capacity and to define the mycorrhizal and/or endophytic/plant pathogen interactions using rapid methods (litter-bags, foliar pH, near-infrared tomography).

## An overview of European Regulatory of biopesticides

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Biopesticides are plant protection products which contain biological control agents (microbials, pheromones, plant extracts, etc.) for agricultural, horticultural and home garden uses. As a PPP in Europe, a biopesticide must be approved before being used, sold, supplied, advertised or stored. The rules and procedures for authorisation of active substances and PPPs are laid down in Regulation (EU) No 1107/2009. European regulations and several guidance documents from OECD (or other institutions) guide and help applicants to prepare, compile and submit required data supporting intended approvals. The evaluation of biopesticides follows the common process of approval of active substance at the European level (assessment by RMS, writing of a DAR...). It could eventually conclude to a low-risk profile, categorising the biopesticide active substance as a low-risk substance. But because criteria could not be completely fulfilled, expert judgement is often involved. Within general requirements common with all pesticides, specific requirements according to category of biopesticides are mandatory. All supporting data must be generated following certain standards; however due to the nature of each biopesticide, there is regularly a need for an accurate characterisation of the substance that can be considered as the keystone of the dossier. The main difficulties encountered for registration of the products containing such substances are the availability of reliable data and the variability of national rules. In the other hand, some advantages are granted for PPP containing such substances (e.g. longest approval period, shorter evaluation by the Authorities...) leading to their current expansion in the majority of European countries.

## Protection of phage applications in crop production: a patent landscape

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In agriculture, the prevention and treatment of bacterial infections represents an increasing challenge. Traditional (chemical) methods have been restricted to ensure public health and to limit the occurrence of resistant strains. Bacterial viruses i.e. bacteriophages could be a sustainable alternative. A major hurdle towards the commercial implementation of phage-based biocontrol strategies concerns aspects of regulation and intellectual property protection. Within this study, two datasets have been composed to analyze both scientific publications and patent documents and to get an idea on the focus of research and development (R&D) by means of an abstract and claim analysis. A total of 137 papers and 49 patent families were found from searching public databases, with their numbers increasing over time. Within this dataset, the majority of the patent documents were filed by non-profit organizations in Asia. There seems to be a good correlation between the papers and patent documents in terms of targeted bacterial genera. Furthermore, granted patents seem to claim rather broad and cover methods of treatment. This research shows that there is indeed growing publishing and patenting activity concerning phage biocontrol. Targeted research is needed to further stimulate the exploration of phages within integrated pest management strategies and to deal with bacterial infections in crop production.

## Evaluating the safety of biocontrol agents for use against plant diseases

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Most microorganisms in the environment are harmless to plants and humans. Plant-associated bacteria that are non-pathogenic to plants have often been deployed in biological control of plant diseases. These bacteria are usually innocuous to humans, but their relative risk will depend on several factors, such as the specific strain of a species, environmental conditions, the susceptibility of human individuals, the microbial dose and the mode of exposure. Countries in the world use slightly different classification systems to rate the relative risk of hazardous microorganisms. Nevertheless, four hazard levels are most commonly utilized, where Class 1 harbors the least harmful and Class 4 the most dangerous human pathogens. The evaluation of an individual biocontrol strain should primarily focus on the examination of its ecology and taxonomic position, and secondly, if it, or its close relative has been reported from a clinical environment, the elucidation of its mode of action(s) in human pathogenesis. Advances in genomics of microbes has clarified the phylogenetic position of many bacteria, and evidenced their potential for harboring mobile genetic elements, another aspect to consider in risk assessment. Species belonging to the genera *Pantoea*, *Pseudomonas* and *Bacillus* are examples of commonly used biocontrol agents, and they have been studied extensively. The same genera are represented among clinical species. The inherent risk of every biocontrol strain has to be evaluated separately. Other risks that are being considered are those connected to the use of

genetically modified microorganisms and the dissemination of bacteria that harbor antibiotic resistance genes.

### Applications of plant beneficial microbes: opportunities and challenges towards sustainable and resilient agriculture

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Growth of the world population requires production of higher quantities of food, feed, fiber and energy. Unfortunately, increasing the production of agricultural commodities also contributes to pollution of the environment, as well as reduction of wild life and biodiversity. The Research and Innovation program Horizon 2020 is included in the EU Commission action plan to accelerate Europe's transition towards a circular economy. One of the goals of this program is to contribute to sustainable and resilient agriculture and therefore to address production, consumption and waste in this area of the circular economy. Microbes can play a significant role to achieve this goal. Research of plant-microbe interactions reveals the importance of microorganisms in adaptation of plants to the environment by protecting them against biotic and abiotic stresses, by improving uptake of certain nutrients and by stimulation of growth and development of crops. Such beneficial microbes can be used as an alternative or as an addition to synthetic pesticides and inorganic fertilizers. Industrial production and application of beneficial microbes is based on scientifically generated knowledge of biology of these organisms and of their mode of action. Therefore, biotechnology industry organizations such as IBMA and EBIC are actively involved in the dialogue with EU policy makers and contribute to adaptation of the current EU legislation to make the process of placing microbial products on the market in agreement with their natural properties.

### New EU regulatory framework for bioprotection agents. IBMA vision on how to improve regulation in the European union

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Bioprotection technologies provide effective and innovative plant protection for modern agriculture. This makes them a vital tool in the EU's work to support farmers and effectively implement the Sustainable Use of Pesticides Directive (SUD, Directive 128/2009/EC). However, the current lack of a specialist bioprotection regulatory body, legislation, procedure and data requirements means that the EU is not fully reaping the benefits of this rapidly growing, predominately SME-based, industry. IBMA therefore propose that by the end of 2020 the EU establishes a bioprotection-specific body that has developed and implemented a short and precise timeline for the evaluation process, with evidence-based procedures and tailored data requirements. Risk-based evaluations should be conducted using high-level scientific expertise and in close communication with applicants. In parallel an interim arrangement in 1107/2009 to protect the viability of biological plant protection product producers until the new Regulation comes into force must be enacted. This new approach would benefit human health, the environment, biodiversity and the bio-based circular economy and, therefore, society as a whole. Please read the White Paper on our website: <http://www.ibma-global.org/en/news/ibma-white-paper-ibma-vision-on-how->

[to-improve-regulation-in-the-european-union%2D%2D-a-new-eu-regulatory-framework-for-bioprotection-agents-](#)

### Technical assistance from agronomists to control bacterial diseases in sustainable agriculture: needs and prospects

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Professional and specialized technical assistance is essential in the planning and management of the processes of the agro-food chain, especially on plant matrices, to guarantee the protection and respect of food safety, public health and the environment. Phytoiatrics (literally "plant care") is dedicated to the prophylaxis and care of plant organisms, both as Consiglio dell'Ordine Nazionale dei Dottori Agronomi e dei Dottori Forestali Autorità di Vigilanza - Ministero della Giustizia Via Po, 22 - 00198 Roma - Tel 06.8540174 - Fax 06.8555961 – [www.conaf.it](http://www.conaf.it) single individuals (e.g. trees of ornamental interest) and as a whole of individuals (crops, woods). It deals with the means, techniques and strategies aimed at protecting plants from adversities, biotic and abiotic. The activities defined by current legislation can be traced back to the definition of "Phytoiatric Act". The introduction of the phytosanitary prescription takes on a decisive role, with a more incisive character, in the health surveillance of the use of plant protection products. Therefore, positive aspects are reliable with regard to the aspects of protection and safeguarding of public health, in particular: 1. Food security of the agricultural product and consumer protection; 2. Protection and safety of the agricultural operator; 3. Protection of the environmental system. The implementation of the phytosanitary prescription, together with the keeping of the treatment register, would allow an analytical control on the food in search of the residue of the product used in the field and therefore an eventual overcoming would be immediately detected, allowing a rapid control of the risk. With the introduction of the phytosanitary prescription, the environment would be subjected to a lower load of chemical substances, with benefits for the soil, surface and deep waters and for the entire ecosystem. The rational use of agrochemicals, dictated by the prescription, would make a significant contribution to reducing water pollution. With the prescription a control system would be introduced, of a preventive nature, which would guarantee the correct management of chemical products, right from the selection stage. The subsequent use of the agropharmaceutical would be related both to the type of crop to be treated and to respecting the existing agro-environmental situation, with reliable direct and immediate effects on the protection of human, animal and environmental health.

### Reorganization of the national phytosanitary service for biological control

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The intense economic relations that led to the globalization of markets and climate change have increased exponentially the risk of importing foreign pests, which can spread with devastating effects on crops and the stability of ecosystems, with consequent considerable economic damage. The new Regulation (EU) 2016/2031 concerning protection measures against plant pests,

together with the Regulations (EU) 652/2014, on interventions funding and 2017/625 concerning official controls, redefines the EU plant protection regime, introducing new elements in every aspect of production, control and surveillance activity, also modifying producers' direct responsibility for plant health and procedures of surveillance implemented by the official services. In the light of the deep transformations that these Regulations introduce, it appears necessary to consolidate the structures of the National Phytosanitary Service to guarantee their application, as well as the performance of controls, sector surveillance and the management of phytosanitary emergencies. The new legislative text redesigns many organizational aspects such as: the structures, the competences of the Central Competent Authority and the Regional Authorities, the establishment of a central Secretariat unit for phytosanitary emergencies, the creation of a national information system, the rationalization of entry points, as well as the definition of uniform control procedures at national level and the training and permanent updating of phytosanitary personnel. Particular importance is given to the scientific and diagnostic aspects with the institution of the national reference institute, identified in the CREA DC - Defense and Certification, which is already a European reference laboratory. The framework of the laboratories is completed by four levels of diagnostic skills and activity coordination. In addition to the Reference Institute there are the national reference laboratories that support the activities of the European laboratories, the official laboratories of the regional phytosanitary services and the laboratories of the national network. These different levels of diagnostic reliability, with their specific skills, define a new framework for the integration of the national scientific network and guarantee the necessary synergy between all the national scientific institutions involved in plant protection.

### **International Year of Plant Health 2020 *Protecting Plants, Protecting Life***

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In December 2018, the United Nations General Assembly declared 2020 as the International Year of Plant Health (IYPH). This is a once in a lifetime opportunity to raise global awareness on how protecting plant health can help end hunger, reduce poverty, safeguard the environment, and boost economic development. Plant health is increasingly threatened by climate change and human activities that have altered ecosystems, reduced biodiversity and created new niches where pests can thrive. At the same time, the huge volume of international travel and trade can quickly spread pests and diseases worldwide. Indeed, up to 40 percent of food crops are lost to plant pests annually. The IYPH emphasizes protection and prevention. As with human health, this is far more cost effective than managing full-blown health emergencies. Because of the strong links between plant health and environmental protection, the IYPH also encourages environmentally friendly ways of dealing with pests such as through integrated pest management. Avoiding poisonous substances helps safeguard the environment, as well as pollinators, natural pest enemies and other beneficial organisms under serious threat. IYPH 2020 will mobilize governments, industries, civic organizations, scientists, and the public to work together to protect the world's plants from

devastating pests; encourage scientific innovation to address pest threats; promote responsible practices to reduce the spread of pests; and increase public and private sector support for sustainable plant health strategies and services.

### **Biocontrol products against bacterial diseases in the NENA region: Egypt as a case study**

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Bacterial diseases continue to threaten the agriculture production in NENA region and reduce quality and quantity of major crops like citrus canker (*Xanthomonas citri*), potato brown rot (*Ralstonia solanacearum*), grapevine crown gall (*Agrobacterium tumefaciens*), soft rot (*Pectobacterium carotovorum*) and fire blight (*Erwinia amylovora*). The agriculture usage of pesticide in NENA region is estimated at total of 53,000 tons of pesticide annually, for which, Morocco, Egypt, Algeria and Saudi Arabia are the top consuming countries in order, representing 65% of NENA usage of pesticide. In Egypt, 45% of pesticides are used to control fungal and bacterial diseases with about 3,600 tons annual consumption. Although the national authorities are generally encouraging farmers to consume less pesticides and rely more on biological products that have lower effect on public health and environment, yet the share of the commercial biocontrol products in the market in country like Egypt represents less than 0.4% of the market value of pesticides that are mainly *Bacillus thuringiensis* products against insect pests. In the database of the Egyptian Agriculture Pesticide Committee, there are only four registered products that contain microbial agents that have been reported as having inhibitory effect against bacterial diseases: *Bacillus megaterium* and *B. subtilis*. There are other three products that contain microbial active ingredient that may show inhibitory effect against bacterial diseases but were registered in Egypt as biofertilizers: *B. cereus* and *B. megaterium*. Referring to internationally published research work available on google scholar database, there are more than 30 articles published by authors affiliated to Egyptian organizations between 2008-2018 on biological control agents against bacterial plant pathogens. Although that, none of those agents were officially registered or commercialized. The challenges that encounter the development, registration and commercialization of biological control agents against bacterial plant diseases are mainly the difficulty of official registration of bioagents, low popularity of biocontrol agents among farmers, weak interest of commercial firms to produce or trade on biocontrol agents and lack of support from official agriculture authorities.

