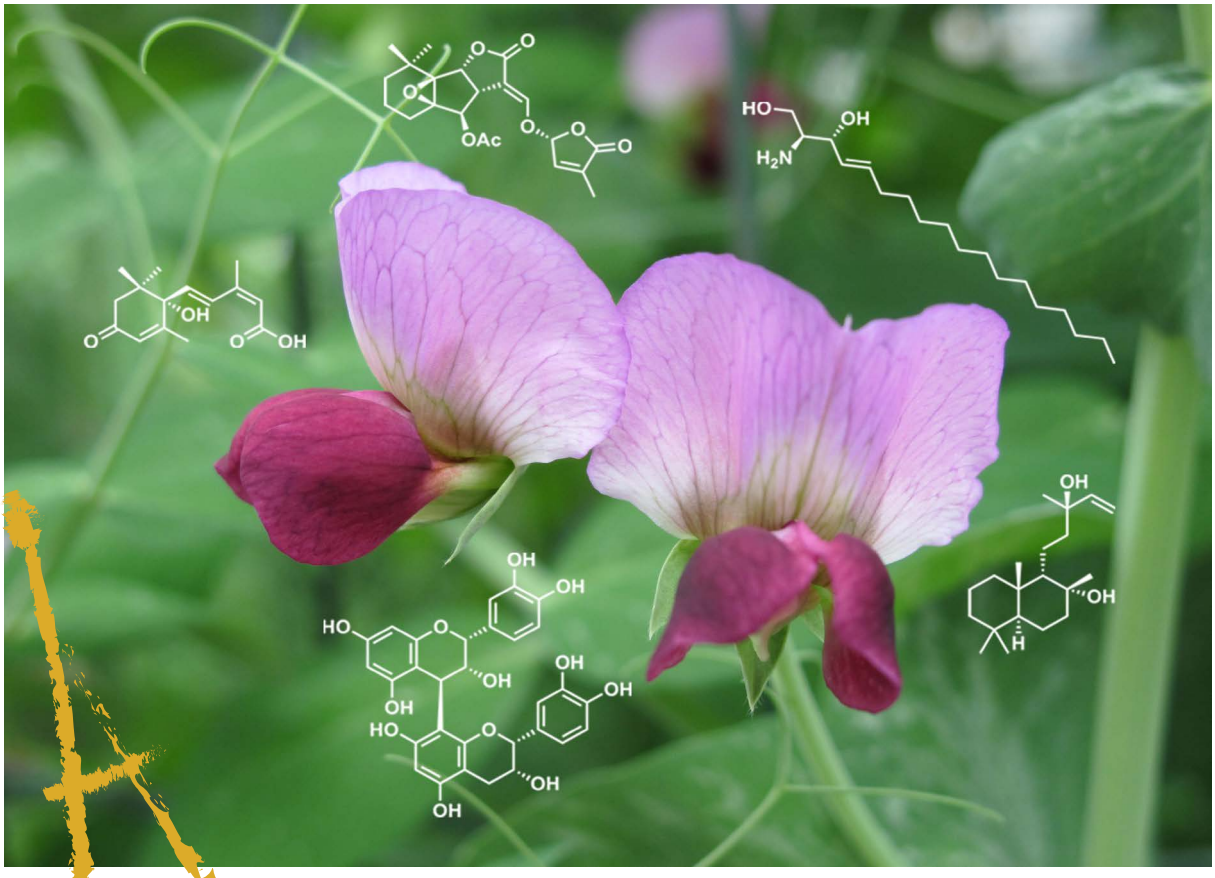
SPS Summer School 2019

June 30th to July 4th, 2019

Versailles - Orsay, France

« SPECIALIZED PLANT METABOLITES: FROM ANALYSIS TO ENGINEERING »

