

### **ABSTRACT BOOK 2025**



from September 23th to 26th, 2025 Perpignan, FRANCE

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### Session 1:

## RMT BESTIM Session 1: Biosolutions in the Field

**GUEST: LOÏC RAJJOU** 

## ORAL GUEST 1: LOOKING FOR EFFICIENT BIOSOLUTIONS TO IMPROVE SEED HEALTH, VIGOUR, AND YIELD: LINKING BASIC RESEARCH TO AGRICULTURAL PRACTICE

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Seed quality is a critical determinant of agricultural success, underpinning germination, seedling establishment, and ultimately crop performance. Conventional seed protection has long relied on chemical treatments derived from synthetic chemistry. However, current societal, environmental, and regulatory pressures demand the development of innovative alternatives that are both effective and sustainable. Fundamental research is essential to unravel the cellular and molecular mechanisms underlying seed defences. Particular attention is directed to the spermosphere, the biochemical microenvironment that shapes microbial dynamics and can favour the establishment of beneficial, non-pathogenic communities capable of protecting seeds and seedlings from biotic stress. Building on these insights, applied research enables the design of seed technologies such as coating, pelleting, or priming, using biosolutions including natural substances, plant-derived extracts, microorganisms, and environmentally compatible formulations. These innovations can act either through biocontrol, by directly suppressing seed- and soil-borne pathogens, or through biostimulation, by improving plant nutrition efficiency, enhancing seed vigour and resilience, and fostering beneficial plant-microbiome interactions. Proof-of-concept studies demonstrate their potential to deliver robust and well-characterised alternatives to synthetic pesticides, while also enabling scale-up and industrial deployment. Crucially, progress in this field requires strong connections between academic research, industry, and farmers in the field, supported by multidisciplinary expertise spanning biology, agronomy, chemistry, and the social sciences. Such collaborations will be decisive for transforming fundamental discoveries into practical, large-scale innovations that address both productivity goals and the broader challenges of the agroecological transition.

Key words: Seed, Seedlings, Damping-off, Seed technology, Basic research, Targeted reserach

## O1: SUBSTANCES ACTIVES DE BIOCONTROLE EN EUROPE : LE POINT DE BASCULE ?

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Les substances de biocontrôle (BCA) à la réglementation pesticides EU progressent en nombre et en pourcentage du nombre total de substance actives depuis 2011 (Fig. 1), l'entrée en vigueur du règlement. Notre analyse de cette évolution, débutée en 2018¹ et poursuivie depuis², montre que même si la progression est régulière, la pente de la courbe décroit au fur et à mesure. De plus, de trop nombreuses BCA (58) ont été abandonnées (Fig. 3), même si certains BCA laissés pour compte, comme le *Bt tenebrionis*, sont candidats de nouveau pour une approbation.

Catégories: L'impact des nouvelles catégories de substance actives créées, comme les substances à faible risque, est essentiel au progrès des BCA dans leur promotion et leur adoption. Jusqu'à présent, 15 ans après le vote du nouveau règlement, toutes les substances à faible risque (75) sont des agents de biocontrôle (Fig. 2), même si l'absence dans cette classification de certaines catégories de médiateurs chimiques (aldéhydes et alcools) reste un mystère.

**Résidus**: Les résidus correspondants à ces substances sont de plus peu impactant et très significativement faibles. Seulement 7.4% des BCA ont des LMR décrites dans l'annexe II ou III, tandis que 92.6% sont sans LMR ou avec la LMR par défaut.<sup>5</sup>

**Futur**: Les perspectives d'avenir des BCA sont contrastées. D'un côté, le positif: elles représentent plus de 70% des substances candidates et aucune substance chimique n'a été approuvée depuis 5 ans. D'un autre côté, elles sont peu nombreuses (56) avec pour certaines, peu de chance d'être approuvées et on observe une tendance à la substance active monomoléculaire qui pourrait être confrontée aux mêmes problèmes de résistances que les substances actives chimiques.

Key words: biocontrôle - substances actives - EU level

<sup>&</sup>lt;sup>1</sup> "Biocontrol active substances: evolution since the entry in vigour of Reg. 1107/2009" D C Robin, P A Marchand, *Pest Management Science*, 2019, 75(4), pp. 950-959, doi: 10.1002/ps.5199

<sup>&</sup>lt;sup>2</sup> "Expansion of the low-risk substances in the framework of the European Pesticide Regulation (EC) No. 1107/2009" D C Robin, P A Marchand, *European Journal of Risk Regulation*, 2022, 13(3), pp. 514–531. doi: 10.1017/err.2021.58

<sup>&</sup>lt;sup>3</sup> "BioControlAgents in Europe: substitution plant protection active substances or a new paradigm?" P A Marchand, *Agrochemicals*, 2023, 2(4), pp. 538–550. doi: 10.3390/agrochemicals2040030

<sup>&</sup>lt;sup>4</sup> "Biocontrol Active Substances in Europe: A Slow Shift Towards Dominance" P A Marchand, *Chronicle of Bioresource Management*, 2023, 7(2), pp 21-23

<sup>&</sup>lt;sup>5</sup> "The major interest for crop protection of agrochemical substances without maximum residue limit (MRL)" M Charon, D Robin, P A Marchand, *Biotechnologie, Agronomie, Société et Environnement*, 2019, 23(1), pp. 22-29. doi: 10.25518/1780-4507.17666

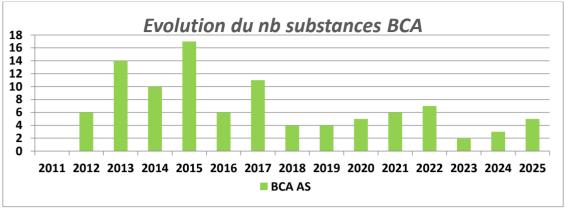


Fig. 1

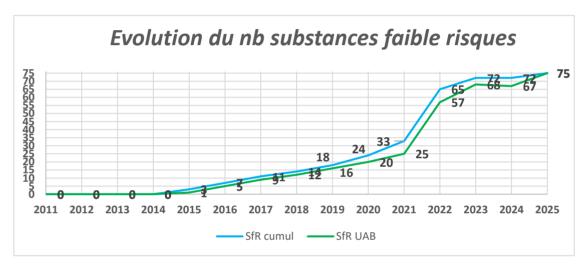


Fig. 2

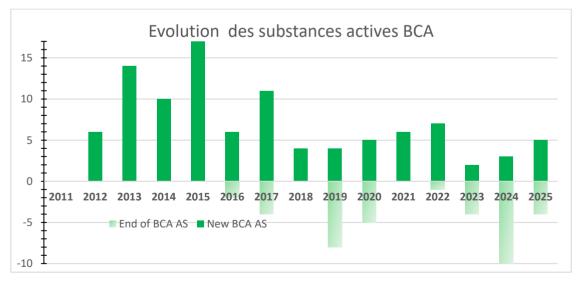


Fig. 3

## O2: LOCAL, SHORT-RUN PRODUCTION OF BIO-BASED PRODUCTS FOR SUSTAINABLE AGRICULTURE

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In 2022, the European Commission adopted a new proposal to provide a framework for the sustainableuse of plant protection products, particularly reducing the use of synthetic pesticides by 50% by 2030. This proposal is set out in the European 'Farm to Fork' strategy. In this context, AkiNaO and Greentechhave partnered to produce two patented bio-based products from local production: an organic fungicide and a mulch, derived from the same plant resource, slimy inula (*Dittrichia viscosa*).

*D. viscosa* is abundant on wasteland in the Pyrénées-Orientales department (south of France). Partnerships have been set up with local farmers to harvest it. Other undesirable plants are managed by sheep grazing on wasteland where the inula content is unsatisfactory. This rational management of the resource also aims to preserve flowering periods for beekeepers, as inula is one of the last speciesto flower in the region. Work is also underway to optimise the cultivation of this plant.

The biofungicide was obtained by extraction of Inula and active compounds identified through biological screening. Trials on peach and apricot trees against moniliasis and others against grapevinedowny mildew showed high efficacy. It is currently being assessed for approval.

*D. viscosa* mulch is obtained by upgrading the extraction by-product. This limits waste generation in a circular economy approach. Its effectiveness has been demonstrated in various experimental campaigns in market gardening, viticulture and horticulture. Trials have shown it to be more than 80% effective in controlling weeds in vineyards, 18 months after application. Moreover, harvested while still green, this mulch benefits from the plant's natural properties, making it wind-resistant and soil- enriching without causing nitrogen starvation.

After several years of R&D, we have succeeded in developing two products from a resource considered undesirable, thereby contributing to the ambitious objectives of the Green Deal for Europe.

Key words: Dittrichia viscosa, circular economy, bio-inputs of plant origin, biofungicide, mulch

## O3: TOWARDS INTEGRATED PROTECTION OF ROSE AGAINST FOLIAR FUNGAL DISEASES: BIOCONTROL AND VARIETAL RESISTANCE?

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The rose is the most important ornamental plant worldwide. Its aesthetic quality can be compromised by pests and pathogens. The regulatory context sets the objective of reducing the use and associated risks of chemicals by 50% by 2030 for producers (Ecophyto plans). In the ornamental sector, significant efforts have to be made to develop alternatives, particularly for public space managers and private gardeners, for whom the use of such products is prohibited (Labbé Law).

The ROBIO project (2022-2024, Action under Ecophyto II+ plan, led by the Ministries of Agriculture, Ecology, Health and Research, with financial support of the French Office for Biodiversity) aimed to assess the efficacy and characterize the mode of action of biocontrol products against the main fungal foliar diseases of rose (black spot disease, powdery mildew, downy mildew and rust) through a stepwise approach (from laboratory tests to field trials under natural epidemic conditions). The project also sought to evaluate the potential influence of varietal sensitivity on product efficacy.

This project led to the development of new tools for rose: (i) the qPFD® chip (Quantitative Low-Density chip), a molecular tool designed to analyze the expression of genes involved in defense pathways; (ii) a pathotest to assess the level of resistance to rust.

The stepwise approach adopted in this project to evaluate the efficacy of biocontrol products has proven effective: the products selected after laboratory screening showed efficacy on whole plants in

greenhouse conditions and are the most promising in the field. The project also integrated varietal resistance by evaluating selected products on five rose genotypes. The results revealed considerable genetic variability, reinforcing the need to combine different strategies to protect roses against foliar diseases.

A field experiment network was established and is being maintained at five sites across the country, each representing different environmental conditions and potentially diverse pathogen pressures.

Key words: rosier - maladies foliaires - biocontrôle - resistance génétique / rose - foliar diseases - biocontrol - genetic resistance

## O4: IDENTIFICATION OF A NETWORK OF CORRELATIONS OF POTENTIALLY INFLUENTIAL VARIABLES FOR BIOSOLUTIONS

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Biosolutions based on plant immunity offer a promising alternative for boosting crop resistance to pathogens, while reducing the use of chemical inputs. Although many formulations have demonstrated their efficacy under controlled conditions, their performance in the field is often disappointing, due to the complexity of interactions between the plant and its environment. In orderto better understand these efficacy losses, we propose an initial approach to identify and characterize the factors and variables influencing the performance of biosolutions. This initial analysis, based on environmental factors, the plant's physiological state and gene expression, aims to better understand the relationships between these different variables in real conditions. The diversity of variables to be considered (environmental conditions, yield, plant condition, transcriptomic data) makes datasets heterogeneous and complex, preventing the use of a single global analysis method. Because of the different scales involved, direct concatenation of all variables would risk introducing bias or masking certain relationships. Data harmonization and a combination of appropriate methods are therefore necessary to draw robust conclusions. Modular and complementary approaches are envisaged, using tools specific to each type of data, before moving on to a joint analysis of the variables.

In this context, we have chosen to mobilize three main methods:

- 1- Bayesian Principal Component Analysis, which explores correlation structures between variables, while integrating uncertainty management. Unlike classical PCA, this approach provides an estimate of the probability densities of the correlation coefficients, making possible to quantify more precisely the robustness of the links between variables in order to build interaction networks. It also provides access to the probability densities of the variables, enabling missing data to be imputed. Finally, the latent spaces obtained can highlight clusters of trials with similar response profiles, opening the way for a partitioning of biosolution effects according to environmental or varietal contexts.
- 2- Gene co-expression network inference, applied to transcriptomic data, which allows the identification of groups of co-regulated genes likely to be involved in responses to biosolutions.

3- Stochastic Block Modeling, which analyzes the structure of the resulting graphs (whether based on Bayesian principal component analysis or gene networks). This approach identifies coherent blocks of highly interconnected variables, making it easier to interpret the networks and sometimes revealing certain temporal dynamics, for example.

This combination of analyses aims to construct graphical and interpretable representations of the most significant interactions between variables, paving the way for a better understanding of the conditions favorable to the effectiveness of biosolutions. Initial models of variable networks will be presented to you.

Key words: Agro-ecology – Biosolutions – Bayesian PCA – Gene Networks - Wheat

## O5: SEEDBIOPROTECT PROJECT: EVALUATION OF SEED TREATMENT TO CONTROL DAMPING OFF IN WHEAT AND TOMATO

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The SeedBioProtect project (2023-2025), funded by the PlantAlliance and the Biocontrol Consortia, explores biocontrol efficacy on seeds by studying interactions between plant genotype and alternative treatments. A key objective is to develop tools to assess the performance of seed-applied biosolutions. In this study, two treatment strategies were tested: biosolutions were either applied to mother plants, with efficacy later evaluated on their seeds, or directly coated onto the seeds before assessment.

Two project partners GEVES and VEGENOV carried out evaluations of treatment efficacy and of

Two project partners, GEVES and VEGENOV, carried out evaluations of treatment efficacy and of genotype tolerance through phenotyping trials (with different pathosystems including *Fusarium graminearum* and *Pythium* spp. on wheat and *Rhizoctonia solani* on tomato). In parallel, protocols have been validated for the qPCR detection of damping-off pathogens in seeds. This project also led to the development of a protocol to study seed elicitation by analyzing the expression of defence-related genes for wheat and tomato. The results suggest that both the production site and plant genotype influence the effectiveness of biocontrol products. Results also indicate an efficacy of the treatment when applied on seeds, but no efficacy of the products when applied on mother plants. The expression of defence-related genes seems to be more impacted by the genotype than by the treatment. The methodologies developed during this project offer a more reliable and efficient framework for evaluating the performance of biocontrol solutions on seeds.

Key words: seeds-- biocontrol - wheat - tomato - SeedBioProtect Project

<sup>\*</sup> Speakers

## O6: THE QPFD® TOOL: 12 YEARS OF OPERATION AND ONGOING DEVELOPMENT FOR RELIABLE EVALUATION OF PLANT DEFENSE INDUCER AND BIOSTIMULANTS ACTIVE SUBSTANCE, FROM THE LABORATORY TO THE FIELD

#### **Emmanuelle Pajot**<sup>1</sup>

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Végépolys Innovation, the R&D center of the Végépolys Valley competitiveness cluster, has been working since 2007 to develop methods for evaluating biosolutions, in partnership with academic laboratories (IRHS, INRAE Angers, Respom). Within this framework, the center has acquired the rights to a patented molecular tool (PCT/FR2011/051470) enabling, via a targeted RT-qPCR approach, the simultaneous analysis of several plant defense metabolic pathways activated in response to biotic or abiotic stresses. After 12 years of use in collaborative research projects and as a service provider, the qPFD® tool has demonstrated its relevance and robustness. Today, it can be used for a wide range of applications: early screening of candidate molecules, understanding of mechanisms of action, optimization of formulations and validation of technical itineraries under real-life conditions. Application cases on different plant species, products (referenced or candidates) and experimental contexts will illustrate its performance. Prospects for further development of the tool, particularly in relation to the integration of high-throughput sequencing technologies (NGS), will also be discussed.

Key words: Elicitor, Plant defence, RT-PCR, défense genes, biostimulants

## O7: ENHANCING APPLE SELF-DEFENSE BY COMBINING METHODS THAT LEVERAGE PLANT IMMUNITY

<u>Matthieu Gaucher</u><sup>1</sup>, Youenn Petite<sup>1</sup>, Terence Mobarak<sup>1</sup>, Romane Lapous<sup>1</sup>, Arnaud Lemarquand<sup>2</sup>, Frédéric Didelot<sup>2</sup>, Julie Ferreira de Carvalho<sup>1</sup>, Mathilde Orsell<sup>1</sup>, Mickaël Delaire<sup>1</sup>, Romain Larbat<sup>1</sup>, Hélène Gautier<sup>3</sup>, Laure Perchepied<sup>1</sup>, Alexandre Degrave<sup>1</sup>, Marie Noëlle Brisset<sup>1</sup>.

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Ensuring effective and sustainable crop protection through approaches that safeguard both environmental and human health, while maintaining producer income, is increasingly vital today. The CAP ZERO PHYTO project, funded under the PPR program "Cultiver et protéger autrement" contributes to experimental research by exploring a range of protection strategies aimed at enhancing the overall immunity of the agrosystem. Focusing on tomato and apple crops, the project investigates both individual and combined approaches that involve quantitative genetic resistance, plant resistance induction (PRI) via biocontrol products or photostimulation, nitrogen fertilization management, and companion plants. Research is conducted from the very molecular scale in controlled greenhouse conditions to a multi-site field network, encompassing both factorial and systems trials. Interesting and promising findings were observed against several economically significant pests when apple trees were treated with a combination of PRI and low-nitrogen fertilization, or PRI applied to genotypes carrying various disease resistance QTLs. Some underlying molecular mechanisms driving this enhanced immunity, along with field trial results for both strategies, will be presented.

Key words: Immunity, protection methods combination, apple, nitrogen fertilization, plant resistance inducers, quantitative resistance.

### Session 2:

## RMT BESTIM Session 2: Biosolutions modes of action

GUEST: MICHELE PERAZZOLI, DOMENICO DESERIO

## ORAL GUEST 2: FUNCTIONAL CHARACTERIZATION OF EFFICACY AND MODE OF ACTION OF A SUSTAINABLE FUNGICIDE AGAINST GRAPEVINE PATHOGENS

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Oomycetes (e.g., Plasmopara viticola) and ascomycete fungi (e.g., Botrytis cinerea and Erysiphe necator) are responsible for significant economic losses in grapevine production. Grapevine disease management involves frequent applications of synthetic fungicides, with potential negative impacts on human health and the environment. This study aimed to investigate the mechanism of action of a new fungicide based on choline pelargonate (CP, patent BE1026779B1) against crop pathogens under controlled and field conditions. CP inhibited mycelial growth and spore germination of B. cinerea and Phytophthora infestans (an oomycete closely related to P. viticola) in vitro, causing leakage of electrolytes, ultrastructural alterations of cellular membranes, and perturbation of lipid content. CP treatments decreased downy mildew and powdery mildew severity on grapevine leaves under greenhouse conditions with no phytotoxic effects. In particular, CP inhibited zoospore release and mobility of P. viticola and decreased spore germination of E. necator, indicating a mode of action mainly based on direct inhibitory effects against phytopathogens. However, translaminar effects and the expression of defense-related genes in CP-treated leaves were assessed to explore the possible priming effects. Field trials confirmed the efficacy of CP in two experimental vineyards and showed high efficacy against powdery mildew under field conditions. To assess potential side effects on non-target microorganisms, bacterial and fungal communities were isolated from grapevine leaves and bunches of control and CP-treated plants. Amplicon sequencing analysis showed that phyllosphere communities differed according to vineyard location and time point. Moreover, CP showed negligible effects on microbial communities that were partially influenced by the composition of the indigenous microbiota. CP is a promising fungicide against grapevine pathogens and represents a viable alternative to reduce the use of conventional fungicides.

## O8: A FREE AMINO ACIDS-BASED BIOSTIMULANT PROMOTES CELL DIVISION AND ALLEVIATES HEAT STRESS EFFECTS IN TOMATO ROOTS PHD GRANT

<u>Loé Malgouyre<sup>1,2</sup></u>, Norbert Bollier<sup>1</sup>, Pascal Martin<sup>1</sup>, Marilise Nogueira<sup>3</sup>, Paul Fraser<sup>3</sup>, RenaudSergheraert<sup>2</sup>, Nathalie Gonzalez<sup>1</sup>, Emmanuelle Mounier<sup>2</sup>, and Michel Hernould<sup>1</sup>

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Biostimulant products enhance plant development by improving nutrient assimilation or increasing tolerance to abiotic stresses. However, their widespread use is limited by a lack of knowledge regarding optimal application methods and the underlying mechanisms of action. A free amino acids-based biostimulant produced by BCF Life Sciences has previously been shown to alleviate drought stress in potted lettuces. To build on this research, we are now investigating its effects on tomato plants, which are increasingly exposed to heat stress during various developmental stages. Our initial analyses focus on root growth under both optimal and high ambient temperatures (HS conditions). Results indicated that amino acid mix treatment improved root development under both normal and HS conditions, resulting in increased meristem size and cell numbers. To better understand the plant responses induced by the treatment, genome-wide expression analyses were conducted. These analyses revealed that the amino acid mix upregulates genes associated with cell division and downregulates heat-stress marker genes. Additionally, hormonal and metabolic profiling showed that the treatment modulates hormone levels and increases the concentration of specific compounds known to enhance stress tolerance through activation of signalling pathways. To further validate the role of these genes in the tomato response to amino acid treatment, additional experiments, including functional analyses, will be performed.

Key words: Heat stress - Biostimulant - Amino acid - Root growth - Cell division.

## O9: NO PAIN NO GAIN, NO GRAINES\* NO PAIN\* (\* GRAINES = SEEDS / \* PAIN = BREAD)

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In the context of ecological and economic considerations, there is a growing interest in alternative methods to enhance plant production, particularly through the use of biostimulation products. These products encompass a range of biostimulants that can enhance responses against abiotic stressors, thereby improving crop assimilation and/or nutrition. Biostimulants may originate from natural, synthetic, or combined sources, including amino acids, enzymes, algae extracts, vegetal hormones, beneficial microorganisms, plant extracts, minerals, and nutrients. Despite their potential, the precise mechanisms of action of these biostimulants remain poorly understood, and certification processes necessitate an evaluation of induced molecular responses.

Our research endeavors to fill this knowledge gap by characterizing molecular responses in pea plants induced by diverse biostimulants which elicit different molecular pathways allowing to improve assimilation, nutrition, increase the capacity to deal with drought and enhance seeds quality. We employ molecular, metabolomic, and physiological approaches to evaluate various products among nine winter pea varieties. Our transcriptomic analysis aims to elucidate molecular pathways activated under specific conditions, considering both the biostimulant used and the genetic background of the plant. Additionally, we seek to establish correlations between physiological observations (such as the Nitrogen Balance Index, root and aerial dry weights, and other physiological traits), molecular responses and metabolomic profiles.

Our work aims to characterize molecular responses induced by different biostimulants within several varieties and species of plant. We performed molecular, metabolomic and physiological approaches to assess eleven products among different categories, several varieties of wheat and of pea. Our transcriptomic analysis allowed us to highlight that certain molecular pathway are induced under specific conditions according to the biostimulant and through genetic background. Moreover, we made some correlation between our physiological observations such as the Nitrogen Balance Index, the root dry weight and the aerial part dry weight and the molecular responses and metabolomic profile.

To facilitate the "lab-to-field" transition, our research extends to assessing the efficacy of molecular responses induced by biosolutions under different stress scenarios, including hydric, nutritional, temperature, and biotic stresses. This comprehensive investigation contributes valuable insights into the mechanisms of biostimulant action and their uses in enhancing plant resilience and productivity.

Key words: biostimulants, plant responses, transcriptomic, physiological responses

## O10: A BETTER UNDERSTANDING OF THE MODE OF ACTION OF A HIGH QUALITY MICROALGAE-BASED EXTRACT BY A TRANSCRIPTOMIC AND PHENOTYPIC APPROACH

Speaker (1), Bachar B, Ph.D, Speaker (2) Francisco Romero Ph.D

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The present speech is intended to release our knowledge on the mode of action of a microalgae-based extract (LRM <sup>TM</sup>) and how it influences plant receptivity and response

In our studies, we characterized the transcriptomic response of the plants, using the model plant *Arabidopsis thaliana* and the horticultural plant *Solanum lycopersicum* (tomato) to a foliar application of a microalgae-based commercial extract preparation (LRM <sup>TM</sup>). We investigated the response over both transcriptomes potentially mediated by various compounds within the (LRM <sup>TM</sup>) influencing salicylic acid biosynthesis processes and drought/heat acclimatization, induced by stomatal control and wax accumulation on cuticle to mitigate abiotic stresses.