### **Competition between pathogenic agrobacteria and root-knot nematodes results in biocontrol effect on tomato**

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Biocontrol exploits antagonistic interactions between pathogens and beneficial organisms. However, beneficial organisms are in this anthropogenic view considered as non-pathogenic agents in relation to plants and humans, while in fact antagonistic

behaviour among pathogens is quite common. In quest of understanding interactions between pathogenic agrobacteria and root-knot nematodes (RKN) we set-up an experiment on tomato. The hypothesis was that incidence of crown gall disease caused by *Agrobacterium tumefaciens* is positively associated with the level of root damage caused by root-knot nematodes, such as *Meloidogyne* spp. The experiment was designed on tomato hybrid plants in hydroponic culture. We assessed plant vitality, nematode reproduction and crown gall incidence after joined activity of both plant pathogens on tomato roots. Results suggest that infection with pathogenic *A. tumefaciens* enhances plant defence against RKN *M. ethiopica* resulting in lower egg and gall counts on roots 45 and 90 dpi. Several genes under plant hormonal control were targeted for evaluation of gene expression with RT-qPCR. Results suggest that the suppression was mediated via systemic acquired resistance by the pathogenesis-related protein 1 (PR1). On the other hand, *M. ethiopica* did not enhance plant defence against *Agrobacterium*. The latter was also demonstrated in pot experiments. Bacterial tumor growth was inhibited by the nematodes in a 45-day experiment if inoculated onto roots before the pathogenic bacteria. The observed antagonism in this tested pathosystem was, according to results, due to an activation of early plant defense mechanism that was later overcome by the invading pathogen and pest.

### Unravelling the impact of plant-arthropod-microbe interactions on plant fitness and performance – a meta-analysis overview

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Direct and indirect interactions between plants, arthropods and plant-associated microorganisms can strongly modify their impacts on plant fitness and performance. Herbivores and pathogens can facilitate each other, causing additional yield loss while beneficial microbes can induce defenses that protect plants against herbivores and promote plant growth and fitness. The outcomes of these interactions are, however, not always obvious and are influenced by a number of factors. To get better insights into such complex relationships we conducted an extensive statistical meta-analysis of relevant literature results on this topic covering the period 1969 – 2016. Meta-analyses are increasingly used in biology and ecology for synthesizing results of a broad spectrum of studies and/or for testing new hypotheses, identifying research gaps and identifying promising further directions of research. For this reason an extensive meta-databases on three-way plant-microbe-arthropod interactions has been compiled by an international consortium of experts. More than 50 different response measures and explanatory variables have been extracted from the studies

which enable us to significantly broaden our knowledge on multi-way interactions in nature. Here, we present our meta-analysis workflow and the overview of tested hypotheses.

The work has been supported by the COST Action FA1405

### Does leaf ionome play a role in the response of olive (*Olea europaea*) to OQDS?

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Olive Quick Decline Syndrome (OQDS) was officially identified for the first time in 2013 in a “core area”, located in south-east of Italy (Lecce, Apulia region) where affected a large number of olive trees. The rates at which syndrome spreads were generally rapid, but occurred with an irregular pattern, affecting some areas more intensely than others. To date, research efforts towards solving the problem have been mainly focused on the bacterium *Xylella fastidiosa* subsp. *pauca*, which has been found in the xylem of many affected trees and, by Koch's postulates, defined the causal agent of OQDS. Although the scientific studies have made considerable progress over the last years in the understanding biology of *X. fastidiosa*, the problem has yet to be solved. It is well known that management of mineral nutrients status of plants is one of the possible mechanisms allowing the reduction of the effects of several abiotic and biotic stress. Among essential elements, sulfur, being required for the synthesis of various compounds, such as thiols, is critical for ensuring normal defense response to environmental stress and pests. The relation between plant nutritional status and the severity of the OQDS symptoms here has been investigated on olive trees from three experimental areas with trees showing severe symptoms (SS), mild symptoms (MS) and with asymptomatic trees (AS). In particular, using inductively coupled plasma-optical emission spectroscopy (ICP-OES), the macro- and micronutrients content of olive leaves was quantified. In addition, we related the different degree of the OQDS symptoms to total S, to thiols accumulation rate and to the activity of O-acetylserine(thiol)lyase, the S pathway-related enzyme directly involved in the synthesis of cysteine. The contents of mineral elements in olive leaves were analyzed according to the principal components analysis (PCA). The first two components used to build both the scatter and loading plots accounted for 72% of the total variance. Along the first component, which described 47% of the total variance, the samples separated in two main clusters and, interestingly, the AS samples were separated from both SS and MS samples. The loading plot showed that the highest positive influence to the distribution along the first component was mainly given by Mn, Zn and Cu. Both AS and MS samples were separated from SS ones along the second component (25% of the total variance) and the highest positive influence was given by Ca, whereas the highest negative contribution was given by S. Results also showed that AS samples were characterized by a reduced activity of the OAS-TL enzyme, compared to the other two plots, and a lower leaf concentration of thiol compounds. These results provide a promising evidence with potential application for dealing with OQDS and in particular the question of whether plant nutritional status plays a role in the

development of OQDS symptoms deserves to be further examined in depth.

### The *Lysobacter brunescens* quorum sensing system diffusible signaling factor, *LbDSF*, regulates anti-*Xanthomonas* XSAC biosynthesis, colony morphology, and surface motility

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*Lysobacter* species are emerging as novel sources of antibiotics, but the regulation of these antibiotics is still poorly understood. In this work, we identified a small diffusible signaling factor (DSF) molecule (*LbDSF*) that regulates the biosynthesis of a novel *Xanthomonas*-specific antibiotic compound(s) (XSAC) in *Lysobacter brunescens* OH23. *LbDSF* was isolated from the culture broth of *L. brunescens* OH23, and the chemical structure was determined by NMR and MS. The *LbDSF* compound induced GUS expression in a reporter strain of *Xanthomonas campestris* pv. *campestris* FE58, which contained the *gus* gene under the control of a DSF (diffusible signaling factor)-inducible *engXCA* promoter. *LbDSF* production was found to be linked to the enoyl-CoA hydratase RpfF and dependent on the two-component regulatory system RpfC (hybrid sensor histidine kinase)/RpfG (response regulator), and *LbDSF* production was increased 6.72 times in the  $\Delta rpfC$  compared to wild-type OH23. *LbDSF*-regulated XSAC production was dramatically decreased in the  $\Delta rpfF$ ,  $\Delta rpfC$ , and  $\Delta rpfG$ . Additionally, a significant reduction in surface motility and changes in colony morphology were observed in the  $\Delta rpfF$ ,  $\Delta rpfC$ , and  $\Delta rpfG$  compared to the wild-type OH23. The exogenous *LbDSF* significantly increased the XSAC production in wild-type OH23 and recovered the XSAC biosynthetic ability in  $\Delta rpfF$ . Together, these results showed that *LbDSF* is a fatty acid-derived DSF that positively regulates XSAC biosynthesis, cell morphology, and surface motility. Moreover, the RpfC/RpfG quorum sensing signal transduction pathway mediates XSAC biosynthesis. These findings may facilitate antibiotic production through genetic engineering in *Lysobacter* spp.

### Introgression of broad-spectrum bacterial blight resistance genes into cultivated rice from *Oryza rufipogon*

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Bacterial blight in rice (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a worldwide disease and seriously restricts the high and stable yield of rice. Enhancing the resistance of rice against bacterial blight by using resistance (*R*) gene is the most effective, economic and environment-friendly measure to control this disease. Wild rice species are important genetic resources for genetic improvement of cultivated rice (*Oryza sativa*). We collected the wild rice *Oryza rufipogon* Griff. in Guangxi province and conducted challenge-inoculation with diverse *Xoo* strains. The accessions, conferring broad-spectrum to BB, were selected and crossed with IR24 or JG30 to transfer the *R* genes from wild rice into cultivated rice varieties. The introgression lines were developed by multiple round back-crossing and

selfing. Evaluation of resistance to *Xoo* and selection of plant type have been carried out successively to obtain the lines with stable resistance and agronomic traits similar to the susceptible recurrent parents (IR24 or JG30). Three excellent lines, CBB23, CBB30 and W6023, have been bred and utilized in breeding of BB resistance rice varieties.

### Possibility to modulate bacterial strain virulence by epigenetically active compounds, as revealed by treatment of *X. campestris* culture

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Epigenetics studies heritable changes in phenotype that are not reasoned by alterations in the DNA sequence. The greatest achievements of epigenetics are recognizable in human medicine, but very little information is available in the field of bacterial epigenetics.

In the present study, a selected strain of *X. campestris* pv. *campestris* (*Xcc*) was treated by 10 different chemical substances with more or less described epigenetic effect. After this treatment individual strains were transferred on standard cultivation media to verify strains remained viable. In this way, the so-called Epi-treated library of 10 differentially treated strains was created. They were subsequently used for inoculation of Chinese cabbage sensitive variety. The speed and vigour of symptoms typical for *Xcc* infection was evaluated in detail 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. The impact of individual epigenetic substances on DNA methylation state of treated strains was also measured by the MSAP method. The 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; WGS of treated strains after bisulfite conversion of methylated nucleotides) or on biological properties (verification of Koch postulates; versatility of promising Epi-treated races in terms of their re-inoculation to the same or other *Brassica* species).

### Molecular fingerprinting and comparison of *Acidovorax citrulli* strains structure in Turkey and USA

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*Acidovorax citrulli* (*Acc*), the causal agent of a bacterial seedling blight and fruit blotch, effects as a serious threat to some cucurbit plant species, including watermelon, melon, cucumber, pumpkin and gourds. Till now,

the bacterium was characterized on the basis of genetic studies with the 16S rDNA, DNA/DNA assays and AFLP analysis, and phenotypic data. Profound knowledge on pathogen diversity is necessary for efficiently disease management and tracking of the pathogen dissemination. In this study, we have compared genetic differentiation between *Acc* isolates collected from Turkey and USA by using selected ISSR primers producing high polymorphic fragments. The results showed that genetically characterized *Acc* isolates of Turkey and USA show genotype flow and similarity. These markers can be selected for analyzing of genetic diversity and tracking of population structure of this pathogen on different hosts.

### Informational war as therapy to silence blackleg and soft-rot diseases

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The means of communication used by several pathogenic bacteria to overwhelm plant defenses have emerged as a target for the development of novel biocontrol strategies. These therapies aim at reducing the expression of virulence systems rather than eradicating the pathogen, using bacteria or enzymes degrading signaling molecules. Unfortunately, these quenching approaches will likely suffer a lack of consistency as observed for most other biocontrol methods. Indeed, the effectiveness of biocontrol treatments greatly depends on the fitness of the protecting agent and its sustainable activity in the vicinity of plant-host. In the fight against soft-rot phytopathogens, we used the *Rhodococcus erythropolis* R138, a biocontrol agent able to degrade signals used by numerous pathogens. Interestingly, the plant protection provided by R138 strain is induced not by the invasion step or the massive presence of the pathogen, but by its quorum-sensing based communication. Therefore, the R138 biocontrol agent was able to suppress effectively the disease during greenhouse and field trials when the  $\gamma$  caprolactone (GCL), a lactone mimicking quorumsensing signals, was spread. GCL is a well-known nutty flavor additive approved for use in foods by the FDA and EFSA, and therefore, could be considered for large-scale crop treatment in sustainable agriculture. Here, we unveil how GCL promotes the elective growth of biocontrol agent and acts as a powerful inducer of biocontrol. The findings presented shows that preventive or curative antivirulence therapies may be triggered and boosted by the addition of cheap biostimulating lactone molecules.

### Isolation and characterization of bacteriophage Xv1

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Viruses are one of the most abundant and diverse entities on the planet. There is a group of viruses that are characterized by infecting bacteria, which are called bacteriophages (phages). Most of the phages described belong to the order *Caudovirales*, this order groups five families: *Ackermannviridae*, *Herelleviridae*, *Myoviridae*, *Podoviridae* and *Siphoviridae*. The objective of this work was to isolate and characterize a phage with lytic phase that can be used as a biocontrol agent. Our experimental strategy allowed the isolation of phage Xv1 from sewage water, this phage showed lytic activity on *Xylella fastidiosa* subsp. *fastidiosa* (ATCC35877), *Xylella fastidiosa* subsp. *multiplex* (ATCC35871), *Xanthomonas axonopodis* pv. *viticola* and 23 strains of *Xylella fastidiosa* isolated from three wine-growing localities in Mexico. Phage is inactivated when exposed to 50 °C for 1 hour and maintains its stability in a pH range from 4 to 12. Electron micrographs show that phage Xv1 has a morphology similar to the *Siphoviridae* family, with a diameter of 60 nm approximately. The assembly and analysis of the genome, and the comparison in the databases allows us to determine that it is a new phage, not reported, double-stranded DNA, with an approximate size of 47407 bp, and a content of C + G of 55.5%. The *in vitro* evaluation demonstrates the lytic capacity of the phage and its potential use as a biocontrol agent of *X. fastidiosa* and *X. axonopodis* pv. *viticola* in vineyards.

### Potential of biocontrol and molecular characterization of a bacterial agent of the *Pseudomonas* genus

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The increasing demand on agricultural products on the world implicates a greater exchange of plant material, and also the possibility of introduction of new diseases for the importing country. The development of alternatives to control plant pathogens, safer for the environment is a priority in the current production systems. The native microbiota of Mexican soils has shown to be a reservoir of microbiological resources with the potential to face this type of phytosanitary risks. The antagonistic potential of the *Pseudomonas* S40 strain isolated from Mexican soils *in vitro* was determined against 13 plant pathogenic bacteria with quarantine status in México. Bioassays were established to assess the effect of the volatile compounds and the diffusible metabolites in solid medium. The inhibitory index against the 13 bacteria were determined using Image J software, as well as the index of biocide capacity, and in relation to this the effect on the colonies growth. For the molecular characterization the genes *16S*, *gyrB*, *rpoB* and *rpoD* were amplified; sequences information were used to construct data bases in conjunction with information of the genes of 221 type strains. The four genes data bases where concatenated and a Multilocus Sequence Analysis (MLSA) were conducted. Volatile compounds produced by strain S40 has bactericidal effect on bacteria belonging to the genera *Clavibacter* and *Curtobacterium* with inhibitory indexes over 97% (Tukey

$P \leq 0.05$ ). Diffusible compounds have significant bactericidal effect only on *Clavibacter nebraskensis* (Tukey  $P \leq 0.05$ ). The MLSA showed that strain S40 belongs to *P. chlororaphis* group with high phylogenetic similarity to *P. protegens*.