Participant's guide



SPS

SACLAY PLANT SCIENCES

Contents

Sponsors and partners

Venue

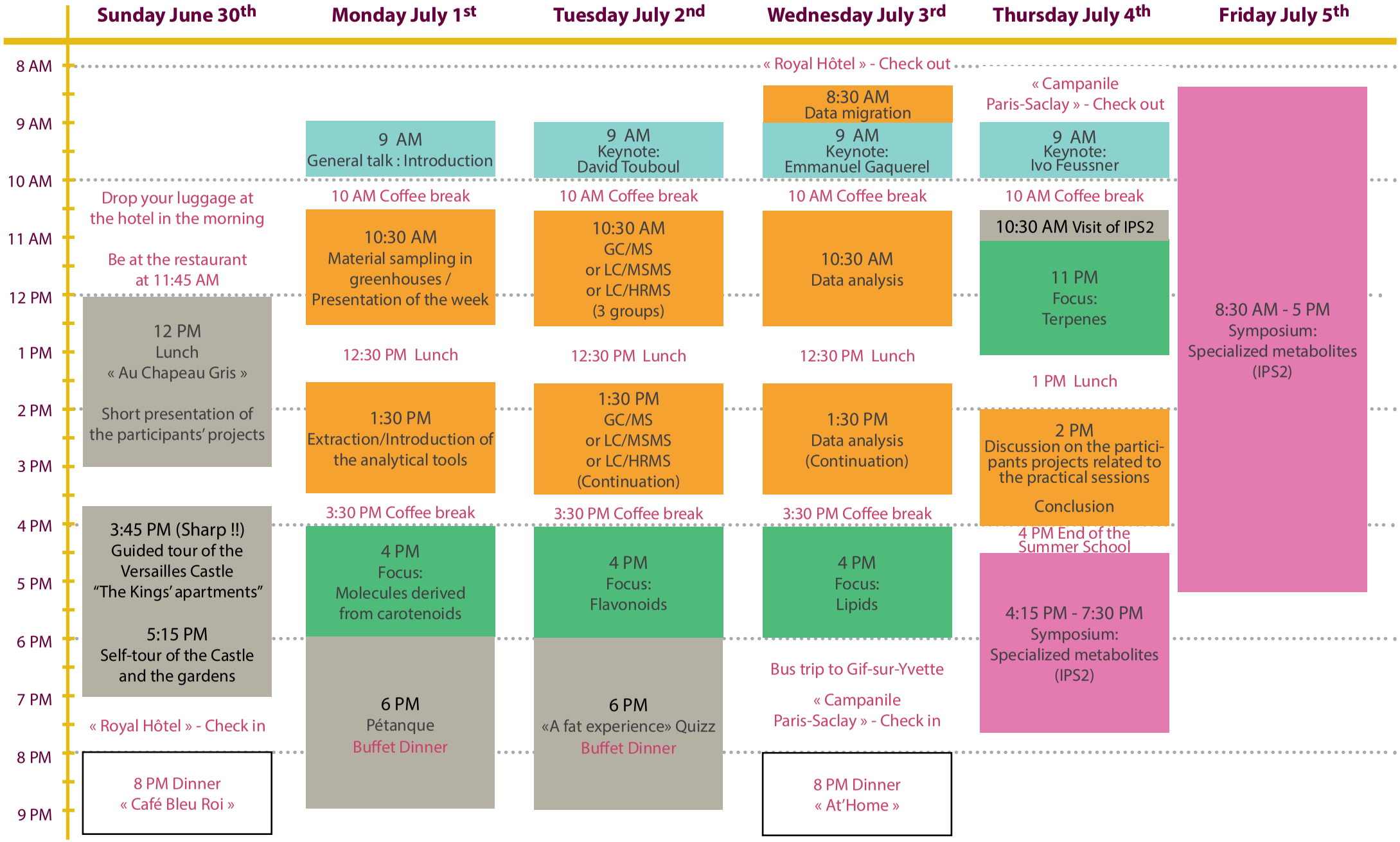
Institut Jean-Pierre Bourgin
INRA Centre Île-de-France Versailles-Grignon
Route de St-Cyr (RD 10)
78000 Versailles

Institut de Sciences des Plantes - Paris-Saclay
Bâtiment 630, rue de Noetzlin
Plateau du Moulon
91190 Gif-sur-Yvette

Summer School e-mail address

SPS-Summer-School@inra.fr

Planning at a glance



Program

Sunday June 30th

Please be at 11:45 AM at the restaurant "Au chapeau gris", 7 rue Hoche, 78000 Versailles.
Don't be late!

Attention :
You will go directly from the restaurant to the Versailles Castle in the afternoon, which means that you must not bring you luggage to the restaurant.
Thus, before coming to the restaurant "Au chapeau gris", you will have to drop your luggage at the "Royal Hôtel Versailles", 23 rue Royale, 78000 Versailles. The hotel check-in will be done in the evening (it cannot be done before 2 PM) but, in the meantime, they will place your luggage in a secured room.

12 PM – 3 PM: Lunch
Introduction (Grégory Mouille)
Presentation of the participants' research (2 to 3 Powerpoint slides, 5 min max.)

3:45 PM – 5:15 PM: Guided tour of the Versailles Castle "The Kings' apartments" - **Don't be late !!!**

5:15 PM – 7 PM: Self-tour of the castle and the gardens

7 PM – 8 PM: Check-in at the "Royal Hôtel Versailles"

8 PM: Diner at the restaurant « Le Café Bleu Roi », 7 Rue Colbert, 78000 Versailles

Night at the "Royal Hôtel Versailles"

Monday July 1st

Institut Jean-Pierre Bourgin (IJPB)
INRA Centre Île-de-France Versailles-Grignon
Route de St-Cyr (RD 10)
78000 Versailles

9 AM – 10 AM: Presentation of the Summer School's Spirit (Building 1 - Library)

10 AM – 10:30 AM: Coffee break (Building 1 - Library)

10:30 AM – 12:30 PM: Practical session (Building 14 – Salle TP):
Stéphanie Boutet, Sylvie Citerne, Gilles Clément, François Perreau, Grégory Mouille
Material sampling in greenhouses /
Presentation of the practical sessions of the week

12:30 PM – 1:30 PM: Lunch at the INRA cafeteria

1:30 PM - 3:30 PM: Practical session (Building 14 – Salle TP):
Stéphanie Boutet, Sylvie Citerne, Gilles Clément, François Perreau, Grégory Mouille
Extraction/Introduction of the analytical tools
(theory Mass Spectrometry and Chemistry)

3:30 PM – 4 PM: Coffee break (Building 1 - Library)

4 PM – 6 PM: Focus: Molecules derived from carotenoids (Building 1 - Library)

"Abscisic acid metabolism, signaling pathways and physiological roles" - Annie Marion-Poll
Beside their function as pigments, carotenoids are the precursors of a vast family of compounds with various biological functions, including the phytohormones, strigolactones and abscisic acid (ABA). Recent studies on ABA metabolism, perception and signaling have contributed to a better understanding of ABA functions in stress responses, as well as in plant growth and development.

"The numerous functions of strigolactones as plant hormones" - Catherine Rameau
Beside their role in repressing shoot branching, strigolactones have now been implicated in controlling a wide range of morphological traits, including leaf senescence; internode growth; shoot branching angle; stem secondary growth and root architecture. In addition species-specific roles are being discovered. Strigolactones do not regulate these processes alone, but in concert with other hormones, either antagonistically or synergistically.

