SFR 4207 Angers
Qualité et Santé du Végétal
Quasav

SFR QUASAV

PhD Students Day

15TH EDITION

PhD students work presentation seminar

Thursday, November 17th
UFR Sciences – Amphitheater D
13h30 – 17h30

anses
ESA École Supérieure d'Agricultures Angers Loire
Nantes Université
INRAE science for people, life & earth
université angers
L'INSTITUT agro Rennes Angers

PLANNING OF THE SFR QUASAV PHD STUDENTS DAY

13h20 Opening of the doctoral students day

13h30 Introduction

FIRST SESSION

13h40 Antoine BODELOT – Functional analysis of the agglutinin gene family in apple/*Erwinia amylovora* interaction.

13h58 Béra LEY-NGARDIGAL – Impact of mechanical stimulation on *Hydrangea macrophylla.*

14h16 Charlotte GAUDIN – Common bean resistance induction studied using transcription activator-like effectors.

14h34 Clovis PAWULA – *Rosa gallica L*. and other Gallica roses, origin and role in the genesis of cultivated roses.

14h52 ADDE – Association Des Doctorant.e.s Etranger.e.s

15h00-15h25 Pause

SECOND SESSION

15h30 Manon MEUNIER – Chemometrics based on laser desorption ionization mass spectrometry assisted by ^{13}C NMR dereplication: an alternative approach to LC-MS^2.

15h48 Marthe MALECANGE – Effects of a free amino acid-rich biostimulant on plant under well-watered and water deficit conditions.

16h06 Sébastien LIGONNIÈRE – Involvement of the nicotinic acetylcholine subunits in modulating insect sensitivity to insecticides.

16h24 Tiffany GARIN – Bacterial Type VI secretion system: a driver of seed microbiota assembly?

16h42 **Outro**

DELIBERATIONS

16h46 Start of deliberations

17h15 Announcements of the deliberations and end of the PhD students day

VIDEO ABSTRACTS

You will find all the video abstracts of the PhD students on the following links:

WEEK 1 : https://youtu.be/ImwHrj7-cgg

- **Antoine BODELOT** (ResPom, IRHS)– Functional analysis of the agglutinin gene family in apple/*Erwinia amylovora* interaction.

- **Manon MEUNIER** (SONAS, UA) – Chemometrics based on laser desorption ionization mass spectrometry assisted by ^{13}C NMR dereplication: an alternative approach to LC-MS^2.

- **Clovis PAWULA** (GDO, IRHS) – *Rosa gallica L*. and other Gallica roses, origin and role in the genesis of cultivated roses.

- **Béra LEY-NGARDIGAL** (STREMHO, IRHS) – Impact of mechanical stimulation on *Hydrangea macrophylla.*

WEEK 2 : https://youtu.be/zyiWjRG-o3w

- **Sébastien LIGONNIÈRE** (SiFCIR) – Involvement of the nicotinic acetylcholine subunits in modulating insect sensitivity to insecticides.

- **Tiffany GARIN** (EmerSys, IRHS) – Bacterial Type VI secretion system: a driver of seed microbiota assembly?

- **Marthe MALECANGE** (STRAGENE, IRHS) – Effects of a free amino acid-rich biostimulant on plant under well-watered and water deficit conditions.

- **Charlotte GAUDIN** (EmerSys, IRHS) – Common bean resistance induction studied using transcription activator-like effectors.

Functional analysis of the agglutinin gene family in apple/*Erwinia amylovora* interaction

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Erwinia amylovora is a Gram negative *Enterobacteria* and the causal agent of fire blight, a disease affecting the members of the *Rosaceae* family, especially apple and pear. Applying a Plant Resistance Inducer called ASM (Acibenzolar-S-Methyl) on apple seedlings reduces the severity of fire blight and induces a transcriptional reprogramming leading to high expression levels of a cluster-organized gene family encoding agglutinins (Warneys et al, 2018). We named these gene family members *MdAGG1* to *MdAGG17* and produced the recombinant protein MdAGG10. This allowed us to show that MdAGG10 is able to agglutinate *E. amylovora* cells *in vitro* by binding to bacterial surface polysaccharides (Chavonet et al, 2022).