On juvenile plants of *Arabidopsis*, Foliar application of the LRM <sup>TM</sup> showed substantial changes in the genes expression compared to control, treated with water. 2 h after application, 399 activated genes and 541 repressed genes were detected in treated plants compared to control plants. This was not transient and was maintained 24h after application, with 339 genes activated and 98 genes repressed.

The results on adults plants was similar, producing a first response detectable after 2 h. Application led to the activation of 116 genes and the expression of 111 genes, that was maintained 24 h later with the activation of 389 and the repression of 436 genes.

Regarding the effect on S. *lycopersicum*, application of LRM on juvenile plants (21DAS) triggered a significant transcriptomic response 2h after application showing 956 genes activated and 1169 genes repressed, compared to control plants treated with water. This effect also was not transient and was maintained 24h after application with 244 genes activated and 637 repressed.

In both plants, the analysis of the biological processes significantly activated 2 h after the application of LRM <sup>TM</sup> allowed for the identification of systemic acquired resistance (SAR) as one of the most significantly enriched process among many others.

When investigating many genes family related to response to abiotic stress, we detect that many processes were significantly activated after the application of the microalgae-based extract, such as response to heat and response to water deprivation, in both juvenile and adults plants.

Specially, the agronomic improvement observed in the treated plants *S. lycopersicum* include an increase in the number of fruits, an acceleration in flowering time and the provision of higher drought resistance by priming effect.

The present study improves our knowledge on the mode of action of the novel high quality microalgae extracts LRM <sup>™</sup> on crops. This approach provides us information to better position our products under practical field conditions and also guide us to develop innovative formulations to target different claims..

Key words: Microalgae-based extract, transcriptomic, abiotic stress, genes expression,

## O11: WILLAERTIA MAGNA C2C MAKY: MODE OF ACTION AND EFFICACY AS A BIO-FUNGICIDE

#### Sandrine TROUSSIEUX (1)

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To ensure crop yield and quality, it is imperative to protect the cultivated plants from diseases. For decades, conventional fungicides have been intensively and almost exclusively used. While their effectiveness is undeniable, their over-consumption can lead to significant risks such as the emergence of resistant strains, environmental impact, residues in agricultural products. Public and farmer concerns about exposure to these products and their toxic residues have driven the search for safer alternatives, therefore increasing the interest in biocontrol solutions. However, few biocontrol products with a broad host range are available today.

Amoéba, a French greentech, has explored the potential of the non-pathogenic, free-living amoeba, Willaertia magna C2c Maky in plant protection. Originally isolated from French Alps thermal water, its efficacy in a lysate form was first demonstrated against grape downy mildew (Plasmopara viticola) in vitro and in planta. The company's research team developed a production process to industrially produce this microorganism and manufacture a plant protection active substance from lysed amoebas. Formulations were developed to cope with environmental conditions such as rainfall.

This innovative active substance has a complementary dual mode of action: it inhibits germination of pathogen spores (a direct effect on the pathogen), and also stimulates plant defenses (an indirect effect on diseases). Up to now, stimulation of plants defenses has been demonstrated in grape, tomato and potato. Recently, this mode of action was studied in wheat and apple. The direct effect was investigated by bio-guided fractionation, revealing evidence of its multi-component efficacy.

SAGROPIA, a European funded project, is dedicated to identifying and developing Integrated Pest Management (IPM) strategies specifically tailored for potato and sugar beet crops. Within this collaborative framework, several partners from various sectors are involved, contributing their expertise to the project's goals. One of the industrial partners of SAGROPIA project is Amoéba. As part of its contribution to SAGROPIA, Amoéba will focus on investigating the mode of action of AXPERA, its commercial biocontrol formulation.

Key words: AMOEBA, Willaertia magna C2c Maky, bio fungicides, mode of action, new active substance.

#### Oral GUEST 3

## ORAL GUEST 3: BIOLOGICAL CONTROL IN THE EU UNDER THE FRAMEWORK OF REG (EC) 1107/2009

**Domenico Deserio (visio)** 

## **Session 3:**

## MiCropBiomes Session 3: Combining Levers to optimise crop stimulation and protection

**GUEST: LUCIANA PAGNUSSAT** 

#### Oral GUEST 4

### ORAL GUEST 4: DOES THE INCLUSION OF MICROALGAE IMPROVE THE EFFICIENCY OF BACTERIAL-BASED INOCULANTS?

#### **Luciana Pagnussat**

Plant-growth-promoting rhizobacteria (PGPR) are beneficial bacteria that have the potential to enhance crop productivity and resilience to abiotic stress through multiple mechanisms. Nonetheless, PGPRs performance may fluctuate due to environmental variables, microbial competition, and agronomical practices. Moreover, a critical aspect of this strategy is that PGPRs inoculated onto seeds must endure on the dehydrated seed surface until suitable conditions arise for root colonization. Consequently, inoculants often require adjuvants or seed-coating treatments to enhance their viability. Despite being less extensively studied than bacteria, single-celled eukaryotic algae (commonly known as microalgae) also exhibit plant biostimulant properties by releasing plant hormones and producing a complex matrix of exopolysaccharides (EPS) that shield co-inhabiting bacteria from desiccation in a microenvironment that supplies nutrients and hormones within microalgae-bacteria consortia. Our multidisciplinary team is dedicated to the study of synthetic consortia of indigenous microalgae and plant growth-promoting bacteria, with a focus on organic agriculture for crops that include wheat, corn, tomato, and lettuce. Our work revealed that microalgae can offer emergent properties to multispecies PGPR inoculants. We explored whether microalgae incorporated into bacterial inoculants can (i) favor the survival of bacteria in the rhizosphere, and (ii) improve crop productivity under field conditions and different tillage managements. Our results demonstrate the potential of microalgae-bacteria synthetic consortia as bioinputs for crops in the field, an area that remains unexplored and holds significant implications for developing novel multi-species inoculants.

## O12: FROM WASTE TO BIOCONTROL: ANTIFUNGAL PROPERTIES OF A LEEK BY-PRODUCT EXTRACT PHD GRANT

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Leek (*Allium ampeloprasum var. porrum*) is widely consumed in Europe, with France and Belgium beingthe leading producers. Prior to commercialization, leeks undergo processing steps that generate approximately 20% of biomass loss, primarily composed of green leaf tips and outer layers of the whitestalk. As a member of the *Allium* family, leek has been recognized for its antimicrobial properties, mostly attributed to the presence of saponins, flavonoids, and organosulfur compounds.

Recovery of these processing by-products as biocontrol agents offers a promising alternative for sustainable agriculture, aligning with circular economy principles. Aqueous extracts obtained from leek by-products have demonstrated fungicidal activity against several phytopathogenic fungi, as well as protective effects on seeds against infection.

Ongoing research focuses on elucidating the molecular mechanisms underlying the fungicidal effect ofthese extracts, particularly against *Alternaria alternata*, selected for its surface adhesion capacity and offering opportunities for biochemical and microscopy approaches. Results highlight the involvement of oxidative stress, membrane permeability disruption, and interference with ergosterol homeostasis.

To ensure the reliable use of such natural substances derived from agriculture, we also pay particular attention to several key factors. These include the identification of extract preparation constraints, and the characterization of antifungal activity variations related to environmental factors such as territory, seasonal conditions, and cultivar diversity. The study shows that leek preparation as well as leek source can impact antifungal activity. This might be the result of qualitative and quantitative variations of active substances and is part of the current research. Addressing these challenges is essential to support the development of consistent and effective biocontrol strategies based on leek by-products.

Key words: Allium, leek, by-products valuation, antifungal, Alternaria alternata, oxidative stress

## O13: DISINHIBITION OF GRAPEVINE IMMUNE RESPONSES: AN INNOVATIVE STRATEGY TO IMPROVE PLANT DEFENSE INDUCERS EFFICIENCY

Chloé Ilbert<sup>1</sup>, Marie-Cécile Dufour<sup>2</sup>, Caroline Bonneau<sup>3</sup>, Sébastien Aimé<sup>1</sup>, Manon Chargy<sup>3</sup>, Emilie Lascaux<sup>3</sup>, Marielle Adrian<sup>1</sup>, <u>Stéphane Bourque<sup>1</sup></u>

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Among the approaches aiming to reduce our reliance on usual phytosanitary products, stimulating plant immune responses is a promising strategy. However, the protection induced by plant defense inducers (PDI) remains often insufficient or inconsistent. In previous fundamental studies carried out on tobacco, we demonstrated that type 2 histone deacetylases (HD2), a family of HDACs specific to plants, act as negative regulators of immune response intensity. We identified a natural, non-toxic molecule (thereafter called « disinhibitor ») that inhibits HD2s, thereby lifting their repressive effect. This led us to develop a "disinhibition" strategy, combining the disinhibitor with a PDI to enhance the level of induced protection.

Under controlled conditions, using grapevine and downy mildew /powdery mildew pathosystems, we showed a synergistic effect between the disinhibitor and the PDI used at reduced doses, achieving full protection while lowering treatment costs. This increased efficacy is notably characterized by stronger and longer-lasting activation of defense genes, such as those involved in the stilbene phytoalexin synthesis. As part of the DEREBIO project (ANR Ecophyto Maturation), in collaboration with Cérience and the BC2Grape platform of UMR SAVE in Bordeaux, we tested this disinhibition strategy together with reduced doses of copper. Experiments were carried in experimental plots and with lead winegrowers across diverse soil and climate contexts during the 2024 vintage, marked by a severe downy mildew pressure. On cv Chardonnay, the combined use of a reduced copper dose (40% below the approved rate), the disinhibitor, and a half-dose of PDI provided a level protection similar to that obtained with the standard copper treatment, without significantly increasing treatment costs.

This innovative biocontrol strategy, for which an international patent has been filed, holds promise for effective disease management against fungal pathogens under field conditions.

Key words: Biocontrol - Plant Defense Inducers - Type 2 HDACs - Disinhibition - Grape - Downy mildew

## O14: MICROBIAL BIO-INPUT ASSOCIATION, A POTENTIAL ALTERNATIVE TO REDUCE CHEMICAL INPUTS IN A LOCAL AGRICULTURAL SYSTEM

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Current agricultural systems heavily rely on chemical inputs such as synthetic fertilizers, pesticides, and fungicides, which, despite boosting crop yields, have disrupted ecological balance and degraded soils. Microbial bio-inputs represent a promising sustainable alternative, reducing dependence on mineral inputs without compromising productivity. In the 3C2B project, we evaluated two such bio-inputs in a winter wheat system: S. sediminicola, a PGPR naturally present in agricultural soils of northern France and known both for promoting growth and stimulating plant defenses, and the cell-free supernatant of B. velezensis GA1, renowned for its production of antifungal lipopeptides. Treatments were applied alone or in combination under conventional and reduced fertilization and/or fungicide regimes, and their ability to sustain yields, modulate plant physiology (via phenotypic traits, chlorophyll content, and untargeted metabolomics), and enhance soil health was assessed. Agronomic performance was measured at harvest, while the long-term persistence of S. sediminicola was quantified by qPCR and shifts in the soil microbial community were analyzed via metabarcoding. This study highlights the potential of bacterial bio-inputs combination to advance resilient, resource- efficient farming.

Key words: Sphingomonas sediminicola, Bacillus velezensis, wheat, biostimulant, biofungicide

## O15: ESSENTIAL OIL MIXTURES AS SYNERGISTIC INSECTICIDAL AGENTS PHD GRANT

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The impact of agricultural pests involves major losses in crop yield and quality. Traditionally, their control has been carried out using synthetic pesticides, however, their use involves risks both to the environment and to human life and the selection of resistance. As an alternative, extracts derived from botanical species are a source of active compounds for the control of pests and diseases in the agricultural sector. In this sense, essential oils (EOs) are extracts with a complex composition that can be used directly or as ingredients in combined biopesticide formulations. An important advantage of EOs in pest control are the synergistic effects between their components, making the selection of pest resistance very difficult.

In this work, we have explored antifeedant synergistic effects against relevant target pests (Spodoptera littoralis and Myzus persicae) for binary combinations of selected EOs in absence/presence of a coadyuvant (an additional EO at 0.1%). These EOs have been obtained from experimentally cultivated plants with stable chemotypes.

Our results showed that the presence of adjuvant modified the activity: more active combinations were found with the adjuvant Rosmarinus officinalis. The effects were species dependent, with the aphid M. persicae being the most sensitive insect to the EO mixtures tested. However, more synergisms were observed against the leat sensitive insect, S. littoralis. The synergistic effects depend on the interactions between the major and minor components of the oils and allow for complex mixtures with strong effect at low concentrations in order to reduce possible pest resistance selection.

Keywords: Essential oils, Synergism, coadjuvant, antifeedant, Spodoptera littoralis, Myzus persicae

## O16: ASSESSMENT OF BIODEGRADABLE BIOPOLYMERS COMBINED WITH PLANT GROWTH-PROMOTING BACTERIA FOR SUSTAINABLE AGRICULTURAL APPLICATIONS

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Microbial biostimulants, composed of plant growth-promoting bacteria (PGPB), play a pivotal role in sustainable agriculture. However, there is a growing need to develop efficient delivery systems to ensure their successful establishment in the rhizosphere and to maximize their beneficial effects on crop productivity. The aim of this study was to evaluate different biodegradable biopolymers as carriers for PGPB.

Two biodegradable materials were developed using pectin, cellulose, and various co-formulants, all fully biodegradable and designed to be produced from food waste within a circular economy framework. When tested alone on Lactuca sativa (lettuce), the biomaterials had no impact on plant development, although pigment content measurements suggested a stress response. To explore potential synergistic strategies, spores of a PGP Bacillus strain were incorporated in the biopolymers. The strain remained viable within the biopolymer matrix up to one month of storage. A second PGP strain from the Rhizobium genus was also tested but could not survive in neither of the two biomaterials developed. Thus, as an alternative delivery strategy, it was encapsulated in alginate beads, a conventional bio-carrier. When applied to soil, the alginate-encapsulated Rhizobium led to significantly higher bacterial recovery from plant roots compared to direct inoculation into soil, indicating improved rhizosphere colonization efficiency. Colonization dynamics of both PGPB were monitored using fluorescently tagged strains.

While both strains induced plant growth-promoting effects when applied directly to the soil, the delivery of the Bacillus strain via the biopolymers and of the Rhizobium strain via alginate beads resulted in a significant decrease of such PGP effect.

These findings highlight the importance of carefully tailoring suitable bio-carriers to deliver viable bacterial biostimulants without affecting plant development, ensuring both sustainability and crop performance.

Key words: biostimulant delivery - biopolymer - PGPB - lettuce

## O17: PLANT DEFENCE ELICITATION DURING DROUGHT IN TOMATO: BRIDGING THE GAP BETWEEN LABORATORY AND FIELD

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Plant elicitors (PE) have the ability to activate pattern triggered immunity (PTI), via their recognition by pattern recognition receptors (PRRs). PE perception results in a series of events including reactive oxygen species (ROS) production, mitogen-activated protein kinase (MAPK) cascades and defense molecules accumulation. An elevated levels of PRR and MAPK transcripts are observed (Beckers et al., 2009; Li et al., 2014) and more generally, a large number of genes are commonly upregulated by diverse PEs (Li et al., 2016). Despite promising results in the controlled environment of the laboratory, many PEs show limited efficacy in the field. This lack of success can be attributed to the exposure of plants to various stresses in dynamic and changing field environments, which impacts the efficacy of applied PEs.

Abiotic stresses, such as drought, can impact plant defence by altering gene expression (Zarattini et al., 2021) and some players of the plant immune system are also implicated in the response to environmental stresses, such as the MAPKs cascades (Shi et al., 2025) or ROS production (Nadarajah, 2020). Therefore, to better understand how the environment influences plant defence elicitation, we studied tomato PTI activation with different PEs under optimal and drought conditions. We analysed ROS accumulation and gene expression level of PRRs, MAPKs and PR proteins. We are also currently assessing tomato resistance to *Pseudomonas syringae* pv. *tomato* DC3000 after PE treatments under both conditions. Our first results, in optimal conditions, shows that treatments with different elicitors (flg22 and chitosan) impact the expression of specific PRR and MAPK genes. Rather than a common response from all elicitors, a distinct response of tomato plants to each PE is observed.

Key words: plant defence, elicitors, drought, pattern recognition receptors, mitogen-activated protein kinase, reactive oxygen species.

## O18: LIPOPEPTIDES: A PROMISING FUTURE ALTERNATIVE TO COPPER AND SULFUR TREATMENTS FOR APPLE SCAB CONTROL

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Professional orchards in organic farming are highly dependent on copper and sulfur for treatments. Few effective alternative solutions to these substances exist, and the sector has high expectations in this regard. Our research aims to develop alternative solutions to copper-based products currently used in organic farming, particularly for disease control in orchards, as illustrated by the studied case: Apple scab disease caused by *Venturia inaequalis*. This ascomycete fungus causes black or brown lesions on the surface of leaves, buds, or fruits. This fungal infection leads to considerable crop losses by reducing yield and fruit quality, inevitably resulting in significant financial losses for agricultural producers. The *in vitro* study of antifungal activity on *Venturia inaequalis* allowed us to select among a set of 11 lipopeptides produced by bacteria or fungi, the six most effective compounds: fengycin, surfactin, mycosubtilin, colistin, polymyxin B and caspofungin. These six compounds were tested *in vivo* on apple seedlings in combination or not with bicarbonates. Mycosubtilin gave the best protection. Fengycin, caspofungin and a mixture of surfactin and fengycin also significantly reduced scab symptoms. Preventive treatments with mycosubtilin in orchard also reduced scab symptoms on foliage compared to the control during the 2023 season. So far, mycosubtilin appears to be the most promising alternative, potentially reducing or even replacing copper applications in organic farming treatments.

Key words: Apple scab, Venturia inaequalis, Lipopeptides, Organic farming, Biocontrol, Copper alternatives, Mycosubtilin, Antifungal treatments

<sup>\*</sup>These authors equally contributed to the work.

## O19: RHAMNOLIPIDS, PROMISING BIOSURFACTANTS FOR SUSTAINABLE AGRICULTURE: PROTECTIVE AGENTS AGAINST BRANCHED BROOMRAPE AND BIOSTIMULANTS FOR BRASSICACEAE.

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Rhamnolipids (RLs) are glycolipids produced naturally by bacteria of the genus *Pseudomonas* and *Burkholderia*. These surfactant molecules have been considered for agricultural applications for several years, due to their ability to stimulate natural plant defense mechanisms and their direct antimicrobial properties. RLs can also help to improve the health and quality of agricultural soils, and the formulation of agricultural products. Their low toxicity and biodegradability support this interest. Produced by fermentation, they have been shown to be effective against a number of diseases affecting vines, rapeseed, tomatoes, peas, maize, etc. Their mode of action, targeting the membrane of micro-organisms, reduces the risk of resistance development.

On rapeseed, GEC and BIOPI laboratories have obtained preliminary data showing that RLs are efficient to control branched broomrape, in particular *Phelipanche ramosa*, an obligate parasitic weed of crops. RLs inhibit the germination of *P. ramosa* seeds and induce the necrosis of the parasite in the first steps of its development in rapeseed-broomrape co-cultures. The evaluation of the protection efficiency of RLs against broomrape on different varieties of this host plant is under progress, while their mechanisms of action, notably on cell wall modifications, are also being studied.

In addition to their value in biocontrol, recent literature suggests that RLs may have biostimulation properties, with beneficial effects on growth and tolerance to abiotic stresses in several plant species. In this project, RLs are being evaluated as biostimulants on the two Brassicaceae plants, rapeseed (*Brassica napus* subsp. *napus*) and *Arabidopsis thaliana*. The effect of RLs is being assessed on seedlings and whole plants under different application conditions with or without abiotic stress. Molecular and metabolism changes in aerial and root parts of plants are being studied to unravel the mechanism of action behind the effect of RLs.

Key words: biosurfactants, rhamnolipids, biostimulation, biocontrol, Brassicaceae, Phelipancheramosa

## **Session 4:**

# Systems and molecular biology, digital tools and AI

**GUEST: TILMANN WEBER** 

#### **Oral GUEST 5**

## ORAL GUEST 5: THE MOLECULAR TREASURE HUNT: GENOME MINING TECHNOLOGIES FOR SUSTAINABLE APPLICATIONS

#### **Tilmann Weber**

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The genetic potential of microorganisms to synthesize specialized metabolites by far exceeds the number of experimentally observed molecules. With decreased costs of obtaining high-quality genome sequences, in silico genome mining has become an indispensable tool to complement the classical chemistry-centered approach to identify and characterize novel secondary / specialized metabolites. Bioinformatics platforms, such as the open-source genome mining pipeline antiSMASH (https://antismash.secondarymetabolites.org)¹, which we develop in collaboration with the group of M. Medema (U. Wageningen, Netherlands) give us convenient access to assess the genetic potential of microbial producers of natural products. Extensions, like BGCFlow² allow to perform comprehensive studies on hundreds to (ten)thousands of genomes. One such dataset are genomes of our proprietary "NBC collection", which contains actinomycetes primarily isolated in Denmark and adjacent countries. We have sequenced more than 1000 species with hybrid illumina/ONP technology that allowed us to assemble high quality genomes³ and perform large-scale pangenomics analyses.⁴

To make practical use of such datasets, efficient engineering approaches for the specialized metabolite producers, in our case filamentous actinomycetes, are required. We have therefore developed an extensive CRISPR-based toolkit for streptomycetes.<sup>5-7</sup>

These tools, in combination with CRISPR-genome editing, BGC cloning and expression methods, allowed us to identify and characterize various BGCs, for example the pteridic acids BGC, which codes for compounds inducing salt- and drought resistance in various plants.<sup>8</sup>

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# O20: STILBENOIDS AS PROMISING ENVIRONMENT-FRIENDLY NATURAL PRODUCTS TO MINIMIZE THE CONTAMINATION OF WHEAT AND GRAPEVINE WITH TOXIGENIC FUNGI AND ASSOCIATED MYCOTOXINS

## Key insights as regards F. graminearum and its mycotoxins

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Fusarium graminearum and Aspergillus carbonarius, are the most frequently pathogens found in cereal and grape crops in Europe, respectively. In addition to direct losses related to yield reduction, these fungi pose potential health risks to animals and humans due to the production of mycotoxins, deoxynivalenol and ochratoxin A, respectively.

The goal of the StilDeTox project\* was to investigate, potentiate and exploit the biological activity of stilbenoid extracts to develop environmental-friendly solutions to counteract the growth of *F. graminearum* and A. *carbonarius* and the production of their mycotoxins. Driven by environmental issues, the project investigated vine by-products as stilbenoid extracts sources with the aim to contribute to the valorization of this biomass, which is currently neglected or burned as an energy source. The project also privileged the use of green extraction procedures.

The *in vitro* efficiency of the eco-extracts obtained from grapevine by-products (canes, trunks, roots) was assessed against fungal growth and mycotoxin production by *F. graminearum*. To identify the compounds responsible for this bioactivity, an original strategy combining both untargeted metabolomics with molecular network and bioguided fractionation was implemented and has led to evidence the key contribution of oligomeric stilbenes, in particular vitisin B.

The antifungal and antimycotoxin activity of stilbenes was then confirmed, using pure molecules (resveratrol, a monomer and vitisin B, a tetramer). The higher efficacy of vitisin B compared to that of resveratrol was demonstrated.

To deeper the knowledge on the mode of action of stilbenes, multi-omics approaches associating mRNA-seq-based transcriptomics and LC-MS/MS-based non-targeted metabolomics have been conducted. Results showed the complexity of the modes of action of vitisin B which affects the primary and secondary metabolism of *F. graminearum*.

Altogether, our data support, on the one hand, the power of metabolomics approaches to identify active molecules occurring in natural extracts and to study their mechanisms of action and, on the second hand, that grapevine by-products could be promising sustainable sources of bioactive compounds for controlling *F. graminearum* and minimizing the contamination of cereals with mycotoxins.

Key words: Fusarium graminearum – Type B trichothecenes – natural extracts – viticulture waste – stilbenoids - biofungicide

<sup>\*</sup> StilDeTox is an ANR project, ANR-19-CE21-0007.

## O21: UNRAVELING BIOFUNGICIDE MODES OF ACTION THROUGH METABOLOMIC PROFILING

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In response to growing concerns about the environmental and health impacts of pesticides, biosolutions are emerging as promising alternatives for crop protection. In this context, AkiNaO developed "Inulagreen", a plant extract derived from *Dittrichia viscosa*. It specifically *targets Monilia laxa*, the fungal pathogen responsible for brown rot fruit trees. However, like many biosolutions, its mode of action (MOA) remains poorly understood, mainly due to the complex chemical composition of natural extracts. Yet, understanding the MOA is essential to anticipate the emergence of resistance, assess potential risks to non-target organisms, and optimize field application strategies.