"Diversity of natural strigolactones, biosynthesis, development of analogs for their studies" - François-Didier Boyer
To date, more than 30 natural strigolactones belonging to different types have been characterized. Many key steps in their biosynthesis are now known. However, the structural complexity of these natural products and their interesting bioactivities and perception mechanism led to the development of numerous analogs and probes.

"Strigolactone perception and signaling » - Alexandre de Saint Germain
By using multidisciplinary approaches (crystallography, enzymology, genetic, biophysics), the SL perception has been elucidated and a unique mechanism of plant hormone reception where the receptor itself performs an irreversible enzymatic reaction to generate its own ligand has been revealed. But this mechanism is still debated and also the ability of the receptor to interact with many partners.

"Strigolactones as complex actors of plant-microbe interactions" - Marie Dufresne
If strigolactones have been well characterized in plant interactions with parasitic plants and arbuscular mycorrhizal fungi, an increasing number of studies try to decipher their role in the interaction of plants with pathogenic microorganisms. However, their exact role is difficult to assess likely because of pleiotropic effects.

6 PM – 9 PM: Buffet dinner (veranda of the INRA cafeteria) and Pétanque tournament

Night at the "Royal Hôtel Versailles"

Tuesday July 2nd

Institut Jean-Pierre Bourgin

9 AM – 10 AM: Keynote: "Mass-spectrometry-based approaches for the characterization of plant secondary metabolites" - David Touboul, ICSN, Gif-sur –Yvette, France (Building 1 - Library)
Analytical chemistry, especially chromatography coupled to mass spectrometry, allows to explore the chemical diversity of complex natural extracts. The lecture will focus on the different tools available to acquire MS data (GC, LC, HRMS, MS/MS) and to analyze them in order to identify chemical features to differentiate groups of samples or to decipher original chemical compounds.

10 AM – 10:30 AM: Coffee break (Building 1 - Library)

10:30 AM – 12:30 PM: Practical session (Meeting point - Building 1 - Library):
> Group 1: GC/MS Part1 - Gilles Clément
> Group 2: LC/MSMS (absolute quantification/structural characterization) - Sylvie Citerne, Grégory Mouille
> Group 3: LC/HRMS (profiling/structural characterization) - Stéphanie Boutet, François Perreau

Group 1	Group 2	Group 3
MAIA Marisa PICHLER Gregor SANDOR Andras WATERMAN Jamie WUYUNTANA	ANGUITA-MAESO Manuel CRUZ Pamela LINGWAN Maneesh LUZAROWSKI Marcin	CORSO Massimiliano HANSEN Cecilie Cetti WANG Jian You

12:30 PM – 1:30 PM: Lunch at the INRA cafeteria

1:30 PM - 3:30 PM: Practical session (Meeting point - Building 1 - Library):
> Group 1: GC/MS Part2 - Gilles Clément
> Group 2: LC/HRMS (profiling/structural characterization) - Stéphanie Boutet, François Perreau
> Group 3: LC/MSMS (absolute quantification/structural characterization) - Sylvie Citerne, Grégory Mouille

3:30 PM – 4 PM: Coffee break (Building 1 - Library)

4 PM – 6 PM: Focus: Flavonoids (Building 1 - Library)

"Flavonoid functions and impacts" - Isabelle Debeaujon
Flavonoids are plant-specific polyphenolic compounds that are widely distributed among land plants. We will describe how these fascinating molecules have evolved to play important roles in plant adaptation to their environment, from development to defense against biotic and abiotic stresses. The impacts of flavonoids as bioactive compounds with both nutritional and medicinal benefits for humans and animals will also be presented.

"Flavonoid chemical diversity and mass spectrometry analyses" - François Perreau
More than 6000 different flavonoids have been found in plants and can be divided in 7 subfamilies. This chemical diversity requires specific methods for quantification and identification. Mass spectrometry-based tools have been developed to tackle these needs, from targeted MRM analysis to profiling and untargeted metabolomics.

"Transcriptional regulation of flavonoid biosynthesis" - Loïc Lepiniec
Flavonoid biosynthesis is largely controlled at the transcriptional level. We will present the key roles of MBW (MYB–bHLH– WDR) protein complexes in developmental and environmental regulations. These proteins are well conserved in higher plants and thus, provide both interesting models for investigating transcriptional regulatory networks and relevant tools for modifying flavonoid content in plant products.

6 PM – 9 PM: Buffet dinner and "A fat experience" Quizz - Marine Froissard (veranda of the INRA cafeteria)

Night at the "Royal Hôtel Versailles"

Wednesday July 3rd

Check-out at the "Royal Hôtel Versailles".
Bring your luggage with you at INRA.

Institut Jean-Pierre Bourgin

8:30 AM – 9 AM: Data migration (Meeting point - Building 1 - Library)

9 AM – 10 AM: Keynote: "Metabolomics-assisted dissection of plants' metabolic innovations" - Emmanuel Gaquerel, IBMP, Strasbourg, France (Building 1 - Library)
The overarching objective of our research is to understand the defensive functions of plant specialized metabolites and their biosynthetic pathways. At the heart of my research agenda lies the idea that such process can be moved up to an unprecedented level through innovations in metabolomics and in the bioinformatics integration of heterogeneous omics data. In this talk, I will present MS metabolomics developments to improve the exploration of intra- and inter-species metabolic variations in the context of plant-insect interactions and how these can be exploited to support gene function discovery.