Our study aims at investigating *in planta* the effective involvement of MdAGGs in apple resistance to *E. amylovora* using transgenic apple Gala lines constitutively overexpressing *MdAGG10* or impaired in numerous *MdAGGs* coding sequences (knock-out -KO- lines) thanks to the CRISPR/Cas9 technology.

Our results show that despite an overexpression and an accumulation of MdAGG10 in leaves tissues comparable to ASM-treated wild-type plants, the constitutively overexpressing MdAGG10 transgenic lines are as susceptible to *E. amylovora* as the untreated wild-type plants. In contrast, ASM failed to protect the KO lines to the same extent than wild-type plants, although the ASM-treated KO lines were more resistant than the untreated wild-type plants. Loss of induced resistance in the KO lines was associated with extensive targeted gene edition ranging from the indel of one or two nucleotides to the deletion of several agglutinin-encoding sequences. These editions greatly affected both the expression level of *MdAGG* genes and protein accumulation upon ASM treatment in comparison to wild-type treated plants.

Overall, these results show that apple agglutinins participate to the resistance mechanisms provided by ASM toward *E. amylovora* but that overexpression of *MdAGG10* alone is not sufficient *per se* to enhance its resistance to fire blight.

Keywords: Malus domestica, fire blight, lectins, transgenesis,

References:

Chavonet Erwan, Matthieu Gaucher, Romain Warneys, Antoine Bodelot, Christelle Heintz, Anthony Juillard, Raphaël Cournol, et al. 2022. « Search for Host Defense Markers Uncovers an Apple Agglutination Factor Corresponding with Fire Blight Resistance ». Plant Physiology 188 (2): 1350-68. https://doi.org/10.1093/plphys/kiab542.

Warneys Romain, Matthieu Gaucher, Philippe Robert, Sophie Aligon, Sylvia Anton, Sébastien Aubourg, Nicolas Barthes, et al. 2018. « Acibenzolar-S-Methyl Reprograms Apple Transcriptome Toward Resistance to Rosy Apple Aphid ». Frontiers in Plant Science 9 (décembre): 1795. https://doi.org/10.3389/fpls.2018.01795.

Impact of mechanical stimulation on *Hydrangea macrophylla*

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Plant compactness is a key feature of ornamentals plants grown in containers. It contributes to plant esthetical value, increases plant robustness, reduces culture and storage surfaces and transportation costs for trading. For a long time, the producers are using some chemical products named plant growth regulators (PGRs) that enable growth modulation, yield improvement and better crop quality [1]. Among PGRs, a major group of compounds named “growth retardants” is particularly used in ornamental crops production to reduce shoot elongation, improve compactness, boost branching and therefore the number of flowers [2]. Despite PGRs benefits for producers, their use has important environmental impacts that has led to administrative withdrawal of several of these products from the market [3]. Alternative methods to growth retardants are thus urgently needed by producers.

Mechanical stimulation (MS) mimicking wind impact on wild plants has proven efficient in reducing growth, increasing diameter and increasing branching in several species, including ornamentals [4, 5]. Industrial solutions are even available for automated mechanical treatment. Yet, not all plant species respond similarly to a given mechanical treatment and desensitization to MS may occur when too frequent stimulations are applied [6]. Therefore, research need to be carried out to find the efficient protocol that meet growers’ objectives for each species.

To-date, little is known on *Hydrangea* and mechanical stimulation in this specie. Using an experimental set-up for mechanical stimulation, we currently investigate the phenotypical and molecular responses of *Hydrangea macrophylla* plants in order to decipher the mechanisms involved.