### Stewart's wilt disease a genomic approach to investigate the likely origin site

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*Pantoea stewartii* subsp. *stewartii* (*Pss*) is the causal agent of the Stewart's wilt disease which is the most serious disease of sweetcorn. The bacteria systemically infect the host plants causing the typical leaf yellow stripes, plant growth reduction, wilting and plant death. *Pss* is an indigenous maize pathogen in the Americas and it is a quarantine organism in the European Union. In the Americas, *Chaetocnema pulicaria* Melsheimer is the only known efficient vector. As part of the surveillance of the Italian territory *Pss* was individuated since 2015 to 2018 by the Plant Protection Services of Emilia-Romagna, Veneto, Friuli Venezia Giulia and Lombardia. *Pss* isolates were characterized by molecular, biochemical and pathogenicity tests according to the EPPO protocol. In order to define their provenience, we used Nanopore technology to sequence eight *Pss* strains deposited at CREA-DC collection. At least 50x coverage for each sample was generated and an assembly was performed. Subsequently, the genome sequences were polished by sequence errors and phylogenetically compared to ~250 genomes of other *Pantoea* spp. deposited at NCBI assembly database. The analysis showed that all Italian strains grouped together with *Pss* strains from different world regions. Moreover, within the *Pss* clade, the Italian isolates grouped with strains isolated in USA from maize, suggesting that the Italian strains are probably imported by the USA. All together these results may suggest that the Italian isolates are imported by seeds trade.

### Detection of virulence factors and varietal sensitivity of *Pseudomonas kairouanensis* and *Pseudomonas nabeulensis*: new pathogenic species isolated from citrus black pit in Tunisia

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A collection of *Pseudomonas* strains was established from fruits and leaves of *Citrus sinensis* cv. 'Valencia Late' and *Citrus limon* cv. 'Eureka' plants with black pit disease from different regions of Tunisia in the period 2016-2017. A phylogenetic analysis of the housekeeping gene *rpoD* was used for strain identification at the species level. These

strains were analyzed morphologically and genotypically by multilocus sequence analyses of the *rpoD*, *gyrB* and 16S rRNA gene sequences, and their phenotypic characteristics by API 20NE and Biolog GEN III. Plant pathogenic properties were confirmed on fruits and detached leaves of *C. limon* cv. 'Eureka'. The genomes of both representatives were sequenced. A genetic screen of 32 type III effector genes was established to identify bacterial genes that are expressed during the infection process. The results revealed a big difference between *P. syringae*, *P. congelans*, *P. kairouanensis* and *P. nabeulensis* representing all citrus pathogenic species responsible of black pit isolated in Tunisia. All strains are able to produce exopolysaccharides (EPSs) and biofilm. The susceptibility of 20 citrus cultivars was evaluated toward six virulent strains on detached fruits. Our results revealed that citrus cultivars 'Eureka' 'Lunario' and 'Star Ruby' were the most susceptible to citrus black pit with a high severity value. However, 'Thompson Navel' 'Sweet Lime' 'Valencia Late' cultivars seemed to be less susceptible to black pit disease. The results showed that strains of *Pseudomonas syringae* are more virulent mainly on *C. limon* cultivars of which KB49 is the most pathogenic. *P. kairouanensis* is the most virulent on 'Star ruby' and 'Valencia late' cultivar was found the most susceptible to *P. nabeulensis*.

### Biochemical properties and metagenomic analysis of bacteria microbiome in soil with olive areas affected by olive quick decline syndrome (OQDS)

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Beginning in 2013, the problem known as the Olive Quick Decline Syndrome (OQDS) hit the olive trees of Salento, becoming the main problem for agriculture in Apulia region, with serious repercussions over all Italian olive growing (of which that Apulian represents around 40%). OQDS, which sees the presence in many trees of the bacterium *Xylella fastidiosa* subsp. *pauca* (strain ST53), shows an infection dynamics partly still unknown and differentiated by incidence even within homogeneous agricultural areas. Most researches on OQDS consider mainly the context of plants vs. pathogen and/or vector insects and few studies analyzed the information about the context of soil and microbial biodiversity in the olive areas affected by OQDS. The main goal of this study was to study the soil properties respect to the diseased olive trees, and characterize the microbial diversity in the context of OQDS. Two olive orchards have been identified in the infected zone with different degrees of OQDS symptomatology; one with severe symptoms of the disease, and the other with just few typical symptoms. Samples of soil (also at rhizosphere level) were collected by each olive orchards. The chemical and biochemical analyses of soil (i.e. total organic carbon, microbial respiration and microbial biomass) and the exploration of soil microbial communities by using the Next Generation Sequencing methods (NGS), were performed in both olive areas. Chemical data and biochemical activities, subjected to Principal Component Analysis (PCA), showed two clusters, one including the asymptomatic plants, and the other the diseased ones, respectively. Microbiome analysis highlighted differences of soil samples collected in the olive areas impacted by OQDS. Here, the *Rubrobacter* and *Gaiellaceae* spp. microbial populations decreased. Specifically, the *Gaiellaceae* were higher in the healthy olive areas showing a good index of mineralization activities. In these semi-arid contexts, a greater abundance of the genus *Bacillus* in the healthy plot was recorded but with species typical of soils in extreme habitats. Current results suggest that the occurrence of OQDS in Apulia might be also helped by a 'poor' soil.

## Genome analysis of strain S1Bt23, a novel subspecies of *Pseudomonas chlororaphis*, reveals an arsenal of antimicrobial gene clusters

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The use of chemical pesticides including antibiotics in plant agriculture has led to antimicrobial resistance with significant potential risks to the environment and public health. Bacterial pesticides are emerging as reliable alternatives. Strains of *Pseudomonas chlororaphis* are reported to be excellent candidates because of their ability to produce antimicrobial compounds. In 2015, a new strain, S1Bt23, was isolated and classified as *Pseudomonas chlororaphis* by multilocus sequence analysis (16S rRNA, *recA*, *gyrB*, *rpoB* and *rpoD*). In preliminary dual culture assays, strain S1Bt23 demonstrated potent *in vitro* antagonistic effects against *Ralstonia solanacearum* and six fungal pathogens. We sequenced, assembled and analyzed the genome of strain S1Bt23 to confirm its taxonomic status and to gain insight of the antimicrobial gene clusters that might be fueling its potent antagonistic activities. Genome-based taxonomic study using *in silico* DNA-DNA hybridization and average nucleotide identity suggests that strain S1Bt23 could be a novel subspecies within *P. chlororaphis*. Genome analysis of strain S1Bt23 revealed an arsenal of antimicrobial gene clusters including phenazines and pyrrolnitrin. The phenazine cluster of approximately 10 kb in size has all the seven genes (*phzA-G*) required for biosynthesis of phenazine-1-carboxylic acid (PCA). A pyrrolnitrin operon, 5.8 kb in size, was identified comprising of the four expected genes (*prnA-D*). Culture extracts of strain S1Bt23 analyzed using thin layer chromatography and High Pressure Liquid Chromatography showed that S1Bt23 secretes phenazine-1-carboxylic acid. In conclusion, strain S1Bt23, a potential novel subspecies, seems to possess the desired characteristics of a potent bacterial biopesticide. These results will be discussed.

## High clonality of strains of *Xylella fastidiosa* subsp. *pauca* from Italian outbreak assessed by tandem repeats analysis

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Italian olive groves in the Apulia region since 2013 are more and more severely threatened by *Xylella fastidiosa* subspecies *pauca*, the causal agent of the Olive Quick Decline Syndrome (OQDS), which represents one of the most severe example of invasive plant pathogen nowadays. This epidemic is attributed to a specific type of this subspecies, the ST53, according to the SNPs detected in the sequences of a pool of specific genes. Being this method quite labor and time consuming, it is not well suited for phytosanitary inspections. This research aimed to develop and test an alternative molecular tool able to steadily identify the type of the pathogen without going into sequencing procedures. The chosen approach refers to VNTR (Variable Number of Tandem Repeats) analysis, which has already been applied on other subspecies of *Xylella fastidiosa* on different hosts. Here, a set of 45 TR loci were identified in both literature and by new search in the genome of De Donno strain (GenBank accession No.

CP020870). They were amplified from the genomic DNAs of a pool of 30 strains isolated from different hosts, sites and time in Apulia. This genotyping basically confirms that the outbreak has a single clonal origin, even if sporadic traces of microevolution are present. The prospective practical use of the assay is discussed.

## Characterization of *Xanthomonas arboricola* pv. *corylina* causing bacterial blight of hazelnut in Oregon, USA

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Bacterial blight [*Xanthomonas arboricola* pv. *corylina* (*Xac*)] of hazelnut (*Corylus avellana* L.) was first reported in Oregon in 1913 and is now recognized as a damaging disease of young hazelnut trees worldwide. In the USA, 99 percent of the national hazelnut crop is grown in Oregon. With the release of hazelnut cultivars resistant to the fungal disease called eastern filbert blight (*Anisogramma anomala*), thousands of new acres of hazelnut are being planted in Oregon. An increase in bacterial blight has been noted on the young hazelnuts, especially in plants grown in marginal sites. Bacterial blight of hazelnut has not been studied in Oregon since the 1970's, so we characterized the pathogen. Bacterial isolates from symptomatic tissues were identified as *Xac* using morphological, biochemical, and molecular assays. Assays included growth on semi-selective media, nutrient utilization profiles, quinate metabolism, copper resistance, hypersensitive response (HR) on tobacco, and pathogenicity on hazelnut. Molecular assays included duplex PCR (*ftsX* and *qumA*), 16S rRNA sequence, and multilocus sequence analysis (MLSA) using *rpoD* and *gyrB*. Pathogenic *Xac* isolates separated into two clades with MLSA, one clade with the type strain and a second clade previously described with isolates from France and Poland. Thus, the phylogenetic diversity of *Xac* observed in other countries also is present in Oregon although there was no difference in pathogenicity between the clades. Future studies will evaluate sensitivity of the newly released OSU hazelnut cultivars to bacterial blight.

## Assessing the variability among *Pseudomonas savastanoi* pv. *savastanoi* strains on farm-scale using MLVA approach

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The endemic disease olive knot has been present in Italy since a long time. The causal agent of this disease is *Pseudomonas savastanoi* pv. *savastanoi*, part of *Pseudomonas syringae* complex. Different genotyping methods have been used to assess the genetic diversity and evolution of this pathogen. To further investigate these aspects, we have developed an MLVA assay based on the analysis of tandem repeat (TR) units at multiple loci of the bacterial genome, amplified by strict PCR protocols. The screening of the genome of *P. savastanoi* pv. *savastanoi* strain NCPPB 3335 (GenBank accession No. CP008742) revealed several

possible Tandem Repeat loci. After a thorough screening, a total number of 15 TRs were selected to investigate the genetic diversity among individuals, and corresponding primers were designed around the flanking region of the selected repeat units. Genomic DNAs from 16 strains isolated from different plants in an olive orchard in Viterbo, Italy, has been amplified in all the TR loci. Then capillary electrophoresis and automated computer analysis were used to obtain an appropriate and definite calculation of the individual numbers of the repeats per locus. The obtained haplotypes were investigated for their reciprocal genetic relationships. As a first assessment of this trial on farm-scale, an unexpectedly high variability among the isolated strain was observed.

### Analogues of a natural *Trichoderma longibrachiatum* peptaibol are ecofriendly molecules effective against the black rot pathogen *Xanthomonas campestris* pv. *campestris*

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Black rot is considered the most important and destructive disease of crucifers worldwide. It is caused by the gram-negative bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*), a seedborne pathogen attacking plants at any growth stage. The identification of new ecofriendly alternatives effective against *Xcc* would allow to reduce copper and chemicals currently used for controlling this microorganism. Fungi belonging to the genus *Trichoderma* are widespread and have been successfully used as biocontrol agents against many crop pathogens. Among the secondary metabolites secreted by *Trichoderma* species, peptaibols are a peculiar family of peptides known for their antimicrobial activity against gram-positive bacteria, yeast-like and filamentous fungi. Analogues of a natural *Trichoderma longibrachiatum* peptaibol, synthesized in our laboratories and previously characterized for their antifungal activity against grape and cereal pathogens, were assayed for antibacterial activity against *Xcc*. Some of the tested peptaibols showed the ability to inhibit bacterial growth. If confirmed for their efficacy at plant seedling stages, these molecules could represent a valuable resource to be used in protecting crucifers from black rot.