In the absence of genomic data on *M. laxa*, a multiplatform metabolomics approach was implemented to explore the biochemical effects of Inulagreen on the pathogen's metabolism. Targeted analyses were performed on lipids (GC-MS, LC-MS, GC-FID) and energy cycle metabolites (IC-MS). In addition, untargeted approaches using UHPLC-HRMS and IC-MS were used to cover the central metabolism of the fungus.

One strategy was to compare the metabolomic profiles of fungi treated with Inulagreen to those treated with conventional fungicides with known MOAs. The hypothesis is that similar MOAs will induce comparable metabolic perturbations. A to)tal of twelve treatments were tested, including Inulagreen, with at least two fungicides per MOA and five biological replicates per condition. The data were analyzed using hierarchical clustering, revealing similarities between Inulagreen and certain fungicides.

The results suggest that Inulagreen interferes with ergosterol biosynthesis, a key component of fungal cell membranes, potentially altering their permeability. Similarities were also observed with a fungicide targeting  $\beta$ -tubulin, suggesting a possible multi-site mode of action.

Keywords: Biofongicide, Monilia laxa, Mode of action, Metabolomic profiles

## O22: REVEALING THE ECOLOGICAL ROLES OF SPECIALIZED METABOLITES IN RAPESEED SEED EXUDATES

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Biocontrol strategies are being developed to identify biobased alternatives to synthetic pesticides for seed treatment. The spermosphere, the microenvironment surrounding germinating seeds, plays a crucial role in early plant development. It comprises primary and specialized metabolites, other seed-exuded molecules such as peptides and microRNAs, and the microbial communities that interact with them

In this study, we comprehensively characterized the seed spermosphere of ten Brassica napus (rapeseed) genotypes grown under two different crop management systems using GC/MS and LC-MS/MS metabolomic techniques. Glucosinolates (GSLs) were among the most abundant metabolites detected in seed spermosphere showing large variations among genotypes. While the role of GSLs in plant-herbivores interactions is well established, their ecological function in seeds and seed exudates remains poorly understood.

We mapped all identified GSLs and their degradation products (isothiocyanates) to their biosynthetic pathways, and quantified their concentrations in both seeds and related exudates. Subsequent bioassays with exudates from rapeseed accessions with contrasting GSLs contents on several seed pathogens (bacteria and fungi) revealed that the high-GSL-concentration genotype exhibited the strongest inhibitory effect against some seed pathogen. Several GSL and isothiocyanate compounds were tested for their specific inhibitory effects on pathogen growth. Furthermore, metabolomic, transcriptomic and proteomic analyses are underway to study genes and enzymes involved in GSL biosynthesis, transport, degradation and decoration during seed exudation.

These data uncovered a large metabolic diversity in rapeseed spermosphere and highlighted a potential ecological role for GSLs and their degradation products in seed-pathogen interactions during germination.

Key words: seed exudates, glucosinolates, biocontrol, specialized metabolites, metabolite distribution

# O23: GENOME MINING REVEALS NOVEL SECONDARY METABOLITES IN NON-RHIZOSPHERIC PSEUDOMONAS STRAINS, UNDERLINING THEIR ROLE AS PLANT GROWTH-PROMOTING BACTERIA (PGPB)

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This work combines genomic and phenotypic analyses to investigate the plant-growth-promoting and biocontrol properties of non-rhizospheric *Pseudomonas* strains isolated from water samples collected from granite soil in the Vosges Mountains of France. Three *Pseudomonas* strains, initially screened for their antioxidant potential, exhibited key plant-beneficial traits, including biofilm formation, phosphate solubilization, phytohormone production, iron chelation, and antimicrobial activity. Genomic analyses revealed biosynthetic gene clusters responsible for the synthesis of several bioactive compounds, including hydrogen cyanide, lipopeptides and siderophores. The production of these secondary metabolites was confirmed through experimental bioassays. The biostimulant effect of those strains was tested on tomato seed germination and the biocontrol activities were assessed against several fungal and bacterial phytopathogens.

Interestingly, our strategy allowed the identification of a single novel pyoverdine variant produced by 2 genetically distinct *P. protegens* strains. In addition, a *P. fragi* strain produced a new siderophore lipopeptide structurally similar to marine amphiphilic siderophores. By focusing on non-rhizospheric bacteria, this research highlights the potential of the aquatic environment as a valuable reservoir of beneficial bacteria with novel bioactive metabolites. These findings demonstrate that *Pseudomonas* strains exhibit unique traits with strong potential for sustainable agricultural applications.

Key words: PGPB, Biocontrol, Genomic analysis, Pyoverdine, lipopeptides, Non-rhizospheric bacteria, Pseudomonas

# O24: A BACTERIAL GENOME-WIDE ASSOCIATION STUDY APPROACH ADAPTED TO INTRASPECIFIC MICROBIAL DIVERSITY IDENTIFIES NEW PLANT GROWTH-PROMOTING FACTORS

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Plant microbiota includes, among others, Plant Growth-Promoting Bacteria (PGPB) that have the potential to mobilize nutrients to the plants, alleviate abiotic stresses, and provide protection against several pathogens. While the beneficial effects of PGPB are highly influenced by climatic conditions, soil conditions, and microbial strain identity, they also depend on the host plant genotype, thereby affecting their use in a wide range of agricultural conditions. While the use of 'omics' methodologies and mutational studies has revealed key genes underlying the benefits conferred by a PGPB on a plant, studies reporting the genetic and molecular mechanisms associated with natural variation of a given PGPB remain scarce.

To better exploit the beneficial effects of members of the microbiota and improve their application, it is necessary to explore the natural genetic variation of the plants-commensal bacteria interactions. By studying native Arabidopsis thaliana - microbiota interactions, we identified strong 'host genotype × bacterial genotype' interactions for diverse vegetative growth-related traits. We set up a Genome-Wide Association study to establish a genomic map of local adaptation of A. thaliana in response to the main members of its leaf microbiota. On the bacterial side, the long-read-based genome of 74 strains of Pseudomonas siliginis revealed extensive genomic diversity. We then developed a new methodology in bacterial association mapping based on multiple reference genomes, which revealed a complex genetic architecture dependent on both A. thaliana genotype and growing conditions.

Our work illustrates the under-exploited potential of intraspecific diversity in plant-microbiota interactions. Our objectives are now to establish a microbial genomic map of regions associated with plant growth promotion, which could in turn open up new prospects for the design of intraspecific microbial synthetic communities and their potential applications.

Key words: Ecological genomics - microbiota - Pseudomonas siliginis - Arabidopsis thaliana - GWA mapping - intraspecific diversity

## O25: PLANT-MICROBIOME INTERACTIONS UNDER A CHANGING WORLD

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Climate change and land-use intensification pose a major threat to many agro-ecosystems, leading to losses in crop yield as well as species loss and alterations in community structure. Plants may adapt to stress situations, but this can lag rapid anthropogenic changes to the environment. Our data show that natural modulations of the microbiome using native, harsh, and wild habitats consortia directly affect plant phenotype and performance, thus increasing plant stress tolerance. The microbiome extends host evolutionary potential and understanding how plant microbiome holobionts function in native, harsh, and wild habitats and how rhizobacteria mediate plant and ecosystem biodiversity in the systems, enables us to identify key factors for plant fitness [1-3]. A systems approach to engineering microbial communities by connecting host phenotype adaptive traits would help us to understand the increased fitness of the crop holobionts supported by genetic diversity.

Identification of genetic loci controlling the interaction of beneficial microbiomes will allow the integration of genomic design into crop breeding programs. It is important to understand the structure of microbial community, functional and environmental patterns, enzyme pathways used and how are they activated. DNA and RNA based microbial community sequencing analysis methods are discussed.

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## Session 5:

# MiCropBiomes Session 5: Plant-microbiome interactions and (a)biotic stresses

**GUEST: VICTOR CARRION** 

## **Oral GUEST 6**

## ORAL GUEST 6: DECODING THE HIDDEN SECRETS OF PLANT MICROBIOMES

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Plant roots harbor an extraordinary diversity of microorganisms, with cell densities often surpassing those of the plant itself. Studies have shown that the plant microbiome plays a key role in enhancing plant tolerance to both abiotic (e.g., drought) and biotic stresses (e.g., disease), while also contributing to nutrition, growth, and development [1]. However, the functions and mechanisms of most plant-associated microorganisms remain poorly understood. Recent advances in 'omics technologies have expanded our knowledge of microbiome diversity and function, shedding light on the complex interactions within plant-associated microbial communities.

This presentation will provide new findings on the role of rhizosphere and endosphere bacteria in protecting plants from biotic and abiotic stresses. Additionally, we introduce bacLIFE [2], a novel bioinformatics tool designed for genome annotation, large-scale comparative genomics, and the prediction of lifestyle-associated genes (LAGs) in bacteria. Using bacLIFE, we analyzed 16,846 genomes from the Burkholderia/Paraburkholderia and Pseudomonas genera, identifying hundreds of genes potentially linked to plant pathogenic lifestyles. Site-directed mutagenesis and plant bioassays confirmed that six predicted LAGs are indeed involved in the phytopathogenic lifestyle, including genes encoding a glycosyltransferase, extracellular binding proteins, and homoserine dehydrogenases. An overview will be given on the wealth of genes and functions of the plant microbiome.

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# O26: CULTURE-DEPENDENT AND METABARCODING CHARACTERIZATION OF THE ENDOPHYTIC MICROBIOTA (FUNGI AND BACTERIA) ASSOCIATED WITH BETHENCOURTIA PALMENSIS AND ITS BIOTECHNOLOGICAL POTENTIAL

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Bethencourtia palmensis (Bees) Choisy (Asteraceae), is a rock-dwelling species endemic to the islands of Tenerife and La Palma (Spain) that contains unique insecticidal silphinene sesquiterpenes (1). B. palmensis hosts a complex fungal endophytic community with demonstrated biotechnological potential (2). This study aimed to characterize the microbiota associated with B. palmensis by metabarcoding (fungal and bacterial) and culture-dependent (fungal) approaches. ITS2 and 16S rDNA metabarcoding allowed the identification of 90 fungal and 553 bacterial ASVs (27 and 142 genera, respectively). A large proportion of the endophytic fungal genera detected by metabarcoding were not recovered by microbiology methods, while some isolates were not detected by ITS2 metabarcoding, highlighting the complementarity and limitations of both methods (3). Among fungi, Alternaria, Stemphylium, and Cladosporium showed the highest overlap between both approaches, confirming their cosmopolitan and ubiquitous biology. Among the bioactive, cultured genera from B. palmensis, some overlapped with those detected by ITS2 metabarcoding, including the most (Alternaria, Aureobasidium and Cladosporium) and the less (Stemphylium or Penicillium) abundant. Additionally, 16S rDNA analysis of bacterial communities revealed genera with recognized potential for the production of antimicrobial, plant growth-promoting, and bioremediation-related metabolites, including Massilia, Pseudomonas, Rosenbergiella, Mesorhizobium, Pantoea, and Sphingomonas, among others. Furthermore, β-diversity analyses suggested that the bacterial community composition may be more sensitive than the fungal microbiota to organ-specific factors. Altogether, these results confirm B. palmensis as a valuable source of microbial diversity with applications in natural product discovery, and support the use of metabarcoding as a tool to guide the selection of promising host plants for future bioprospecting efforts.

<u>Keywords:</u> plant endophytes, bioprospection, metabarcoding, diversity

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## O27: RHIZOSPHERIC PSEUDOMONAS: BIOLOGICAL WEAPONS AGAINST OROBANCHES?

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Broomrapes are obligate root holoparasitic plants belonging to Orobanche and Phelipanche genera in the Orobanchaceae family. They cause significant damage in Europe, particularly in Eastern Europe and in the Mediterranean region, leading to important yield and economical losses on a wide range of crops worldwide (rapeseed, sunflower, tobacco, tomato, etc). So far, crop protection strategies against parasitic weeds are mostly based on the use of non-selective chemical pesticides in combination with peculiar plant genotypes 'with good behavior' (tolerant) or of non-host plants in rotation to induce Orobanche seed suicidal germination. In order to contain the levels of broomrape seeds in field, there is an urgent need to develop microbial solutions of biocontrol for reducing broomrape seed stocks in fields. During the WeedsBiocontrol project (ANR- ECOM-2019-0002 Ecophyto-Maturation-2019), we explore the use of biocontrol rhizobacteria of the Pseudomonas genus as a biological weapon to limit broomrape infestation. We identified candidate strains with Orobanche Germination Inhibition (OGI) activity. By combining comparative genomics and metabolomic profiling, some of these OGI metabolites were identified. Biocontrol strains with the greatest OGI activity were then produced first in lab-scale fermenters, followed by scaling up to a 100L production pilot. Various growth conditions were tested to optimize cell biomass. Subsequently, greenhouse assays evaluated the protective effects on rapeseed against P. ramosa and on sunflower against O. cumana, allowing the selection of the most effective strain for optimized pilot production. Practical application methods (product quantity, number of applications, optimal plant stage) were defined. Then, field experiments were conducted in Western (rapeseed) and Southwest France (sunflower), assessing efficacy in naturally contaminated fields. While the selected Pseudomonas strain showed a significant reduction of parasitism on rapeseed or sunflower, under greenhouse conditions (in more than 10 assays), field trials were less conclusive. The high variability of broomrape infestation levels within a same plot or territory, and across years, presents significant challenges for field experiments, complicating efforts to demonstrate the effectiveness of agroecology strategies.

Key words: Broomrape, Biocontrol, Pseudomonas, Metabolomics, Field trials

# O28: DECIPHERING THE POTENTIAL OF BACILLUS STRAINS IN PROMOTING WHEAT GROWTH UNDER DROUGHT STRESS AND FUSARIUM BIOCONTROL

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Drought stress and Fusarium diseases are among the primary factors limiting wheat yield. Concerns over the risks that chemical fungicides pose to human, animal, and environmental health have prompted the scientific community to explore safer and more sustainable alternatives. Recently, the use of drought-tolerant plant growth promoting bacteria with biocontrol potential has been promoted as a promising and ecofriendly practice. In this sense, the Bacillus genus is of particular interest due to its stress tolerance, production of antifungal metabolites, and plant growth promoting capabilities. This research evaluated the potential of three Bacillus strains to promote wheat growth under combined drought stress and Fusarium infection. Three Bacillus strains (TII-19, TCG-6, and TMG-6), isolated from Cuban wheat varieties, were characterized in vitro for their drought tolerance by assessing growth under different concentrations of PEG-6000 and their biofilm production under drought stress. Moreover, the biocontrol potential of theses strains and their metabolites was assessed by evaluating their effect on reducing Fusarium symptoms on wheat spikelets and leaves, as well as on the germination of Fusarium graminearum and Fusarium culmorum. Additionally, The Bacillus strains, along with a consortium of two strains, were tested in pot experiments for wheat growth promotion under stress conditions. Results demonstrated that the strains TII-19, TCG-6, and TMG-6 could grow in Tryptone Soy Broth amended with 30% (w/v) PEG-6000, exhibiting higher biofilm production under these conditions. The strains and their metabolites effectively reduced the severity of F. graminearum and F. culmorum on wheat spikelets and leaves. In vitro, bacterial metabolites delayed the germination of both fungal strains. In planta, bacterized plants showed the highest average plant size and increased leaf and root production, both under irrigated and drought stress conditions. Under drought stress, plants treated with TMG-6 and the bacterial consortium had a size similar to control plants under irrigated conditions. The detached leaf bioassay confirmed that Bacillus strains reduced F. graminearum lesions on wheat leaves. These findings highlight the beneficial effects of Bacillus strains in promoting wheat growth and providing bioprotection under stress conditions.

Key words: Bacillus, drought stress, Fusarium, combined stress, wheat

# O29: TEMPERATURE-DEPENDENT EFFECT OF CYCLIC LIPOPEPTIDES PRODUCED BY PSEUDOMONAS FUSCOVAGINAE UPB0736 IN BIOCONTROL

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Pseudomonas fuscovaginae UPB0738 was originally isolated from sheath rot-infected rice plants from the highland in Madagascar. It belongs to the Pseudomonas asplenii subgroup under the Pseudomonas fluorescens group, known for plant-beneficial rhizobacteria and biocontrol strains. Analysis of the disease-suppressive Cocoyam rhizosphere from Cameroon identified closely related members of the P. asplenii subgroup as beneficial rhizobacteria. Thus, the P. asplenii subgroup harbours both pathogens and biocontrol agents based on plant niche and environmental conditions. Pseudomonas strains produce different bioactive metabolites like Cyclic Lipopeptides (CLPs). CLPs are composed of an oligopeptide macrocyclic ring linked with a fatty acid tail and are involved in pathogenicity, antimicrobial activity, mobility, and biocontrol. Chemical analysis of UPB0736 has revealed the presence of three CLPssyringotoxin, fuscopeptin, and asplenin. These CLPs are produced at a higher concentration at a lower temperature. The function of UPB0736's CLPs was evaluated at different temperatures, using bean as a model plant and Rhizoctonia solani as a pathogen. It was observed that at lower temperature (18°C) UPB0736 offers good biocontrol, which is syringotoxin-mediated in vitro and both syringotoxin and fuscopeptin-mediated in plants. However, this biocontrol capacity declined with increasing temperature and was completely lost at high temperature (28°C). The loss of biocontrol at 28°C was CLP-mediated, as the mutants lacking CLP production provided improved protection to the plants from the pathogen stress. These findings reveal a temperature-dependent role of UPB0736 CLPs, providing biocontrol at low temperatures but masking biocontrol at higher temperatures.

Key words: Pseudomonas fuscovaginae, Cyclic lipopeptides (CLPs), Biocontrol, Pathogenicity, Temperature-dependent behavior

## O30: THE EFFECT OF RHIZOSPHERE ON THE RESISTANCE OF WILD TOMATO RELATIVES TO VERTICILLIUM DAHLIAE

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The soilborne phytopathogenic fungus Verticillium dahliae (Vd) is difficult to control as it infects many economically important crops and chemical control is inefficient. Exploring the dynamics of the rhizosphere, the narrow zone of soil directly influenced by root secretions and associated soil microbes, is a promising approach to develop strategies against this pathogen. Here, we aim to characterize what makes two wild tomato relatives - Solanum pimpinellifolium (W1) and S. lycopersicum x S. cheesmanii (W2) - resistant to Vd, compared to cultivated S. lycopersicum tomato cv. Moneymaker (Mm). First, we confirmed that W1 and W2 are more resistant to Vd race 1 than Mm when grown in potting soil. We collected root exudates from both uninfected and Vd-infected Mm, W1, and W2 plants to assess their role in resistance. Metabolomic analysis showed that metabolite profiles differed markedly across treatments. Notably, Vd showed a reduced chemotropic response to W2 exudates, and exudates from Vd-infected W2 plants significantly inhibited Vd spore germination. Next, we evaluated if resistance is maintained when plants are grown in sterile potting soil amended with field soil, and if it is effective against Vd races 1, 2 and 3. When infected with races 1 and 2, Mm displayed the highest susceptibility, W1 showed lower disease severity and W2 exhibited the lowest disease severity, indicating the higher resistance levels of W1 and W2 in microbe-rich conditions. Growth parameters such as plant height and root/shoot ratios supported these observations. Vd race 3 reduced plant height, but caused few visible symptoms in all cultivars. Next, we will collect rhizosphere samples from W1, W2 and Mm to test their effect on tomato and Arabidopsis, and isolate microbes contributing to disease resistance. Our results highlight that wild crop relatives possess untapped plant traits and beneficial microbes that can be harnessed to sustainably enhance resistance to soilborne pathogens.

Key words: metabolomics - plant disease - rhizosphere - Verticillium dahliae - Verticillium wilt - wild tomato relatives

# O31: TOWARDS A NOVEL BIOPESTICIDE FROM BACILLUS VELEZENSIS 32A: UNVEILING THE SYNERGISTIC POTENTIAL OF DUAL PURIFIED POLYKETIDES AND A SIDEROPHORE FOR CROWN GALL BIOCONTROL WITH INSIGHTS INTO THEIR LOW CYTOTOXICITY

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Risk assessment of microbial-derived biopesticide is essential for international and EU approval processes of plant protection products. In recent investigation using deficient mutants, we highlighted for the first time the strong synergetic effect of difficidin and macrolactin A, as well as the significant implication of bacillibactin, a siderophore, in the management of crown gall disease caused by *Agrobacterium tumefaciens*. Therefore, the current work aims to purify these molecules from *B. velezensis* 32a to confirm their anti-*Agrobacterium* effect and evaluate their cytotoxicity on Vero cells, a kidney cell line derived from the African green monkey.

Initially, to enhance the purification of active metabolites, a combined ultrafiltration/diafiltration process was performed, followed by semi-preparative-reversed phase high-performance liquid chromatography (RP-HPLC-UV) fractionation. Secondly, the resulting fractions were tested for their antagonistic activity using the agar well diffusion assay and analyzed by RP-HPLC coupled with mass spectrometry. Results showed that none of the other fractions exhibited activity; only the fraction containing bacillibactin was active when tested alone. However, a combination of fractions containing macrolactin A and oxydifficidin revealed a significant inhibitory effect on the pathogen. Therefore, these findings support our previous results from mutants, which demonstrated a synergistic effect between macrolactin A and oxydifficidin, and the role of bacillibactin in the antagonistic activity. Finally, cytotoxicity assessment of the individual fractions containing the three active molecules, as well as their mixtures was conducted on Vero cells. Results revealed very low cytotoxicity for both the individual compounds and their combinations, with IC<sub>50</sub> values exceeding 500 mg/L.

These results highlight the safety of the tested compounds and offer insights to inform future regulatory decisions regarding their use as biopesticides.

Key words: Bacillus velezensis - Agrobacterium tumefaciens - Oxydifficidin - Macrolactin - Bacillibactin - Cytotoxicity

## O32: PRIMING WITH BIOLOGICALS TO SUPPORT DROUGHT STRESS TOLERANCE OF CEREALS

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In grain cultivation, both biotic and abiotic stresses can lead to substantial losses in yield and quality. Thereof, drought is a major contributor, responsible for yield reductions of up to 50% (Mirgol et al., 2020). Maintaining grain quality amid unstable climate conditions, coupled with the decreasing availability of synthetic chemical plant protection agents, poses a significant challenge for farmers. As a result, thorough testing of biologicals and biostimulants for their ability to combat abiotic stress and support overall plant health has become urgently necessary. Although a few biologicals and biostimulants were shown to positively affect plant health and resiliance already, the underlying physiological background and genetic determinants are not well understood yet.

Therefore, the aim was to analyse the effects of different biologicals on drought stress for a diverse set of spring barley (*Hordeum vulgare* L.) genotypes. Eight biologicals (Alginure, ASL Kombi Power, CropCover, ErosionControl, Burdock, Giant knotweed, FytoSafe, and Bioplantol mycos V forte) showed positive effects on physiological and yield-related parameters under early drought stress compared to the untreated control. Four biologicals were selected for two-year validation trials with ten genotypes in greenhouse and field experiments. Drought stress started at BBCH 13 for six weeks with and without spray-application of the different biologicals. Under greenhouse condition, one biological significantly (p<0.05) increased chlorophyll content in four genotypes. Another notable result was the significant (p<0.05) increase of grain biomass among all genotypes after treatment with three of the biologicals under drought stress. The raw protein content was significantly (p<0.05) increased by the use of one biological under drought stress. For all traits, the genotype showed significant (p<0.001) effects and genotypes responded differently to the biological application. Our results provide valuable insights into the genotype specific effects of biologicals and indicate a potential strategy for reducing the yield losses caused by early drought stress.

Key words: barley, drought, biologicals, biostimulants

## **Session 6:**

## MicropBiomes Session 6: Management of plant microbiota and integrated strategies

**GUEST: MONICA HÖFTF** 

## O33: ACTION OF PYTHIUM OLIGANDRUM ON GRAPEVINE TRUNK DISEASES AND ITS IMPACT ON MICROBIAL COMMUNITIES

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Grapevine Trunk Diseases (GTDs) have become a major challenge for viticulture.

Since the ban of sodium arsenate in 2001, the need for alternative methods, such as biocontrol, has become increasingly urgent. One promising microorganism is the oomycete *Pythium oligandrum*, which is known to improve plant health by increasing natural defences and reducing disease incidence by up to 40%. In order to use this microorganism in vineyards, it need first to be formulated and tested to ensure its safety.