Keywords: *Hydrangea, mechanical stimulation, alternative growth regulators, thigmomorphogenesis, plant compactness*

References:

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Common bean resistance induction studied using transcription activator-like effectors

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Common bacterial blight of bean (CBB) is an economically important bacterial disease caused by *Xanthomonas citri* pv. *fuscans* (*Xcf*) and *X. phaseoli* pv. *phaseoli* (*Xpp*). Common bean resistance to CBB is intricate as it involves at least 14 quantitative resistance loci scattered throughout the genome. To better understand the molecular pathways leading to resistance in common bean, we previously performed RNAseq analyses on both susceptible and resistant genotypes after inoculation by *Xpp* strain CFBP-6546R. This highlighted candidate genes whose induction was linked to resistance against CBB. To test if the induction of these genes can trigger resistance to *Xpp* in common bean, artificial transcription activator-like effectors were built to specifically induce each candidate gene, and introduced in strain CFBP-6546R. Complemented strains were then used for pathogenicity tests and qRT-PCR assays, the results of which will be presented. This study highlighted *PvOvate7* as a major contributor to CBB resistance.

Keywords: Common bean, common bacterial blight, resistance, TALE.

Rosa gallica L. and other Gallica roses, origin and role in the genesis of cultivated roses

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Rosa gallica L., an endangered tetraploid rose species, protected in France and historically used in traditional medicine, is known to have a hybrid origin (Debray et al. 2022; Raymond et al. 2018). It is one of the main ancestors of the modern perfume and ornamental cultivars. This species was one of the first among the genus *Rosa* to be bred by humans in Europe (Gardes et al. 2005; Liorzou et al. 2016). The evolutionary history of the species has been scarcely investigated and studies on the subject have yielded incongruent results (Debray et al. 2022; Raymond et al. 2018).

For the first time, we try to decipher this question by implementing a comprehensive approach. First, we characterise the diversity of the species giving insight into the origins of French wild populations. Second, we evaluate the relationship between wild populations and old cultivated roses. Finally, taking advantages of these results we will be able to build a robust subsample to identify probable parental species of *R. gallica*.

We first gathered roses samples from France and abroad increasing the number of sampled populations from 90 to 248 including 124 from abroad. In parallel, we developed genotyped by sequencing microsatellite markers adapted to the genus *Rosa*. Subsequently, 1520 individuals regrouping wild and cultivated *R. gallica* as well as individuals belonging to other *Rosa* species were genotyped. Preliminary analyses revealed that several French wild populations are not so much wild as feral. We will test for the existence of several genetic groups by carrying diversity and structuration analyses. Clone correction will allow us to identify clonal varieties. Parentage and relatedness analyses could show that only few varieties represent a bridge between wild and cultivated compartments.

The identity of the parental species will be investigated by sequencing 96 single copy orthologous loci on individuals representing the diversity of *R. gallica* and on individuals belonging to other *Rosa* species. Phylogenetic network inference will then be performed to shed light on the hybrid origin of the species.

Finding answers to these questions will improve the conservation of the species and the management of the *R*. *gallica* cultivated genetic resources.

Keywords: *Rosa gallica, Population genetics, Phylogenetic network, Diversity*

References :

Debray, Kevin, Marie-Christine Le Paslier, Aurélie Bérard, Tatiana Thouroude, Gilles Michel, Jordan Marie-Magdelaine, Anne Bruneau, Fabrice Foucher, et Valéry Malécot. 2022. « Unveiling the Patterns of Reticulated Evolutionary Processes with Phylogenomics: Hybridization and Polyploidy in the Genus Rosa ». Systematic Biology 71 (3): 547‑69.

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Raymond, Olivier, Jérôme Gouzy, Jérémy Just, Hélène Badouin, Marion Verdenaud, Arnaud Lemainque, Philippe Vergne, et al. 2018. « The Rosa Genome Provides New Insights into the Domestication of Modern Roses ». Nature Genetics 50 (6): 772‑77.