### Genome mining reveals a cyclic lipopeptide gene cluster of *Pseudomonas* sp. S58 is involved in induced resistance in *Nicotiana benthamiana*

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*Pseudomonas* strain S58 was isolated from tobacco rhizosphere in Yunnan Province, China. In *in vitro* assays, it showed noteworthy antagonistic activity against many plant pathogenic fungi and

bacteria except *Pseudomonas syringae*. However, strain S58 could elicit cell death-like immunity in *Nicotiana benthamiana* and control wild-fire disease caused by *P. syringae* pv. *tabaci* through induced resistance. The complete genome of strain S58 was sequenced and analyzed in large. None of type III secretion system (T3SS) related genes were found from the genomic sequence, which suggests the cell death phenotype triggered by strain S58 is not a T3SS-dependent non-host resistance. The pipelines of antiSMASH and PRISM were deployed to identify secondary metabolite biosynthesis gene clusters, and no polyketides but seven hypothetical cyclic lipopeptides (CLPs) were discovered from the genome. All of the seven CLP clusters were deleted individually and only the mutant of the fourth cluster lost the ability to trigger cell death. But the fourth mutant still retained antagonistic activity against to plant pathogens, which indicates that the fourth CLP is a unique plant resistance elicitor. The whole cluster four has a length of 79.6 kb including 39 open reading frames, which was predicted to encode a peptin-like CLP. The CLP elicitor is being isolated and characterized by matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) analysis of the cell-free culture filtrates from strain S58 and its mutant.

### Effects of cultivation conditions on *Bacillus amyloliquefaciens* activity against *Pectobacterium carotovorum* subsp. *brasiliense*

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In 2018, *Pectobacterium carotovorum* subsp. *brasiliense* (PCB) was detected at several locations in Serbia causing blackleg and soft rot of potato which resulted in severe losses on potato fields. An isolate of PCB obtained from potato grown on these fields was used in this study and antimicrobial activity of *Bacillus amyloliquefaciens* (BA), cultivated under different conditions, was tested against the isolate. BA was cultivated in flasks in media containing different carbon sources for 96 h. As glycerol proved to be one of the most suitable sources of carbon, ensuring high antibacterial activity of BA against PCB, in both tested forms - as cultivation liquid containing BA cells and cell-free supernatant, further studies were conducted in lab-scale bioreactor in a medium containing glycerol as carbon source. BA cultivation liquid produced in bioreactor was tested for antibacterial activity, every 12 h, in three forms: cultivation liquid containing cells, cell free supernatant and pure biomass suspended in sterile saline, set to the same concentration as in cultivation liquid. The results showed that increase in cultivation duration increases antibacterial activity of cultivation liquid containing cells, as well as of biomass saline solution, being the highest after 96 h of cultivation. No antibacterial activity was registered for cell free supernatant, regardless cultivation duration. Results suggest that in scale-up production process of BA, antibacterial extracellular metabolites production was reduced, probably due to different aeration and mixing conditions compared to spontaneous aeration and mixing in flask production. However, cells developed mechanisms to keep and even significantly improve antibacterial properties against PCB.

## Evaluation of six medicinal plant extracts against *Acidovorax citrulli* causing bacterial blotch of watermelon

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Antibacterial activities of six medicinal plants were tested against *Acidovorax citrulli*, the bacterial blotch pathogen of watermelon (*Citrullus lanatus*). Isolation of the pathogen was made from infected fruits on Nutrient Agar (NA) and pathogenicity was carried out on healthy watermelon fruits. The extracts from the leaves of these plants (*Entada africana*, *Vitex doniana*, *Lawsonia inermis*, *Azadirachita indica*, *Acalypha hispida* and *Nauclea latifolia*) were prepared using ethanol, cold and hot water. Phytochemicals analysis of each extract was done using standard methods. Antimicrobial activities of each extract was evaluated at 50 mg/ml using agar well diffusion techniques with bacterial inoculum of  $2 \times 10^6$  cfu/ml. Control experiments were set up using sterile water as negative control and Gentamicin (10 µg/ml) as positive control. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by a modification of the agar well dilution method. The results on phytochemical analysis revealed the presence of either one or more of the alkaloid, saponin, tannin, proanthocyanin, steroid and flavonoid in the six plants evaluated. Alkaloids, tannins and flavonoids were found in all the plant extracts. The six plants exhibited varying degrees of antibacterial activity against the test organism. Ethanol extracts of *Azadirachita indica* and *Vitex doniana* gave the highest inhibition of 81% and 73% respectively against the pathogen. The ethanolic extracts of *Lawsonia inermis*, *Azadirachita indica* and *Nauclea latifolia* showed a broad spectrum of activity with MIC of 1.5 mg/ml against the tested pathogen. Therefore, field trial of these medicinal plants against *Acidovorax citrulli* is recommended.

## Integrating copper and a microbial consortium for an effective control strategy against the bacterial blight of walnut

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The bacterial blight of walnut is a disease that may cause fruit losses up to 60% during epidemic years. The causal agent is *Xanthomonas arboricola* pv. *juglandis*, a Gram-negative organism, divided into several, different genetic populations. The most important trait of such organism is its ability to detoxify copper, even at high concentration, making its control in walnut orchards quite cumbersome. Experiments have been done in three commercial orchards during three years in Romagna (Italy), to implement an orchard management strategy able to reduce the copper input by using a consortium of microbial biocontrol agents as well. The product chosen was MICOSAT F<sup>®</sup>, composed by several beneficial microorganisms, such as *Agrobacterium radiobacter*, *Bacillus subtilis*, *Streptomyces* spp., which previously confirmed their effective antagonism *in vitro* against the pathogen. MICOSAT F<sup>®</sup> was used in its powdery form both as root inoculant and as

suspension sprayed on the canopy, from the phenological phase called “prayer’s stage” to the endocarp hardening (a total of six sprays, bi-weekly). A field comparison was made with the conventional integrated control, which is based on several copper+mancozeb treatments per season, copper sprays alone and not treated plots. Results highlighted the excellent performance of the microbial consortium, able to reduce the disease by approximately 85%, and increasing fruit production by approximately 45% (both when compared with the untreated plots). Therefore, the copper+mancozeb sprays in autumn, to reduce the overwintering bacterial populations, may be excellently integrated with spring-summer sprays using a prospective microbial consortium to trigger plant’s defence mechanisms.

## Set up of a collection of tomato root-associated bacteria for the biological control of bacterial diseases

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Tomato is subject to several bacterial diseases that affect both field- and greenhouse-grown crops. To select cost-effective potential biocontrol agents, we used laboratory throughput screening to identify bacterial strains with versatile characteristics suitable for multipurpose uses in tomato cultivation. Approximately 400 bacterial strains were selected on different culture media during a population study on tomato-root associated bacteria. Bacterial strains were stored long-term in Nutrient broth + 30% glycerol in 96-wells, and subcultures were obtained by replica plating. Strains were analysed for antagonistic activity against strains of representative tomato pathogens, namely the Gram-positive bacterium *Clavibacter michiganensis* pv. *michiganensis* (*Cmm*), and the Gram-negative *Pseudomonas syringae* pv. *tomato*, *P. corrugata* (*Pco*) and *Xanthomonas euvesicatoria* pv. *perforans*. The antagonistic bacteria were also tested for antagonistic activity against *Fusarium oxysporum* ff.ssp., as well as other traits such as the production of siderophores, solubilisation of phosphates, and tolerance to NaCl. The highest and lowest number of antagonistic bacteria were detected against *Cmm* (88%) and *Pco* (40%). Approximately 30% (129 strains) of the root-associated bacteria showed antagonistic activity against all tested bacterial strains: 42%, 26% and 31% from rhizosphere ecto- and endo-rhizosphere compartments, respectively. All but three isolates also showed antifungal activity. Most of the bacterial isolates were Gram-negative regardless of the compartment of isolation (average 75%). Twenty-three bacterial isolates produced siderophores, and were able to solubilise phosphates and grow on NA with 8% NaCl. Seven of them isolated from the endosphere based on 16S rDNA sequencing belong to the orders *Bacillales*, *Enterobacteriales* and *Pseudomonadales*.

## Delivery of antimicrobial peptides from wild plants into solanaceous cultivars using different chitosan nanoparticles

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Nanoparticles (NPs) have a number of unique properties associated with their ultra small size and applied delivery of polypeptides, RNA and DNA of various sizes into the plant cells. Our data demonstrate a potential of delivery of antimicrobial peptide biomolecules that have been previously isolated from wild species and structure-functional characterized into above-ground organs (in particular, leaves) using various types chitosan NPs and methods of low (ultra low)-volume spraying or infiltration. The data demonstrate physical characteristics of NPs coated with recombinant antimicrobial peptides (AMPs) that generated by heterologous expression in prokaryotic expression system. Three molecules possess different molecular mechanisms of action (with membrane-active, associated with microbial cell wall or intercellular localized) toward main economically important diseases of Solanaceae caused by filamentous fungi, oomycetes and bacteria. AMP from wild plants loaded into chitosan nanoparticles (CS-NPs) could increase peptide's antimicrobial (preliminary antifungal) activity, while reducing its phytotoxic potential. Additionally, the nanocarrier evidenced a sustained antimicrobial action against various plant pathogens compared to plain CS-NPs. The antimicrobial evaluation tests demonstrated that the gradual release of AMPs reduced the plant damage and ensuring a long-lasting antimicrobial effect. The developed nanocarrier is eligible for the administration of several AMPs with antiviral, antifungal and antibacterial effect.

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### A novel *Pseudomonas fluorescens* strain as a versatile measure against different bacterial plant diseases

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In this study, the efficacy of the strain BB20 of *P. fluorescens* (Pf) bv. 1 (Patent No. PCT/IB2012/050694; European Application No./Patent No. 12706932.6 – 1401) was *in vitro* and *in vivo* evaluated against several bacterial pathogens, agents of disease of different plant species. Moreover, preliminary solid and liquid formulations of the antagonist were firstly assayed for their time stability when stored at 4 °C and assayed against bacterial pathogens in *in vivo* experiments. In *in vitro* experiments BB20 inhibited the growth of several bacterial pathogen species by producing a peptidic antibacterial compound. Moreover, the antagonist suspension was able to prevent infections of *Erwinia amylovora* (Ea), *Xanthomonas vesicatoria* (Xv) and *X. arboricola* pv. *pruni* (Xap) by providing a relative protection of 62%, 64% and 58% when applied on pear, tomato and peach plants, respectively, under greenhouse and outdoor conditions. The solid and liquid formulations resulted stable and maintained the initial concentration for 6 months at 4°C. The *in vivo* efficacy of both solid and liquid formulations was comparable to that of fresh bacterial suspensions and resulted able to significantly reduce the fire blight incidence on pear plantlets and to statistically decrease the bacterial leaf spot severity on tomato plants and peach one-year-scions. The *in vitro* efficacy against a broad spectrum of bacterial pathogen species and the ability to prevent bacterial plant infections under controlled and field conditions pose the strain BB20 as a versatile tool in the frame of integrated control measures.

### *Bacillus* sp. strains, an effective tool to face out the bacterial canker of kiwifruit

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In the last decade, bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), provoked heavy economical losses since its pandemic spread in 2008. The control of disease is achieved through integrated methods, by combining appropriate cultural practices and chemical treatments as cupric salts/antibiotics, and biocontrol agents (BCA). In this study, two strains QST713 and D747 of *Bacillus* sp., active principle of commercial biofungicides, were investigated for their ability to reduce the *in vitro* Psa growth. Both bacterial strains were assayed *in planta* for their capacity in directly reducing the severity of bacterial canker on green- and yellow-fleshed kiwifruit plants under greenhouse and open field conditions. Moreover, D747 strain was studied for its ability to induce resistance against Psa in yellow-fleshed kiwifruit plants after its application at the roots by irrigation with a suspension of the antagonist. In *in vitro* experiments the strains QST713 and D747 resulted similar in reducing Psa growth. Under greenhouse conditions, when applied on kiwifruit plants 48 h before the Psa inoculation, only the strain D747 resulted able to significantly reduce the disease severity, and in open field it prevented the natural Psa infection and lowered the disease severity. D747 was also able to reduce the disease severity by inducing plant resistance after its application at the roots. These are the first results which concern the ability of a BCA in directly reducing the pathogen growth and its disease severity, as well as its indirect capacity to induce resistance in *Actinidia* spp. plants towards Psa.

### Antagonistic soil-borne *Bacillus* spp. and *Pseudomonas* spp. bacteria against late blight and other potato pathogens: from laboratory to field

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Potato crop is facing, worldwide, pests and diseases. Biocontrol solutions as alternative plant protection measures to the use of chemically based pesticides are needed. With this idea, up to 2,800 *Bacillus* spp. and *Pseudomonas* spp. strains were isolated from composts, manure, potato plants, and soils, targeted for their localisation in Belgium. Their antagonistic activities were assessed *in vitro* against pathogens like *Alternaria solani*, *Fusarium solani*, *Pectobacterium carotovorum*, *Phytophthora infestans* and *Rhizoctonia solani*, five potato pathogens. Activities have also been detected against *Streptomyces scabies*, causal agent of common scab of potato. Following this preliminary screening, 52 *Bacillus* spp. and eight *Pseudomonas* spp. with major *in vitro* inhibition, especially against

*P. infestans*, were selected for further characterized. The bacterial isolates were then identified, based on their 16S rRNA gene sequence. They were also genetically characterized for their production of potential bioactive secondary metabolites. The metabolites such as bacilysin, bio-surfactants and siderophores were identified as possible drivers of biocontrol activities. The *in vivo* antagonistic effect against potato late blight was evaluated under glasshouse conditions, for the further selection of eleven strains of interest. Such bioassays were followed by a pilot field trial which highlighted one *Bacillus subtilis* able to decrease significantly late blight severity throughout the crop season. The question of the impact of the large-scale application of such biocontrol approach will be discussed. Overall, this study revealed the potential of using antagonistic indigenous soil bacteria as alternative biocontrol measures to the indiscriminate use of pesticides in potato agro-systems.