10 AM – 10:30 AM: Coffee break (Building 1 - Library)

10:30 AM – 12:30 PM: Practical session (Meeting point - Building 1 - Library):
Data analysis GCMS - Group 1 - Gilles Clément
Data analysis LCMS – Groups 2 and 3 - Stéphanie Boutet, François Perreau

12:30 PM – 1:30 PM: Lunch at the INRA cafeteria

1:30 PM - 3:30 PM: Practical session (Building 10 – Computer room):
Data analysis (continuation) - Groups 1 / 2 / 3
Stéphanie Boutet, Gilles Clément, François Perreau

3:30 PM – 4 PM: Coffee break (Building 1 - Library)

4 PM – 6 PM: Focus: Lipids (Building 1 - Library)

"Metabolic engineering for oil improvement – Principle and applications" - Jean-Luc Cacas
Roughly eighty percent of the plant oil market (200 hundred million tons) is dedicated to food industry and the yearly-growing twenty percent left is a supply for other industrial applications, mainly linked to bio-based production. The huge diversity of fatty acids found in plants (around three hundred molecular species) is associated with as much properties-driven applications. This potential likely explains, in part, the increasing request for biotechnological usage. It has thus become obvious that improving oil yield and modifying its fatty acid composition is of utmost importance. Metabolic engineering is a powerful approach that can help with these two tasks, especially when it comes to accumulate low abundance or ectopic fatty acids in oils.

"Gene editing for breeding new oil profile" - Jean-Denis Faure
Engineering gene dosage with genome editing provides an efficient tool for plant breeding and metabolic engineering. Large collection of combinatorial mutants in delta-12-desaturase (FAD2) genes involved in omega6 fatty acid synthesis was generated allowing fine-tuning of mono/polyunsaturated fatty acid ratio. Lines with specific fatty acid profile are valuable tool to investigate the role of specific fatty acids in plant development and in the response to environmental changes.

"Biotechnological strategies for the production of omega-7 fatty acids in seeds" - Sébastien Baud
Omega-7 monoenoic fatty acids are increasingly exploited both for their positive effects on health and for their industrial potential. Some species produce fruits or seeds with high amounts of these fatty acids. However, the low yields and poor agronomic properties of these plants preclude their commercial use. As an alternative, the metabolic engineering of oilseed crops for sustainable omega-7 production is emerging.

6 PM – 8 PM: Bus trip to Gif-sur-Yvette
Check-in at the hotel "CAMPANILE Paris-Saclay", 3 rue Joliot-Curie, 91190 Gif-sur-Yvette

8 PM: Diner at the restaurant "L'AT HOME", 9 Bis rue Joliot-Curie, 91190 Gif-sur-Yvette

Night at the "Campanile Paris-Saclay" hotel

Thursday July 4th

Check-out at the "Campanile Paris-Saclay" hotel.
Bring your luggage with you at IPS2.

Institut de Sciences des Plantes - Paris-Saclay (IPS2)
Bâtiment 630, rue de Noetzlin
Plateau du Moulon
91190 Gif-sur-Yvette

9 AM – 10 AM: Keynote: "What the transcriptome does not tell – the metabolome is closer to the phenotype" - Ivo Feussner, Goettingen University, Germany (Primevère room)
The metabolome of the plant provides a wealth of additional information on plant–microbe interactions since it not only represents an additional level of regulation, but often harbors the end products of regulatory processes. Therefore, it is closest to observed phenotypes. Metabolomics has developed into a powerful approach to discover the role of small molecules during plant–microbe interactions involved in systemic acquired resistance and the precursors for the formation of molecules that provide physical barriers to prevent spreading of pathogens were identified.

10 AM – 10:30 AM: Coffee break (Primevère room)

10:30 AM – 11 AM: Visit of IPS2

11 AM – 1 PM: Focus: Terpenes (Primevère room)

"Combination of sub-cellular metabolic activity investigation and mutagenesis to improve specialized metabolites production"- Adnane Boualem
"13C-NMR analysis to understand specialized metabolite pathways" – Caroline Mauve
Plants synthesize a vast number of diverse, low-molecular-weight molecules (known as specialized/secondary metabolites), many of which exhibit important biological properties and have industrial applications. Terpenoids are the largest and most diverse group of plant specialized metabolites. Their structural complexity makes the total chemical synthesis expensive, uneconomical and difficult. A major factor constraining the effective improvement of plant specialized metabolites production is our poor understanding of the biochemistry and gene regulatory pathways required to synthesize the target metabolites and the molecular mechanisms controlling the development of the metabolite-producing organ. Our research aims to improve our understanding of the specialized metabolite biosynthesis and regulation and also to deliver the tools and the necessary expertise to engineer high productive plants by manipulating the specialized metabolism pathways.

1 PM – 2 PM: Lunch (Lunch-boxes - IPS2 cafeteria)

2 PM – 4 PM: Discussion on the participants' projects related to the practical sessions (Primevère room)
- Stéphanie Boutet, Sylvie Citerne, Gilles Clément, François Perreau, Grégory Mouille
Conclusion

4 PM: End of the Summer School

Abstracts of the participants

Differences in xylem sap composition of distinct olive tree varieties: a metabolomic approach

Manuel Anguita-Maeso, Carmen Maria Haro, Juan Carlos Rivas, Juan A. Navas-Cortés, Blanca B. Landa

Instituto de Agricultura Sostenible (IAS), Spanish National Research Council (CSIC), Spain

manguitamaeso@gmail.com

The importance of determining xylem sap composition is vital to obtain information for understanding the nutritional requirements of xylem-limited microbiome. Branches from Picual and Arbequina cultivated trees and plants grafts grown into rootstocks of Olea europaea var. sylvestris (Ac13 and Ac18) were selected to characterise the chemical composition of xylem sap. A Scholander pressure chamber was used to perform xylem sap extraction with an external port allowing branches inclusion up to 60 cm long. Metabolome analysis of xylem sap was performed by proton nuclear magnetic resonance (NMR) spectroscopy-based study and ionome analysis was performed by inductively coupled plasma with optical emission spectroscopy (ICP-OES). A total of 30 metabolites were identified in xylem sap through NMR including amino acids (alanine, arginine, aspartate, glutamate, glutamine, isoleucine, leucine, methionine, proline, threonine, tyrosine, and valine), sugars (glucose, fructose, mannitol, myo-inositol, and sucrose), organic acids (formic, fumaric and succinic acid), alcohols (ethanol and methanol), and other molecules. Furthermore, ICP-OES allowed the detection of 14 elements and 5 inorganic anions. Knowledge of the chemical composition of xylem sap will lead to a better understanding of the nutritional requirements of olive xylem-inhabiting microorganisms and help in the design of artificial growing media for olive microbiome cultivation.