The aim of this study is to demonstrate the efficiency of the bio-fungicide at different spatial scales (from the greenhouse to the vineyard, including the nursery grapevine propagation) but also to better understand the effects of microorganism-based treatments on existing microbial communities, essential to ensure sustainable and safe vineyard management practices.

In a greenhouse under controlled conditions, the treatment applied showed reduced necrosis induced by the pathogens P. chlamydospora and N. parvum by 47% and 67% respectively.

In nursery, with commonly used cultivars, these solutions consistently demonstrated lower levels of natural contamination by GTD-plant pathogenic fungi monitored by qPCR analyses compared to the untreated control.

The *P. oligandrum* biopesticide had minimal effect of on the total microbial community of the vine rhizosphere. However, the treatment appeared to enhance several beneficial taxa that play a role as plant growth promoting rhizobacteria (PGPR) or biocontrol agents (BCA).

In the vineyard, 3 years of treatments were managed (2021- 2022 and 2023) with different formulations (solid or liquid). For one of the field trial (at Château Luchey), a diminution of Esca symptomes had taken place for the vines treated with the liquid biofungicide, while with the solid formulation, the disease has not progressed but on the contrary seems to reverse (vines went from symptomatic to healthy).

Key words: Grapevine Trunk Diseases - biocontrol - biopesticide - Pythium oligandrum - microbial communities

# O34: NITRATE AS A KEY NITROGEN SOURCE TO ALLOW TRICHODERMA HARZIANUM AND BACILLUS VELEZENSIS COMPATIBILITY IN BIOSOLUTION DEVELOPMENT

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The co-application of compatible microbial strains offers a promising, eco-friendly alternative to chemical pesticides in sustainable agriculture. This study investigates the interaction between Trichoderma harzianum MUCL29707 and Bacillus velezensis GA1 to develop a stable, nitrate-based co-formulation.

While B. velezensis inhibits T. harzianum in nutrient-rich media through the production of antifungal lipopeptides, both strains were able to coexist under nitrate-limited conditions. T. harzianum metabolized nitrate into amino acid-like compounds that supported the growth of B. velezensis, which cannot directly assimilate nitrate. Under these conditions, B. velezensis suppressed lipopeptide production—not due to limited growth—but as a result of direct physical contact with the fungus, as confirmed by assays using Trichoderma supernatant.

Based on these findings, a formulation including both strains and nitrate was developed and patented. The biostimulant potential of this formulation was evaluated using tomato (Solanum lycopersicum) under water stress conditions. Seeds treated with the microbial consortium showed significantly improved germination rates, as well as enhanced root and stem elongation, compared to untreated controls and treatments using either microorganism alone.

In parallel, bioprotection assays were performed against two major tomato pathogens: Botrytis cinerea and Oidium neolycopersici. Treatment with the co-culture formulation provided notable protection against B. cinerea when applied via seed coating and root inoculation. In contrast, no significant protective effect was observed against O. neolycopersici. Furthermore, protective effects were substantially less important when the strains were applied individually, underscoring the synergistic role of the co-culture under nitrate-facilitated conditions. These differential outcomes suggest that the formulation's efficacy depends on the trophic lifestyle of the pathogen, with necrotrophs being more susceptible to the defense-priming effects induced by the microbial consortium.

Key words: Coculture, Bacillus velezensis, Trichoderma harzianum, Nitrate, Biocontrol

## O35: PREBIOTICS AS A SOIL BIOSOLUTION: SHAPING MICROBIAL COMMUNITY STRUCTURE AND FUNCTION

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Soil fertility and productivity are increasingly threatened by degradation, overexploitation, and climate change, highlighting the need for innovative agroecological practices. Among these, prebiotics, a class of biostimulants, are gaining attention for their potential to enhance soil health by modulating microbial communities and supporting plant growth. This study evaluates the functional and structural effects of two commercial prebiotics, K1® and NUTRIGEO L® (referred to as SPK and SPN, respectively), on agricultural soil cultivated with Zea mays L., compared to untreated control soil (SP). Analyses were conducted at two post-application time points: three weeks (D1) and ten weeks (D2). The evaluation included plant growth measurements, soil properties, microbial community composition, enzyme activity, and metabolic diversity.

Both prebiotics significantly altered the soil's physicochemical profile and induced distinct shifts in microbial community structure, enriching beneficial bacterial and fungal taxa associated with plant growth promotion, saprotrophic activity, and symbiosis. SPK favored microorganisms such as Caulobacter and Mortierella globalpina, whereas SPN enriched for Neobacillus and Mortierella minutissima. Functionally, prebiotic application enhanced soil enzymatic activity, with SPN promoting  $\alpha$ -glucosidase and  $\beta$ -galactosidase, and SPK increasing arylsulfatase and phosphatase activities. These microbial and enzymatic changes were linked to improved plant biomass and elevated soil organic and total carbon content, particularly under SPN treatment. Metabolic profiling further revealed functional diversification: SPK stimulated degradation of carbon sources across five chemical groups, while SPN enhanced utilization in three groups.

These findings demonstrate that prebiotic amendments can shape both the structure and function of soil microbial communities, highlighting their potential as biosolutions for enhancing soil fertility and sustainability in agricultural systems.

Key words: Prebiotics, Biostimulants, Soil Fertility, Microbial Community, Enzymatic Activity, Metabolic Diversity

## O36: HARNESSING VERMICOMPOST-BASED BIOSTIMULANTS FOR SOIL REGENERATION AND CROP RESILIENCE

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The Ver'Repair project aims to evaluate the potential of vermicompost-derived biostimulants for soil regeneration and as crop support. Rooted in an agroecological transition framework, the project investigates the agronomic and ecological efficiency of these products, focusing on the enhancement of soil ecosystem services.

A micro-plot field trial on lentils, known for their high agronomic and environmental potential, was conducted under a conventional farming system. The experimental design included plant growth monitoring, assessment of plant-microorganism interaction and a comprehensive characterization of the rhizosphere environment, combining chemical and biological analyses targeting microbial community. The application of biostimulants demonstrated slight but measurable effects on plant growth and rhizobial symbiosis establishment. Biostimulant application also induced significant shifts in rhizospheric microbial communities, favoring the recruitment of beneficial microbial taxa and enhancing microbial activity, by modulating enzymatic activities linked with nutrient cycles. These findings suggest a possible mode-of-action through the stimulation of microbial-mediated nutrient mineralization and availability. The magnitude and nature of these effects varied with the biostimulant formulation, underscoring the complexity of plant-soil-microbiome interactions.

A complementary microcosm experiment was conducted to assess the impact of a biostimulant on crop residue degradation, through the monitoring of carbon and nitrogen mineralization dynamics. The results highlight a differentiated response depending on the biochemical nature of the residues, illustrating the dependence of biostimulant efficacy on the quality of organic substrates. This highlights the capacity of the biostimulant to activate microbial processes even in contexts where organic matter turnover is constrained.

Overall, the Ver'Repair project emphasizes the potential of vermicompost-based biostimulants to enhance soil health and sustain crop growth. It also shows the need for deeper mechanistic understanding and context-specific efficiency assessment, through systemic and multi-scale approach, to foster their thoughtful integration into farming systems and maximize long-term benefits.

Key words: biostimulation, microbial communities, microbial functions, ecosystem services, regenerative agriculture, soil health, crop growth

## Oral GUEST 7

## **ORAL GUEST 7: BIOSOLUTIONS 2025: KEY TAKEAWAYS**

## **Monica Höfte**

## **Posters**

## P1: AGROECOLOGY IMMUNITY FRESK, THE SERIOUS GAME

RMT BESTIM – Training & Communication Working Group

Serious games represent valuable tools for diversifying educational methods, combining learning with enjoyment. Their popularity has grown significantly due to their ability to engage learners and enhance their educational experience. Within this context, a working group from the RMT developed "The Agroecological Immunity Fresk," a serious game designed to raise awareness about the various factors influencing agroecological immunity. The game promotes a systemic, integrated approach, incorporating contextual elements from regulation to consumer awareness. It emphasizes the interconnectedness of plant immunity and development, prompting players to reflect on interactions among various factors affecting plant health and resilience. Participants gain insight into strategies for deploying biocontrol and biostimulant solutions effectively.

This card-based game comes in two distinct versions tailored to different audience levels. The first version, comprising 34 cards, targets the public familiar with basic concepts of plant immunity. The second, more detailed version, is intended for advanced learners or experts.

Testing of the first version began in late 2024 and continues into spring 2025, collecting user feedback to refine the game's experience and educational content. Early testing sessions revealed common misconceptions about biocontrols and biostimulants even among informed participants, demonstrating the game's potential to clarify these concepts interactively.

Structured into four phases, the game addresses plant-soil interactions, plant immunity strategies (including diagnostics and preventive practices), corrective input solutions, and external regulatory and contextual constraints.

French and English editions of the public version are undergoing testing in 2025. The game, along with facilitator guidelines, will be freely downloadable and printable from the RMT website, ensuring broad accessibility and effective educational outcomes.

Key words: serious game - agroecology - immunity - fresk

## P2: LATE BLIGHT, WHEN PLANT EXTRACTS TAKE THE FIGHT!

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Late blight, caused by *Phytophthora infestans*, is a highly damaging disease of tomatoes and potatoes. Due to environmental and health concerns, the use of synthetic fungicides has become increasingly restricted, further complicated by the emergence of fungicide-resistant strains. Therefore, sustainable alternatives for crop protection are urgently required. Biocontrol products derived from plant extracts represent a promising option. This study evaluated the *in vitro* inhibitory effects of a plant extract-based biocontrol product, developed by Biom InnoV, on various stages of the *P. infestans* lifecycle, including mycelial growth, sporangia & zoospore formation and germination. Additionally, nontarget effects were assessed on ubiquitous micro-organisms: four fungi, including some isolated from the phyllosphere and two common bacteria.

Bioassays compared the formulated product (FV) with the plant extract (PE) and a concentrated plant extract (CPE). Results demonstrated complete inhibition of *P. infestans* mycelial growth by FV, while CPE showed moderate effects. Infection structures (sporangia and zoospores) were more susceptible to PE, but FV consistently displayed the highest inhibitory potency. Notably, even at sub-inhibitory concentrations, PE disrupted zoospore germination, causing abnormal phenotypes.

Overall, the plant extract exhibited substantial inhibitory activity against *P. infestans*. FV was notably effective at extremely low concentrations (0.01–0.05%), significantly inhibiting mycelium, sporangia, and zoospores, indicating an enhanced activity of the formulated extract. Non-target microorganisms were affected only at much higher concentrations than those required to control *P. infestans*.

This study underscores the efficacy of plant extract-based biocontrol products in managing late blight with minimal non-target effects, supporting their potential inclusion in integrated disease management strategies.

Key words: biocontrol, Phytophthora infestans, plant extract, sporangia, zoospores, fungi, bacteria

## P3: VITIGUARD: REINFORCING THE VINE'S IMMUNITY FOR MORE SUSTAINABLE VITICULTURE

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The wine industry is currently facing major challenges, including the need to reduce the use of synthetic phytosanitary products. Nevertheless, alternative and sustainable production strategies are not yet widely adopted in french winegrowing systems. Thus, the aim of the project Vitiguard, is to propose innovative practicies for controlling downy mildew and powdery mildew, that are based on schemes that will be validated in the vineyard.

These management strategies will combine biocontrol products, especially plant defense inducers (PDI), and vine varieties resistant to the targeted diseases in order to reduce the use of pesticides, and ultimately help to improve the image of winegrowing.

The technical objectives of the project are as follows:

- Increase the efficacy and optimise the use of PDIs (Messager and Belvine) by combining them with biostimulant products (BS) or biofungicides (BF),
- Facilitate the combination and improve the efficacy of PDIs, by developing an extemporaneous adjuvant with an innovative formulation,
- Characterise by molecular markers the response to PDIs of new varieties resistant to fungal diseases,
- Define the technical itinerary for application of PDIs (+ BS or BF) to resistant varieties,
- Validate the performance of the innovative itinerary on a vineyard plot scale.

The project is supported by France2030.

Key words: biocontrol, plant defense inducer, biostimulant, adjuvant, grapevine, resistance varieties, powdery mildew, downy mildew.

## P4: THE GRAPEVINE LYSM RECEPTOR KINASE VVLYK4-2 IS A KEY PLAYER OF CHITOSAN RECOGNITION

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In a context of sustainable agriculture, the use of biocontrol products is an alternative strategy to protect crops against pathogens. Among these compounds, chitosan has gained notable traction as a natural fungicide and elicitor of plant immunity. This biopolymer is obtained through deacetylation of chitin derived from crustacean shells and fungal cell walls and its dual action is related to its degree of polymerization. Whereas good protection levels against grapevine pathogens as *Plasmopara viticola* and *Erysiphe necator* can be obtained in controlled conditions, the chitosan efficacy is lower in vineyard thus preventing the generalization of this plant protection product. Studying the mechanisms allowing the perception of chitosan by grapevine cells is a key point to understand this partial lack of effectiveness.

Contrary to chitin, the mechanisms underlying the recognition of chitosan, remain poorly investigated. Chitooligosaccharides are typically detected by LysM receptor-like kinases (LYK) at the plasma membrane. Here, we investigated the role of the two grapevine orthologs of *AtLYK4*, component of the tripartite complex for chitin perception in the plant model *A. thaliana*. Using a dual approach consisting of the functional complementation of the *atlyk4/5* double mutant and CRISPR-Cas9 genome editing in *Vitis vinifera*, we showed that *VvLYK4-2* was involved in both chitin- and chitosan-induced immune responses. Furthermore, grapevine *in vitro* plantlets lacking *VvLYK4-2* exhibited a significantly reduced response to chitosan. These findings indicate that this pattern recognition receptor plays a crucial role in the perception of chitosan oligomers and has thus potential for selective breeding purposes.

Key words: grapevine, chitosan, biocontrol, pattern recognition receptors (PRR), immune responses

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## P5: BIOSTIMULANTS AS A LEVER TO DRIVE GRAPEVINE ROOT HOLOBIONT IN RESPONSE TO WATER STRESS

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In a context of climate change, cultivated vines are subject to numerous abiotic stresses and, in particular, to frequent water constraints. The root system is a major component in the plant physiology since it is essential for plant water and nutrient uptake. Its development and architecture can be impacted by abiotic stress and lead to plant decline situations. On the other hand, it could be improved by the use of biostimulants, facilitating root implantation and access to hydro-mineral resources.

The response of the vine to biostimulants can be genotype- and product-dependent, and the effects induced by water constraint and by biostimulation on the vine root microbiota remains less studied. This is why we have phenotyped, in 5 different genotypes of rootstock, the interest of biostimulant commercial products in the grapevine root holobiont functioning and its resistance to water stress.

In our experimental conditions, we demonstrated strong genotypic responses and characterized, for the 3309 C rootstock, molecular effects of both water stress and the biostimulation (in response to a water stress or not). We observed that Osiryl (Frayssinet) induces a clear accumulation of root mineral ions (iron, manganese, magnesium and bore, in particular) associated with a modification of the leaf phenotype (more green leaves). We also observed that Osiryl-biostimulated plants appear to be more resistant to water stress than control (i.e., non-biostimulated) ones, both in terms of phenotype (lower mortality and lesser impact on root dry weight) and physiological functioning (i.e. reduction in carotenoid and optimization of chlorophyll b contents).

Finally, we determined how such physiological modulations can be perceived by the microorganisms associated with the plant (i.e., in the rhizosphere and roots). Indeed, in the context of the development of new agroecological practices, the microbiota (as a part of the plant holobiont) represents an additional source of plant genetic and phenotypic variabilities, which could be exploited by agricultural practices and which it is important to take into consideration.

Key words: grapevine - root holobiont - biostimulation - water stress

## P6: FIELD EVALUATION OF BIOSOLUTIONS-INDUCED PLANT DEFENSES AGAINST WHEAT SEPTORIA TRITICI BLOTCH

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Septoria tritici blotch (STB) is a major fungal disease affecting wheat crops worldwide, leading to significant yield losses. Traditional management of STB relies heavily on fungicides, but their extensive use raises concerns about health and environmental impact and pathogen resistance. An alternative approach is to enhance the plant's natural defenses through the application of biosolutions and biocontrol products. While numerous products have shown promise under controlled conditions, field data remain scarce.

In this study, we aim to evaluate the induction of plant defenses following the application of various biosolutions products in field conditions. Over two years and across different locations, we applied a range of biosolutions and assessed their effectiveness in inducing plant defenses. Various sampling methods were tested, including dry ice, RNA Later, and FTA cards, to ensure robust data collection.

Although our experiments are ongoing, preliminary observations suggest potential benefits in using biosolutions for STB management. These experiments aim to control the increase in the expression of plant defense genes in the field and to evaluate the possibility of using alternative sampling methods to dry ice. This study will contribute to the understanding of biosolutions efficacy in real-world agricultural settings and support the development of sustainable disease management strategies.

Key words: RT-qPCR, biosolutions, fields conditions, defense gene expression

# P7: UNDERSTANDING THE EFFECT OF BIOSTIMULANTS USED IN SEED TREATMENT ON THE EARLY STAGES OF DEVELOPMENT FOR TOMATO AND OILSEED RAPE CROPS

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Biostimulants can be used to enhance metabolic efficiency and thus improve germination or promote plant growth in different crop species (Gupta et al., 2022). The effectiveness of biostimulants often depends on biostimulant type, concentration, and plant species but also on the plant stage when the application is done. In this study we investigated the effects of three commercial biostimulant solutions applied as seed treatment.

Preliminary tests were carried out using imaging tools to identify the concentration at which the solution had the greatest effect on germination. The development of the seedling and its different organs helped to understand the effect of biostimulants. Especially, studying root architecture is very interesting, and it was assessed in the laboratory by Winrhizo, an image analysis software package coupled with a scanner that can be used to measure various parameters such as root length, volume, surface area, diameter, etc... In order to identify the effect of biostimulant solutions on the development of the root system, various samples were taken between 4 and 21 days after sowing. The seedlings were evaluated according to the ISTA rules. The results showed a significant response from some solutions for the two studied species: tomato as vegetable and oilseed rape as agricultural crop.

One solution increased the length of the rape root system by an average of 22%, while another one increased the length of the tomato root system by 34% after just a few days of growth and on the different varieties which were tested.

Biostimulants could have a great impact in seedling root development when applied on seeds but should be specific to crop species and to seedling stage.

Key words: biostimulation - seed treatment - germination - root system development

## P8: AN INNOVATIVE 96-BIOMARKER APPROACH TO RAPIDLY CLASSIFY BIOSTIMULANTS AND NATURAL DEFENSE STIMULATORS

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Current biostimulant classifications related to nutrient use efficiency and the availability ofsoil elements rely on regulatory frameworks originally developed for fertilizers. However, for other categories of claims—such as enhanced tolerance to abiotic stress and improved crop quality—mechanistic characterization tools remain limited. RNAseq-based omics approaches have been proposed, but their high cost and limited scalability restrict their applicability in large-scale, multivarietal, and multi-environment studies. While INRAE has developed and patented a qPFD chip for biocontrol applications targeting defense gene expression in potato and grapevine, no equivalent tool exists for broader biostimulant characterization.

To address this gap, we developed a high-throughput qRT—PCR platform based on a custom 96-gene Fluidigm chip designed for wheat and pea. The selected genes cover key physiological functions, including nutrient assimilation, photosynthetic activity, hormonal signaling, responses to abiotic stress, and defense-related pathways. Marker selection was informed by both literature and RNAseq analyses of diverse biostimulant formulations tested across multiple cultivars. The goal is to identify specific molecular signatures associated with each product. These markers have been validated through a combination of laboratory and fieldexperiments, where biostimulant efficacy was evaluated under controlled biotic and abiotic stress conditions.

This tool enables the functional profiling of biostimulants by monitoring their impact on gene expression across multiple biological processes. It offers an innovative, cost-effective, and scalable alternative to RNAseq for mechanistic studies. Moreover, this platform facilitates the differentiation between biostimulants and plant defense inducers, supporting accurate product positioning within the current EU regulatory framework (Regulation EU 2019/1009), which requires scientific justification for claims but does not yet provide standardized criteria or molecular benchmarks for such assessments. This methodology not only aids manufacturers in obtaining product authorization but also strengthens the case for biostimulant use under field conditions through mechanistic validation.

Key words: Fluidigm chip, biostimulants, gene markers, pea, wheat, classification

# P9: EVALUATION OF THE EFFECT OF A BIOSTIMULANT ON TOMATOES UNDER WATER STRESS CONDITIONS, USING A COMBINATION OF APPROACHES: PROTEOMIC ANALYSIS AND AGRONOMIC TRIAL

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Agriculture has to meet growing food demand while coping with the impact of climate change, in particular the intensification of environmental stresses. These constraints negatively affect plant growth and productivity. In this context, the development of biostimulants, especially plant-based extracts, represents an innovative approach to improve crop resilience. Biostimulants stimulate natural plant processes to improve nutrient uptake or stress tolerance. The aim of the trial was to assess the effects of the AXIOMA Fruit biostimulant in boosting tomato tolerance to water stress across different developmental stages. To achieve this, a non-targeted proteomic analysis (LC-MS/MS) was carried out to identify the biochemical variations induced by application of the product. The results show that the biostimulant promote tomato plant growth and development, notably by enhancing carbon fixation and glucose production. It also promotes the activation of key metabolic pathways, such as the precursor amino acids of the shikimate pathway and the biosynthesis of terpenes, involved in plant defense. Furthermore, the increased abundance of proteins associated with redox homeostasis (thioredoxin reductase and enzymes involved in glutathione metabolism) observed at both developmental stages analyzed, suggests that the biostimulant activates antioxidant defense mechanisms. This modulation of protein expression reveals a better regulation of oxidative stress, thus reinforcing the crop's tolerance to adverse abiotic conditions. These modifications then lead to improvements in plant physiological parameters and better agronomic performance.

Key words: Volatile Organic compounds (VOCs) – grapevine – vineyards - elicitors

# P10: CABIOSE: COMPARATIVE ANALYSES OF BIOSTIMULANT EFFECTS ON TOMATO CROP AND THEIR ASSOCIATED MICROBIOTA, UNDER WATER STRESS CONDITION

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The aim of the project is to study the response mechanisms induced by biostimulants (BS) on tomato plants under semi-controlled conditions. In particular, the objective is to determine the direct contribution of BS on plants and their indirect contribution, through the variations in rhizosphere and phyllosphere microbiota.

We planned a three-stage approach: Plant – Product – Soil. The product approach aims at screening the most effective products for biostimulation in tomato under water stress. BS of various natures are tested: algae extract, vegetal extract, mineral. The soil approach is designed to assess whether selected BS at the end of the screening influence the microbiota of various types of soil.

The first experiment in 2024 under water stress conditions allowed selecting the two BS among seven, under semi-controlled conditions, in a greenhouse. Another experiment in 2025 under water stress conditions and integrating two different soil conditions is dedicated to study the effects of these two BS on plants and plant microbiota. Phenotypic observations and measurements are planned togenerate a growth curve. Samples will be taken from leaves and fruit at different stages of growth formetabolomic analysis and from soil and plant tissue for analysis of the rhizosphere and phyllosphere microbiota. The aim of combining metabolomics and metagenomics analyses is to assess the influenceof BS on the phyllosphere and rhizosphere microbiota communities and on their functions.

Thanks to this project we hope to gain insights into the mechanisms by which plants develop resilience to abiotic stress in response to BS applications directly and on the indirect contribution *via* the microbiota.

Mots-clés / Key words: biostimulant - plant development - water stress - microbiota - nutrition.

## P11: IDENTIFICATION OF A BIOSTIMULANT ENABLING POLLEN PROTECTION AND PREVENTING YIELD LOSS UNDER HEAT STRESS

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The project ST POL (Carnot Agrifood Transition Institute 2024-2026) aimed to 1/ establish a comprehensive study protocol for thermal stress 2/ evaluate a potential correlation between pollen quality and fruit development in order to predict yield at early stage and 3/ identify biostimulant able to alleviate plant thermal stress by maintaining good pollen quality

The protocol was developed on a vegetable crop with fruit valorisation (tomato) and a broadacre crop (spring wheat). Three types of stress were applied per crop, modulated in time (duration and photoperiod) and intensity (temperature), with a maximum temperature of 40°C.

The stress intensity was characterized by several biochemicals markers: electrolyte leakage, proline, MalonDiAldehyde, Abscisic acid contents. The use of the qPFD® chip (INRAE license) also allowed monitoring the impact of thermal stress, with overexpression of genes related to the ethylene pathway.

