

# SFR QUASAV

## PhD Students' Day

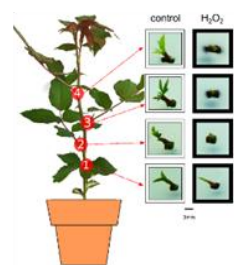
### 12<sup>TH</sup> EDITION

PhD students' work presentation seminar

Tuesday, the October 8th

UFR Sciences – Building L - Amphitheater L004

08h45-17h00





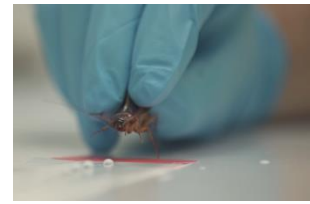
## Planning of the SFR QUASAV PhD students' day

8h45- 9h00	Welcome	
9h00 - 9h15	Introducing talk of the PhD Day	
9h15 - 9h50	180" Talk (1 <sup>st</sup> session)	Alexandre Bantz Alexis Porcher Caroline Lacault Diana Lopez-Arias Elise Bizouerne Enora Dupas Juliette Benejam Justine Foucher
9h50 - 10h15	Coffee or tea break	
10h15 - 11h50	Poster (1 <sup>st</sup> session)	
11h50 - 13h30	Lunch	
13h30 - 14h10	180" Talk (2 <sup>nd</sup> session)	Louise Paillat Michela Skopikova Ning-Ning Zhou Sabine Tourneur Timothée Cherièrè Wilfried Chevalier Zhijuan Chen Wujian WANG
14h10 - 15h40	Poster (2 <sup>nd</sup> session)	
15h40 - 16h00	Coffee or tea break	
16h00 - 16h45	Jury deliberation	
16h45	Award ceremony	



## **Molecular and physiological changes induced by exposure to a sublethal dose of imidacloprid lead to an acclimation of american cockroaches to their environment**

A. Bantz, D. Goven, V. Raymond



*Laboratoire SiFCIR « Signalisation Fonctionnelle des Canaux ioniques et Récepteurs », UPRES-EA2647, USC INRA 1330, SFR 4207 QUASAV, Université d'Angers, Angers, France*

To ensure that enough food is available to meet the needs of a growing population, insecticides have been used. However, widespread insecticide treatments have led to resistant insect emergence. Studies were focused mainly on mechanisms involved in resistance. We know now that exposure to sublethal doses of insecticide can induce physiological changes favouring the development of adapted insects<sup>1</sup>. Indeed, as insecticides degrade over time or can volatilize with wind until their concentration becomes sublethal, insects may be exposed to different doses. Therefore, the aim of our study is to identify cellular and molecular changes induced by subchronic exposure to a sublethal dose of insecticide and to explore if these modifications are maintained over time to promote the development of resistant insects. In this project, we demonstrated that the subchronic exposure to a sublethal dose of imidacloprid during 30 days reduces sensitivity to imidacloprid of treated cockroaches compared to control cockroaches. This decrease of sensitivity to insecticide was also observed on treated cockroaches after the 30-day resting period. Moreover, studies of detoxification enzyme activities and expression of the insecticide targets show a link between cellular and molecular changes and acclimation mechanisms. Further transcriptomic studies will allow us to determine more precisely molecular actors involved in this adaptive response. All results should be considered to develop innovative strategies to circumvent insecticide resistance and to improve pest control.

### **References:**

1 : Bantz et al, 2018. "Exposure to sublethal doses of insecticide and their effects on insects at cellular and physiological levels", *Current Opinion in Insect Science*.

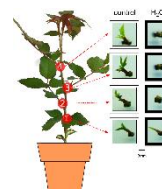


## Implication of ROS homeostasis in the mechanism of axillary bud outgrowth in rosebush (*Rosa* 'Radrazz')

Alexis PORCHER<sup>a\*</sup>, Jérémy LOTHIER<sup>a</sup>, Vincent GUERIN<sup>a</sup>, Françoise MONTRICHARD<sup>a</sup>, Anita LEBREC<sup>a</sup> and Alain VIAN<sup>a\*</sup>

(\*: corresponding authors) : [alporcher@etud.univ-angers.fr](mailto:alporcher@etud.univ-angers.fr), [alain.vian@univ-angers.fr](mailto:alain.vian@univ-angers.fr)