Chemometrics based on laser desorption ionization mass spectrometry assisted by ^{13}C NMR dereplication: an alternative approach to LC-MS^2

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Natural Products (NPs) are known for a wide range of most interesting biological effects[1]. In order to avoid the repetitive isolation of previously described compounds and time-consuming bioassay guided fractionation[2] strategies, the early-on identification of active metabolites from complex mixture has become a key element in NPs research. Merging chemical profiling with biological data, chemometrics[3] represent a cornerstone of this strategy. In the process Ultra High-Performance Chromatography (UPLC) coupled with High Resolution Mass Spectrometry (HRMS) is considered as a method of first choice for data acquisition. Despite its indubitable assets, UPLC-HRMS may nevertheless encounter certain limitations linked to solvent limitation, ionizability of analytes and the differentiation of (stereo)isomers. Addressing these issues, matrix free laser desorption ionization-HRMS (LDI-HRMS)[4] assisted by ^{13}C NMR and the MixONat ^{13}C dereplication software[5] may provide an alternative approach. As a working example, LDI-HRMS assisted by ^{13}C NMR was used successfully applied for the identification of secondary metabolites with anti-AGEs* activities [6] from *Garcinia parvifolia* bark extracts and results were thoroughly compared with concurrently performed UPLC-HRMS experiments.
*The used assay evaluates inhibition of the formation of advance glycation end products.

Keywords: Natural Products, Chemometrics, Mass spectrometry, NMR

References:
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Effects of a free amino acid-rich biostimulant on plant under well-watered and water deficit conditions

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Water deficit leads to substantial yield losses, which will be more problematic due to climate change. In order to improve plant tolerance to abiotic stress, plant biostimulants are increasingly used in agriculture. However, more research is needed to clarify the mechanisms of action of plant biostimulants, including protein hydrolysates.

In this study, we examined, for the first time, effects of a commercially available free amino acid-rich biostimulant (from BCF Life Sciences) on greenhouse lettuce (*Lactuca sativa* L.) grown under well-watered and water deficit conditions. In order to characterize the mode of action of this biostimulant, we analyzed physiological and metabolomic water deficit responses of lettuce treated with biostimulant or not.

We found that foliar application of tested biostimulant was less effective compared to root application. Indeed, the root application of this biostimulant increased both shoot fresh biomass of well-watered (+40%) and deficit-irrigated (+20%) lettuces. These results are explained by an increase in total leaf area rather than an enhancement of leaf production (number of leaves). Moreover, the Shoot: Root ratio has also been increased by root application of this biostimulant, irrespective of the water condition. Data of metabolic profiling have revealed that, under water deficit condition, the better growth of biostimulant-treated lettuces could be associated to the accumulation of osmolytes (proline, soluble sugars, polyols). We also observed that, under water deficit condition, biostimulant application has led to the accumulation of compounds involved in tolerance to oxidative stresses (polyamines).

All these findings indicate that the tested biostimulant is a powerful product, which improves and secures yield, irrespective of the water condition, through adjustment of plant metabolism.

Keywords: *Agronomy, biostimulant, water deficit*

Involvement of the nicotinic acetylcholine subunits in modulating insect sensitivity to insecticides