IFC technology (impedance flow cytometry) was used to monitor the quantity and viability of pollen. The different tested temperatures highlighted a loss of viability ranging from 10% to 100% in flowers subjected to stress. The correlation between pollen quality, fruit development, stress markers and yield is being analysed.

A biostimulant product was identify, able to alleviate the heat stress by protecting pollen quality and reducing the intensity of stress markers

Key words: Heat stress, Pollen quality, Yield

## P12: BACILLUS SUBTILIS SUPERNATANT, FROM GENOME TO PROTEOMIC CONTENT

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To overcome the challenges brought by the evolution of modern agriculture, slowly moving toward greener practices, the development of BioSolutions, inspired by natural soils bacteria, has emerged. Among them, widely studied plant growth promoting bacteria (PGPB) of the *Bacillus* genus display a high potential in being the source of future biostimulation products.

A supernatant of Bacillus subtilis was designed to achieve stable content and efficiency of its secondary metabolites through foliar application. All steps have been optimized, from the natural genetic modification of our strain with high-throughput screening to the highly controlled fermentation and purification process. The product is commercially available as Novastim<sup>®</sup>.

The presence of secondary metabolites—particularly lipopeptides and volatile compounds, was closely studied during product development and appears to account for a significant part of the product's efficacy. However, *in planta* efficacy tests comparing artificial mixtures of lipopeptides and volatile compounds to the supernatant-based product showed that the latter was more effective. This led to the hypothesis that the remaining efficacy may be attributed to the proteomic content of the supernatant.

To further understand the product's mode of action, a study of its proteomic content was initiated. As a first step, the Plant Growth Promoting (PGP) traits of the Novastim®-producing bacteria were predicted using the PlaBase platform. These predictions were then compared with the results of a proteomic analysis of the supernatant used in the final product.

The study indicates that protein presence may also contribute to the biostimulant activity observed in the Novastim® product, enables the identification of candidate proteins responsible for this efficacy, and highlighted the value of using the complex mixtures present in supernatants.

Key words: Bacillus subtilis - Biostimulation - Proteomics

## P13: MASSIVE BACTERIAL GENOME MINING FOR IDENTIFICATION OF NOVEL LIPOPEPTIDES WITH HIGH POTENTIAL USE IN BIOCONTROL

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Cyclic lipopeptides (CLiPs) are secondary metabolites mainly produced by environmental bacteria from the *Pseudomonas, Burkholderia*, and *Bacillus* genera. These bioactive molecules have various properties as antimicrobial activities against plant pathogens, surfactant properties or biostimulation activities by inducing plant systemic resistance and promoting plant growth. In a context of chemicals reduction use in the fields, lipopeptides are promising compounds in Biocontrol applications for sustainable agriculture.

CLiPs are synthesized by modular mega-enzymes so-called NonRibosomal Peptide Synthetases (NRPS), encoded by large biosynthetic gene clusters (BGCs) spanning over dozens of kb in the genomes. NRPS work as assembly lines that recruit amino-acids and catalyse peptide bond formation between them. This modular mode of synthesis leads to a huge structural biodiversity that we propose to explore.

For a decade, bioinformatics tools have been developed to facilitate the identification of BGCs within bacterial genomes and to predict the structure of potentially produced nonribosomal peptides (NRPs). Based on sequence specificities, modules and domains of synthetases can be sorted in different classes allowing the prediction of peptide moiety structures of NRPs. Moreover, some NRPs are well described and classified in families according to common features like the number and the nature of amino acid monomers.

In this work, we developed a bioinformatics pipeline for large-scale bacterial genome analysis allowing to identify BGCs involved in lipopeptides synthesis. From 22528 bacterial genomes of *Pseudomonas* genus and 11479 genomes of *Bacillus* genus available in public databases, we explored the diversity of lipopeptides using parallel analysis with antiSMASH software (Blin *et al.*, 2023). Subsequent data extraction based on cluster region type, synthetases domain signatures or peptide monomer motifs offers an overview on the bacterial goldmine of natural lipopeptides with potential beneficial applications in biocontrol.

Key words: Lipopeptides – genome mining – Pseudomonas – Bacillus – Biocontrol

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## P14: HETEROLOGOUS EXPRESSION OF BANANAMIDE AND PUTISOLVIN FOR THE BIOCONTROL OF GRAY MOLD DISEASE IN GRAPEVINE

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Grapevine (Vitis vinifera L.), one of the most widely cultivated fruit crops, is highly susceptible to fungal diseases such as Botrytis cinerea, the causal agent of gray mold. This disease compromise both yield and fruit quality. In the context of increasing regulatory and societal pressure to reduce reliance on chemical fungicides, cyclic lipopeptides (CLiPs) of microbial origin are emerging as promising biocontrol agents due to their potent antimicrobial and plant defense inducer activities, and biodegradability.

In this study, we evaluated the antifungal properties of semi-purified extracts of supernatants containing the lipopeptides bananamide and putisolvin produced respectively by Pseudomonas azadiae SWRI103 (LMG 32047) and Pseudomonas ekonensis COR58 (LMG 32175). Their efficacy was assessed both in antagonism assay in vitro and on detached grapevine leaves against B. cinerea. In the detached leaf assay, a clear dose effect was observed. Treated leaves exhibited a significant reduction in necrotic lesion areas and a delayed onset of necrosis up to 96 hours post-inoculation, compared to untreated controls. Sporulation was also notably reduced. Complementary in vitro diffusion assays further confirmed the strong inhibition of fungal development, with spore germination significantly reduced or completely halted in the presence of bananamide and putisolvin, underscoring the specificity and potency of the CLiPs in controlling B. cinerea infection. To support scalable and controlled production of these bioactive compounds, we are employing synthetic biology strategies under the HETEROCLIPS project. These efforts focus on reengineering regulatory elements within the bananamide and putisolvin biosynthetic gene clusters, alongside the development of optimized microbial chassis for efficient heterologous expression. These strategies aim to overcome production bottlenecks and support downstream applications in structural characterization and phytopathogen control. Our findings highlight the biotechnological potential of bananamide and putisolvin as eco-compatible alternatives to conventional fungicides, supporting the integration of microbial metabolites into sustainable viticulture practices.

Keywords: HETEROCLIPS Project, lipopeptides, Bananamide, Putisolvin, Grapevine, Gray mold, Biocontrol.

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### P15: PRIMING BY BACTERIAL SEED COATING REDUCES DISEASE SYMPTOMS IN BARLEY

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Depending on the severity of the infection and environmental conditions fungal diseases can reduce barley yield by up to 50%. Plant-associated beneficial microbes can initiate induced systemic resistance (ISR) and thereby can support plants to cope with biotic stress. We have previously identified primable and non-primable genotypes in a set of 200 barley accessions under greenhouse conditions. To validate priming effects, 10 selected barley accessions were analysed in two-year field experiments. As a priming approach seed coating was applied with 108 CFU/g seed of two Bacillus species: FZB42-Rif (B. velezensis) and ABi11-Rif (B. pumilus), or with H2O in the control treatment. Primed seeds were sown in three replications in a randomised complete block design. Several agronomical important traits, such as plant developmental stage, plant height, grain yield and thousand grain weight (TGW) were scored. The estimation of soil coverage and disease symptoms (Blumeria graminis and Puccinia hordei) was carried out both, visually and with multispectral measurements of unmanned aerial vehicles (UAV). ANOVA revealed significant effects (p<0.001) of the priming treatment for TGW. Additionally, the priming with ABi11-Rif (B. pumilus) significantly (p<0.001) decreased the susceptibility of barley to P. hordei and B. graminis. The effects of treatment with FZB42-Rif (B. velezensis) on symptoms of both diseases depended on the year. Fourteen weeks after sowing, Box PCR fingerprinting confirmed the presence of the applied bacteria in the rhizosphere of the primed plants, but not in the control plots. This demonstrated that the bacteria were capable of colonising the roots and surviving in the rhizosphere until the grain developmental stage. The third year of the experiment is currently running. Our final aim is to develop molecular markers to select for primable genotypes in barley breeding programs.

Key words: barley, priming, diseases, ISR

### P16: BIOCONTROL OF THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI USING THE BACTERIAL-DERIVED CYCLIC LIPOPEPTIDE FENGYCIN

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Wheat is one of the most widely cultivated cereal crops worldwide. However, it is affected by several diseases, including Septoria tritici blotch, caused by the hemibiotrophic fungus Zymoseptoria tritici, the most frequently occurring pathogen on this crop. The management of Z. tritici still relies on the use of chemical fungicides and to a lesser extent on varietal resistance. Nevertheless, the current context promoting agroecology and the frequent development of resistance to fungicides in fungal population need the development of sustainable crop protection alternatives, such as biocontrol. Here, we evaluated the biocontrol potential of fengycin, a cyclic lipopeptide produced by Bacillus subtilis, against Z. tritici. Three independent in vitro bioassays performed in solid medium revealed that fengycin exhibits a significant antimicrobial activity towards the pathogen, with a half-maximal inhibitory concentration of 1.52 ± 0.45 mg/L. Interestingly, microscopical observations under white light and fluorescence showed that the biomolecule induces morphological changes on the fungus, especially the formation of chlamydospores-like structures that occurred in the presence of fengycin. The highest rates of these structures were observed at the highest concentrations of the biomolecule. Further investigations are in progress to assess the protection efficacy of fengycin on the pathosystem wheat-Z. tritici in planta and to characterize both direct and indirect modes of action of the molecule using molecular analyses. This study provides new insights into the use of fengycin as a biocontrol compound on wheat towards Z. tritici.

Key words: Wheat, Zymoseptoria tritici, Biocontrol, Lipopeptide, Fengycin

### P17: STUDY OF THE IMPACT OF LIPIDS IN THE BIOCIDAL MODE OF ACTION OF RHAMNOLIPIDS AND FENGYCINS IN SCLEROTINIACEAE

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Rhamnolipids and fengycins are very promising natural antifungal metabolites secreted mainly by *Pseudomonas* and *Bacillus* bacteria, respectively. They are biodegradable, weakly ecotoxic and they induce plant defense mechanisms while having direct antifungal properties. As amphiphilic compounds, and due to their affinity for lipids, they are proposed to target the plasma membrane of fungi. To better understand the effect of rhamnolipids and fengycins on mycelial fungi, we investigate the relationship between fungal sensitivity and lipid composition. We chose *Sclerotinia sclerotiorum* and *Botrytis cinerea*, the white and gray mold agents, two major phytopathogenic fungi responsible for significant economic losses in more than 400 plant species.

To elucidate the mode of action of rhamnolipids and fengycins, we study their cytological effects on fungal hyphae using different microscopy techniques. We are carrying out lipidomic analyses of fungal phospholipids, sterols and sphingolipids. We are also studying the role of ergosterol and sphingolipids in fungal susceptibility using inhibitors of their biosynthetic pathways and mutants. Molecular simulations on biomimetic lipid bilayer help us to study the interaction between rhamnolipids and fengycins on fungal membrane models. By better understanding the mode of action of these promising natural compounds, our work will help to improve the use of rhamnolipids or fengycins as antifungal solutions to protect crops.

Mots-clés / Key words: rhamnolipids – fengycins - biopesticides – fungi – membrane lipids

### P18: RHAMNOLIPIDS, PROMISING PROTECTIVE AGENTS AGAINST BRANCHED BROOMRAPE ON HEMP

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Hemp is a high value-added crop, which is recently confronted with emerging infestation by the parasitic plant *Phelipanche ramosa* (branched broomrape), in various production areas in France. This parasite has become a major problem in infested areas since herbicide use is prohibited on this crop. Moreover, there is no effective method for controlling this parasitic plant, although the existence of more or less resistant varieties has been proposed as a solution. Another promising alternative in the fight against broomrape would be the use of natural molecules stimulating plant defenses, such as rhamnolipids (RLs), which are protective agents against different phytopathogens. RLs are glycolipids produced naturally by bacteria of the genus *Pseudomonas* and *Burkholderia*. They are well known as promising biofongicides.

The GEC and BIOPI laboratories have obtained preliminary results showing that RLs are efficient to control this parasitic plant on rapeseed. RLs inhibit the germination of *P. ramosa* seeds and induce the necrosis of the parasite in the first stages of its development in rapeseed-broomrape co-cultures. The evaluation of the protection efficiency of RLs against broomrape on hemp varieties with different level of resistance is underway. We are also analysing the early stages of the infestation of this pest, such as the stage of attachment to the roots of the host plant in order to have a good understanding of this pathosystem. Indeed, these initial phases involve pectin-modifying enzymes (PREs: Pectin Remodeling Enzymes) synthesized by the parasitic plant, allowing colonization of host plant tissues. In this project, we propose to study the role of PREs in the interaction hemp-*P.ramosa* in the presence or absence of RLs, on the greenhouse patho-system. The analysis of the parietal changes in hemp roots in response to broomrape and the application of RLs, is being carried out using complementary approaches that integrate phenotyping, transcriptomic and biochemical analyses.

Key words: Biocontrol, Hemp, Phelipanche ramosa, Rhamnolipids, Rapeseed

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## P19: BACTERIAL LIPOPEPTIDES AS PROMISING BIOCONTROL MOLECULES AGAINST THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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Septoria tritici blotch, caused by the hemibiotrophic fungus Zymoseptoria tritici, is one of the most frequently occurring diseases on wheat crops worldwide, especially in western Europe where the environmental conditions are suitable for disease development. Wheat protection against Z. tritici relies mainly on the use of chemical synthetic fungicides. However, because of the potential toxicity and ecotoxicity of these chemicals, there is an increasing need for the development of alternatives plant protection methods against this parasite. Biocontrol, based on the valorization of biological resources and natural mechanisms to reduce the pressure of plant diseases, is one of the most promising tools to address such a challenge. Here, the biological activities of lipopeptides on the wheat-Zymoseptoria tritici pathosystem, obtained from different bacterial strains of the genera Bacillus and Pseudomonas, were examined both in vitro and in planta. The results showed overall significant antimicrobial activity of the tested cyclic lipopeptides towards the phytopathogenic agent. The mode of action of certain lipopeptide biomolecules was characterized by microscopic analyses and cytological assays. The observations revealed that these biomolecules probably act by interacting with the cytoplasmic membrane of both the fungal pathogen and the host plant. Further works are in progress to produce lipopeptides heterogously using chassis bacterial strains and to characterize deeply their mode of action on the targeted pathosystem. This study sheds light on the importance of bacterial metabolites in the biocontrol of Z. tritici.

Key words: Wheat, Zymoseptoria tritici, Biocontrol, lipopeptides

## P20: AN INTEGRATIVE FRAMEWORK FOR DECIPHERING METHYLOBACTERIUM SYMBIOTICUM SB23 PERFORMANCES IN WHEAT AND MAIZE HOLOBIONTS

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Plant-associated microorganisms are of considerable practical importance in agriculture, especially in the context of the agroecological transition (Thérond *et al.*, 2017). Some of those microorganisms can enhance nutrient availability (biofertilizers), stimulate plant growth through hormonal effects, or improve plant resilience to various biotic and abiotic stresses (biostimulants). These beneficial traits make them promising for the development of biosolutions as sustainable alternatives to synthetic inputs (O'Callaghan *et al.*, 2021).

However, despite the attention paid by the scientific community and companies to microbial inoculants in recent decades, their effectiveness in agroecosystems shows considerable variability (Triverdi *et al.*, 2017; van Lenteren *et al.*, 2018). Indeed, several key factors influencing their performances remain often unknown or insufficiently characterized. This includes in particular their mechanisms of action, their ecological competence and their compatibility with the plant holobionts to which they are applied, i.e., their ability to establish and interact positively with host plants and their associated microbial communities (Yakhin *et al.*, 2017; Sessitsch *et al.*, 2019; Zboralski *et al.*, 2023).

In collaboration with Corteva Agriscience, our project aims to gain a deeper understanding of the mechanisms of action of *Methylobacterium symbioticum* SB23 across diverse wheat and maize holobionts. This facultative endophytic bacterium, registered for use on a wide range of crops under the commercial names Utrisha® N and BlueN®, is currently marketed for its biofertilizing and biostimulating properties.

To establish an integrative profile of the phytobeneficial and adaptative traits of strain SB23, a combination of genomic, functional (e.g., enzymatic activities related to growth and nutrient mobilization, phytohormones production) and ecophysiological (e.g., growth kinetics, carbon and nitrogen assimilation profiles) analyses are used. To assess its performances *in planta*, an inoculation kinetics of strain SB23 tagged with a GFP derivative is currently monitored over a two-month experiment on various plant holobionts.

This kinetics aims to investigate how physiological and ecological contrasting plant species associated with diverse endophytic communities – maize and soft winter wheat – presenting different degrees of varietal selection – ancient varieties selected during the post-war period of agricultural

intensification and modern varieties developed in the 2020s – will influence the colonization of strain SB23 and the expression of its phytobeneficial effects. Several eco-physiological traits (leaf gas exchange, total carbon and nitrogen measurements...), concentration of central and specialized metabolites (using a targeted metabolomics approach) and endophytic bacterial communities (using a metabarcoding approach) will be analyzed within the aerial and root compartments.

Functional characterization data obtained and colonization studies are expected to yield a comprehensive understanding of the performances of *M. symbioticum* SB23 across diverse wheat and maize holobionts, thereby supporting its more targeted and efficient application in agroecosystems.

Key words: Bacterial inoculants – Methylobacterium symbioticum SB23 – Mechanisms of action – Colonization – Plant holobionts

### P21: MICROBIAL ALLIES ACTIVATED: PSEUDOMONAS RESPONDS TO ROOT INFECTION WITH A POTENT ANTIMICROBIAL ARSENAL

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Legume root rot caused by the oomycete *Aphanomyces euteiches* is a major constraint to legume crop production, particularly in Europe. One promising yet underexplored strategy for disease control is to investigate the role of the rhizosphere microbiota during infection and to enhance its potential antagonistic activity against *Aphanomyces*.

As a first step toward this goal, a synthetic microbial community (SynCom) was constructed from the rhizosphere microbiota of the model legume *Medicago truncatula* using a culturomics approach, yielding 1,364 bacterial isolates. Illumina 16S rRNA gene metabarcoding combined with the UCLUST algorithm (97% identity threshold) allowed the identification of 812 pure isolates, corresponding to 79 unique operational taxonomic units. These isolates were assembled into a SynCom. In a gnotobiotic assay, this SynCom conferred partial protection against *A. euteiches*. Metabarcoding analysis revealed an enrichment of Pseudomonas strains during infection, along with the production of the broad-spectrum antimicrobial compound 2,4-diacetylphloroglucinol (DAPG).

Genome sequencing revealed that two out of five SynCom-associated *Pseudomonas* strains carried a DAPG biosynthetic gene cluster similar to those found in well-characterized species, and these two exhibited *in vitro* inhibition of *A. euteiches*. Together our results suggest that root infection by *A. euteiches* leads to the recruitment of Pseudomonas strains producing the antimicrobial product DAPG. Ongoing investigations aim to elucidate the mechanisms underlying *Pseudomonas* recruitment to the rhizosphere and the induction of DAPG production. We propose that enriching the legume rhizosphere with selected DAPG-producing *Pseudomonas* strains may offer a promising strategy to enhance resistance against root rot pathogens.

Key words: pseudomonas - Aphanomyces- 2,4-diacetylphloroglucinol (DAPG)-root microbiota

## P22: HARNESSING PLANT-MICROBIOTA INTERACTIONS TO IMPROVE CEREAL RESILIENCE UNDER LOW-INPUT SYSTEMS: COMBINING MODELING, IN VITRO AND IN PLANTA APPROACHES

Salomé Lengrand <sup>1</sup>, Anne Legrève <sup>1</sup>, Xavier Draye <sup>1</sup> and Claude Bragard <sup>1</sup>

Currently, there is an urgent need to improve cereal crop resilience and productivity under reduced chemical inputs and changing climatic conditions. A central objective is to develop innovative breeding and agronomic strategies by selecting environment-resilient plant ideotypes combined with beneficial plant-microorganism interactions. Our focus is on the microbiome as a key driver of this transition, with the aim to screen bacterial and fungal strains to identify candidates capable of enhancing nutrient use efficiency under low-nitrogen conditions. Bacterial and fungal strains selected through in vitro screening for IAA production, nitrogen fixation, and phosphate solubilization are first evaluated for their ability to enhance nutrient use efficiency in planta, both individually and in combination. In parallel, the mechanisms underlying their activity is investigated by analyzing their metabolic pathways through genome-based modeling and by assessing their metabolic responses under different nutrient input conditions. Based on these data, in silico simulations helps predicting interactions between strains and exploring how microbial behavior is influenced by both environmental factors and the presence of other community members. Ultimately, we aim to integrate experimental results, both in vitro and in planta, with predictive modeling to assess whether we can accurately forecast the dynamics and effectiveness of synthetic microbial communities designed to improve wheat nutrient efficiency. Our final objective is to propose a synthetic microbial community specifically designed to stimulate wheat growth and improve wheat nutrient use efficiency, supported by a deep understanding of its mode of action. In addition, we aim at developing guidelines for the screening and evaluation of microbial consortia with biostimulant potential.

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## P23: SPECIES-LEVEL MICROBIOME SHIFTS FOLLOWING BIOCONTROL AGENT INTRODUCTION IN WHEAT: INSIGHTS FROM THE PRONAME PIPELINE

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In the search for sustainable alternatives to chemical pesticides, biological control agents (BCAs) represent a promising strategy for crop protection. We investigated the impact of introducing the candidate BCA *Pseudomonas sivasensis* CF10PS3 on the wheat phyllosphere microbiome, compared to a conventional chemical fungicide program. Taking advantage of the innovative PRONAME bioinformatics pipeline for species-level resolution community profiling using long-read Nanopore metabarcoding (16S-ITS-23S operon) developped by Dubois (2024)\*, we tracked microbial community dynamics on wheat leaves after treatments. *Alpha* and *Beta* diversity indices showed no major disruption of overall community structure following either BCA or chemical treatments. However, deeper analysis to the species-level revealed that chemical treatment induced only transient effects, with few impacted taxa and a balanced number of promoted and depleted species by the end of the trial. In contrast, CF10PS3 introduction led to an initial, short-term decline in the abundance of many microbial species, followed by recovery and ultimately promotion of numerous taxa, including known beneficial genera with biocontrol abilities. Whereas the microbial community quickly recovered its previous state after chemical treatment, it remains to be investigated whether the new equilibrium following BCA application harbors distinct functional properties.

This work highlights the utility of PRONAME for high-resolution microbiome analysis and demonstrates the potential of BCAs as targeted microbiome modulators. Besides, this study provides a robust metagenomic method for studying cereal-associated bacteria.

Keywords: Pseudomonas sivasensis CF10PS3; microbiome; PRONAME; metabarcoding; microbial community dynamics

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<sup>\*</sup> Dubois B, Delitte M, Lengrand S, Bragard C, Legrève A and Debode F (2024) PRONAME: a user-friendly pipeline to process long-read nanopore metabarcoding data by generating high-quality consensus sequences. Front. Bioinform. 4:1483255. doi: 10.3389/fbinf.2024.1483255

## P24: DIVERSITY AND ANTAGONISTIC ACTIVITY AGAINST XYLELLA FASTIDIOSA OF CULTURABLE BACTERIA COMMUNITY OF PHILAENUS SPUMARIUS

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*Xylella fastidiosa* affects a great diversity of plant species, both forestry and of high agronomic and economic interest. This bacterium colonizes host xylem and is transmitted by xylem-sap feeders including cicadellids and aphrophorids, such as *Philaenus spumarius*. Control measures are currently insufficient, relaying mainly in eradication and vector control with insecticides, and it is necessary to search new strategies such as biological control agents (BCA) or synthetic communities (SynComs).

Aiming to find bacterial strains candidates against *X. fastidiosa*, a study of the diversity of culturable bacteria of *Philaenus spumarius* was carried out. Its antagonistic activity was evaluated against three different *X. fastidiosa* subspecies. Bacterial strains isolated from eggs, nymphs and adults were identified by 16S rDNA sequencing. Although culturable bacterial microbiota differed between the three stages, the genera *Pseudomonas*, *Rhizobium*, *Microbacterium* and *Stenotrophomonas* were present in all of them.

In antagonistic activity evaluation by *in vitro* dual culture tests, some isolates belonging to different genera clearly inhibited *X. fastidiosa* growth. These strains showed an interesting potential to control infection by *X. fastidiosa* in host plants, either directly or by inhibiting the acquisition of the pathogen by the vector.

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Mots-clés / Key words: culturable bacteria, Philaenus spumarius, antagonistic activity, Xylella fastidiosa.