<sup>a</sup> : IRHS, INRA, AGROCAMPUS-Ouest, Université d'Angers, SFR 4207 QUASAV, 42 rue Georges Morel, 49071 Beaucauzé cedex, France



Branching of aerial part is a major component of plant shape and directly related with axillary bud outgrowth process. This process is regulated by different internal actors to the plant (hormones, sugars, nitrogen...) themselves in interaction with many environmental factors. Reactive oxygen species (ROS), initially considered as toxic bioproducts, have recently emerged as important actors in plant physiology, particularly in responses to biotic stresses or variations of environmental conditions. Hydrogen peroxide ( $H_2O_2$ ), the most abundant and stable form of ROS in plant tissue, had already been involved in plant growth and development processes. Previous studies suggest that  $H_2O_2$  is involved in shoot branching process in tomato (Sagi *et al.*, 2004; Chen *et al.*, 2016). However, the relationships between  $H_2O_2$  and axillary bud outgrowth have not yet been clearly established and remain to be elucidated.

In our study, we showed the existence of a strong interaction between  $H_2O_2$  and axillary bud outgrowth in the growing young rose bushes (*Rosa* 'Radrazz'). First, we established that a significant and continuous decrease of  $H_2O_2$  levels in median buds during bud outgrowth in favorable branching condition. This decrease results of a modification of the balance of  $H_2O_2$  metabolism: a decrease in the accumulation of *RBOHD* and *RBOHF* genes transcripts, NADPH oxidases that are involved in the production of apoplastic  $H_2O_2$ . In the same time, we observed the activation of scavenging pathway of  $H_2O_2$  through the ascorbate-glutathione cycle by measuring both transcript accumulations and enzyme activities. In particular, we have identified a modification of glutathione content during bud outgrowth. In addition to its scavenging capacity, this antioxidant compound is also involved in the cell cycle control (Diaz Vivancos *et al.*, 2010) that is tightly controlled during dormancy breaking. In parallel of these results obtained *in planta*, we have demonstrated that  $H_2O_2$  (5 mM) is able to strongly to inhibit bud outgrowth process of isolated nodes cultivated *in-vitro* by decreasing the and the subsequent axis elongation. In this condition, we identified that  $H_2O_2$  can modify the expression of genes previously identified as being involved in bud outgrowth process.

To go further in the comprehension of hydrogen peroxide involvement in bud outgrowth, we investigated the implication of  $H_2O_2$  metabolism in the photocontrol of bud outgrowth. Indeed, in many plants, including rose bushes, bud break is tightly dependent upon light and does not occur in darkness (Girault *et al.*, 2008). We showed that bud outgrowth prevention in darkness is related with the maintenance of a high content of  $H_2O_2$  in buds. Our data suggest that maintaining a high content of  $H_2O_2$  could be the consequence of the significant decrease in the expression of various genes involved in the scavenging of  $H_2O_2$ , especially those involved in the ascorbate-glutathione cycle (*APX*, *DHAR*, *MDHAR*, *GR*).

All these results showed the implication of the  $H_2O_2$  metabolism in the determinism of the bud outgrowth process, as well as in its photocontrol. They will be integrated into the global mechanistic model of the control of bud burst in the rosebush.

**Keywords :** Branching ; Bud outgrowth ;  $H_2O_2$  ; Ascorbate-glutathione cycle ; cell cycle, photocontrol

### References :

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**Zucchini vein clearing disease is caused by *Pseudomonas syringae* pv. *peponis* pv. nov. and pv. *cucurbitacearum* pv. nov.**

Caroline Lacault, Martial Briand, Marie-Agnès Jacques and Armelle Darrasse  
[caroline.lacault@inra.fr](mailto:caroline.lacault@inra.fr)

IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, 49071, Beaucouzé, France



Zucchini (*Cucurbita pepo*) is worldwide affected by *Pseudomonas syringae* strains inducing vein clearing, stunting and necroses during plantlet development. A collection of 58 *P. syringae* strains isolated from diseased zucchini plantlets between 2005 and 2014 was characterized by multilocus sequence analysis (MLSA). A subset of 23 strains responsible for vein clearing of zucchini (VCZ) were evaluated for pathogenicity on eleven cucurbit species and cultivars and their genomes were sequenced. Most VCZ strains belonged to clades 2a and 2b-a within phylogroup 2 of *P. syringae* species complex and were closely related to other strains previously isolated from cucurbits. These VCZ strains had a clearly distinct host range and symptomatology on cucurbits, which is leading here to the description of two pathovars. *P. syringae* pv. *peponis* pv. nov is pathogenic on squashes (*C. pepo*, *Cucurbita moschata*, and *Cucurbita maxima*) and watermelon (*Citrullus lanatus*), while *P. syringae* pv. *cucurbitacearum* pv. nov is pathogenic on squashes, melons (*Cucumis melo*), cucumber (*Cucumis sativus*) and watermelon (*C. lanatus*). Based on draft genome sequences, sets of Type III effectors (T3Es) were identified that correlated with and hence might explain host ranges of these two pathovars. Overall, these two pathovars that are responsible for VCZ represent different epidemic threats in cucurbit producing areas, as a consequence of their different host ranges. In order to monitor the different *P. syringae* epidemics in cucurbit producing areas, specific identification tools were designed and will be used to evaluate the impact of vertical versus horizontal transmission of the pathogens in outbreaks.

**Keywords** : *Pseudomonas syringae*, *Cucurbita pepo*, cucurbits, bacterial seedborne disease



## Resistance to black spot disease in diploid rose

Diana Carolina Lopez Arias, Annie Chastellier, Julien Jeauffre, Tatiana Thouroude, Martin Leduc, Vanessa Soufflet-Freslon, Laurence Hibrand-Saint Oyant, Fabrice Foucher

Contact information: [diana.lopez-arias@inra.fr](mailto:diana.lopez-arias@inra.fr) ; [vanessa.Soufflet-Freslon@agrocampus-ouest.fr](mailto:vanessa.Soufflet-Freslon@agrocampus-ouest.fr) ; [laurence.hibrand-saint-oyant@inra.fr](mailto:laurence.hibrand-saint-oyant@inra.fr) ; [fabrice.foucher@inra.fr](mailto:fabrice.foucher@inra.fr)

IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, 42 Rue Georges Morel, Beaucouzé, France



Black spot disease (BSD), caused by the fungus *Diplocarpon rosae* Wolf, is one of the most important and widespread disease of rose in outdoor landscaping. Up to now, an efficient control of BSD still requires intensive use of fungicides. However, new laws to decrease agrochemical use <sup>[1]</sup> and consumer expectations have encouraged breeders and researchers to study this disease and to develop varieties with higher level of resistance. As most of the rose cultivars with the highest economic impact are susceptible to black spot disease, a better understanding of disease resistance and the use of resistant roses would help to reduce the application of chemicals. Qualitative resistance conferred by major genes (*Rdr* genes) have been widely described <sup>[2-5]</sup> whereas genetic basis of quantitative resistance has not yet been elucidated. The objective of our project is to study the genetic resistance to BSD and to identify genes involved in quantitative resistance to it. The project focuses on the resistance observed in a hybrid of *Rosa wichurana* (RW) that was used as a male parent to develop three populations (OW, HW, and FW). RW was crossed with three female parents (*Rosa chinensis* 'Old Blush', H190 genotype and *Rosa polyantha* 'The Fairy') known to have different degrees of resistance to BSD. To identify genes involved in the resistance of RW, two approaches are adopted: (1) a genetic study with QTL and Meta-QTL analyses of the three connected populations and (2) a transcriptomic study of genes differentially expressed between the resistance genotype RW and a susceptible one (*Rosa chinensis* 'Old Blush') at different time points of the pathogen infection. Candidate genes underlying these QTLs will be explored thanks to rose genome sequences <sup>[6,7]</sup> and further validations of candidate genes will be done by qPCR and transient expression.

**Keywords:** *Rosa wichurana*, *Diplocarpon rosae*, natural Infection, genetic map, quantitative resistance, Meta-QTL analysis, transcriptomics, candidate genes