### Isolation and screening of beneficial bacteria associated to ancient tetraploid wheat landraces

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Wheat is one of the most important crops in the agro-food sector and tetraploid wheat production has an important role in Italy where is cultivated mainly in the southern regions, including Sicily. Recently, there was a rising interest in the valorization of local ancient wheat landraces as an important source of genetic and microbial diversity. The influence of the plant microbiota on plant physiology and health condition has become particularly important. In particular, plant growth-promoting rhizobacteria (PGPR) are widely studied for their ability of promoting plant growth through several mechanisms, including increasing soil nutrient availability and biocontrol activities. Furthermore, the study of endophytes could be of particular interest for their ability of establishing a mutualistic interaction with the plant. Recently, technologies based on spectral data acquisition, including proximal and remote sensing, are acquiring great importance in agriculture for the possibility of measuring parameters associated to plant physiology and health through hyperspectral vegetation indices (HVI). Therefore, a multidisciplinary approach was used to isolate and select a group of potential PGPRs from two ancient field-grown tetraploid wheat landraces, Perciasacchi and Tumminia, based on their *in vitro* activities and 16S ribosomal sequence data. In addition, a set of HVIs related to plant growth and the presence of plant growth promoting activities was identified. The effects of the selected PGPR isolates on plant growth were also evaluated *in vivo* on modern and ancient wheat varieties in a controlled environment allowing to identify one strain showing significant effects on wheat ear biomass.

### Evaluation of antibacterial products for the control of *Xanthomonas euvesicatoria* pv. *perforans* in organic tomato farming

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Control of bacterial plant pathogens in tomato organic cultivation still relies on the use of copper compounds. The demonstrated noxious effect of this heavy metal on soil microbial communities has led to regulatory restrictions of its use in the EU, now permitted at a maximum rate of 4 kg/ha/yr. Taking into account future restrictions in its use and possible emergence of copper-tolerant strains due to its continuous use, research is necessary to screen alternative products able to reduce or gradually phasing out copper in organic farming. In this work the efficacy fertilizers, resistant inducers with low copper content, essential oils and biocontrol agents was evaluated for their inhibitory efficacy against *Xanthomonas euvesicatoria* pv. *perforans*, recently reported in Sicily in tomato greenhouses as agent of bacterial leaf spot and pith necrosis. The antibacterial activity was evaluated *in vitro*, showing to be proportionally decreasing according to the reduced concentration applied in each treatment. The *in vivo* tests were carried out with tomato plants cv. Sir Elyan, first treated with the products which showed *in vitro* the best activity, and after 24 h inoculated with the pathogen. Among them, two fertilizers/resistance inducers with low copper content showed excellent results after 20 days of incubation, comparable with copper oxychloride and copper hydroxide used as control, and were able to reduce disease incidence and disease severity in tomato leaves as well as pith necrosis in tomato stems. This study highlights the possibility to replace or reduce the commonly used copper compounds to control *X. euvesicatoria* pv. *perforans* in organic tomato farming.

### *Lactobacillus plantarum* strains as biological control agents of bacterial plant diseases. Improvement of their fitness

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Fire blight of apple and pear, bacterial canker of kiwifruit, bacterial spot of stone fruits, and angular leaf spot of strawberry are plant diseases responsible for important crop losses worldwide. There is the necessity to develop novel efficacy and sustainable management tools. Lactic acid bacteria (LAB) are good candidates as microbial biopesticides. They show broad-spectrum antagonism against several phytopathogenic bacteria and fungi due to different mechanisms of action. In addition, LAB are ubiquitous members of many plant microbiomes and are generally considered as safe by food safety agencies. Five LAB strains were selected for showing broad-spectrum antagonism *in vitro*. Particularly, *Lactobacillus plantarum* PM411 and TC92 prevented infections of *Erwinia amylovora*, *Pseudomonas syringae* pv. *actinidiae*, *Xanthomonas arboricola* pv. *pruni*, and *Xanthomonas fragariae*. The biocontrol performance of both strains was comparable to reference products in the semi-field and field experiments performed. However, the fitness of *L. plantarum* on the plant surface is limited under unfavourable low relative humidity (RH) conditions. Consequently, we physiologically improved the fitness of PM411 and TC92 by means of increasing their tolerance to desiccation stress and low relative humidity conditions. We used a procedure consisting of the adaptation of cells to stressing conditions during inoculum preparation. The combined-stress adaptation treatment in PM411 provided a better survival under harsh conditions in the aerial plant surfaces. This beneficial effect is related to a more consistency in bacterial disease suppression.

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## Development of new delivery systems based on derivatized cellulose nanocrystals

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The emerging effects of global warming has prioritized the development of a new circular and green economy based on sustainable natural resources. Renewable materials, such as cellulose, can be easily extracted from natural wastes in the form of cellulose nanocrystals (CNC). CNC have been proposed as carriers for active substances to be employed in agriculture and human health. The work aimed at studying CNC functionalization using two different methods to produce derivatized CNC (dCNC) for active compounds delivery in agrifood applications. CNC were produced from MCC by sulfuric acid hydrolysis. Green methods were chosen to label amino groups and  $\beta$ -cyclodextrins ( $\beta$ -CD) on CNC. Surface functionalization was determined by FT-IR, zeta potential and conductivity tests, and UV-vis spectroscopy. Ferulic acid, caffeic acid, and quercetin were loaded in dCNC and spray-dried to form microparticle (MP) dry powders. MP morphology and *in vitro* release were investigated by scanning electron microscopy (SEM-EDX) and UV/Vis spectroscopy. Cellulose nanocrystals were successfully derivatized by adding amine and  $\beta$ -CD centers with a derivatization yield of about 0.75%. The obtained CNC-NH<sub>2</sub> entrapped successfully quercetin but to a lower extent ferulic and caffeic acid. The obtained MP showed a different behavior and morphology of  $\beta$ -CD-CNC and CNC-NH<sub>2</sub>. An almost zero order sustained release of the active molecules was recorded. The characteristics of the obtained dCNC seem to support their potential as versatile carrier systems for the controlled release of active molecules. The choice of active molecules to be delivered should account for their physicochemical properties and chemical nature.

## Screening of natural active compounds as an eco-sustainable weapon against different bacterial and fungal plant pathogens

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Bacteria and fungi represent a wide range of plant pathogens, affecting several agronomic crops. Nowadays, bacterial pathogens are mainly contrasted by prevention, adequate agronomic practices and use of copper salts. On the other hand, a wide range of synthetic chemical substances are available to control fungal pathogen, in particular molecules belonging to inhibitors of ergosterol and strobilurins. Since European Community is restricting the use of copper salts and synthetic pesticides are affecting soil, human, animal and environmental safety, it results urgent to formulate new eco-friendly methodologies contrasting plant pathogens. In the present study, we tested several natural molecules (gallic, ellagic, caffeic acids, chitosan hydrochloride, esculetin, oleuropein and lignin) in a range of different concentrations (0.01% - 2%) against several bacterial (*Xanthomonas campestris* pv. *campestris*, *X. axonopodis* pv. *vesicatoria*, *Pseudomonas syringae* pv. *actinidiae*, *P. syringae* pv.

*tomato*, *P. savastanoi* pv. *savastanoi*, *Agrobacterium thumefaciens*, *Erwinia carotovora* and *Pectobacterium carotovorum* subsp. *odoriferum*) and fungal (*Fusarium graminearum* and *Verticillium dahliae*) plant pathogens. The *in vitro* assays were performed in broth medium using 96 microtiter plates: bacterial colonies ( $1 \times 10^6$  cfu/ml) and fungal spores ( $1 \times 10^5$  s/ml) were treated with natural compounds at known concentration and then transferred to the medium broth. Cells vitality was evaluated measuring the OD<sub>600</sub> and OD<sub>450</sub> for bacteria and fungi, respectively. Results were expressed as percentage of growth inhibition, comparing the values with ones from mock control. Among the different natural molecules tested, chitosan hydrochloride (0.1 – 0.5%) and lignin (1%) resulted able to better contrast both bacteria and fungi showing a growth inhibition of 90% and 70 – 80%, respectively.

## Characterization of the *Xanthomonas euvesicatoria* specific bacteriophage KΦ1 and its potential in control of pepper bacterial spot

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*Xanthomonas euvesicatoria* phage KΦ1, a member of *Myoviridae* family, was isolated from the rhizosphere of pepper plants showing symptoms of bacterial spot. The phage strain expressed antibacterial activity to all *X. euvesicatoria* strains tested and did not lyse other *Xanthomonas* spp., nor other less related bacterial species. The genome of KΦ1 is double-stranded DNA of 46,077 bp including 66 open reading frames and an average GC content of 62.9%. Forty-four (67%) predicted KΦ1 proteins shared homology with *Xanthomonas* phage OP2, while 20 genes (30%) were unique to KΦ1. The strain KΦ1 is resistant to chloroform, stable in different media and buffers, sustain at pH 5-11, and can be stored at 4°C at least two years without decreasing of titer. Copper compounds reduced the phage vitality proportionally to the used bactericide concentration. UV light was detrimental to the phage, but skim milk plus sucrose formulation extended its survival. Results of three repeated experiments showed that foliar applications of the unformulated KΦ1 phage suspension effectively controlled pepper bacterial spot compared to the standard treatment and the untreated control.

## Efficacy of novel *Podoviridae* phage as biocontrol agent against *Pectobacterium atrosepticum*

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A new virulent *Pectobacterium atrosepticum* phage that belongs to the family *Podoviridae* was isolated from Egyptian soil, and its morphological and epidemiological characteristics were studied. The phage was

isolated and purified following single plaque isolate approach, then propagated in bacterial cell suspension inoculated by the optimal multiplicity of infection (MOI) of phage. The phage produced a plaque size of 2.0–4.0 mm in diameter. Morphological examination by electron microscope showed that phage has icosahedral head of 60 nm in diameter and a short tail of 15 nm in length, belongs to the family *Podoviridae*, order *Caudovirales*. The molecular weight of the major head protein is approximately 38 kDa, detected by SDS–PAGE. The optimal MOI value of the phage was 0.1 based on the average count of plaque forming units (PFU). Single-step growth curve indicated that the latent period of infection was 60 min, while the complete cycle of infection was 180 min. The phage has an average burst size of ~27 virions per bacterial cell. The specificity of the phage was tested against 15 isolates; two *P. atrosepticum* target isolates and 13 non-target bacterial isolates including other potato pathogens and antagonistic bacteria. The *in vitro* experiment showed that the phage was able to retain activity at temperatures up to 60 °C but lost at 70 °C or higher. The phage showed optimal activity at pH 7, while lytic stability was maintained at pH range 5–9.

### Effect of some aqueous plant extracts antibacterial activity on tomato bacterial leaf spot disease

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One of the most important tomato bacterial diseases is bacterial leaf spot caused by *Xanthomonas vesicatoria* (Xv). In this study, the antibacterial effect of 29 different medical and aromatic plants extract on the disease were investigated in *in vitro* and *in vivo* conditions. Among the aqueous plants extracts, *Allium sativum* and *Eucalyptus camaldulensis* extracts were effective on the pathogen. *Allium sativum* extract inhibited Xv growth with the mean inhibition zone of 20.5 mm in diameter. Minimum Inhibition Concentrations (MIC) value of *Allium sativum* was 3 µl/ml for the pathogen. In seed experiments, effect of the aqueous plants extracts as seed treatments on disease incidence, disease severity and seed germination were recorded. Disease severity was evaluated in cotyledon leaves three weeks after germination, using a 0–3 scale, taking as a reference the necrotic symptoms developed by positive control seedlings. As a result of *in vivo* experiments, *Allium sativum* and *Eucalyptus camaldulensis* aqueous plant extracts inhibited the disease incidence and severity by 100% and 96.7–98.0%, respectively. Seed treatments did not reduce seed germinations. The results of this study indicate *Allium sativum* and *Eucalyptus camaldulensis* aqueous extracts as promising in biological seed treatments, and could be used for the control of bacterial spot disease of tomato. In general, plant extracts are promising for the development of new pesticides against bacterial diseases of tomato.

### Antimicrobial activity of natural organic products and potential use against phytopathogenic bacteria

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Phytopathogenic bacteria are responsible for a large number of diseases, some of which are devastating on various vegetal crops. Control strategies against these pathogens are mainly based on the adoption of preventive measures as use of healthy propagation material and application of copper-based agrochemicals. However, such products, having no endotherapeutic action, are ineffective against vascular wilts caused by xylem colonizing bacteria. Furthermore, copper products are increasingly limited by legislation because of their environmental and toxicological risks. For these reasons, there is a need of new environmental-safe bioactive compounds as alternative tools to control phytopathogenic bacteria. Under this scenario, in the present research we evaluated the antibacterial activity of new organic compounds against common phytopathogenic bacteria. The results of experiments conducted *in vitro* evidenced an interesting dose-dependent and broad-spectrum inhibitory activity of some vegetal extracts against *Pectobacterium carotovorum* subsp. *carotovorum*, *Pseudomonas avellanae*, *Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *pelargonii*, and *Xylella fastidiosa* subsp. *pauca*. In *in vivo* assays, two selected products (AG and BioFlav) were tested by soil and/or foliage applications at different concentration. These products were compatible with tomato, pepper, olive and ornamentals crops and also evidenced biostimulant effect on the plant. On pot grown tomato plants artificially inoculated with *P. syringae* pv. *tomato*, AG significantly reduced tomato speck symptoms. Results are discussed in relation to the possible mechanisms of action involved in AG and BioFlav activity as well as to the potential for their use to prevent bacterial diseases in organic and integrated crop systems.