A new role for flavonoids in micronutrients homeostasis and in plant adaptation to extreme metal environment

Massimiliano Corso[1,2], Friederike Mey[1], Gilles Bruylants[3], Christine Delporte[4], Kristin Bartik[3], Sylvain Merlot[2], Sébastien Thomine[2], Marc Hanikenne[5], Nathalie Verbruggen[1]

[1] *LPGMP, ULB, Belgium*
[2] *I2BC, CNRS, Université Paris-Saclay, France*
[3] *EMNS, ULB, Belgium*
[4] *LPC, ULB, Belgium*
[5] *InBioS-PhytoSystems, ULg, Belgium;*

massimiliano.corso@i2bc.paris-saclay.fr

The fine regulation of micronutrient homeostasis is of vital importance for all living organisms. Flavonoids are phenolic compounds which play a pivotal role in human health and diet. I recently described an emerging role for flavonoids in plant adaptation to extreme environment. Ionomic, transcriptomic and metabolomic data from *Arabidopsis* species showing contrasting metal tolerance and accumulation highlight a new role for flavonoids in the regulation of metal homeostasis and adaptation to extreme metallic environments. Moreover, Nuclear Magnetic Resonance data also support flavonoids ability to form a complex with metals.
Are flavonoids (and other secondary metabolites) important for metal homeostasis in plants? What is the mode of action of flavonoids in metal binding and antioxidant stress responses? What are the flavonoid-related genes that play a major role during micronutrient excess or deficiency? My research aims to answer these questions by studying physiological, molecular and biochemical processes that plants use to regulate micronutrient homeostasis. I envisage that a greater understanding of secondary metabolites in the regulation of metal homeostasis will contribute to the enhancement of sustainable agriculture impacting on food security and food quality, and to the identification of new solutions to detoxify contaminated environment.

Elucidation and engineering of the biosynthesis of alkaloids of pharmaceutical interest originating from Apocynaceae

Pamela Cruz, Vincent Courdavault, Marc Clastre

EA2106 Biomolécules et Biotechnologies Végétales, Université de Tours, 37000 Tours, France

pamela.lemoscruz@etu.univ-tours.fr

The Madagascar periwinkle (*Catharanthus roseus*) is a model medicinal plant of the Apocynaceae family producing indole monoterpene alkaloids (MIA) which became the reference in the study of a specialized metabolism. The increased knowledge on this metabolism has been driven by the development of transcriptomic resources combined with new methods of functional characterization of genes.
The objective of the thesis subject is to elucidate the biosynthetic pathways of MIAs of interest in *C. roseus, Vinca minor* and the genus *Rauwolfia* by the identification of enzymatic steps that have not yet been elucidated through gene coexpression studies by analyzing the transcriptomic data to select candidate genes. Functional validation of these genes will be performed by loss of function studies using the VIGS (virus-induced gene silencing) technique. Finally, gene function will be characterized by biochemical studies performed on the corresponding recombinant enzymes.
These experiments are prerequisites for implementing enzymatic modules within the yeast *Saccharomyces cerevisiae* to produce the MIAs of interest by metabolic engineering. This will make it possible to propose a new way of sourcing these molecules without the use of the plants. In addition, this work can also go farther by exploiting enzymatic promiscuity of certain enzymes to manufacture, by metabolic engineering, non-natural biogenic MIAs, thus offering innovative perspectives for the development of new active substances.

Orchestration of cyanogenic glucoside biosynthesis in *Eucalyptus*

Cecilie Cetti Hansen[1,2,3], Mette Sørensen[1,2], Birger Lindberg Møller[1,2,3], Elizabeth H. J. Neilson[1,2]

[1] *Plant Biochemistry Laboratory, Department of Plant and Environmental Sciences, University of Copenhagen*
[2] *VILLUM Center for Plant Plasticity, University of Copenhagen*
[3] *Center for Synthetic Biology, University of Copenhagen*

cich@plen.ku.dk

The genus *Eucalyptus* (+700 species) is rich in structurally diverse natural products including terpenes, phenolics, formylated phloroglucinols and cyanogenic glucosides (CNglcs). CNglcs are amino acid-derived specialized metabolites produced by species throughout the plant kingdom, including certain *Eucalyptus* species. Cell disruption results in hydrolysis of CNglcs and release of toxic hydrogen cyanide providing an effective chemical defense against generalist herbivores. The formation of CNglcs is typically catalyzed by two multifunctional cytochromes P450 (CYPs) and an UDP-glucosyltransferase. We demonstrate that this orchestration is different in *Eucalyptus*. Twenty-three cyanogenic *Eucalyptus* species have been identified [1], and these species all produce phenylalanine-derived CNglcs, primarily prunasin. Using analytical chemistry, transcriptomics and biochemical analysis, we have recently characterized prunasin production in *E. cladocalyx* and identified the biosynthetic genes [2]. In contrast to other known CNglc biosynthetic pathways, formation of the cyanohydrin in *E. cladocalyx* requires the sequential action of three CYPs including a unique CYP706. The involvement of a CYP706 in CNglc biosynthesis is specific to *Eucalyptus* and represents a fascinating example of convergent evolution in this widespread chemical defense system in plants. By investigating de novo assembled transcriptomes from additional cyanogenic *Eucalyptus* species, we also identified an interesting biosynthetic difference at the UDP-glucosyltransferase level revealing pathway heterogeneities between species within a single genus.