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- <sup>2</sup>Hattendorf, A., M. Linde, L. Mattiesch, T. Debener, et H. Kaufmann. 2004. « Genetic Analysis of Rose Resistance Genes and Their Localisation in the Rose Genome ». *Acta Horticulturae*, no 651 (juin): 123-30. <https://doi.org/10.17660/ActaHortic.2004.651.14>.
- <sup>3</sup>Kaufmann, H., D. Terefe, A. Yasmin, A. Biber, A. Kuehr, et T. Debener. 2010. « Cloning and Analysis of *Rdr1*, a Black Spot Resistance Gene from Roses ». *Acta Horticulturae*, no 870 (juillet): 191-96. <https://doi.org/10.17660/ActaHortic.2010.870.25>.
- <sup>4</sup>Terefe-Ayana, Diro, Aneela Yasmin, Thanh Loan Le, Helgard Kaufmann, Anja Biber, Astrid Kühr, Marcus Linde, et Thomas Debener. 2011. « Mining Disease-Resistance Genes in Roses: Functional and Molecular Characterization of the *Rdr1* Locus ». *Frontiers in plant science* 2 (août). <https://doi.org/10.3389/fpls.2011.00035>.
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- <sup>6</sup>Hibrand Saint-Oyant, L., T. Ruttink, L. Hamama, I. Kirov, D. Lakhwani, N. N. Zhou, P. M. Bourke, et al. 2018. « A High-Quality Genome Sequence of *Rosa Chinensis* to Elucidate Ornamental Traits ». *Nature Plants* 4 (7): 473-84. <https://doi.org/10.1038/s41477-018-0166-1>.
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## Effect of light during tomato fruit ripening on seed vigour

**Elise Bizouerne, Julia Buitink, Jérôme Verdier, Benoit Ly Vu, Joseph Ly Vu, Olivier Leprince**



*UMR 1345 IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 Quasav, 42 rue Georges Morel, 49071 Beaucouzé, France*

Seed companies invest considerable resources to produce seeds of high vigour. This is particularly important for high value seeds such as tomato. Seed vigour is a multifactorial trait determined by complex gene by environment interactions. It is sequentially acquired during development according to genetic programs that are poorly understood. The objective of this study is therefore to identify the regulatory genes controlling the acquisition of seed vigour depending on fruit maturity and environmental conditions, such as light. To study the effect of dim light during fruit ripening on seed vigour, tomato plants cv. Moneymaker were grown in the greenhouse and fruits were excised at breaker stage and subjected to dim light. They were then compared to two other ripening conditions: excised fruits ripened in the light or fruits ripened on the mother plant. At fruit maturity (red fruit), dim light decreased the speed of germination compared to the two control conditions. Transcriptomic analysis carried out on the 3 seed tissues highlighted a signaling pathway involving C-REPEAT BINDING FACTOR-type transcription factors, one paralog of *DELAY OF GERMINATION 1 (DOG1)* and several *GA OXIDASE*, all of them being implicated in seed dormancy. Changes in differential gene expression in dim light might explain the observed differences in the speed of germination. They appeared to be embryo specific.

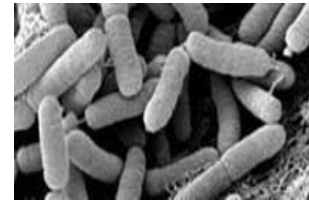


## Improving *Xylella fastidiosa* subspecies identification by qPCR

Enora Dupas<sup>1,2</sup>, Sophie Cesbron<sup>2</sup>, Martial Briand<sup>2</sup>, Marie Agnès Jacques<sup>2</sup>

[enora.dupas@inra.fr](mailto:enora.dupas@inra.fr)

[enora.dupas@anses.fr](mailto:enora.dupas@anses.fr)



<sup>1</sup> IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, 49071, Beaucouzé, France