### Isolation of active bacteriophages against phytopathogenic bacteria *Erwinia carotovora* and their study for bio-control purposes

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Phytopathogenic bacteria of *Erwinia* type damage extensively strategic agricultural crops - potatoes. In the arsenal of the fight against potato bacteria, biological means have a small place. Different types of agrotechnical, physical or chemical methods, which are currently used, require significant expenditures of labor resources; they often cause environmental pollution and threaten human health. Therefore, in developed countries, to fight the pests the advantage is given to biological methods which are based on bacterial antagonists, entomopathogenic bacteria and viruses, specific enzymes, phytoncides, pheromones and other biologically active substances. Fighting against plant bacterial diseases through specific bacteriophages and their complex mixtures is also quite promising. The aim of the research was to identify and characterize specific bacteriophages against the potato bacterial pathogens, as well as conduct their experimental study as the biological means to combat bacteriosis. Two active phages (Pp1 and Pp2, which belonged to *Myoviridae* family) of bacteria *Erwinia* sp. were selected from the separated bacteriophages on the basis of the stability range and high indicator of lysis spectrum. To test antibacterial effectiveness of the isolated bacteriophages *in vitro* tests in liquid/fluid area and model experiments were conducted. In the tuber discs simultaneous inoculation of phytopathogenic bacterial strains and corresponding phages (or mixture of phages) was done by modifying infectious doses and phage titer. The best result was recorded using phage suspension three times over the standard dose of the pathogen. The average rate of disease development was 30% and 35%

respectively in the individual phage Pp1 and Pp2 groups after 96 h. Therapeutic effectiveness has increased significantly while using combination of bacteriophages: the disease development rate has been reduced three times than in the control group. The obtained results serve as a basis for the use of specific bacteriophages and their active mixtures against *Erwinia carotovora* in field tests at the next stage to avoid the emergence of bacterial rot and spread caused by *E. carotovora* in potato storage.

### Spraying of *Pseudomonas* supernatants controls *Xanthomonas axonopodis* pv. *phaseoli*

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The potential of *Pseudomonas* isolates (PI) DFs513 and DFs831 to control the common bacterial blight has been demonstrated through seed microbiolization. However, the behavior of these isolates when applied on leaves is still little explored. The objective of this study was to evaluate the effect of the application of PI supernatants on the biocontrol of *Xanthomonas axonopodis* pv. *phaseoli*. For this, different bacterial treatments were sprayed on the leaves in the following modalities: alive, dead, alive+chitin, supernatant and supernatant+chitin. Control treatments were water and liquid medium. The pathogen was inoculated by scissors (10 cuts per trifoliate leaf). At the first symptoms the incidence and severity were analyzed every two days (five evaluations to calculate AUDPC). ANOVA and mean comparison (Duncan 5%) were used to analyze the data. The experiment was set up in DIC with six replicates. It was observed that when DFs513 was sprayed, only the supernatant and alive+chitin, and all treatments derived from DFs831 reduced the disease severity in relation to both controls and their efficacy were at the same level of control when chitin was used to induce resistance. In the evaluation of the incidence, it was observed that for DFs513 only the application of the supernatant provided significantly lower averages than the control, for DFs831, the application of the living bacteria, supernatant and the alive+chitin, differed from the control. These results confirm the potential to control common bacterial blight by DFs513 and DFs831 supernatants.

### Sensitivity of various *Xanthomonas campestris* pv. *campestris* strains to bacterial and fungal antagonists

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*Xanthomonas campestris* pv. *campestris* (*Xcc*) causing black rot of crucifers is a phytopathogen responsible for serious damage of vegetable crops. Bioprotection employing antagonistic microorganisms can be a promising approach to decrease the infections and reduce the economical losses. The aim was to compare the sensitivity of various *Xcc* strains to selected antagonistic bacteria and fungi. Growth inhibition was measured in dual cultures on Bushnell-Haas (BH) agar medium (pH 7.0) at  $Fe^{3+}$  concentrations of 8 and 200  $\mu M$ . Grown in liquid BH medium at 200  $\mu M$   $Fe^{3+}$ , the doubling times of the collection strains *Xcc* HRIW 3811, HRIW 1279A and HRIW 3871A were 1.1-1.3-fold compared to a strain *Xcc* SU that was isolated from a cabbage field. All *Xcc* strains were extremely sensitive to growth inhibition by *Bacillus amyloliquefaciens* at both  $Fe^{3+}$  concentrations. The sensitivity of *Xcc* to *Pseudomonas aeruginosa* PAO1, *P. putida* IBU, *P. fluorescens* Flu,

and *Pseudomonas* sp. DF1 strains ranged from medium- to no inhibition; the sensitivity was influenced by the concentration of  $Fe^{3+}$ . Inhibition by *Trichoderma harzianum* could not be evaluated in dual agar cultures due to much faster growth of *Xcc* compared to the fungus. The fungus was able to colonize the agar surface despite the presence of robust *Xcc* biofilms colonizing the agar surface before the fungus. The analyses of the metabolomic profile of the dual culture *B. amyloliquefaciens/Xcc* SU are ongoing.

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### Essential oils and plant extracts with *in vitro* inhibitory capacities on *Pseudomonas syringae* pv. *actinidiae*, the causal agent of kiwifruit bacterial canker

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Bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* (*Psa*) has been defined as a real pandemic causing severe losses on yellow (*Actinidia chinensis*) and green (*A. deliciosa*) kiwifruit. Control strategies have shown the potential to slow down disease progress, by decreasing the number of successful infection events and reducing the bacterium colonization. For this purpose, the identification of novel compounds, that can contribute to the integrated control of bacterial canker is a major objective. This study investigated the *in vitro* inhibitory capacity of thirty plant essential oils (EOs) and of several plant extracts (roots of *Hypericum perforatum* and *Polygonum cuspidatum*, root bark of *Morus nigra*, mesocarp of *Punica granatum*, seeds and pomace of *Vitis vinifera*). A screening of EOs with the strongest inhibitive capacities was performed by *in vitro* assay. Among them, eight were found to be particularly suitable. This latter and the plant extract, characterized by the presence of different classes of polyphenols, were evaluated for their inhibitory capacities by the standard broth microdilution method (in accordance with Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI M07-A9. 2012) and by calculating the MIC and MBC. EOs showing the strongest capacity to inhibit bacterial growth at the lowest concentrations were clove bud (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), cinnamon (*Cinnamomum zeylanicum*) and to a lesser extent, garlic (*Allium sativum*). About plant matrices extracts, the most promising results were obtained from the roots of *Polygonum cuspidatum* and from seeds of *Vitis vinifera*.

### New control strategies against *Erwinia amylovora* and *Monilinia laxa* - bacteriophages and nanocomposites derived from herb extracts

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*Erwinia amylovora* and *Monilinia laxa* are among the economically most significant plant pathogens infecting pome and stone fruits, respectively. Three *E. amylovora* specific bacteriophages belonging to the family *Myoviridae* were used in this study. They were isolated from *E. amylovora* infected trees grown in Hungary and their capability to elimination of *E. amylovora* was tested on apple flowers under *in vitro* conditions. Antimicrobial activities of different nanocomposites of three essential oils were tested *in vitro* and *in planta* against both pathogens. The plant essential oils used were extracted from oregano (*Origanum vulgare* L.), English thyme (*Thymus vulgaris* L.), and summer savoury (*Satureya hortensis* L.). Three preparations (NANOIL02, NANOIL11 and NANOIL12) tested differed in particle size and amount of active ingredients. The formulations' effect on pathogens was evaluated *in vitro* in shaken liquid cultures and poisoned agar plates, then based on the results of phytotoxicity tests, on detached apricot and apple flowers. Bacteriophages applied in combination reduced the infection index on apple flower by 16%. Among the nanocomposites tested, the best effect against *M. laxa* was conferred by formulation NANOIL11 at 1.0 mg/ml, since it reduced the infection index on flowers of sour cherry by 45%. The most effective preparation against *E. amylovora* was the NANOIL12, which contained 0.6 mg/ml of essential oil and was supplemented with 0.8 mg/ml zinc hydroxide. This treatment decreased the infection index on apple flowers by 44%.

### Isolation and mycelial growth of endophytic fungi from Thai lotus, Bua Pathum Chart (*Nelumbo nucifera*)

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The fungal endophytes have profound impacts on plant communities such as conferring abiotic and biotic stress tolerance, increasing biomass, plant growth and yield by increasing nutrient uptake or suppressing pathogen via antimicrobial activity. The purpose of this study was to isolate the endophytic fungi from fresh water plant (Bua Pathum Chart, *Nelumbo nucifera*) at Thailand, and to investigate their mycelial growth of fungi on solid agar. The endophytes were isolated by surface sterile method, morphology and mycelial growth was conducted by using potato dextrose agar (PDA). Nine part of plant were selected for isolation; root, seed, petal, vein, intervein, pollen, stolon, stem and receptacle, respectively. Six fungal strains were isolated, namely, ST 2.1, RE8.1, RE3.1, PT1.1, RT1.1, IT3.1. After cultivation, endophyte strain RT1.1 showed the highest mycelial growth with 8.83±0.28 cm in 14 days, while RE3.1 displayed the lowest mycelial growth with 4.27 ±0.37 cm in 21 days at 30°C. Further studies to identify fungal species and biological activity are required.

### Biological effectiveness of *Bacillus subtilis* on common bacterial blight on bean in *in vitro* and *in vivo* conditions

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Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) is an economically important seed borne bacterial disease of beans. Currently, management of this disease is a challenge due to the limited efficacy of control strategies, as the pathogen acquires resistance to antibiotics and fixed copper compounds. Therefore, development of eco-friendly alternative disease management strategies is essential. In this study, a first time *in-vitro* and *in-vivo* conditions experiment was conducted to assess the effectiveness of *B. subtilis* strain QST 713 against CBB in a susceptible dry bean cultivar in comparison with copper hydroxide (CH) and their mixes. A weekly three times foliar spray were applied for each treatment with three replications and repeated twice. There was a significant control of disease infection rate and disease incidence by all treatments as compared to the controlled experiment. The lowest values of percent severity index (PSI) (37.50%), disease incidence (35.07%) and area under the disease progress curve (AUDPC) (586.60%-days) were observed from plants that are treated by mixes of strain QST713 and CH followed by strain QST713 and CH alone. The experiment which was treated with mixes and strain QST 713 showed reduced infection rate by 51.21 and 40.36 %, and disease incidence by 53 and 52.25%, respectively. The lowest bacterial populations were also recorded in plants that had been treated by mixes ( $1.38 \times 10^5$  CFU g<sup>-1</sup> of fresh weight of leaves) and strain QST713 ( $2.84 \times 10^5$ ) compared to untreated experiment ( $1.49 \times 10^6$ ). The mix treatment reduced the bacterial population on the surface of bean leaves by far (90.72%) over control treatment. The foliar application of strain QST713 and mixes of strain QST713 and CH showed a good progress on some measured agronomic parameters by increasing plant height, primary branches per plant and leaves per plant as compared to control and monk plants. These results suggest that the application of the commercial bio-bactericides may have not the only potential for managing CBB alone or in combination with CH, but may also reduce the rate of CH application in sustainable CBB disease management. Molecular characterization of the plant defense related genes expression before and after application of these treatments upon bacterial challenge and the mechanism of synergetic effect and compatibility between strain QST 713 and CH should be further investigated.

### Biological control of plant bacterial pathogens by a beneficial *Streptomyces* sp.

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In the present study a set of beneficial microorganisms were evaluated for their *in vitro* antimicrobial activity on various phytopathogenic bacteria, like *Xanthomonas vesicatoria*, *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*), *Acidovorax citrulli* and *Ralstonia solanacearum*. A prospective antagonist, strain SA51 (*Streptomyces* sp.) was selected based on its remarkable *in vitro* antibacterial activity and was further studied under greenhouse conditions on tomato (*Solanum lycopersicum*) seedlings as a model plant against *X. vesicatoria*. From *in vitro* results, it was clearly evident that SA51 was very active against bacteria like *Cmm*, *Cms*, *R. solanacearum* and *X. vesicatoria* compared to other antagonists. Tomato plantlets were inoculated with SA51 at the roots and, thereafter, they were sprayed with *X. vesicatoria*. Protection against *X. vesicatoria* by the bacterized tomato plants was confirmed in the greenhouse: disease was reduced by approximately 96%. Additionally, plants bacterized by strain

SA51 showed significant plant growth, particularly in aerial parts as compared to un-bacterized controls. Finally, benefit was seen in inoculated healthy plants in terms of a significant increase in dry weight and length of roots and shoots, as compared to the uninoculated controls. A GFP mutant of strain SA51 was produced to study its endophytic colonisation in tomato plants: results confirmed that SA51 was able to efficiently colonise tomato endophytically, from the roots to the leaves. Field experiments confirmed the ability of strain SA51 to act as plant growth promoting agent: such promoting activity was also reflected into an increase of fruit production by approximately 7%.