## P25: EVALUATION OF ENDOPHYTIC BACTERIA AND THEIR METABOLITES FOR CONTROL OF S. SCLEROTIORUM IN CABBAGE

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Endophytic bacteria colonize host tissues internally without causing damage. In some cases, they promote plant growth and confer protection against pathogens. Previously, we selected three isolates with plant growth-promoting traits: *Pantoea agglomerans* Bru13, *Bacillus velezensis* Bro5, and *Bacillus subtilis* Bro11. This study aimed to test them as whole cells and their cell-free supernatants (CFS) as biocontrol agents against *Sclerotinia sclerotiorum* on cabbage plants, and to explore their genomes for genes related to antimicrobial compound production.

First, antagonism was evaluated in dual culture assays using the isolates and their CFS against *S. sclerotiorum*. Next, disease suppression was assessed *in planta* by measuring necrotic lesions at 24 and 48 hours post-inoculation (hpi) on cabbage leaves. Finally, genomes were analysed for biosynthetic gene clusters (BGCs) using AntiSMASH 2.0 and BAGEL4.

In vitro, the isolates inhibited pathogen growth by 30–75%, while CFSs had stronger effects, in some cases fully inhibiting growth. In planta, Bro5 showed the greatest protection, reducing necrotic lesions by 70% and 35% at 24 and 48 hpi, respectively. CFSs also showed protective effects: Bro5's CFS reduced lesions by 66% at 48 hpi; Bro11's CFS reduced lesions by 60% and 25% at 24 and 48 hpi, respectively; and Bru13's CFS was protective only at 24 hpi. Genomic analysis revealed nine BGCs in Bru13, including antibiotic and siderophore clusters. Bro5 and Bro11 harboured 13 and 11 BGCs, respectively, many with antimicrobial potential. Regarding bacteriocins, Bru13 contained genes for bottromycin and carocin D, Bro5 for amylocyclicin and LCI, and Bro11 for competence, subtilosin, and sporulation killing factor.

Our findings highlight our isolates as promising biocontrol agents against *S. sclerotiorum*, supported by their antagonistic activity and genomic potential for antimicrobial production. We are currently assessing their fungal inhibition effects in co-culture against *Fusarium oxysporum*, *Alternaria alternata* and *Botrytis cinerea*. The inhibition zones will be analysed to search for potential bioactive compounds and the active isolates will be fermented in liquid medium to provide organic extracts for further studies on their bioactive metabolites.

Key words: endophytes - antagonism - antimicrobial compounds

### P26: DEVELOPMENT OF COMPATIBLE BACTERIAL SYNCOMS FOR BIOSTIMULANT FORMULATIONS

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Agriculture today encounters significant challenges that require innovative approaches to enhance both the productivity and quality of crops while ensuring sustainable management practices. Synthetic communities (SynComs) can act as biostimulants or biofertilizers and represent promising biological products. They can improve water absorption, nutrient acquisition, tolerance to abiotic stresses, plant vigor, quality, and crop yield. However, assessing the compatibility between the microbial components of the SynCom is crucial for providing effectiveness as biostimulant or biofertilizer.

The aim of this study was to determine the compatibility of several bacterial strains to construct a SynCom using a bottom-up strategy. These bacterial components were previously isolated from plant environments and selected based on important treats such as nitrogen fixation, phosphate solubilization, hormone production, ACC deaminase activity, and siderophore production.

The compatibility of a strain collection was studied through dual in vitro assays. Four bacterial strains from different species (S1, S2, S3 and S4) that exhibited compatibility and relevant traits, were selected for the construction of a bottom-up SynCom. We evaluated the performance of combinations of two, three and four of these strains, as well as the individual strains in a substrate potting mix under different humidity conditions and in the roots of tomato plants. This assessment was conducted using plate counting techniques with antibiotic-resistant strains.

The kinetics of populations indicated that the strains selected were highly compatible. Then, we are currently conducting plant experiments using tomatoes and wheat plants to study beneficial effects.

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Key words: Microbial consortia, Bottom-up construction, Compatibility

# P27: XYLEM CORE ENDOPHYTOME AND ITS ROLE IN BACTERIAL DISEASES OF SALICACEAE: INTERACTIONS WITH XYLELLA FASTIDIOSA AND BRENNERIA SALICIS AND POTENTIAL FOR DYSBIOSIS MITIGATION

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Xylella fastidiosa and Brenneria salicis are xylem-limited pathogens responsible for vascular diseases in Salicaceae, yet infected plants often show contrasting outcomes ranging from severe decline to complete asymptomatic persistence. The underlying drivers of this variability remain unclear, but increasing evidence points to the critical role of the xylem core endophytome in modulating disease expression. In this study, we explore the interplay between endogenous xylem-associated bacterial endophytes and these two pathogens, focusing on their capacity to inhibit pathogen colonization and reduce xylem dysbiosis. A total of over 400 cultivable endophytic strains were isolated from willow and poplar species, complemented by metagenomic amplification to refine microbiome profiling. Through in vitro confrontation assays against X. fastidiosa KLN59.3 and B. salicis LMG2698, we identified several antagonistic endophytes with promising biocontrol potential. Selected isolates were subjected to genome sequencing and phenotypic characterization, including transformation with fluorescent markers to enable in planta tracking of colonization and interactions within xylem tissues. Co-inoculation experiments in Salicaceae and Nicotiana tabacum models further elucidate the dynamics between endophytes and pathogens in planta. Our findings highlight the functional role of the xylem core endophytome in shaping host-pathogen outcomes and support its potential application in sustainable disease management strategies targeting vascular pathogens in woody plants.

Key words: Microbiome, endophyte, Salix, Populus

### P28: ASSESSING CYANOBACTERIAL AND MICROALGAL POTENTIAL AS BIOSTIMULANTS AND BIOCONTROL AGENTS

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The global demand for food is growing in parallel with the human population. However, agriculture faces increasing challenges, such as diminishing arable land, chemical soil burnout, climate change, water shortages, and increasing resistance to plant diseases.

Cyanobacteria and microalgae are ubiquitous photosynthetic organisms and rich bioresources with great potential for agriculture. They are a source of nutrients, carbon sequesters, some are able to fixate nitrogen. Moreover, they produce a wide range of natural compounds (exopolysaccharides, phytohormones, peptides) with different applications for agriculture.

The project aims to explore the potential of cyanobacteria and microalgae as sources of biostimulants and biocontrol agents, as green alternatives for agriculture. Strains isolated from different crops in North Portugal were cultivated in controlled conditions and extracted using different methodologies. The biostimulant activity of crude extracts was evaluated *in vitro* by exposing tomato and lettuce seeds to various concentrations of different crudes, either through 24-hour exposure prior to incubation or during a 7-day incubation period. The parameters assessed after incubation were the index of germination, root length, and number of lateral roots.

Biocontrol potential is currently being assessed through preliminary susceptibility assays against phytopathogenic bacteria (*Erwinia amylovora*, *Pseudomonas syringae pv. actinidiae*, Xanthomonas *euvisicatoria*). The microorganisms are exposed to different concentrations of extracts prepared with different solvents.

Future work includes *in vivo* assays to evaluate physiological performance and leaf gas exchanges using the most promising extracts, testing extracts potential against phytopathogenic fungi and fractioning the antimicrobial extracts.

Key words: cyanobacteria - microalgae - biostimulant - biocontrol

## P29: UNCOVERING THE ROLE OF VERTICILLIUM ISAACII IN VERTICILLIUM WILT SUPPRESSION ACROSS CROPPING SYSTEMS

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Globally, Verticillium wilt diseases pose a major threat to agricultural productivity across diverse crop species. These diseases are primarily caused by Verticillium dahliae, a highly virulent pathogen with a broad host range, and the diploid hybrid Verticillium longisporum, which specifically affects Brassica species. Their soil-borne, xylem-colonizing nature, combined with the limited efficacy of conventional fungicides, underscores the urgent need for sustainable integrated pest management (IPM) strategies. In the context of disease-suppressive soils, several Verticillium isolates were recovered from both soil and plant material. Molecular identification revealed these endophytic isolates to be Verticillium isaacii, a highly diverse and largely understudied species. Due to its endophytic lifestyle, V. isaacii has emerged as a promising biocontrol agent (BCA) against pathogenic Verticillium species. This study aims to characterize the intraspecific diversity of V. isaacii and evaluate its biocontrol potential against Verticillium wilt. Isolates are being screened across several crops, including flax, cauliflower, and pepper, with future research extending to eggplant and ornamental species. The effectiveness of different inoculation methods (seed treatment and/or root dip) is also being assessed. Notably, one V. isaacii strain shows strong disease suppression in cool-season crops (e.g., flax and cauliflower), but not in warm-season crops such as pepper, suggesting a potentially temperature-dependent efficacy. However, this remains a working hypothesis, as other factors – such as host root physiology, root exudate composition or other interactions – may also contribute to the observed variability in biocontrol performance. Mode-of-action studies revealed no evidence of antibiosis or plant growth promotion, suggesting the involvement of alternative mechanisms such as competition for nutrients, space, or colonization sites, or induced resistance. Future research will explore these mechanisms using fluorescence-based microscopy and specific competition assays (e.g., BIOLOG profiling of carbon/nitrogen source utilization). Altogether, these findings support the development of crop- and climate-adapted biocontrol solutions against Verticillium wilts within IPM frameworks.

Key words: Verticillium wilts – Verticillium isaacii – endophytes – biocontrol

### P30: MICROBIAL IMPRINTING FOR CROP RESILIENCE

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Climate change and land-use intensification pose a major threat to many agro-ecosystems, leading to losses in crop yield as well as species loss and alterations in community structure. Plants may adapt to stress situations, but this can lag rapid anthropogenic changes to the environment. Our data show that natural modulations of the microbiome using native, harsh, and wild habitats consortia directly affect plant phenotype and performance, thus increasing plant stress tolerance. The microbiome extends host evolutionary potential and understanding how plant microbiome holobionts function in native, harsh, and wild habitats and how rhizobacteria mediate plant and ecosystem biodiversity in the systems, enables us to identify key factors for plant fitness [1-3]. A systems approach to engineering microbial communities by connecting host phenotype adaptive traits would help us to understand the increased fitness of the crop holobionts supported by genetic diversity. Identification of genetic loci controlling the interaction of beneficial microbiomes will allow the integration of genomic design into crop breeding programs. It is important to understand the structure of microbial community, functional and environmental patterns, enzyme pathways used and how are they activated.

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## P31: EFFECTS OF REPLACING CHEMICAL FERTILIZER WITH BIOINPUTS BASED ON BENEFICIAL NATIVE MICROORGANISMS AND BIOCHAR IN POTATO CULTIVATION (SOLANUM TUBEROSUM. L)

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A study was conducted between 2022 and 2024 to evaluate the effects of replacing chemical fertilizer with bioinputs based on beneficial native microorganisms MAB and biochar on potato cultivation (Solanum tuberosum L.).

The experiment was conducted at the Grass and Forage Experimental Station Indio Hatuey, University of Matanzas, Cuba. The variety Alouette was used, with a population density of 370,037 plants/ha.

Experimental treatments were compared under a randomized block design.

T1: 1.5 t/ha NPK + 0.22 t/ha Urea + 3 t Biochar

T2: 1.125 t/ha NPK + 0.165 t/ha Urea + 2.5 t C + 3 t Biochar

T3: 0.75 t/ha NPK + 0.11 t/ha Urea + 5 t C + 3 t Biochar

T4: 0.375 t/ha NPK + 0.055 t/ha Urea + 7.5 t C + 3 t Biochar + 3 t Biochar

T5: 10 t/ha + 3 t Biochar

TC: 1.5 t/ha NPK + 0.22 t/ha Urea

Plant growth, primary and secondary metabolism, and crop yield indicators were evaluated. Several instruments and devices were used: a millimeter ruler, a digital caliper, a KERN CXB 15K1 scale, a Dualex sensor (Cerovic et al., 2012), a pH sensor HANNA HI 9813-5, and a sensor of redox potential LUTRON YK-23RP.

Statistical analysis ANOVA and comparison of means using the Tukey test (P≤0.01) were performed using Infostat 2008 software.

In the first year of evaluation, the plants with the greatest growth were in treatments T1, T2, T4, and T5. Primary metabolism was best in T2 (chlorophyll content 28.96  $\mu g/cm^2$  and NBI® 30.6), with no difference compared to T3. Secondary metabolism (flavonoid and anthocyanin content) did not differ between treatments. The highest yields were obtained in treatments T1, T3, and T5 (0.91; 0.90; 0.86 kg/plant), coinciding with better plant growth and metabolism.

In the second year, the greatest growth in height and girth was observed in treatments T2, T3, and T5, and coincided with the best primary metabolism indicators in T2 (chlorophyll content 32.30  $\mu$ g/cm² and 24.03 NBI®) and T3 (31.05 and 23.83 NBI®). The highest anthocyanin values were in T4, T5, and TC, while the highest flavonol values were in T1 and T4, TC. The highest yields were obtained in T3, T2, T5, and T1 (0.88; 0.67; 0.62; 0.62 kg/plant).

The effects of the treatments on soil quality changes were not detected. The indicators averaged: total salts (267.75 ppm); pH 7.48; electrical conductivity 0.38 dS/cm; and redox potential 654.10 mV.

It is concluded that the use of compost-based bioinputs with beneficial native microorganisms has a significant effect in replacing 50% of the chemical fertilizer NPK + urea; and can achieve complete replacement.

Keys word: growth and metabolism of plants, agricultural yield, biofertilization, soil qualities, IHPLUS \*BF

## P32: RESPONSE OF RHIZOSPHERE MICROBIOME TO BIOAUGMENTATION WITH POLLUTANT-DEGRADERS FOR PETROLEUM HYDROCARBON BIOREMEDIATION

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The restoration of large, contaminated sites relies on cost-effective strategies like phytorhizoremediation that is based on plant ability to recruit and biostimulate degrading microbial communities, able to remove the pollutants and alleviate phytotoxicity.

Here, metataxonomic, culturomic and qPCR analyses were applied in two experimental approaches evaluating different bacterial resources for rhizoremediation practices aimed to decrease hydrocarbons (HCs) concentration in a polluted soil.

In the first set-up, the strains *Acinetobacter calcoaceticus* P320 and *Rhodococcus jostii* B12, originally isolated from an historically polluted industrial site, were exploited as allochthonous microbial resources for single strain applications on sunflower plants cultivated in the HC contaminated soil. 16S rRNA Illumina sequencing data showed that the sunflower rhizosphere microbiome was shaped both by the plant (biostimulation) and the inoculation of the degrading strains (bioaugmentation). The enrichment of degrading population, monitored through qPCR targeting HC catabolic genes, and soil chemical analysis showed that the two degrading strains impacted the structure and functionality of the sunflower rhizosphere microbiome leading to a decrease in the concentration of different HC fractions.

In the second approach, the microbial recruitment ability of the sunflower root system was exploited to select native degrading rhizobacteria from the HC polluted soil. A four-members SynCom was designed to steer the bioremediation potential of the native rhizomicrobiome. The SynCom application significantly boosted sunflower growth in the polluted soil and altered the structure of the rhizosphere microbial communities, although HC removal was not significantly impacted.

These findings reveal that the selection of strain/SynCom used for bioaugmentation can strongly influence the response of the rhizosphere community to the bioremediation treatment, thus affecting the pollutant clean-up.

Key words: Bioremediation, biostimulation, bioaugmentation, hydrocarbon degrading potential, SynCom

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### P33: THE 'MICROBIOME AND STRESS RESILIENCE ACADEMY' PROJECT

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Agriculture has a huge impact in land management for the preservation of natural resources and soil fertility. To sustain environmental-friendly approaches, the exploitation of the plant-associated microbiome can represent a valuable strategy to promote plant resilience to stresses.

Despite the great potential of adopting microbiome-based strategies, it requires familiarising a wider audience to the microbiome concepts and its benefits for plant fitness. Therefore, it is necessary to train the whole chain of stakeholders and producers by providing appropriate and reliable pedagogical materials. The project MSRA 'Microbiome and Stress Resilience Academy' aims to develop high-quality scientific and pedagogical digital training courses for a vast array of users, including, students, trainers, farmers.

The pedagogical material will be prepared by partners involved in research, science, academia and digital learning and include: My Green Training Box (MGTB), a provider of digital learning solutions (France); the University of Malaga (Spain); the Agricultural University of Athens (Greece); the University of Milan (Italy) and the French SME AkinaO.

The project will provide training material about the contribution of the plant microbiome to enhance sustainable solutions in agriculture and preserve ecosystem biodiversity by: 1) generating 4 digital training courses that will be released for free as micro-learning videos, pdf documents, podcasts and will be available for free in 5 languages in the MGTB digital platform; 2) by organizing 8 webinars, treating specific case studies about microbiome benefit for plant fitness that will be released for free as web-series; 3) by animating 8 pilot training courses to gain practical experience on microbiome applications.

The project will offer to the final users access to qualitative and reliable digital contents, promoting knowledge on the plant microbiome and its role in supporting plant fitness under stress.

### Key words: digital training, agriculture sustainability, plant microbiome, stress resilience

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### P34: ECOPHYTOPIC, A TOOL FOR A BETTER UNDERSTANDING OF BIOSOLUTIONS

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EcophytoPIC (www.ecophytopic.fr) is the French national website regarding Integrated Pest Management (IPM). It aims to transfer and valorize the references of IPM. It gathers and relays national and European information, such as projects funded by the European Union and regulatory information and makes it available mainly to farmers, advisers, and trainers, with the ambition of reaching other targets, which is why this website is completely free. Created in 2012 and concerning all crop sectors, this tool offers various resources to learn about biosolutions in integrated pest management practices: synthesis about biostimulants, articles about different biosolutions, pest and beneficials identification sheets, and many others.

With the objective of promoting biosolutions, a section dedicated to biocontrol is under development, featuring an interactive list of biocontrol solutions available for each crop in France, a description for each of them, information about the pest targeted by each biocontrol solution and links to useful resources. It aims to make biocontrol easier to understand and to choose as a viable option. This work has been completed for vineyards, orchards and arable crops and will be available for vegetables, horticulture and tropical crops by the end of 2025.

Key words: biocontrol, integrated pest management, resource center

# P35: CPER BIHAUTS ECO DE FRANCE PLANT AND MICROBIAL BIORESOURCES AND TECHNOLOGICAL INNOVATION FOR THE DEVELOPMENT OF INNOVATIVE BIOCONTROL PRODUCTS IN THE HAUTS-DE-FRANCE REGION.

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The CPER BiHauts Eco de France is a research program aims to actively contribute to the competitiveness of the Hauts-de-France region in the field of bioeconomy. It is based on 4 strategic areas: agro-ecological transition, valorization of agro-resources, development of the circular economy, development of eco-processes, control of food quality and safety. This program involves partners from 11 laboratories and 4 research platforms in the Hauts-de-France region including Univ. Lille, Univ. Liège, Univ. Artois, ULCO, UPJV, UTC, CNRS, INERIS, INRAE, Centrale Lille, ESCOM, JUNIA and UniLasalle. The CPER BiHauts Eco de France mobilize, structure and perpetuate, in the Hauts-de-France region, multidisciplinary skills and expertise (digital, biology, agronomy) to develop biocontrol and varietal resistance, two agroecological levers recognized for their importance in bio-protection. Biocontrol involves making the most of natural resources, such as beneficial microorganisms and natural substances, to combat crop pests sustainably. In this sight the CPER BiHauts Eco de France support several projects aiming to use of plant extracts, consortia of microorganisms and microbial metabolites for their bioherbicide, biofungal and biostimulant effects. Four specific projects will be highlighted in this poster: (i) the use of walnut and juglone extracts as herbicides, (ii) the study of the mode of action of lipopeptides from Bacillus strains against wheat septoria and/or apple scab, (iii) the evaluation of a patented formulation of a product containing Trichoderma harzianum, Bacillus veleznesis and nitrate as a biostimulant for tomato growth and in field test of Bacillus velezensis for the biocontrol and the biostimulation in wheat cultures.

Key words: bioherbicide – biofungicide- biostimulant - biomolecules

### P36: MICROPBIOMES COST ACTION CA22158: EXPLOITING PLANT-MICROBIOMES NETWORKS AND SYNTHETIC COMMUNITIES TO IMPROVE CROP FITNESS

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Plants and its microbiome will be subjected to several stresses the coming years, either abiotic such as drought and heat, and/or biotic including new plant pathogens. Current agricultural practices in Europe depend on agrochemicals and high amounts of water and will therefore not be sufficient to face the upcoming stresses. Founded in 1971, COST is the oldest and widest European intergovernmental framework for transnational Cooperation in Science and Technology. Running from 2023 to 2027, the COST Action MiCropBiomes (CA22158) grounds on the urgent need for a transition to sustainable agriculture to ensure food security and safety, by coordinating and developing knowledge on crop microbiomes (and holobiomes) for application in precision sustainable agriculture. First, we coordinate the available knowledge and datasets on microbiome assemblies and will decipher the phytobiome functional and molecular signaling through metagenomics techniques. Second, the Action will coordinate the available knowledge and datasets on the microbiome dynamics and the relation under specific environments with a focus on abiotic stress (drought and/or heat) and growing media (soil versus soilless systems). Third, we aim to understand the ecological processes that govern plant microbiome assemblies and functions and how these are disrupted by pathogens. Finally, the Action will explore the plant microbiome as a source of beneficial associations of microorganisms, in which we make the transition from current microbial inoculants to synthetic communities in relation to resilience against biotic and abiotic stresses in agriculture.

Keywords: Crop microbiomes, synthetic community, climatic changes, beneficial associations

## P37: TOWARDS SUSTAINABLE AGRICULTURE: THE WALLOON DIGIBIOCONTROL NETWORK UNITING DIGITAL AND BIOLOGICAL SOLUTIONS

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In alignment with the European Commission's Smart Specialization Strategy (S3), the Walloon region launched the Strategic Innovation Initiative (IIS) Digibiocontrol in 2023 to promote sustainable innovation by leveraging synergies between digital technologies and biocontrol solutions in agriculture. This strategic initiative supports the development of dynamic, innovation-driven ecosystems that reinforce regional competitiveness and scientific excellence, while responding to the the growing demand for environmentally friendly agricultural practices.

Indeed, despite their recognized potential, biocontrol products remain underutilized due to various limitations, including challenges in formulation, storage, application methods, and the limited availability of effective products. The integration of advanced digital technologies, such as sensors, robotics, data analytics, and decision-support tools, offers promising avenues to overcome these barriers and optimize the use of biocontrol solutions. Digibiocontrol initiative aims to connect two traditionally separate ecosystems to effectively tackle these issues and foster innovation.

The initiative operates along three complementary pillars: fostering research and innovation, strengthening education and skills development, and promoting entrepreneurship. It enables collaboration through thematic working groups, knowledge-sharing sessions, dedicated training activities, and support for innovative project development. In addition, the initiative actively contributes to European policy dialogue and seeks to reinforce international partnerships, positioningWallonia as a reference region for biocontrol-integrated digital agriculture.

Currently, more than 70 public and private stakeholders, including universities, research centres, private companies, and non-profit organizations, are involved in the network. These actors collaborateto co-create knowledge, launch pilot projects, and develop new technologies and services that support the green transition in agriculture. Recruitment of new partners remains ongoing to further broaden the initiative's impact and accelerate its contribution to sustainable agricultural transformation at both regional and European levels.

Mots-clés / Key words: Biocontrol, Biostimulant, Digital tools, Walloon Network

### P38: MICROSOS: PLANTS CRYING FOR MICROBIAL HELP IN A CHANGING WORLD

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Agriculture is continuously affected by environmental cues due to climate change. Therefore, developing novel sustainable approaches to improve plant resilience is crucial. The EU funded MSCA-MicroSOS project aims to study and exploit plant beneficial microbes to strengthen health of tomato plants. These efforts are directly aligned with the needs of farming communities, ensuring the practical relevance and eco-sustainability of the scientific breakthroughs generated through the project. Via international collaboration and knowledge exchange, MicroSOS empowers academic and non-academic partners to realise a breakthrough multidisciplinary research.

After the standardization and protocol establishment to be used in all work packages, the project plans to generate a microbial synthetic community (SynCom) to inoculate on tomato plants, apply (a)biotic stresses on tomato, and perform molecular analysis to assess the impact of the SynCom on plant's ability to cope with stresses and the perturbation of root microbiome by the Syncom. By analyzing tomato response to different abiotic and biotic stress on plants with and without SynCom will allow us to deeply understand the role of developed SynCom in modulating the plant defense response mechanisms.