<sup>2</sup> Anses Laboratoire de la santé des végétaux, F- 49044 Angers

*Xylella fastidiosa* is a quarantine plant pathogenic bacterium originating from the Americas that has been first detected in Europe in 2013. *X. fastidiosa* is a genetically diverse species that is divided in three well supported subspecies (*fastidiosa*, *multiplex* and *pauca*), which display pathogenicity on various but overlapping host ranges. Since the management and the regulation of *X. fastidiosa* outbreaks in Europe depend on the subspecies, it is of major importance to accurately identify the subspecies as early as possible in the monitoring of the detection of *X. fastidiosa*. Limited to xylem vessels, *X. fastidiosa* has a wide host range estimated to encompass more than 560 plant species. As isolation from plant material remains long and fastidious, the detection of *X. fastidiosa* is made directly from plant macerate. Some *X. fastidiosa* host plants are rich in polyphenols and polysaccharides, which can act as PCR inhibitors and therefore increase the detection threshold. One aim of my PhD thesis was to improve the detection of *X. fastidiosa* in plants rich in PCR inhibitors by directly detecting and identifying the subspecies *via* the development of qPCR tetraplex assays. Primers and probes were designed using Sklf, a tool based on k-mers, to detect specific signatures of species and subspecies from a dataset representative of *Xf* diversity including 47 genomic sequences. Assays were tested on 39 target and 30 non-target strains, as well as on 13 different spiked plant species and on 10 different environmental plant samples. Sensitivity and specificity were compared to those obtained with current protocols. *In silico* and *in vitro* analysis revealed that the six new qPCR primers and probes developed in this study were species- and subspecies-specific. The qPCR assays allowed the detection of *X. fastidiosa* in all spiked plant samples with a similar detection threshold compared to those obtained with the reference qPCR assay, with a limit of detection of up to  $10^3$  b.mL<sup>-1</sup>. In environmental plant samples, the tetraplex qPCR approach has identified subspecies where the typing scheme using MLST failed. Artificial mixed infections of two or three subspecies could also be detected in the same sample with the tetraplex assays. The qPCR assays described here is a reliable and effective tool for simultaneously detecting and identifying *X. fastidiosa* subspecies directly from plant samples. Rapid, sensitive, specific and easy to perform, qPCR represented a good alternative to the actual MLST scheme used to identify the subspecies.

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**Keywords:** leaf scorch diseases, *X. fastidiosa* detection, subspecies identification, Sklf, diagnostic.



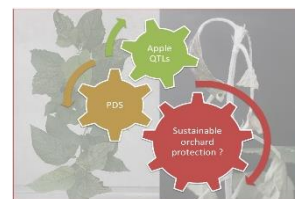


# Exploring interactions between genetic resistances and defenses induced by Plant Defense Stimulators in apple

Juliette Bénéjam\*, Marie-Noëlle Brisset, Charles-Eric Durel, Laure Perchepped

juliette.benejam@inra.fr

UMR 1345 IRHS, 42 Rue Georges Morel, 49070 Beaucouzé



Finding new solutions to reduce the use of pesticides is a major challenge to move towards productive fruit orchards respectful of the environment and human health. The creation of new varieties naturally resistant to bio-aggressors [1] has long been used, but a rapid overcome of genetic resistances is often observed [2]. Another method is the use of plant defense stimulators (PDS) ([3],[4]), however, the instability of their efficacy [5] does not yet allow this biocontrol method to be considered as a single solution. Combining such control methods could provide a more sustainable protection, especially because the diversification of selection pressures exerted on pathogen populations should disadvantage their adaptation [6]. This combining strategy raises several questions: 1- Is the effect of these two control methods synergistic? 2- Does genetic resistance interact with the efficacy of PDS? 3- If so, can we identify predictors of interactivity?

My PhD thesis project aims at answering these questions on apple, the first fruit species in France and Europe. Most of apple commercial varieties are highly susceptible to diseases (in particular, scab disease caused by the ascomycete fungus *Venturia inaequalis* [7] Bowen et al. 2011, Mol. Plant Pathol. 'Venturia Inaequalis: The Causal Agent of Apple Scab'. and fire blight caused by the bacteria *Erwinia amylovora* [8]). Disease susceptibility and PDS protection tests have been carried out on a wide range of apple genotypes (traditional varieties and a F1 progeny representing a high genetic diversity), in order to evaluate the interaction between genetic resistances and PDS-induced defenses. In parallel, putative predictors of this interaction have been investigated by measuring the expression of constitutive defenses and the composition of defense metabolites in the same genotype range.

The first results support the hypothesis of an interaction between the genotypes and the efficacy of PDS, with a differential response of genotypes to PDS. Through a genetic mapping approach, quantitative trait loci (QTLs) for scab or fire blight resistance are currently being identified in a F1 progeny pre-treated or not with PDS. Modulation of QTL effects after PDS treatment compared to QTL effects after water treatment (control) will be explored. Additional QTLs could correspond to specific response to PDS. QTL modulation/appearance may be explained by an interaction between the defense pathways activated by both the genetic and PDS mechanism. Involved metabolites will be further explored in a subset of F1 individuals.

**Keywords :** *Apple, Genetic resistances, Plant defense stimulators, Constitutive defenses, interaction mechanism*

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# Comparative transcriptomics profiling of resistant and susceptible common bean genotypes in response to *Xanthomonas phaseoli* pv. *phaseoli*.