### Biochar as carrier for bioinoculants for the control of *Pseudomonas syringae* pv. *tomato* populations on tomato plants

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Biochar is defined as charred organic matter, generated from waste biomass via pyrolysis, a carbonization process. Due to the evidence of beneficial properties on the soil, biochar has always been used worldwide as a soil improver and since 2015 it has been accepted as an amendment in Italy. In addition to the improving action on the soil, the activity towards plant diseases, in particular towards some phytopathogenic bacteria, is also well recognized. In the present work, we investigated the ability of an EURIFINS certified biochar obtained from olive plants pruning, to act as a carrier for plant beneficial bacteria and fungi and the direct activity for the control of the bacterium *Pseudomonas syringae* pv. *tomato* (PST), the causal agent of bacterial speck of tomato. The results of the *in vitro* tests show that biochar is an excellent matrix that supports colonization by bacterial (*Bacillus* spp.) and fungal (*Trichoderma* spp.) inoculants, ensuring both an uniform and localized distribution of plant beneficial microorganisms in the soil. The tests carried out *in vivo* on tomato plants, grown on substrates containing different combinations of biochar, beneficial microorganisms and peat, show that the biochar used mixed only with peat (from 1% to 10% dw/dw) did not control PST populations. A positive and significant effect in the containment of PST populations was instead demonstrated both in the theses where tomato plants were grown on a substrate formed by peat and biochar (10%) added with *Trichoderma* spp. or *Bacillus* spp. These results provide a basis for further investigations on new combinations of biochar and beneficial bacteria or fungi for the control of tomato diseases.

### Effects of *Bacillus subtilis* and *Glomus mossae* on tomato bacterial canker control

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*Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is a bacterial disease that occurs worldwide and causes severe reduction in fruit yield

and quality on tomato. As a biological control agent *Bacillus subtilis* is suitable for organic farming and its low toxicity enables application until the day of harvest. Mycorrhizal activity plays important roles in soil biology and soil chemistry. In the study, the effectiveness of biocontrol agent *Bacillus subtilis*, arbuscular mycorrhizal fungus *Glomus mossae* and copper compounds [copper sulfate (CS) and copper hydroxide (CH)] were compared on disease severity (%) on three popular tomato cultivars 'Newton', 'Orient' and 'Vuslat'. *G. mossea* was spread onto seed bed as 1000 spore kg<sup>-1</sup> soil before the planting and the bacterium was inoculated to two-week-old plants. After bacterial inoculations, *B. subtilis* strain QST 713 (1%) was applied to the plants as it will be two times before two days and later two days. Copper compounds, CS and CH, were sprayed to the plants in two times with one week interval after the bacterial inoculations. The plants were inoculated with bacterial suspensions (10<sup>8</sup> CFU ml<sup>-1</sup>) of the virulent *Cmm* isolate, Kcm27. To determining the severity of the diseases, 0-5 scale was used. According to the findings, the lowest disease severities were obtained by *B. subtilis* strain QST 713 (51-57%), followed by *G. mossea* (38-42%), CS (27-32%) and CH (22-30%) as compared with 76-83% of the streptomycin treatment. It is thought that beneficial microorganisms are biological and environmentally friendly preparations suitable for organic and sustainable agriculture, and they can be used as an effective factor within a disease control program.

### Biological control of *Pseudomonas syringae*, the causal agent of 'citrus blast' and citrus 'black pit' by epiphytic bacteria under field conditions

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Citrus fruits have an important place in the agricultural sector and are among the strategic products. Despite the use of several chemical products, including copper, citrus fruits continue to face some biotic problems such as bacterial infections. Citrus blast and black pit caused by the phytopathogen *Pseudomonas syringae*, have become more important in recent years. The spread of the disease can be reduced by other epiphytic bacteria able to minimize phyllosphere colonization and inhibit the parasitic phase. Therefore, a collection of epiphytic bacteria was isolated from fruits of *Citrus limon* cv. 'Eureka' and *Citrus sinensis* cv. 'Maltaise'. Strains were evaluated for their antagonistic activities *in vitro* (agar diffusion method) and *in vivo* (detached fruits and leaves). Five different non-phytopathogenic strains found to be antagonistic *in vitro* as well as *in vivo* were selected for field experiments conducted in July, September and December 2018. The last treatment was accompanied by superficial wounds at 100 *Citrus limon* 'Eureka' fruits to promote the black pit. Among this strains, *Bacillus velezensis* which provided the highest mean reduction in disease severity (85 %), followed by *Bacillus amyloliquefaciens* (79%) and *Pseudomonas grimonti* (29%) while the efficiency of copper was 57%. The PCRs detection of antibiotic biosynthesis genes confirmed the presence of genes for biosynthesis of fengycin, bacillomycin, bacilysin, surfactin and iturin A. The API-ZYM and API20NE tests showed also the detection of several enzymatic activities essential for antagonistic bacteria.

## Isolation and characterization of bacteriophages specific to *Acidovorax citrulli*

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Bacterial fruit blotch and seedling blight, caused by *Acidovorax citrulli*, is one of the most destructive diseases of melon and watermelon worldwide. Biological approach in the disease control might be a potential solution and substitute for available bactericides of poor efficacy. Therefore, we isolated twelve bacteriophage strains specific to *A. citrulli* from rhizosphere of watermelon plants showing symptoms of the disease. The strains were characterized based on host range, plaque and virion morphology, thermal point of inactivation, adsorption rate, one step growth curve, and RFLP analysis. All phages lysed 30 out of 32 tested *A. citrulli* strains isolated in Serbia, and did not lyse other less related species. They produced clear plaques on bacterial lawn of different *A. citrulli* strains after 24 h of incubation. Examination by transmission electron microscopy of three phage strains indicated that they belong to the order *Caudovirales*, family *Siphoviridae*. The thermal inactivation point of phages was 66 or 67 °C. They were sensitive to chloroform, stable in pH 5-9, but inactivated after 5-10 min exposure to UV. RFLP analysis using *EcoRI*, *BsmI* and *BamHI* enzymes did not show genetic differences among the tested phages.

## *In vitro* evaluation of the antibacterial activity of some essential oils and the antagonistic activity of Algerian *Trichoderma asperellum* isolates against three gram negative phytopathogenic bacteria

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The biocontrol of the three gram-negative phytopathogenic bacteria: *Erwinia amylovora*, *Pectobacterium carotovorum* and *Pseudomonas* sp. isolated from potato is based on the search for biomolecules with bactericidal potentialities and antagonistic isolates. The essential oils of five medicinal plants of *Pelargonium graveolens*, *Mentha pulegium*, *Mentha rotundifolia*, *Origanum glandulosum* and *Eugenia caryophyllus* extracted by hydrodistillation were tested *in vitro* using the aromagram technique at concentrations of 5%, 2.5%, 1.25% and 1%. The antagonism focused on the direct confrontation of 13 Algerian isolates of *Trichoderma asperellum* against the three studied phytopathogenic bacteria, but the antibacterial activity of the methanolic extracts of their culture filtrates was carried out according to the same study technique as that for essential oils. The results showed a variability in bacterial growth inhibition rates according to plant essential oils, their concentrations, antagonist isolates and extracts of their culture filtrates. This study confirmed the effectiveness of certain essential oils of plants and certain antagonists isolates of *Trichoderma asperellum* to integrate them in the biocontrol of the studied phytopathogenic bacteria.

## Study of *Erwinia* phage-host interactions through quantitative real-time PCR

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The goal of many biocontrol programs is the use of bacteriophage to target and repress the population of a specific bacterial pathogen. Regardless of the environment in which the treatment will be applied, the study of the interactions between the phage and its host is primarily performed using plaque assays, spot tests, and optical density measurements: all techniques which have remained vastly unchanged over the last 70 years. Using a combination of chloroform-based sampling, centrifugation, DNase treatment, and quantitative real-time PCR, the stage of the lytic phage lifecycle an individual *Erwinia* phage genome can be determined. Monitoring the rate of transition between these stages in a population then allows one to calculate adsorption rate, burst size, and the latent period of a phage-host combination within a single experiment. The characteristics of four different genera of *Erwinia* phage on their ideal hosts were determined using this technique. The *Ea214virus* and *Sp6virus* genera are able to adsorb to their hosts at a rate up to 6.6 times faster than *Ea92virus* and *Agrican357virus* while producing a comparable phage output over time suggesting they may make more effective phage-mediated biocontrol agents.

## Antibacterial *in vitro* activity of experimental and formulated essential oils towards *Erwinia amylovora*

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The control of *Erwinia amylovora* (*Ea*), causal agent of bacterial fire blight, is one of the major challenges in pome fruit production. Nowadays, the main control strategy of this disease is based on the use of integrated methods: the infections are mainly controlled through the use of chemical treatments, mainly based on heavy metals/antibiotic compounds and resistance inducers, and by biological control agents (BCA). Among BCA, antagonistic bacteria and natural compounds as essential oils/plant extracts were studied to find more eco-friendly control tools targeted to the control of *Ea* infections. In the present work, experimental and formulated essential oils were tested for their antimicrobial activity against *Ea* in *in vitro* experiments. The synergistic effect of the experimental oils was also evaluated by checkerboard assay, and the Fractional Inhibitory Concentration index was calculated; the synergistic effect of oil combinations was assayed by macro-dilution assay as well. Among the experimental oils tested, those of *Origanum compactum* and *Satureja montana*, carvacrol chemotyped (48.13% and 54.86%, respectively),

and *Thymus vulgaris*, thymol chemotyped (46.84%), showed antibacterial activity; the minimal inhibitory concentration (MIC) resulted 0.03% for all oils. VitiBioSap 458 Plus® and VitiBioSap ES Plus®, whose formulated oils mixture activity is enhanced by the presence of the MicroSap® component (Patent N° EP 3071039–28.09.2016), significantly reduced the *Ea* population after 24 h and resulted to be bactericidal at the concentrations 1% and 2%, respectively. Also Prevam Plus® (6%), an orange essential oil based compound, was shown to be able to inhibit the growth of *Ea* after 24 h.

## Analysis of *Erwinia amylovora* isolates from Korea

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Recently, the outbreak of fire blight disease caused by *Erwinia amylovora* has been resulting in serious economic loss in apple and pear production in Korea. The present study was carried out to analyze *E. amylovora* isolates obtained from 40 apple and pear farms where fire blight occurred in 2015 in Korea. For genetic analysis, the pattern of clustered regularly interspaced short palindromic repeats (CRISPR) and variable number of tandem repeats (VNTR) were analyzed. When 64 Korean *E. amylovora* isolates were compared with the known 122 isolates from 14 countries, all the Korean isolates were classified as the CRISPR genotype 2-22-38 which included four isolates from USA and Canada. When the nucleotide sequences of six loci in VNTR were analyzed, five sorts of genotypes were found in the 64 Korean *E. amylovora* isolates. Interestingly, among the five sorts of genotypes, the genotype 2 agreed with two isolates from USA and Canada which belong to CRISPR genotype 2-22-38. Further analysis of the five sorts of genotypes using 20 different nutrient substrates showed that there was no apparent difference among them in the use of substrate utilization. In the disc-diffusion assay of antibiotics, all of them showed sensitivity to streptomycin and no difference was found in the degree of sensitivity to the antibiotics among the five sorts of genotypes. The present analytical information on the *E. amylovora* isolates would be applied to the development of control strategies for fire blight disease in Korea.

## *Erwinia horticola* and its two temperate related bacteriophages 49 and 59

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*Erwinia horticola* isolated from beech trees with fire blight symptoms was identified as the causative agent of beech black bacteriosis in Ukraine. The recent data of 16S rRNA gene

sequencing indicated 98–99% similarity with typical strains of *E. amylovora* and *E. pyrifoliae*, but the relationships between *E. horticola* and other erwinias require additional studies. Here, we report complete nucleotide sequences of two temperate phages 49 and 59 that infect *E. horticola*. Previous studies have classified them as the members of the *Siphoviridae* family propagating exclusively on the *E. horticola* strains. These viruses possess unique structure organization: their heads and tails are completely identical while base plates differ. Phages 49 and 59 do not have significant similarity to previously reported viruses of *Enterobacteriaceae*. However, the revealed synteny and gene order indicate them as the novel members of the lambdaoid phage group. Comparative genome analysis showed that phage 49 and 59 genomes are 47% similar and share 29 genes with up to 100% nucleic acid sequence identity essentially over the DNA packaging, virion morphogenesis and lysis regions. Intact prophages were found in genomes of *E. persicina* B64 and *E. billingiae* Eb661, but not in *E. amylovora* genomes, that have extensive sequence homology to phage 49 and 59 chromosomes. This points out that *E. horticola* is more distantly related to *E. amylovora* than to *E. persicina* or *E. billingiae*. The obtained data may be helpful for better understanding of relationships among *Erwinia* species.

## Biocontrol of soft rot and blackleg diseases caused by *Pectobacterium* in Morocco

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Soft rot of tubers and blackleg of potato plants are two bacterial diseases caused by several species of the genera *Pectobacterium*. The contaminations which occur in both the field and after harvest cause important economic losses of potato production. In this study we have tested the antagonistic activity against *Pectobacterium* sp. of several bacterial cultures isolated from potato tubers and rhizosphere soils obtained from different locations across Morocco. Principally, we have found that 40 strains, all originating from tubers, inhibited growth of the pathogen on potato slices. The symptoms suppression of the tested strains on potato slices was superior to 50%. Among the 40 isolates, 10 were found to have a symptom suppression higher than 90%. The antagonistic activity of the 10 strains was demonstrated, *in vitro*, by the appearance of clear zone in the agar medium. The inhibition indicated the production of diffusible antibacterial substances which probably inhibited the growth of the bacteria. *In planta*, significant differences in symptoms including blackleg and vascular discoloration were observed between plants inoculated with *P. carotovorum atrosepticum* and those non-inoculated. This research suggests the potential of biocontrol against soft rot and blackleg of potato using antagonistic bacteria.