[1] Gleadow *et al.* (2008) *Phytochemistry* 69, 1870-74
[2] Hansen *et al.* (2018) *Plant Physiol.* 178, 1081–95

Decoding the essential oil metabolomes and associated pathways in plants using NMR and GC-MS

Maneesh Lingwan, KVK Linga Rao, Yogesh Pant, Shyam K. Masakapalli

BioX Centre, School of Basic Sciences, Indian Institute of Technology Mandi, Himachal Pradesh- 175005, India

maneesh.iitmandi@gmail.com

Essential oils (EOs) are aromatic, volatile secondary metabolites that play significant ecological and biological roles in Plants. Decoding the EOs metabolomes and associated pathways in plants comprehensively will teach us their regulation and in identifying repertoire of phytochemicals with pharmaceutical, agricultural and cosmetic applications. The efficiency of EOs for various applications depends mainly on the composition of characteristic metabolites that are present. In this study, 18 essential oils from aromatic plants of Himalayan and Indian origin were profiled for their metabolome analysis using NMR and GC-MS. We profiled more than 250 metabolites (such as Asarone, pinene, Limonene, Linalool, Linalyl Acetate, Myrcene, p-Cymene, Geraniol, Ocimene, Cedrol etc.) which further correlated the similarities and variations among EOs using standard multivariate statistical analysis (PCA, PLS-DA). The metabolite identities were confirmed collectively from three databases (fragrance and flavour, FIEN20, NIST2017) and commercially available standards. The metabolites profiles allowed us to overlay them to various secondary metabolic networks in plant systems. Mainly the terpenoid indole alkaloid (TIAs) pathways contributed to a range of metabolites. Future work is ongoing towards identifying plant/tissue specific metabolites that might be of relevance to correlate with biochemical evolution, robustness of secondary metabolism and chemical signatures. In addition, we are building up mass spectroscopy library and online database, which would be beneficial EOs-based research and other applications.

Untargeted map of yeast Sacharomyces cerevisiae protein-protein-metabolite interactome reveals unprecedented complexity of the protein-protein and protein-metabolite interactions

Marcin Luzarowski[1], Andrei Kiselev[1], Ruben Vicente Perez[1], Izabela Wojciechowska[1], Urszula Luzarowska[2], Lothar Willmitzer[1], Aleksandra Skirycz[1]

[1] *Max Planck Institute of Molecular Plant Physiology, Golm, Germany*
[2] *Ben-Gurion University of the Negev, Be'er Sheva, Israel*

luzarowski@mpimp-golm.mpg.de

Query of the experimentally validated protein-metabolite (PMIs) and protein-protein interactions (PPIs) reveals a huge discrepancy in number. In the model yeast *Sacharomyces cerevisiae*, there is six times more reported PPIs than PMIs. As the complexity of metabolome compares the one of proteome, we expected that analogously complexity of the protein-metabolite will equal the complexity of protein-protein interactome. To test our assumption, we resorted to a recently developed method, called PROMIS, which exploits size separation for the cell wide, untargeted analysis of PPIs and PMIs. As anticipated, we detected hundreds of metabolites separating with protein complexes, revealing unprecedented complexity of the protein – metabolite interactome. By interpolating PROMIS with the list of predicted PMIs and PPIs retrieved from respectively the STITCH and STRING databases, we provided experimental validation for 225 PMIs and 14,949 PPIs of the predicted interactions. Finally, by combining PROMIS with affinity purification and thermal proteome profiling we delineated a list of high confidence binders of Ser-Leu dipeptide, implicating leucine containing dipeptides in control of amino acid metabolism and protein processing. Moreover, we could demonstrate that Ser-Leu binds and consequently inhibit activity of the glycolytic enzyme phophoglycerate kinase (PGK).

Mapping the functional proteome and metabolome of grapevine towards the discovery of disease-associated biomarkers

Marisa Maia[1,2,3], Andreia Figueiredo[3], Marta Sousa Silva[1,2]

[1] Laboratório de FTICR e Espectrometria de Massa Estrutural, Faculdade de Ciências da Universidade de Lisboa, Portugal
[2] Centro de Química e Bioquímica (CQB), Faculdade de Ciências da Universidade de Lisboa, Portugal
[3] Biosystems & Integrative Sciences Institute (BioISI), Faculdade e Ciências da Universidade de Lisboa, Portugal

mrmaia@fc.ul.pt

Grapevine (*Vitis vinifera* L.) is the most important fruit plant cultivated worldwide due to its economic importance in the wine industry. However, all *Vitis vinifera* cultivars frequently used for wine production are susceptible to fungal diseases causing highly economical losses. Some strategies have been implemented to cope with the threat caused by fungi, including the intensive use of fungicides or phytochemicals and the introduction of resistant grapevine varieties. My research project aims to identify metabolites or metabolic pathways that can be used as biomarkers for grapevine resistance to fungal pathogens such as downy mildew (caused by the oomycete *Plasmopara viticola*). Currently, I am following a systems biology approach, by integrating the metabolic profiling of different grapevines with the information from transcriptomics and hyperspectral imaging analysis, to enlighten the molecular basis of grapevine resistance. Finally, the uncovered compounds/pathways will be tested and validated in new grapevine bred cultivars, with high resistance against fungal pathogens, to understand if they can be used as biomarkers.