Justine FOUCHER<sup>1</sup>, Mylène RUH<sup>1</sup>, Anne PREVAUX<sup>1</sup>, Sébastien CARRERE<sup>2</sup>, Martial BRIAND<sup>1</sup>, Marie-Agnès JACQUES<sup>1</sup>, Nicolas W.G. CHEN<sup>1</sup>.

<sup>1</sup>IRHS, INRA, AGROCAMPUS OUEST, Université d'Angers, SFR4207 QUASAV, 42, rue Georges Morel, 49071 Beaucouzé, France.

<sup>2</sup>CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR 2594, Castanet-Tolosan, France

Common bean (*Phaseolus vulgaris*) is a major legume crop consumed worldwide. Two main gene pools have been identified for cultivated common bean in South America (Andean gene pool) and in Mexico and Central America (Mesoamerican gene pool). Common bacterial blight affects common bean crops everywhere where beans are cultivated and causes up to 40% yield loss in the most severe cases. *Xanthomonas phaseoli* pv. *phaseoli* is one of the agents responsible for this disease. In order to investigate its impact on common beans originating from the two major gene pools, we inoculated plants from genotypes BAT93 (Mesoamerican) and JaloEEP558 (Andean) with *X. phaseoli* pv. *phaseoli* strain CFBP6546R. Pathogenicity tests revealed that BAT93 was resistant to *X. phaseoli* pv. *phaseoli* while JaloEEP558 was susceptible. To characterize the genes differentially expressed during the interaction with *X. phaseoli* pv. *phaseoli*, we performed RNAseq experiments 48h after inoculation with strain CFBP6546R on these two common bean genotypes. First, to describe the general expression pattern of common bean during the interaction, we analyzed the core transcriptome of these two genotypes. Then, we searched for genes specifically induced or repressed in resistant and susceptible background. Our study provides the first description of common bean transcriptomes after inoculation with *Xanthomonas*, and brings preliminary information potentially important for further management of common bacterial blight of bean.

**Keywords** : Common bean, transcriptomics, resistance.



# Crossing contrasted growing media and organic fertilizers affects microbial activities and nutrient availability in soilless horticulture

Louise Paillat<sup>a,b,\*</sup>, René Guénon<sup>a</sup>, Lydie Huché-Thélier<sup>c</sup>, Fabrice Barraud<sup>b</sup>, Patrice Cannavo<sup>a</sup>



\* Corresponding authors: louisepaillat@gmail.com (L. Paillat)

<sup>a</sup> EPHOR, AGROCAMPUS OUEST, 49045 Angers, France

<sup>b</sup> PREMIER TECH GHA SAS, Le Ciron, 49680 Vivy, France

<sup>c</sup> IRHS, INRA, AGROCAMPUS-Ouest, Université d'Angers, SFR 4207 QUASAV, 42 rue Georges Morel, 49071 Beaucouzé cedex, France

Increasing concerns over environmental impact of plant production leads to a diversification of growing media (GM) and fertilizers in favour of sustainable organic materials. If mineral fertilization is well controlled, organic fertilization and how microorganisms mediate its mineralization are less understood. Contrasted properties of GM (e.g. water holding capacity (WHC), aeration) are suspected to drive microbial activity in response to new resources input. The fertilizer quality should modulate these responses. We conducted a lab-experiment to investigate the effect of microbial activity on nutrient release.

We crossed three GM (peat, coir and composted bark) and three organic fertilizers (applied at 300 mg N L<sup>-1</sup>), compared to control without fertilizer. We selected horn as animal based and high N fertilizer (13% N w:w) and two well NPK-balanced plant-based fertilizers (NPK 6-1.3-3.3 and 2-0.2-2.1). Each GM-fertilizer combination was incubated at 25°C and 60 % WHC. After 7, 14, 28 and 56 days, we measured pH, electrical conductivity, nutrient contents (NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, PO<sub>4</sub><sup>3-</sup>-P), and enzymes activities (β-1.4-glucosidase, urease, acid phosphatase, arylsulfatase).