## Isolation and characterization of bacterial pathogen causing soft rot disease of potato in Tunisia

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Potato (*Solanum tuberosum* L.) is cultivated in many countries and is one of the most common and important vegetables listed among the five principal and popular crops grown in the world as well as in Tunisia. Different bacterial diseases have been reported to attack potatoes leading to a high economic loss in yield and quality under favorable environmental conditions. However, the potato soft rot is one of the most important diseases of potatoes causing great reduction in yield resulting in economic losses in the field and even under storage conditions. Depending on the mode of infection, the rot begins at the end of the stolon or at the lenticel level, but the development of the disease depends largely on climatic factors, the tubers can be infected in the field before harvest or storage. In recent years, there has been an increase in diseases due to *Pectobacterium* bacteria. This group of bacteria is classified as one of the most economically important pathogens for potato cultivation. The main objective of the present study was to isolate and characterize the bacterial causal agents of the soft rot disease of potato and evaluate their pathogenic degree. Potato samples (tubers and stems) were collected from several sites in northern Tunisia, including stem and tubers suspected to be infected with soft rot, grown in the fall and spring seasons and cold storage chambers. A collection of about 70 isolates were established from stem and potato tubers. They were identified by phenotypical (morphological, physiological and biochemical features) and molecular tools (sequencing 16SrDNA gene). Preliminary results showed a predominance of *Pectobacterium* spp. isolates which were extremely virulent on both stem and potato tubers. All isolates were able to produce plant cell-wall degrading enzymes such as pectate lyase, cellulase and protease.

### Screening of antagonistic organism from soil against *Ralstonia solanacearum* complex species

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In Guangdong province, China, bacterial wilt caused by *Ralstonia solanacearum* complex species is considered as the major limiting factor for commercial crop production, such as tomato, pepper, eggplant, ginger, tobacco and peanut, causing about 35 to 90 per cent crop loss. An attempt was taken to control bacterial wilt by antagonists. A total of 67 bacterial isolates were collected from soil of Guangdong province and were screened against the selected virulent isolates of *R. solanacearum* strains Ssf-4 and GMI1000 on TZC media plates. Seven isolates appeared as the most effective antagonist in inhibiting the growth of the selected *R. solanacearum* strains Ssf-4 and GMI1000 by using Kirby-Bauer disk diffusion method. Diameters of inhibition zone were from 24 to 36 mm after co-culture for 48 h. Seven isolates were identified as *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus velezensis* and *Bacillus* spp. base on phylogenetic analysis results of 16S rRNA gene. These results provide promising hope to develop biological agents for control of *R. solanacearum* in Guangdong province, China.

### Investigation of endophytic bacteria from different olive varieties in Apulia region

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*Xylella fastidiosa* subsp. *pauca* ST53 is the causal agent of the Olive Quick Decline in Apulia region, whose control is still lacking. The primary aim of the ongoing research is therefore oriented to the control of the pathogen and its vectors. Olive varieties show a different susceptibility to *Xylella* infection which is also associated with the presence of resistance genes in those with mild or no symptoms. Recently, the study of plant microbiota has raised particular interest because of its influence on plant growth and health. Furthermore, promising results have been obtained using manipulated microbiota that can lead to the formulation of biocontrol products. The purpose of this study is therefore to isolate and characterize populations of endophytic bacteria in different varieties of Apulian olive trees. In the period 2018 - 2019, seasonal sampling was carried out in the demarcated areas of Apulia region, choosing symptomless varieties of olive trees. Isolation of the bacterium was done from 2-3 year old twigs using the patented CIHEAM-IAMB method of crude sap extraction (WO2017017555A1) and the direct print method. One hundred and twenty isolated endophytic bacteria were morphologically identified and biochemically characterized. Seventy isolates with morphological and biochemical differences were analyzed by PCR. The amplification of 16rRNA confirmed the variability of the endophytic bacterial population in different seasons and between different olive varieties. These preliminary results could lead to the identification of endophytic olive bacteria that may have a role of interaction with *Xylella fastidiosa* and the OQD disease.

### Improvement of the real time LAMP protocol for the detection of *Xylella fastidiosa* in *Philaenus spumarius*

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The epidemic spread of *Xylella fastidiosa* in southern Italy, with serious economic repercussions mainly for the olive trees, has highlighted the effectiveness of the approach with spy insects in early monitoring the infection before symptoms development in host plants. This approach is based on molecular tests, among which the real time LAMP is the most suitable for mass monitoring of the bacterium. The improvement of real time LAMP sensitivity for the detection of *X. fastidiosa* in *Philaenus spumarius*, the most epidemiologically relevant vector of this bacterium in southern Italy, was carried out using the commercial kit (Enbiotech, Italy). Approximately 525 specimens of *P. spumarius* were sampled in late summer in an infected olive grove in Lecce. The total incidence of infection, ranged from 13% to 16%, using single entire insect and single heads, respectively; this incidence was confirmed with grouped heads to assess the diagnostic sensitivity of the real time LAMP test and a progressive increase in bacterial detection was observed by analyzing a higher number of heads (up to a pool of four tests). Spiked samples were then tested using the entire insect and grouped insect heads artificially inoculated with a serial dilution (from 10<sup>6</sup> to 10<sup>0</sup> CFU) of *X. fastidiosa* ST53 isolated from olive tree. The lowest bacterial dilution was revealed with no difference among the entire insect or 2 pooled heads. This result will be validated next summer by monitoring the pathogen through the analysis of *P. spumarius* in a recently infected area with a low bacterial inoculum pressure.

## Trend analysis of pesticide residue bioassay in Kalimati vegetable market, study on pesticide utilization and poisoning in Nepal

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The haphazard use of insecticides and fungicides still exists in the farmer's field of Nepal. The extensive use of pesticide and incognizance regarding withdrawal period had left their noxious residues in the vegetable products available in consumer market is a matter of serious concern. The study was conducted from 2014 to 2018 in Rapid Bioassay Pesticide Analysis Laboratory, Kalimati Fruit and Vegetable Market among 7445 vegetable samples belonging to nine different families that were imported from 49 locations of Nepal and neighboring countries. The rapid bioassay is based on the inhibition of the enzyme acetylcholinesterase (AChE) by organophosphate or carbamate insecticides based on absorbance percentage which is calculated using spectrophotometer at 412 nm. Meanwhile, tomato, cauliflower, bean, and potato products were reported with the highest insecticide residue, mostly among samples imported from Kavre followed by eastern region (major Sarlahi district) of Nepal and India. The analysis illustrates the exponentially decreasing trend among insecticide use in vegetable from 2014 to 2018. From the study in farmer's field during 2018 in Morang District in eastern Nepal, the awareness of the color-coding mark pertaining to the insecticide and fungicide level was significantly different (5.91\*\*,  $p < 0.05$ ) among 40 commercial and 15 subsistent vegetable farmers under study, and still 90.90% use the pesticides. People are discouraged using IPM practice because of its confounding procedure and lack of training - trained ones (21.81%) are more concerned while non-trained are neglecting the method and dose of application. Whilst they felt an acute effect, 72% reported the spraying time to be 10 am - 2 pm in the day and 80% reported higher pesticide use during the winter season. Similarly, patient information derived from emergency department of Bharatpur Hospital of Central Region (2015-2019) depicts highest pesticide poisoning occur in the vegetable pocket region between 30-40 age group. FGD and KII suggests awareness program on impact of chemical pesticides, application of integrated pest management technique (that gave good result regarding awareness in Eastern Nepal), proper quarantine, disposal, field inspection and alternative plant protection measures like bio-pesticides are urgent need to protect the consumers and environment from hazards of pesticides in both short and long terms.

### Surfactin and iturin produced by *Bacillus amyloliquefaciens* Jt84 play an important role in biocontrol of rice blast disease

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The rice blast caused by fungus *Magnaporthe oryzae* is a severe production constraint worldwide. Biological control of rice blast using plant-

associated microorganisms is an efficient approach to disease management and is regarded as environment friendly. *Bacillus* sp. can produce a broad array of antifungal lipopeptides, making this species a biological control agent. In a previous study, *Bacillus amyloliquefaciens* strain Jt84 was found to possess biocontrol activity against rice blast disease. The antifungal compounds were isolated by acid precipitation of cell-free supernatants, extracted with methanol. Lipopeptides were tested for antagonistic activity against *M. oryzae*, which showed a strong inhibitive activity. The inhibitory effect was observed in abnormal mycelial morphology when treated with lipopeptides extract. Further analysis using PCR and HPLC-ESI-MS confirmed the presence of surfactin, iturin and fengycin. Pot trials suggest that surfactin and iturin play an important role in biocontrol of rice blast, and the results were supported by mutagenesis analysis, targeted to suppress the biosynthesis of surfactin and iturin A production. *B. amyloliquefaciens* Jt84 significantly reduced the incidence of rice blast disease, controlling efficacy ranging from 79.2% to 85.6%, i.e. better than that afforded by tricyclazole in field experiments. We conclude that *B. amyloliquefaciens* Jt84 might be a promising biocontrol agent and should be further studied.

### Introducing next-generation phage biocontrol in agriculture

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Bacterial infections in agriculture are estimated to cause losses up to 10%. Relevant crops for agriculture in Belgium (Flanders) include *Pseudomonas syringae* pv. *porri* (*Pspo*) and *Xanthomonas campestris* pv. *campestris* (*Xcc*). These bacteria are known to cause bacterial blight in leek and black rot in *Brassica* spp., respectively. Until recently, bacterial infections were treated using copper-based chemicals and antibiotics like streptomycin. However, the use of these compounds is not sustainable since they are prone to resistance development and have a detrimental impact on the environment. A valuable alternative is the use of biocontrol agents like bacteriophages to fight bacterial infections.

In this regard, different phages have been isolated to tackle both *Pspo* and *Xcc* infections. In the case of *Pspo*, five different phages, KIL1-KIL5 (KU Leuven-ILVO), along with one host range mutant KIL3b, were found that are able to collapse 88% of *Pspo* strains found in Flanders. For *Xcc*, we isolated eight different phages that can lyse 71% of the strains relevant for agriculture in Belgium (SoPhi1-7 and Phibonacci). These phages are being investigated both genetically and microbiologically to determine their host range, infection efficiency, biosafety and potential to be used in phage biocontrol. Preliminary field trials have shown promising results. Currently, evolution experiments are being performed to select for phages with higher *in planta* survival.

### Ability of Chilean bacteria to control *Pseudomonas syringae* pv. *actinidiae* in kiwifruit by antibiosis and induction of resistance

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Bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidia* (*Psa*), has caused severe losses worldwide. Developing new strategies to control this disease are required. Forty-seven bacteria were assessed *in vitro* to determine their ability to reduce *Psa*. Eighteen strains from the *Pseudomonas fluorescens* group produced inhibition halos on bacterial lawn of *Psa* on King B medium. Four strains were assessed comparatively with a control inoculated with *Psa*, antibiotics mix, and acibenzolar-S-methyl (ASM, Bion 50 WG, 0.2 g L<sup>-1</sup>). Experiments were conducted in three-month-old plants obtained from 'Hayward' seeds in a plant growth chamber (22 °C under light:night time of 12 h with LED lights). In an antibiosis experiment, plants were inoculated with *Psa* at the same time of the antibiotic and the antagonistic bacteria treatments. Result showed that both strains reduced necrotic spots caused by *Psa* after 30 days ( $P < 0.05$ ). Strain Ca2 reduced between 85.4 and 96.6% the level of infection, while ChC7 reduced the disease between 85.4 and 84.7% with respect to the *Psa* inoculated control. In an induction of resistance experiment, plants were treated on leaves or roots with ASM and the bacterial strain and after seven days were inoculated with *Psa*. Results showed that necrotic spots were reduced by the antagonistic strains, while when they were applied in roots, spots were reduced in a 47.4% by strain Ca2, 82% by ASM, and ChC7 did not reduce the disease. Chilean *Pseudomonas* strains are promising alternative to control *Psa* in kiwifruit.

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### **Assessment disease resistance genes expressions associated with bacteria with antimicrobial activity in kiwi (*Actinidia deliciosa*)**

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*Pseudomonas syringae* pv. *actinidia* (*Psa*), causal agent of bacterial canker of kiwifruit, is controlled with acibenzolar-S-methyl (ASM), a chemical inducer of resistance. However, there are few information about the impact to use antagonistic bacteria to control this disease by induce systemic acquire resistance (SAR). Relative expression of genes *pr1* (pathogenesis-related protein 1), *ICS* (Isocorismate sintase), *PAL* (Phenilalanine amonia lyase), *lox* (lipooxygenase), *TLP1* (Thaumatococcus-like protein), *APX* (L-ascorbate peroxidase), *pr4* (pathogenesis-related protein 4), *CNP60* (Chaperone-60), *Serpina* (Serpine ZX), and *Actine* as a house-keeping were assessed in *Actinidia deliciosa* plants at 1, 7, and 15 days after inoculation with the bacterial strains ChC7, Ca2, and ASM (Bion 50WG, 0.2 g L<sup>-1</sup>). Treatments were applied on the foliage or in roots. Results showed that bacterial strains showed patterns of gene expression relative similar with ASM, when they were applied to the foliage. Overexpression of *pr1*, *PAL*, *pr4*, *TLP1*, and *lox* was very similar between strain Ca2 and ASM, while strain ChC7 overexpressed gene *pr4*. ASM increased the expression of *ICS1*, gene associated with salicylic acid production. When treatments were applied to the roots, strain Ca2 increased the expression of *pr8*, gene associated with expression of a chitinase type II enzyme, and maintained the expression of *TLP1* along the period of assessment. *TLP1* gene expression was increased by ASM along the time, while strain ChC7 only increased expression of this gene the day after the application. Ability to induce different plant defense genes in kiwifruit suggests that these bacterial strains have SAR activity to control *Psa*.

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