The lichen symbiosis: Metabolites involved in lichenization

Gregor Pichler[1], Fabio Candotto Carniel[2], Erwann Arc[1], Wolfgang Stöggl[1], Lucia Muggia[2], Alberto Pallavicini[2], Mauro Tretiach[2], Ilse Kranner[1]

[1] *Department of Botany, University of Innsbruck, A-6020 Innsbruck, Sternwartestraße 15*
[2] *Dipartimento di Scienze della Vita, Università degli Studi di Trieste, Italy*

gregor.pichler@student.uibk.ac.at

Lichens are a complex symbiosis between a fungal mycobiont and one or more microalgal and/or cyanobacterial photobiont(s). The intricate process by which free-living fungi and algae form a lichen is termed «lichenization», but the factors determining this process are poorly understood. We are dedicated to studying the chemical cross talk between mycobiont and photobiont prior to lichenization. Using untargeted GC-MS-based metabolite profiling in conjunction with targeted LC-MS/MS-based phytohormone measurements we will compare the sets of metabolites released by photobionts and non-lichen-forming algae. The effects of environmental stimuli will also be defined. Unknown compounds will be identified by high resolution MS and NMR Spectroscopy. Furthermore, we will treat mycobiont cultures with metabolites found in exudates of photobionts and non-compatible algae, and assess changes in the metabolite profile and transcriptome of the mycobionts, the latter based on genome sequencing. Our project will help to identify genes and metabolites, which are potentially key to lichenization or are an important source between interkingdom signalling. New knowledge in the field of symbiotic plant-fungus interactions, could lead to a downstream effect to agriculture and biotechnology.

Creating and functionalising a synthetic vesicular compartment in plants

Andras Sandor, Lee Sweetlove, Mark Fricker

University of Oxford, Department of Plant Sciences & Doctoral Training Centre

andras.sandor@plants.ox.ac.uk

The production of industrial or pharmaceutical proteins in plants present a range of highly desirable properties, such as low cost, high potential yields, scalability, lower ecological footprint and lower risk of contamination. However, widespread commercial exploitation is hampered by a few key limitations. Sequestration of proteins of interest in an easily purifiable intracellular compartment would alleviate most of these problems. To this end, we are creating a new synthetic intracellular compartment based on the findings of Dr Ian Moore and Dr Marketa Samalova that polymerising transmembrane protein scaffolds targeted to the endoplasmic reticulum can form a large selforganising synthetic membrane-bound compartment. As part of the project, we are characterising the properties of this *de novo* compartment in depth, and improving it using rational design, by modifying the protein scaffold to generate novel forms of the compartment to allow a range of functionalisations and streamlined purification. The second part of the project focuses on functionalising the synthetic compartment. This includes intracellular storage of high-value biochemicals (such as carboxylic acids succinate and fumarate), purification of toxic membrane proteins (e.g.: ompG porin). Further applications include sequestering metabolic pathways in the lumen of the compartment to improve pathway efficiency and to reduce toxicity or host background effects, and to use the protein polymers as a scaffolding to generate synthetic microdomains, for which proof of principle studies would utilise flavonoid synthesis.

Zaxinone, an apocarotenoid metabolite required for normal rice growth and development*

Jian You Wang, Imran Haider, Muhammad Jamil, Pei-Yu Lin, Abdugaffor Ablazov, TingTing Xiao, Ikram Blilou, Salim Al-Babili

King Abdullah University of Science and Technology, Division of Biological and Environmental Science and Engineering, 23955-6900, Saudi Arabia.

jianyou.wang@kaust.edu.sa

Carotenoids are precursors of plant hormones and signaling molecules that play important roles in regulating plant growth and developments. Recently, we have identified zaxinone, an apocarotenoid metabolite formed by the rice carotenoid cleavage dioxygenase (OsZAS), as a novel signaling molecule that is involved in root and shoot development and which down-regulates strigolactone biosynthesis and release, as demonstrated by the phenotypes of the corresponding loss offunction mutant (zas; Wang *et al.*, *Nature Communications*, 10:810, 2019) Confirming the biological function of zaxinone, application of this compound restored different zas phenotypes, and reduced strigolactone levels and promote root growth in wild-type seedlings. To understand the effect how zaxinone promotes root growth, we conducted metabolomics and transcriptomics studies on zaxinone-treated wild-type seedlings in a time course experiment. Interestingly, Metabolomics pre-analysis revealed that zaxinone triggers plant central metabolism in root tissues, which was in line with transcriptomics data. In summary, the aim of this project is to investigate the effect of zaxinone on metabolome, transcriptome, and hormone composition, and to deeply uncover the mechanism of zaxinone being a key regulator in plant development and metabolism.

*Correspondence concerning the abstract: salim.babili@kaust.edu.sa

The Mechanistic Role of Silicon in Plant Defence Against Insect Herbivores

Jamie M. Waterman[1], Scott N. Johnson[1], Christopher I. Cazzonelli[1], Susan E. Hartley[2]

[1] Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith NSW2751, Australia
[2] York Environment and Sustainability Institute and the Department of Biology, University of York, York YO10 5DD, United Kingdom

j.waterman@westernsydney.edu.au

Silicon (Si) is the second most abundant element in earth's crust and in thus ubiquitous in soil systems. This considered, certain classes of plants have evolved the ability to uptake and transport Si between tissues. Nevertheless, the functional role of Si in plant biology remains ambiguous. Although as it has been well documented that Si may modify the chemical defence capabilities of plants, the mechanisms through which this is achieved have not been well documented. When a plant is attacked by an insect herbivore, numerous stimuli are introduced such as mechanical stimulation, wounding, and chemical elicitation from both insect- and microbial symbiont-derived compounds. Using simulated herbivory techniques, I will investigate the role of Si in defence responses against each herbivore-associated stimulus in order to understand the mechanisms through which Si helps to confer resistance against herbivore attack. Additionally, Si might also assist with defence against herbivory by acting as a physical barrier, abrading the insect alimentary tract and/or hindering nutrient absorption; beyond solely chemical defences, I will attempt to realise the influence of Si on the nutritional qualities of plant tissue. By understanding the functional role of Si in mediating plant defences, it may be possible to determine the evolutionary basis for the accumulation and transport of Si by plants and may help to inform useful pest mitigation strategies that avoid the use of costly and potentially harmful chemical treatments.