Furthermore, collecting a wide range of data from phenotypic to metabolomics, will give a comprehensive and deep knowledge of the SynCom effectiveness in enhancing plant responses to stress. This knowledge has a great potential to be translated to sustainable agricultural practices, such as development of bioformulates.

### P39: MICROSOS PROJECT: MULTI-OMICS INTEGRATION

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Agriculture is continuously affected by environmental cues due to climate change. Therefore, developing novel sustainable approaches to improve plant resilience is fundamental. At the heart of the EU funded MCSA-MicroSOS project is a strong focus on integrating diverse research breakthroughs to agronomic practices to address the complex interactions between crops and beneficial microbes.

After the standardization and protocol establishment to be used in all work packages, the project plans to generate a microbial synthetic community (SynCom) to inoculate on tomato plants, apply (a)biotic stresses on tomato, and perform molecular analysis to assess the impact of the SynCom on plant's ability to cope with stresses and the perturbation of root microbiome by the Syncom. The analysis includes different features from plant fitness and phenotype, to metabolomics, nutrient content and gas emissions. Data integration represents a crucial step, being a powerful approach to obtain wide spectrum results.

Indeed, it is particularly useful to analyze complex dynamics such as plant-microbiome interactions in field conditions. This approach allows the development of holistic agricultural models and decision-making tools rooted in both research evidence and field-tested practices to enlarge our knowledge of the SynCom effectiveness in enhancing plant responses to stress. This knowledge has a great potential to be translated to sustainable agricultural practices, such as development of bioformulations.

## P40: MICROSOS PROJECT: FOSTERING COLLABORATION AND CAREER DEVELOPMENT IN PLANT MICROBIOME AND AGRICULTURAL RESEARCH

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Agriculture is increasingly challenged by stress factors due to climate change. Developing innovative sustainable management practices to improve plant resilience is essential. The EU funded MCSA-MicroSOS project fosters a unique collaboration between academic institutions and non-academic partners from the agricultural and environmental sectors to enable reciprocal knowledge exchange and staff mobility. Furthermore, this project aims to strengthen the bridge between plant microbiome research and its practical applications in the field. This partnership ensures that scientific innovation is informed by real-world agricultural needs.

After the standardization and protocol establishment to be used in all work packages, the project plans to generate a microbial synthetic community (SynCom) to inoculate on tomato plants, apply (a)biotic stresses on tomato, and perform molecular analysis to assess the impact of the SynCom on plant's ability to cope with stresses and the perturbation of root microbiome by the Syncom. After that, data will be integrated to obtain an holistic and deeper understanding of plant-microbiome dynamics and to assess the effectiveness of the SynCom in enhancing plant responses stress.

This knowledge has a great potential to be translated to sustainable agricultural practices, such as development of bioformulates. These exchanges not only support the development of practical, climate-resilient solutions but also strengthen career prospects for young researchers from Europe and beyond, ensuring long-term impact through a well-connected and interdisciplinary research community.

## P41: MOLECULAR MODELLING TO OPTIMIZE AND DEVELOP TOOLS FOR EVALUATING BIO-SOLUTIONS FOR SOIL AND PLANT HEALTH - MAMBO

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Over the years, it has become obvious that there is a loss of effectiveness of biocontrol (BC) and biostimulation (BS) solutions during the technological transfer from the laboratory to the field. The MAMBO project aims at addressing this issue through a systemic approach that does not limit itself to study the effects of bio-solutions on the plant alone, but encompasses the holobiont, its environment, and agricultural practices. MAMBO has been funded by the french "Grand défi Biocontrôle et Biostimulation pour l'Agroécologie", and gathers together 11 academic and private partners. It will form one component of the future national, distributed infrastructure, and will offer an unprecedented range of services based on high-throughput multi-omics approaches combined withstatistical analyses and modelling to understand, evaluate, and optimize the field effectiveness of all types of bio-solutions. MAMBO will provide high-throughput, multi-scale phenotyping services and facilities to the entire community involved in bio-solutions and agroecology. These services and areas of expertise will be available without restriction regarding crops, agricultural supply chains, and BC and BS methods employed. The consortium will be able to develop and implement various omics approaches to evaluate plant and soil health, representing two central pillars of agroecology, using samples provided by stakeholders in the field, which will be accompanied by (eco)physiological and pedoclimatic data. By integrating these heterogeneous datasets using robust bio-statistical analyses and modelling methods, the project will identify molecular signatures and predictive biomarkers of the effectiveness of bio-solutions, soil microbiological quality, and plant products, including proteins and metabolites.

This will be achieved through (1) exploratory data analysis methods, variable selection, and predictive integration techniques, (2) modelling rhizosphere and phyllosphere microbial communities, and (3) multi-scale modelling of plants (e.g., roots, leaves, fruits, seeds). The methods, approaches, and data generated will contribute to understanding the modes of action of BC and BS systems, as well as other agroecological levers, including alternative agricultural practices. They will not only underpin the development of decision support, phenotyping, and diagnostic tools applicable in diverse contexts, but also help refining the claims of bio-solutions.

Key words: Metabolomics, Proteomics, Ionomics, Metagenomics, Enzymomics, Plants, Soil, Phytobiome, Modelling, Biomarkers, Efficacy prediction

### P42: METABOLOMIC CLUSTERING OF ESSENTIAL OILS PROVIDES INSIGHT INTO INSECTICIDAL POTENTIAL

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Considering the growing importance of EOS as insecticidal ingredients, the domestication and cultivation of Aromatic and Medicinal Plants (AMPs) to produce chemically stable EOs contributes to species conservation, provides sustainability of the production and lower variations in active ingredients (Andrés et al., 2024).

Previously, AMPs from 22 species belonging to different genera have been adapted to cultivation in preliminary field trials located in Aragon, Spain (Navarro-Rocha et al., 2023). In this work, their EOs obtained by a steam distillation pilot plant, were analysed by GC-MS and tested against a stored product pest model (*Tribolium confusum*) by repellence and direct contact bioassays. Fourteen of the EOs showed at least one type of bioactivity against the pest insect *T. confusum*. Using the chemical profiles of these essential oils, the hierarchical cluster analysis effectively categorized them into five groups represented by common major compounds. Out of the five groups, two demonstrated the strongest insecticidal activity: group 3 (represented thymol, carvacrol and p-cymene) and group 2 (represented piperitenone and piperitenone oxide).

Furthermore, major and minor components were evaluated to determine synergistic or antagonistic effects within selected EOs. Several of the minor compounds showed similar bioactivity to the original essential oil and similar results were observed for some combinations of compounds, indicating synergistic effects. Therefore, differential components that discriminate oils into different groups may help to identify potential synergistic activity. In conclusion, this composition-based clustering analysis offers valuable information to organize and understand the similarities and distinctions in essential oil composition and to assess potential bioactivity.

Key words: Essential oils - Tribolium confusum - Clustering - insecticidal activity

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Navarro-Rocha, J., Gimeno, D. and González-Coloma, A., 2023. Production of essential oils from different species in two localities of Aragón, Spain. Acta Hortic. 1358, 243-250. DOI: 10.17660/ActaHortic.2023.1358.32

## P43: ANTIFUNGAL ACTIVITY OF SIX ESSENTIAL OILS AGAINST ZYMOSEPTORIA TRITICI, THE CAUSAL AGENT OF SEPTORIA TRITICI BLOTCH

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Zymoseptoria tritici, the causal agent of Septoria tritici blotch, is one of the most frequently occurringfungal pathogens on wheat crops worldwide, causing yield losses of up to 50% when the environmental conditions are suitable for disease development. Currently, the management of this pathogen relies mainly on synthetic fungicides. However, due to their negative effects on both the environment and human health, and the frequent development of fungicide resistance, looking for sustainable disease control methods is strongly encouraged. Biocontrol of plant pathogens using plant-derived metabolites is one of the most promising alternative tools. Here, we assessed the potential activity of six essentialoils from different plant species against Z. tritici. The experiments were performed in vitro in liquid glucose peptone medium amended or not with different concentrations of each essential oil and using a fumigation bioassay where the fungus was exposed to different concentrations of essential oil vaporsin Petri dishes. From the obtained dose-response curves, minimum fungicidal concentration (MFC), half-maximal inhibitory concentration (IC50), and the concentration inhibiting 90% of fungal growth (IC<sub>90</sub>), were determined for each essential oil. The results revealed that the antifungal activity varies depending on the essential oil, with IC50 values ranging from 10 mg/L to 276 mg/L in liquid medium and from 1.5 mg/L to 38 mg/L of air in fumigation assay. Moreover, cytological observations showed that the tested essential oils cause marked morphological changes on fungal mycelium in liquid medium. Further assays are in progress to assess the protection efficacy of the six essential oils in planta and tocharacterize the modes of action of the most promising on the targeted pathosystem. This study highlights the potential of botanicals for the biocontrol of Z. tritici.

Key words: Biocontrol, Zymoseptoria tritici, Essential oils, Antifungal activity

## P44: ESSENTIAL OILS AS BIOCONTROL AGENTS AGAINST LEIODES CINNAMOMEUS, AN EMERGING PEST OF BLACK TRUFFLE IN SPAIN

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Leiodes cinnamomeus, a beetle species endemic to truffle-producing areas of southern Europe, has recently emerged as a notable pest of black truffle (*Tuber melanosporum*) cultivation in Spain, particularly in key production regions such as Aragón, Catalonia, and Comunidad Valenciana. Its life cycle is closely aligned with the truffle's maturation period, and the lack of effective natural enemies in cultivated systems makes management challenging. Additionally, the limited availability of authorized plant protection products has sparked growing interest in biocontrol strategies based on natural products, such as essential oils and plant extracts, which may act as repellents or inhibit larval development without compromising the quality of the truffle or the integrity of the mycorrhizal system.

This study investigates the effectiveness of Pilot Plant steam-distilled selected essential oils (EOs) from experimentally cultivated aromatic plants against a laboratory model beetle (*Tribolium confusum*) and field-collected *L. cinnamomeus* adults. Fourteen EOs were tested for toxicity and repellency on *T. confusum*. Among these EOs, five were selected for further tests on *L. cinnamomeus* based on their effects. The overall repellency and toxicity of these five EOs was lower for *T. confusum* than for *L. cinnamomeus*, suggesting that these species-related differences might be related to their specific feeding adaptations and ecology. Additionally, while *T. confusum* is a well recognized stored-product pest with a long history of pesticide exposure, *L. cinnamomeous* is an emerging pest that has never been exposed to any insecticidal compound.

These findings highlight the potential of essential oils as effective biocontrol agents specifically tailored to emerging pests like *L. cinnamomeus*. The observed differences in sensitivity between the two beetle species underscore the importance of selecting target-specific bioactive compounds, particularly when dealing with pests that have not yet developed resistance due to prior chemical exposure. This research provides a promising foundation for the development of environmentally friendly, selective management tools for black truffle cultivation, aligning with current efforts to reduce reliance on synthetic pesticides in high-value agroecosystems.

Key words: Leiodes cinnamomeus - Black truffle - Biocontrol - Essential oils - Emerging pest

### P45: DESIGNING ARTIFICIAL VOLATILE BIOSTIMULANTS TO ENHANCE CROP RESILIENCE TO CLIMATE STRESS

Céline Alexis-Montrosier, Frederic Delmas, Nathalie Gonzalez, Michel Hernould, Théo Poucet

Heat stress, drought stress, and their combined effects—becoming increasingly common due to global warming—pose a major threat to agriculture by negatively impacting plant growth and productivity. In this context, biostimulants of natural origin, such as essential oils, represent a promising strategy to enhance plant tolerance to abiotic stresses. While their bioactivity is already well documented and increasingly used in agriculture, our understanding of their allelopathic potential linked to volatility remains limited. We hypothesize that volatile organic compounds (VOCs) naturally present in certain essential oils can act as chemical signals, enabling crops to anticipate and better respond to environmental stresses.

This study explores the biostimulant potential and priming effects of essential oil volatiles against combined heat and drought stress in Arabidopsis thaliana and tomato. In a first step, we conducted a large-scale screening of essential oils from various botanical families to assess their biostimulant activity on Arabidopsis. Plants were exposed to essential oil vapors for three days, followed by seven days of combined heat stress (35 °C day / 28 °C night) and water deficit. Biomass production and other physiological parameters were measured. Results showed that pre-exposure to specific volatile compounds significantly improved both biomass accumulation and survival under combined stress, suggesting a priming effect mediated by VOCs.

This screening led to the identification of 11 essential oils of interest, and GC-MS analysis of charcoal filters placed in the exposure chambers allowed us to identify 15 majors candidates volatile molecules likely responsible for the observed bioactivity. These molecules were subsequently tested under in vitro, controlled, and semi-controlled conditions, using plant species to validate their bioactivity. This approach enabled the design of fully defined and reproducible artificial essential oil blends, that displayed at least equivalent biostimulant activity to that of natural essential oils.

Keywords: VOCs, ensential oils, abiotic stress, arabidopsis, tomato

## P46: MOLECULAR IDENTIFICATION OF ALSEIS YUCATANENSIS AND NEMATICIDAL EVALUATION OF AQUEOUS EXTRACTS FROM SERIAL COLLECTIONS AGAINST MELOIDOGYNE INCOGNITA AND M. JAVANICA

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Alseis yucatanensis, a native species to southern Mexico and Central America, has shown nematicidal potential (Aviles-Gomez et al., 2022), although its phytochemical variation has not yet been studied in depth. This work, confirmed its molecular identity by sequencing the rbcL gene and registering it for the first time in GenBank. In addition, the nematicidal activity of aqueous extracts (3% and 6% w/v), obtained from quarterly collections during one year, was evaluated against juveniles (J2) of Meloidogyne incognita and *M. javanica*.

The extracts showed variable efficacy depending on the time of collection. Against M. incognita, collections in January, April and July showed outstanding mortalities. At 6% w/v, mortalities were 77%, 71% and 86%, respectively, while at 3% w/v, 73% (January) and 60% (April and July) were observed. Against M. javanica, the most effective collections were observed in January, April and August, with mortalities up to 96% at 6% w/v and 74% at 3% w/v. The lowest DL<sub>50</sub> were detected in July (6.02% w/v) and April (4.58% w/v).

These results suggest that the collection time influences the biological activity, possibly due to changes in the phytochemical composition of the extracts, and support the potential of *A. yucatanensis* as a source of sustainable bio inputs for agricultural nematode management.

Keywords: extract, Alseis yucatanensis, bioassay, serial collections

Aviles-Gomez, J., Cristóbal-Alejo, J., Andrés, M.F., González-Coloma, A., Carnevali, G., Pérez-Brito, D., Moo-Koh, F.A., and Gamboa-Angulo, M. (2022). Nematicidal screening of aqueous extracts from plants of the Yucatan Peninsula and ecotoxicity. Plants, 11(16), 2138.

# P47: VALORIZATION OF CO-PRODUCTS FROM ESSENTIAL OIL DISTILLATION FOR THE BIOPROTECTION OF MAIZE AGAINST THE FALL ARMYWORM SPODOPTERA FRUGIPERDA, A SIGNIFICANT PEST IN MADAGASCAR AND RÉUNION, IN A CIRCULAR ECONOMY PERSPECTIVE

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An invasive species recently introduced to Madagascar and Réunion, the fall armyworm (FAW) Spodoptera frugiperda causes significant damage to maize crops. Biosolutions such as co-products from essential oil distillation could provide promising and sustainable alternatives to synthetic pesticides, in a circular economy perspective. We evaluated the potential of liquid residues (hydrosols) from the distillation of aromatic plants, in tests on maize leaf disks in Petri dishes, under artificial infestation by neonate FAW larvae (3 per leaf disk for 24h). We assessed, on the one hand, in both Madagascar and Réunion, 0.4% essential oil solutions produced in the Highlands of Madagascar, and on the other hand, solely in Madagascar, 10% and 30% solutions of floral waters from several Malagasy sources. The essential oils evaluated were lemon eucalyptus, geranium, citronella, rosemary, and camphor. The hydrosols were those of geranium, citronella, and clove. The tests highlighted higher larval mortality and lesser leaf damage with the essential oils of citronella, lemon eucalyptus, and geranium, while the essential oil of camphor ("Ravintsara") had no effect. The tests with hydrosols established citronella as the most promising in terms of effects on larval mortality and on reduction of leaf damage. These tests will be complemented by the evaluation of the essential oil of lemon eucalyptus and the characterization of molecules and mechanisms of action (biocidal, repellent, and/or antifeedant) involved. We will also examine the effect of incorporating the solid residues from essential oil distillation into the soil on maize growth and reaction to FAW attacks. Finally, these results will be confirmed in a field trial with leaf spraying of essential oil solutions, under natural FAW infestation.

Key words: floral waters, aromatic plants, lemongrass, geranium, clove, lemon eucalyptus

# P48: EXPLORING THE USAGE OF LEEK BY-PRODUCT EXTRACT FOR BIOLOGICAL APPLICATIONS: ANTIFUNGAL EFFECTS ON PHYTOPATHOGENS AND SOIL MICROBIOME MODULATION

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The added value of biosolutions compared to synthetic phytopharmaceutical products lies in their ability to protect plants from pathogens while having a low impact on biodiversity. Leek cultivation generates substantial by-products, largely underexploited, containing antimicrobial metabolites. This study evaluates an aqueous extract derived from leek by-products for its antifungal activity and its impact on soil microbiota.

The extract caused strong inhibition of a wide spectrum of phytopathogenic fungi, notably Alternaria alternata, subsequent to the rapid induction of an oxidative stress leading to the loss of membrane integrity. In parallel, soil samples treated with the extract were analyzed using both a culturomic approach and 16S rRNA gene sequencing. The extract reduced fungal abundance while modifying bacterial diversity, with an increase in abundance of some families as oxalobacteraceae, lachnospiraceae and corallolubacterium and a decrease in others including chitinophagaceae. While these shifts suggest potential for bacterial enrichment, the observed fungal decrease by culturomic underlines the need for careful assessment of long-term ecological impacts on soil microbial biodiversity. Overall, leek by-product extract represents a promising biocontrol agent with a rapid and effective antifungal mechanism. However, further field studies are required to confirm its safety and compatibility with soil biodiversity preservation strategies.

Key words: Allium, leek, by-products, antifungal, Alternaria alternata, microbiota, soil, diversity

<sup>\*</sup>These authors contribute equally to the study

<sup>\*\*</sup>These authors co-supervise this study

# P49: EVALUATION OF THE EFFICACY OF BIOPESTICIDES BASED ON THE LEAVES OF PLANT SPECIES IN THE CONTROL OF THE MILLET EARWORM (HELIOCHEILUS ALBIPUNCTELLA DE JOANNIS) (PENNISETUM GLAUCUM (L.) R. BR) AND A PEST OF MILLET STOCKS (CORCYRA CEPHALONICA STAINTON) IN THE GROUNDNUT BASIN

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Millet (Pennisetum glaucum) is a major crop in Senegal's central groundnut basin. The aim of this study is to contribute to a better understanding of the millet cropping system in the groundnut basin. Specifically, the aim will be to understand the area's millet cropping system, identify crop and stock pests and the control methods used, and assess the effect of extracts of neem (Azadirachta indica), datura (Datura metel) and papaya (Carica papaya) leaves on the millet earworm. A questionnaire survey was carried out among 378 millet growers in Nioro du rip (280), Koungheul (19), Fatick and Foundiougne (79). The trial was conducted at the Centre d'Application et de Production (CAP) of the Institut Supérieur de Formation Agricole et Rurale (ISFAR) in Bambey using a randomised complete block design with three (3) replications and five (5) treatments. The treatments included absolute and reference controls (acetamiprid and lambda-cyhalothrin) as well as neem, datura and papaya leaf extracts. The results of the field survey showed that during millet production, the most frequent pests in millet-growing fields are Heliochelus (97%) and birds (78%); the most frequent stock pests are rodents (93%) and Corcyra (84%). The most common pest control methods used by growers are chemical in Foundiougne (92%), Fatick and Koungheul (83%), and 31% in Nioro. The methods of burning used tyres and plant or tree leaves (neem) are most common in Foundiougne (69%), Koungheul (67%) and Nioro (60%). The biological method (use of biopesticides) is only well developed in Koungheul (83%) and Foundiougne (46%), while in Fatick it is 17% and in Nioro 9%. The results of the experimental field study at Bambey revealed a highly significant effect for caterpillar incidence between the control and treatments with datura (p = 0.003) as well as the pesticide (p = 0.007); moreover, a significant effect was observed between the control vs papaya (p = 0.012) as well as control vs neem (p = 0.031) treatments. Regarding severity, the analysis of variance also showed a highly significant effect between the control and treatments including datura (p = 0.004), papaya (p = 0.008) and the pesticide (p = 0.009). On the other hand, there was no significant difference between neem and the control. Comparison of the averages enabled the treatments to be subdivided into two homogeneous groups (A and B) for incidence, and into three groups for severity, including two distinct groups (A and B) and an intermediate group (AB). The analysis of variance revealed a very highly significant effect on the number of larvae between the control and the different treatments, i.e. datura, pesticide and papaya, with p-values of 0.001, 0.004 and 0.008 respectively. In addition, a significant effect was observed between the control and neem, with a p-value of 0.023. Comparison of the means enabled us to divide the treatments into 2 homogeneous groups (A and B). Group A, which is the first group, is made up of TO (absolute control) with the highest average number of larvae (6.56) and group B which includes treatments with neem (T2), papaya (T3), pesticide (T1) and datura (T4) with respective averages of 4; 3.56; 3.28 and 2.89. The effect of the different treatments on yield revealed a

highly significant difference between the Datura vs Papaya (p = 0.009) and Datura vs Pesticide (p = 0.007) treatments. There was also a significant difference between the Datura and control treatments (p = 0.045). On the other hand, there was no difference between the Datura vs Neem treatments (p = 0.445). Comparison of the means with the LSD test enabled us to group the treatments into 3 groups, including 2 homogeneous groups A and B and an intermediate group AB. Group A, which includes T4 (datura), recorded the highest yield of 2.15 t. ha-1; T2, the only treatment in group AB, recorded an average yield of 1.96 t. ha-1. Treatments T0 (absolute control), T3 (papaya) and T1 (pesticide) make up group 3 (B) with respective averages of 1.66 T.ha-1, 1.50 T.ha-1 and 1.49 T.ha-1.

Mots-clés / Key words: Millet, groundnut basin, pests, biopesticides, Heliochelus albipunctella, Datura metel, Azadiracta indica, Carica papaya

### P50: AXPERA: A NEW BIOFUNGICIDE BASED ON A LYSATE OF THE NON-PATHOGENIC AMOEBA WILLAERTIA MAGNA C2C MAKY

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AMOEBA is a French greentech founded in 2010 developing applications based on the of the non-pathogenic amoeba *Willaertia magna* C2c Maky. Discovered in the thermal waters of Aix-Les-Bains (France) in 2001, this amoeba is a natural predator of water-borne micro-organisms and was initially used in a biocide application.

Amoéba later discovered that the components of the dead amoeba have their own biological activity. In fact, the amoeba lysate, whether in liquid or dry form, can prevent the release and germination of fungi/oomycetes spores, and particularly phytopathogenic fungi. The company has used this property to develop a biocontrol product against fungal plant diseases, AXPERA, the first biofungicide based on the lysate of an amoeba.

The culture process of this unique amoeba has now reached industrial scale. Amoeba is the first company in the world to grow an amoeba strain in industrial-scale bioreactors using a continuous process. The cells are harvested by centrifugation and the culture medium is removed. The concentrated cells are then mechanically lysed to obtain the active substance.

This very innovative active substance has a complementary dual mode of action: it stimulates plant defenses (an indirect effect on diseases) and also inhibits germination of pathogenic fungal spores (a direct effect on the pathogen). The broad spectrum of efficacy of the formulated product AXPERA was demonstrated against inter alia tomato downy mildew (*Phytophthora infestans*), tomato powdery mildews (*Oidium neolycopersici* and *Leveillula Taurica*), cucurbitaceous powdery mildew (*Golovinomyces cichoracearum*), cucurbitaceous downy mildew (*Pseudoperonospora cubensis*), lettuce downy mildew (*Bremia lactucae*), grapevine downy mildew (*Plasmopara viticola*), grapevine powdery mildew (*Erysiphe necator*) and banana black Sigatoka ( *Mycosphaerella fijiensis*). The overall efficacy has been demonstrated through 600 trials since 2019 under both greenhouse and field conditions.