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One of the new challenges of the contemporary agriculture is to produce enough quality food to meet the food needs of a constantly growing population. For several decades, the widespread use of insecticides has allowed the protection of crops against pest insect to increase their yields. However, their massive use has led to the development of resistant insect species resulting in an overall decrease of their effectiveness. Thus, as modern agriculture evolves towards an agroecological crop protection, the study of the molecular and cellular mechanisms involved in insecticide resistance is crucial to allow a more sustainable agriculture. Previous studies have shown the contribution of sublethal doses of insecticides in the development of resistance. In particular, it has been shown that cockroaches *Periplaneta americana* exposed to sublethal doses of imidacloprid present a decreased sensitivity to this insecticide as well as modifications of their nicotinic acetylcholine subunit expression pattern. Thus, the decrease or increase of some nicotinic subunit expression impacts the sensitivity of insects to insecticide. In a first part of this work, we have been focusing on the involvement of the α7 nicotinic subunit in the cockroach sensitivity to imidacloprid. The expression of this subunit was found to be decreased after an exposure of the cockroaches to a sublethal dose of imidacloprid. So, our hypothesis is that the α7 nicotinic subunit is involved in the imidacloprid sensitivity of the cockroaches. First, we mimicked the decrease of α7 nicotinic subunit expression observed in the terminal abdominal ganglia (TAG) of cockroaches exposed to sublethal doses of imidacloprid using RNA interference. Acute imidacloprid intoxication experiments on dsRNA-α7 treated cockroaches allowed us to observe a decreased sensitivity to the insecticide showing a direct or indirect involvement of the α7 nicotinic subunit in imidacloprid sensitivity. As insecticide sensitivity can be influenced by different molecular or cellular mechanisms such as the modification of target proteins or calcium signaling, we then sought to identify the underlying mechanisms responsible for the decreased sensitivity of dsRNA-α7 treated cockroaches. We have shown that the decreased α7 nicotinic subunit expression induced a compensation mechanism by altering the expression of other nicotinic subunits. Finally, as the mammalian homomeric nicotinic receptor α7 is highly permeable to calcium, we assess the impact of dsRNA-α7 on the calcium response of the cockroach nicotinic acetylcholine receptors (nAChRs) using calcium imaging. In this first study, the use of dsRNA targeting the α7 nicotinic subunit allowed us to make cockroaches less sensitive to imidacloprid. In a future study, we will study the impact of dsRNAs targeting other nicotinic subunits in order to make the cockroach more sensitive to an insecticide or to restore the sensitivity to an insecticide of cockroaches that has become resistant. These results which will increase our knowledge on cellular and molecular mechanisms involved in insecticide sensitivity could contribute to develop sustainable insect resistance management strategies.

Keywords: *Insecticide resistance, Imidacloprid, RNA interference, α7 nicotinic acetylcholine subunit.*

Bacterial Type VI secretion system: a driver of seed microbiota assembly?

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Plant seeds harbor a diversified microbiota that contributes to plant health. The seed habitat is quite limited in space and resources and, consequently, the seed microbiota is reduced with few dominant microbial taxa in comparison to other plant habitats. The competition for resources partially explains the composition of microbial assemblages. Microbial interactions also include intermicrobial competition through specific antimicrobial weapons such as the type VI secretion system (T6SS) which is widespread among seed associated bacteria. We hypothesize that such bacterial T6SS contributes to the assembly of the seed microbiota especially during the transmission of microbiota from seed to seedling. We chose a strain of a *Stenotrophomonas rhizophila* isolated from radish microbiota with a high transmission efficiency from seed to seedling and with a strong antimicrobial effect towards the phytopathogenic bacteria *Xanthomonas campestris pv. campestris* 8004 (Xcc8004). After the description of one complete T6SS cluster in *S. rhizophila* genome, we constructed two T6SS deletion mutants and demonstrated that its T6SS was involved in an *in vitro* contact antibiosis towards Xcc8004. Then, we confronted different bacterial communities to the *S. rhizophila* wild type and its T6SS deletion mutant *in vitro* and described the resulting community composition with *gyrB* metabarcoding. The dynamics of community assembly totally differed between those with the wild-type strain and those with the T6SS-mutant. T6SS of *S. rhizophila* is therefore a strong driver of *in vitro* seed microbiota assembly and has a widespread effect on diversified bacterial taxa. We now investigate the role of T6SS in the transmission of *S. rhizophila* from seed to seedling and also in shaping transmitted bacterial communities *in planta*.

Keywords: *T6SS, Stenotrophomonas rhizophila, Xanthomonas campestris pv. campestris 8004, intermicrobial competition, seed, microbiota.*