Herbivory of "Living Fossils": Chemical and structural control factors

Wuyuntana

Estonian University of Life Sciences Institute: Institute of Agricultural and Environmental Sciences

wuyuntana@emu.ee

Interactions between herbivores and plants play a vital role in terrestrial ecosystems and underlie some of the key evolutionary and ecological processes in nature. Most plants rely at least in part on physical defense, such as oil bodies, resin ducts or trichomes to deter herbivores. The primary function of plant secretory structures is related to defense responses, both constitutive and induced, against herbivores. Also, plants elicit changes in the bouquet of secondary metabolites, especially volatile organic compounds after attacked by herbivores. Different species emission response is different under herbivore attack, phylogenetically related plants have similar reaction towards herbivory stress. The focus all around the world has been so far on angiosperm and gymnosperm herbivory, but if we want to understand volatile organic compound emission evolution in plants affected by herbivory, we need detailed studies on herbivory induced stress responses through different groups of plants. "Living fossils" including mosses, Equisetum, Psilotum, Selaginella, lycopods, Araucarias etc. are ideal materials for inferring evolution of plant-herbivore interaction, because there is a novel class of terpene synthases that occur widely and exclusively in nonseed plants, but are absent in seed plants. Therefore, in this project, we will study interactions between terrestrial herbivores and plants from "Living fossil" point of view and identify patterns of structural and chemical emission responses to herbivory across different evolutionary taxa.

Speakers

Emmanuel GAQUEREL
Institut de Biologie Moléculaire des Plantes, IBMP, Strasbourg
emmanuel.gaquerel@ibmp-cnrs.unistra.fr

Ivo FEUSSNER
Goettingen University, Germany
ifeussn@gwdg.de

David TOUBOUL
Institut de Chimie des Substances Naturelles, ICSN, Gif-sur-Yvette
david.touboul@cnrs.fr

Sébastien BAUD
Institut Jean-Pierre Bourgin, IJPB, Versailles
sebastien.baud@inra.fr

Adnane BOUALEM
Institute of Plant Sciences Paris-Saclay, IPS2, Gif-sur-Yvette
adnane.boualem@inra.fr

Stéphanie BOUTET-MERCEY
Institut Jean-Pierre Bourgin, IJPB, Versailles
stephanie.boutet@inra.fr

François-Didier BOYER
Institut Jean-Pierre Bourgin, IJPB, Versailles
Institut de Chimie des Substances Naturelles, ICSN, Gif-sur-Yvette
francois-didier.boyer@cnrs.fr

Jean-Luc CACAS
Institut Jean-Pierre Bourgin, IJPB, Versailles
jean-luc.cacas@inra.fr

Sylvie CITERNE

Institut Jean-Pierre Bourgin, IJPB, Versailles

sylvie.citerne@inra.fr

Gilles CLEMENT

Institut Jean-Pierre Bourgin, IJPB, Versailles

gilles.clement@inra.fr

Isabelle DEBEAUJON

Institut Jean-Pierre Bourgin, IJPB, Versailles

isabelle.debeaujon@inra.fr

Alexandre DE SAINT-GERMAIN

Institut Jean-Pierre Bourgin, IJPB, Versailles

alexandre.de-saint-germain@inra.fr

Marie DUFRESNE

Institute of Plant Sciences Paris-Saclay, IPS2, Gif-sur-Yvette

marie.dufresne@ips2.universite-paris-saclay.fr

Jean-Denis FAURE

Institut Jean-Pierre Bourgin, IJPB, Versailles

jean-denis.faure@inra.fr

Marine FROISSARD

Institut Jean-Pierre Bourgin, IJPB, Versailles

marine.froissard@inra.fr

Loïc LEPINIEC

Institut Jean-Pierre Bourgin, IJPB, Versailles

loic.lepiniec@inra.fr

Annie MARION-POLL

Institut Jean-Pierre Bourgin, IJPB, Versailles

annie.marion-poll@inra.fr

Caroline MAUVE

Institute of Plant Sciences Paris-Saclay, IPS2, Gif-sur-Yvette

caroline.mauve@ips2.universite-paris-saclay.fr

Grégory MOUILLE

Institut Jean-Pierre Bourgin, IJPB, Versailles

gregory.mouille@inra.fr

François PERREAU

Institut Jean-Pierre Bourgin, IJPB, Versailles

francois.perreau@inra.fr

Catherine RAMEAU

Institut Jean-Pierre Bourgin, IJPB, Versailles

catherine.rameau@inra.fr

Websites

Transports in Paris area

http://www.ratp.fr/
https://www.vianavigo.com/en/home

SPS

www.saclayplantsciences.fr

LabEx Sciences des Plantes de Saclay (SPS) - Institut Jean-Pierre Bourgin - Bâtiment 2 - INRA Centre de Versailles-Grignon
Route de St-Cyr (RD 10) - 78026 Versailles Cedex - France - Tél: + 33(0)1 30 83 30 82 - sps@versailles.inra.fr