AXPERA (SC 215 Gg/L) is not intended to be used only as a stand-alone product, but rather in combination. For example, AXPERA combined with a reduced dose of copper (30-50% of the authorized application rate) is as efficient as copper at its maximal authorized application rate against downy mildew. The product can be mixed with copper, sulphur, and chemical fungicides. A first product (100% active substance) has already been authorized by EPA in the USA (2022). In Europe, the dossier for the active substance approval is in the final administrative phase after we received the positive and definitive report from EFSA. Officila registration of the active substance is expected mid of 2025. In France, we just received an emergency market authorisation (4 months period for use against downy mildew on grapevines).

An upcoming market launch: the first marketing authorizations for AXPERA in Europe are expected end of 2025, firstly on grapevines and vegetable crops (downy and powdery mildew).

Key words: AMOEBA, Willaertia magna C2c Maky, bio fungicides, Downy, powdery mildews, new active substance.

#### P51: ROMEO®, A WELL-CHARACTERIZED PLANT DEFENSE STIMULATOR, IS A BIOCONTROL SOLUTION FOR EFFECTIVELY MANAGING TREATMENT STRATEGIES FOR VEGETABLE CROPS, VINES AND BERRIES AGAINST THE MAIN FOLIAR FUNGAL DISEASES

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Agrauxine by Lesaffre, a business unit of Lesaffre group, develops, produces and markets biosolutions derived from microorganisms to improve plant health in accordance with sustainable agriculture technologies. Based on this expertise, Agrauxine by Lesaffre developed a non-living natural active substance named cerevisane® (composed by cell walls of Saccharomyces cerevisiae yeast strain LAS117) and the related biocontrol product Romeo®. cerevisane® acts preventively, and its mode of action consists in the stimulation of plant defense mechanisms. This active substance activates complementary plant defense metabolic pathways, which explains its multi-disease efficacy against downy mildew, powdery mildew and Botrytis. Agrauxine has acquired a wealth of knowledge to clarify cerevisane's mode of action, as well as the factors influencing its efficacy. Under field conditions, Romeo® demonstrated significant efficacy on grapevine, berries and also vegetable crops (lettuce, strawberry, tomato, cucurbits). Romeo® biofungicide efficacy is maximized when the product is integrated into a pest management program. This solution helps growers to reduce significantly the use of copper, sulfur and actives substances from chemical origin. Its innovative multi-target positioning allows flexible integration into conventional or sustainable treatment programs. The regular use of Romeo® optimizes the applied quantities of conventional phytosanitary products and limits the risk of residues while ensuring a satisfactory efficacy level. Cerevisane® is registered in Europe and in USA, where it is classified as a lowrisk substance with no MRLs. Romeo® is compatible with organic farming and is certified by Ecocert, OMRI and is in compliance with the NOP. The plant bioprotection product is already commercialized for example in France, Italy, UK, Germany, Austria, Belgium, the Netherlands and in USA.

Keywords: biocontrol - elicitor - grapevine - vegetable crops - yeast

## P52: MYCOTERROIR®: LOCAL ARBUSCULAR MYCORRHIZAL FUNGI (AMF) SUPPORTING SOIL AND PLANT HEALTH

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MYCOTERROIR® is an innovative service that leverages local arbuscular mycorrhizal fungi (AMF) communities to naturally enhance soil and plant health. Relying on an internally developed national reference database of AMF communities and a process that enables simultaneous multiplication of a wide diversity of local species, MYCOTERROIR® is adapted to environments with low levels of AMF.

Each MYCOTERROIR® is custom-made from local AMF communities and contains a high diversity of species adapted to the specificities of the soil that need to be restored. In the field, a preliminary assessment of AMF abundance and diversity is conducted on the site to be restored. The local AMF communities to be multiplied are collected from our so-called 'reference' sites containing a great diversity of species and located within the same geographical area as the site where they will be applied.

Referring upon our national reference database of AMF communities, our approach ensures a rich local diversity, while maintaining species cohesion and their adaptation to environmental conditions. The AMF communities collected are then propagated in a growth chamber. Each MYCOTERROIR® is highly diverse with at least 20 different species, dosed with viable spores and stored for up to 18 months. The three formulations developed allow each MYCOTERROIR® to be applied at planting, sowing, or on existing plants. MYCOTERROIR® quickly restores a high diversity of AMF adapted to each site, accelerates the transition to practices that rely less on inputs, and improves soil and plant health both rapidly and sustainably. Internal biomolecular and bioinformatics analyses developed by our team also enable monitoring of local AMF communities.

Key words: local arbuscular mycorrhizal fungi communities - diversity of local species - environmental conditions - soil and plant health

### P53: JDS1, A NEW BIOFUNGICIDE WITH HIGH POTENTIAL AGAINST DOWNY MILDEW ON GRAPEVINES

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Grape downy mildew (*Plasmopara viticola*) is one of the most common diseases in vineyards. Protection against downy mildew remains a major challenge for winegrowers, especially in a context of a high phytosanitary treatment frequency indicator (FTI), and few alternatives to chemical or mineral fungicides. Moreover, in organic farming, copper is the main available fungicide, but with increasing concerns regarding its polluting effects on soils.

Cerience has developed a new product, JDS1, a natural substance obtained from grape seeds. The grape seed extract JDS1, is a polyphenol-based extract, with a high content of proanthocyanidins. The European Commission approved recently the grape seed extract (*Vitis vinifera*) as a basic substance (Regulation (EU) 2025/96).

Under controlled conditions on grapevine/downy mildew pathosystem, we demonstrated that JDS1 has a dose effect and a cumulative protection efficiency, with a protection level superior to 85% when applied in one preventive application, and of 100% with 2 applications. The protection was maintained up to 12 days post treatment.

Studies of its mode of action, demonstrated that JDS1 has a direct effect on *Plasmopara viticola*, with a high inhibitory effect on release, mobility and encystment of downy mildew zoospores.

In-depth plant defense induction ability was assessed by qPCR on a set of marker defense genes. Our data demonstrated that JDS1 exhibits a moderate induction of pathogenesis-related proteins genes and of the secondary metabolic pathway.

Different positionings of JDS1 as an alternative for copper treatment were studied under field conditions. Protection efficacy of JDS1 was confirmed as a partial substitute from copper treatments.

This innovative product, acting as a biofungicide, offer new perspectives for both conventional and organic farming management to fight against downy mildew of grapevines.

Key words: downy mildew, grapevine, basic substance, biofungicide, protection

#### **P54: BIOSOLUTIONS FOR OILSEED RAPE**

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Oilseed rape is an important crop throughout the world. Only few efficient biosolutions are however available until now. In regards to sclerotinia disease by example, varietal resistance are coming out but their efficiency is still partial. It requires the combination with biocontrole products. This crop also suffers of many biotic and abiotic stresses at early stage, during autumn season. Biostimulants would be of great help for the plants to start the winter in good conditions. Pathologists of Bio-Eva helps companies in the development of their biosolutions for oilseed rape from the beginning of the adventure in the lab until field assays for final validations. This poster presents experimentations performed to support the development of such products.

Key words: praestitutum - gloriosam - rumigerulos

## P55: EFFECT OF LONG-TERM STORAGE ON THE ACTIVATION EFFICIENCY OF FERMENTED FOREST LITTER FOR LIQUID BIOFERTILIZER PRODUCTION

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Fermented forest litter (FFL) represents a low-cost, low-tech, nature-based solution for the production of biofertilizers. This study investigates the impact of storage duration on the activation efficiency of FFL for liquid biofertilizer production. A Cuban FFL formulation (IH+) was produced by fermenting forest litter (*Terminalia buceras, Quercus alba, Calophyllum antillanum Britt.*) with rice bran, cow milk, and water under anaerobic conditions at room temperature for one month. Two batches were compared: IH+22 (stored for 30 months) and IH+24 (stored for 1 month). Activation was performed by mixing 25 g of IH+ with 25 g of sugarcane molasses in 1 L of water, incubated anaerobically for 8 days.

Fermentation was monitored through pH, sugar consumption, and metabolite production (lactic acid, ethanol, and acetic acid). Both IH+ samples showed a pH drop from 4.7 to 4.2; however, IH+22 displayed a 3-day lag phase. In IH+24, sucrose was depleted by day 4, followed by rapid glucose and fructose utilization. Similar profiles were observed for IH+22, with delayed onset. After 8 days, lactic acid reached  $^{\sim}50$  mM in both treatments, with kinetic profiles accurately described by a Gompertz model ( $R^2 > 0.988$ ):

[Lactic acid] = 
$$\alpha \exp(-\beta \exp(-kt))$$

where  $\mathbb{P}$ ,  $\mathbb{P}$ , k are fitting parameters, allowing to calculate a maximum productivity ( $V_{max}$ ). In both cases, the  $V_{max}$  values were similar: 16.9 mM/day for IH+22 and 15.1 mM/day for IH+24.

Ethanol concentrations reached 40.9 mM (IH+22) and 48.2 mM (IH+24). Acetic acid production followed linear kinetics in both cases.

These results demonstrate the resilience of FFL technology: despite a moderate lag phase, long-term stored FFL remained effective in producing key fermentation metabolites, supporting its practical value for decentralized, sustainable agricultural practices

Key words: Fermented forest litter, biofertilizer, storage stability, Gompertz model, nature-based solution

### P56: SELF PRODUCTION OF BIOINPUTS OR BIOFACTORIES: A TASTE OF FUTURE FOR SMALLHOLDERS?

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The aim of a biofactory is to empower the local development of agriculture. To get an overview of such infrastructures, a non-exhaustive inventory has been drawn up in Latin America, sub-Saharan Africa, and Asia, using literature, websites, and contacts. They vary widely in size, from individual production of bioinputs to village communities trained by Paysans Relais (in Madagascar), University structure, and crop-protection firm-type entities. The bioinputs produced, given or marketed to smallholders, are mainly organic fertilizers (compost-based biofertilizers such as 'Bokashi'), substitutes for synthetic pesticides such as plant-based preparations, and microbial solutions (e.g., biostimulants). Beneficiaries often practice organic or agroecological farming. To help set up a biofactory, guides and videos are available. Diverse factors influence the adoption or rejection of the produced bioinputs. Among the constraints to be overcome before a biofactory can be truly deployed are the availability of technical and plant materials, the time required to prepare and store bioinputs, their effectiveness in relation to their application frequency (for botanical extracts), and the product cost when marketed. A main important factor for their implementation is their promotion by institutions, governments (as in Colombia), and organizations (e.g., ESSOR, Ethiquable) some being non-profit. Research is called upon at every stage to validate the bioinput value chain.

Key words: natural preparation, biofertilizer, plants extract, botanicals, biological input, biofactory

## P57: MYCEA: AN INNOVATIVE SOURCING, PLATFORM, AND PIPELINE TO DISCOVER AND SELECT NEW BIOCONTROL SOLUTIONS

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As part of the agroecological transition, Mycea conducts research and develops natural alternatives to synthetic plant protection and fertilizers. Our innovation lies in harnessing the biochemical and biological properties of fungi. These organisms represent one of the largest reservoirs of biodiversity— estimated at around 8 million species— but remain largely understudied and undervalued.

Mycea have built a collection of over 200 fungal strains isolated from natural environments. After cultivation in axenic conditions, extracts are produced from these strains, providing a platform for the discovery of new bioactive compounds. Currently, the focus is exclusively on identifying biofungicidal properties. The extracts are tested *in vitro* to identify fungicidal effects against major crop pathogens including downy mildew of grapevine, late light of potato, gray mold of tomato, scrab on pome fruits, and Septoria leaf blotch of wheat.

Promising active substances are identified through a selection pipeline driven by the characterization of their efficacy *in vitro*, *in planta*, and *in campo* on the pathosystem, along with their chemical composition and toxicological and ecotoxicological profiles. Selected substances are then formulated for field trials, and the production process is optimized for industrial-scale development. To date, six promising extracts have passed through the selection pipeline. For one of these extracts, field trials are currently being conducted to assess efficacy against downy mildew of grapevine and potato.

Key words: Biofungicidal - Bioactive compounds - Selection pipeline - Fungal strains - Biofungicidal properties

## P58: CONSPECIFIC DNA AS A BIOCIDE: A NOVEL AVENUE FOR PHYTOPROTECTION

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The search for new bioactive agents in phytoprotection is a major challenge in reducing the environmental impact of conventional pesticides while addressing key requirements such as specificity and sustainability. Among emerging approaches, conspecific DNA—that is, DNA originating from the same species as the target organism—is drawing increasing interest due to its inhibitory effects on various organisms.

First described by Mazzoleni et al. (2015), this phenomenon of **induced autotoxicity** has been observed in plants, microorganisms, and certain insects. Exposure to conspecific DNA can slow growth, disrupt the cell cycle, or even induce lethal effects, depending on the species and DNA concentrations. However, many aspects remain unknown, particularly the underlying mechanism of action.

In this context, conspecific DNA appears to be a promising candidate for the development of phytoprotective agents that are species-specific, non-toxic, and biodegradable.

This presentation will provide a brief synthesis of current knowledge on this phenomenon and explore its potential applications in biocontrol. In addition, we will present **preliminary results** from our work on the spruce budworm—the most destructive forest pest in North America—highlighting the concrete phytoprotective potential of conspecific DNA.

Key words: conspecific DNA, biocide, phytoprotection

## P59: EVALUATION OF THE ENVIRONMENTAL FATE AND IMPACT OF BIOPESTICIDES USING AN INNOVATIVE APPROACH COUPLING HIGH-THROUGHPUT METHODS

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Biopesticides are complex substances that are derived from natural sources (e.g., plants, animals, microorganisms), and recently offer a promising alternative to traditional pesticides, but it is still unknown how long biopesticides and their residues remain in the environment then how long they impact organisms living in soil (prokaryotic and microeukaryotic species).

In this context, we conducted an experiment to evaluate the environmental fate and impact of Beloukha, a bioherbicide containing Pelargonic acid as active substance. A kinetics study was performed over 57 days in soil microcosms comparing treated and non-treated conditions. The samples were analyzed using high-throughput omics techniques (metabolomics UHPLC-HRMS and 16S and 18S rRNA gene metabarcoding).

Thanks to the metabolomics data, we could determine the dissipation time. It corresponds to the time required for the dissipation of the biopesticides compounds (i.e. the Beloukha extract with the formulating agents). Bioinformatics approaches allowed us to separate the soil metabolites and the degradation products from the pesticide components. Subsequent kinetic modeling revealed that 99% of the biopesticide compounds have half-life below 19 days and 94.9 % of metabolites have time degradation of 99% of their initial quantity below 125 days.

Metabarcoding analyses revealed biodiversity changes over time (with alpha and beta diversity) aswell as the impact of the biopesticide and its degradation products on bacteria. In particular, we found that bacterial assemblages were impacted at the first-time, TO2 and T14 steps of the experiment. Moreover, correlation analyses revealed high correlations between biopesticide components and bacteria genera that play important roles in plant growth, plant hormones regulation and azote fixation.

In essence, this work establishes a robust workflow applicable for studying the dissipation of biopesticides in the environment and determining their profound impact on biodiversity.

Key words: metabolomics, metabarcoding,fate; dissipation time, impact, data integration,biopesticides, modeling

### P60: WHEN PLANT NUTRITION IS THE BASIS OF PLANT HEALTH: THE FRAY7 INNOVATION!

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Although it is increasingly recognized that the level of fertilization plays a major role in the plant health and their capacity for adaptation and self-protection, the technical programmes of plant production are still disconnected. Nitrogen is known to be an essential macronutrient for plant nutrition, which also plays a key role in plant defense signaling pathways (Verly et al., 2019; Fagard et al., 2020) and in pathogenicity (Ballini et al., 2013; Huang et al., 2016; Jeon, 2019). Results of 5 years of research to select and associate the best organic components in one ready to use solution for users, we have elaborated a specific organic fertilizer improving crop development and enhancing global plant health: the Fray7 product (Frayssinet®). This innovative organic product combines several physiological mode of action due to its complex composition. Numerous field experiments demonstrated its efficacy both in plant nutrition and vigor to help plant in adverse environmental conditions. Following the great efficacy of Fray7 product in real conditions, we introduced the developped solution in a global one health approach on grapevine (Chaire AgroLab Biomed), apple (CTIFL, lanxade) and wheat to come (Stimcross, PHIM partnership). These experiments in farmers' conditions will lead to a complete and synergistic program for plant production, improving plant nutrition while decreasing the use of protection products or helping biocontrol products. Results from previous and current trials will be presented.

Keywords: Fray7 - organic fertilization - plant productivity - plant health - global approach

## P61: IMPROVING THE SHELF LIFE OF SUGARLOAF PINEAPPLE (ANANAS COMOSUS) IN BENIN: PRE AND POST-HARVEST PHYSIOLOGICAL INTERVENTIONS

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Sugarloaf pineapple (Ananas comosus) is the third most cultivated crop in Benin with a high market potential. Despite such specific attribute, the fruit remains highly perishable (short shelf life) due to physiological sensitivity, and rapid physicochemical changes. This study aimed to explore strategies to extend the shelf life of Sugarloaf pineapple in Benin using pre harvest (evaluation of neem oil-coated urea fertilizer on fruit quality and productivity, use of cumulative thermal time to determine optimal harvest maturity) and postharvest (assessment of postharvest damages and application of active coatings and elicitors to improve postharvest preservation). Key physicochemical parameters, including firmness, pH, total soluble solids, and moisture content, were measured to assess fruit quality. The findings revealed the prevalence of postharvest disorders such as translucency, black rot, vitreous core, Brown spot, insect damage, brown heart, crown rot and sunburned. A higher K2O: N fertilization ratio significantly increased fruit weight and sugar content. The use of neem oil-coated urea improved nitrogen use efficiency, reduced nutrient loss, and contributed to better performance and economic returns. Significant differences were observed in physicochemical properties and shelf life of fruits harvested at various maturity stages. Harvesting at 2200°C.days and 2300°C.days extended the shelf life to 21 days while maintaining export-grade quality. Treatments involving active coatings and elicitors also contributed to preserving fruit quality during storage.

Based on these results, it is recommended that farmers apply a fertilization scheme with a  $K_2O:N$  ratio of 0.35 reducing then the amount of urea. Harvesting within the thermal window of 2200-2300°C.days ensures optimal fruit quality and shelf life, enhancing the competitiveness of Benin's sugarloaf pineapple in both domestic and international markets.

Keywords: Sugarloaf pineapple, postharvest damage, elicitors, edible coating, neem oil, thermal time, shelf life, physicochemical properties, Benin.

## P62: PRELIMINARY STUDY: COPPER STRESS BIOMARKERS RESEARCH ON A MODEL PLANT, FALSE YELLOWHEAD (DITTRICHIA VISCOSA)

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To fight the downy mildew, winegrowers are using Bordeaux mixture, in which copper sulphate is the active substance. This has led to copper contamination of vineyards soils which can cause disruptions and physiological imbalances in the plant. Grubbing-up premiums were introduced to help farmer which vineyard plots were no longer cost-effective. A presence of false yellowhead was observed on these agricultural wastelands. The aim of this preliminary study is to look for copper stress biomarkers on the false yellowhead using a non-targeted metabolomics approach, coupled with the characterization of rhizosphere bacteria based on a 16S rRNA metabarcoding approach.

To achieve this, 15 plants of false yellowhead were cultivated in a phytotron with original soil (not spiked with copper) (5 plants), spiked soil with 50 mg/kg of bioavailable copper (5 plants) and spiked soil with 500 mg/kg of bioavailable copper (5 plants). After 2 months, leaves and rhizospheric soils were collected. An optimisation of the extraction of the leaves metabolites was realised using a Box-Behnken design of experiments. Leaves metabolites were extracted using MeOH/ $H_2O$  (70/30) and analysed with an UHPLC-HRMS system. Rhizospheric bacterial DNAs were extracted using a DNA extraction kit. A sequencing of the DNAs was performed.

Multivariate analyses revealed an impact of copper on the metabolome of the false yellowhead. Propositions of structure or chemical family (flavonoids, sesquiterpenes...) were made on the chemical biomarkers of the copper stress. This families could have antioxidants activities. An impact of copper on the bacterial community was also observed with multivariate analysis on the metabarcoding data. Bacterial biomarkers (Phyla Actinobacteria and Proteobacteria) were found using linear discriminant analysis (LDA) of effect size (LEfSe). On another study, the monitoring of these biomarkers will be used to test the positive effect of bio-intrants on a copper stressed false yellowhead.

Key words: Environment - copper stress - metabolomics - microbiology - geochemistry - bioinformatics

## P63: ENVIROTYPING: A TOOL TO CONTEXTUALIZE AND STRENGTHEN THE AGRONOMIC EVALUATION OF BIOSOLUTIONS

Florence PICAULT, Stéphane BATON, Thomas GUILLET, Laure REZE, Jeanne SALE

The efficacy of biosolutions, such as biocontrol products and biostimulants is highly variable across environments. This is influenced by soil characteristics, climatic conditions, crop management history, and the presence of biotic or abiotic stresses. This variability challenges the interpretation of trial data and limits the extrapolation of results across locations and seasons.

Envirotyping offers an integrative framework to characterize the environmental context of field trials by analyzing pedoclimatic, agronomic, and stress-related variables. By contextualizing crop responses, envirotyping enhances the robustness of agronomic evaluations, improves reproducibility, and facilitates cross-site and multi-year comparisons.

The poster will present the main envirotyping tools and methods available, detailing their implementation, complexity, and cost.

Applied examples will illustrate how envirotyping can be integrated into trial management, including use cases related to drought stress response, biocontrol product efficacy, and varietal performance under different environmental scenarios.

## P64: GLOBAL ENVIRONMENTAL CHALLENGES: CAUSES AND CONSEQUENCES

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Climate change poses a great threat not only to ecosystems and animal life, but also to human well- being and society. Because of that this paper present the impact on the environment through the prism of the literature and when it was possible also through the sources. Environmental change refers to the alterations that occur in the natural world as a result of various factors, including human activities and natural processes. These changes encompass a wide range of phenomena, such as climate change, deforestation, habitat destruction, pollution, and species extinction. With the growing recognition of the significant impact of human actions on the environment, understanding and addressing environmental change have become crucial for the sustainability of the planet. One of the most pressing environmental challenges is climate change. Human activities, particularly the burning of fossil fuels, have led to a substantial increase in greenhouse gas emissions, trapping heat in the Earth's atmosphere and causing a rise in global temperatures. This phenomenon has far-reaching consequences, including more frequent and intense extreme weather events such as hurricanes, droughts, and heatwaves. Additionally, climate change disrupts ecosystems, leading to shifts in plant and animal species distributions, altered rainfall patterns, and rising sea levels, which pose threats to coastal communities.

Deforestation is another significant driver of environmental change. Forests play a vital role in maintaining the planet's ecological balance. They act as carbon sinks, absorbing and storing large amounts of carbon dioxide from the atmosphere. However, rampant deforestation for agricultural expansion, logging, and urbanization has resulted in the loss of vast forested areas worldwide. This destruction not only releases carbon dioxide back into the atmosphere but also diminishes biodiversity, disrupts ecosystems, and contributes to soil erosion and water scarcity. Habitat destruction, often linked to deforestation, poses a severe threat to countless species. Pollution is another significant factor contributing to environmental change. The release of pollutants into the air, water, and soil has detrimental effects on both human health and the natural environment. Air pollution, primarily caused by industrial emissions, vehicle exhaust, and burning fossil fuels, leads to respiratory problems, cardiovascular diseases, and even prematured.

Keywords: climate change, pollution, forests, ecosystems

## P65: MISTIC: EVALUATING BIOSOLUTIONS FOR IMPROVED DOWNY MILDEW MANAGEMENT IN GRAPEVINES

#### **Claire JAMET**

The MISTIC project explores innovative strategies to manage grapevine downy mildew by combining biostimulants (BS) and biocontrol (BC) products. The objective is to enhance plant tolerance through sustainable approaches and to develop physiological and molecular tools for assessing product efficacy.

Candidate products were evaluated under controlled hydric stress and infection conditions. Physiological responses and gene expression (via qPCR) were analyzed, focusing on markers related to plant defense (e.g., PR1, GST) and nutrition (e.g., NRT2, AS). FTA® cards were used in field trials to monitor transcriptomic responses on-site.

Results showed that certain biostimulants (e.g., BS1 - plant extract-based) significantly reduced water stress, and combinations like BC + BS3 (oligosaccharides and seaweed extracts) improved protection against downy mildew compared to individual treatments. Molecular analyses revealed enhanced expression of defense and nutrition genes in treated plants, validating physiological observations.

These findings highlight the complementary effects of BS and BC products and support the use of molecular diagnostics for in-field monitoring. This project paves the way for more efficient, sustainable, and scalable vineyard protection strategies.

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