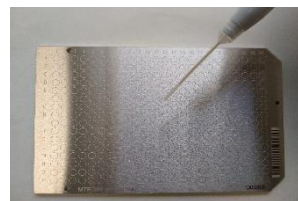
There was a significant interaction between GM and fertilizer type on several microbial functions indicating that GM type affected microbial response to fertilizer. Indeed, nitrification was the fastest in bark and the slowest in peat, the latter resulting into high ammonia content for the plant-based fertilizers. Nitrification was delayed by 7 days in coir but recovered after 28 days. Phosphate content was low in bark throughout the experiment as long as phosphatase activities. Phosphate content increased in coir and peat with plant-based fertilizer whereas phosphatase activity only increased in coir.

Each GM presented contrasted microbial activities. However, are these bulk materials adapted for the plant growth conditions? There is a future challenge in mixing these materials in order to combine their positive effects.

**Keywords:** Agronomy, C N P S mineralization, enzyme activities



# Application of laser desorption ionization mass spectrometry in the study of *Malus x domestica* infected by *Erwinia amylovora* and treated by a plant resistance inducer



M. Skopikova<sup>a\*</sup>, M. Gaucher<sup>b</sup>, S. Boisard<sup>a</sup>, M.-N. Brisset<sup>b</sup>, P. Richomme<sup>a</sup>, A. Schinkovitz<sup>a</sup>

<sup>a\*</sup>University of Angers, EA 921 SONAS/SFR 4207 QUASAV, Campus du Végétal, 42 rue Georges Morel, 49070 Beaucouzé, France

<sup>b</sup>INRA, UMR1345 IRHS, 42 rue Georges Morel, 49071 Beaucouzé, France

*Erwinia amylovora* is one of the major pathogens of apple trees. In order to prevent infections by this bacterium, pesticides are commonly used in industrial agriculture. This however, poses a heavy burden on the environment, so alternative methods of crop protection are being widely explored. In this regards, the induced formation of natural defense compounds represents a promising alternative to conventional methods. The present work therefore evaluates the impact of Bion® 50 WG plant resistance inducer (PRI) on the formation of phenolic compounds in apple seedlings infected with *E. amylovora*. Water treatment was used for the control group.

Usually the detection of small molecules is facilitated by techniques such as liquid or gas chromatography coupled with mass spectrometry (LC-MS, GC-MS). However, due to their close structural vicinity to MALDI matrices, these compounds show a strong tendency of being ionized by simple laser radiation (LDI), allowing their direct detection from crude extracts. With this in mind, *Malus* extracts were analyzed by high resolution LDI-MS.

Results showed different chemical profiles for treated and non-treated plants, with strong variations of relative intensities of detected compounds. Observed differences between samples were further confirmed by principal component analysis. Moreover, molecular formulas for several of the detected signals could be proposed. Requiring very little experimental time and hardly any sample preconditioning or method optimization, LDI-MS may provide interesting supplement or alternative to classical analytical approaches.





## Genetic of prickle density in rose

ZHOU N N<sup>1,2\*</sup>, Hibrand-Saint Oyant L<sup>1</sup>, Foucher F<sup>1</sup>

[ningning.zhou@inra.fr](mailto:ningning.zhou@inra.fr) ; [laurence.hibrand-saint-oyant@inra.fr](mailto:laurence.hibrand-saint-oyant@inra.fr) ;  
[fabrice.foucher@inra.fr](mailto:fabrice.foucher@inra.fr)

<sup>1</sup> IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, Beaucouzé, France.

<sup>2</sup> Flower Research Institute of Yunnan Academy of Agricultural Sciences, 650205 Kunming, Yunnan, China



**Abstract:** Rose is one of the most important ornamental plants, with a great variety of prickles. Cut-flower roses with prickles are more difficult to handle, harvest and transport. Furthermore, it also brings safety hazards for public and workers. Therefore, there is a strong demand from breeders to develop roses without prickle. Prickles are thought to be originated from epidermal cells, only few studies were made concerning the genetics and molecular determinism of prickles on stem and petiole. Our objective is to decipher the genetic determinism of prickle initiation and development, and to characterize genes involved in these processes.

To study the genetic determinism, we used 151 F1 individuals obtained by a cross between *Rosa chinensis* 'Old blush' (OB) and *Rosa x wichurana* (RW). Plants were scored for the number of prickles on the stem, Shapiro-wilk normality test and comparison of normality test for residuals showed that the phenotype data was not normally distributed. Non-prickle or prickle low-density (Number < 2 on 4 internodes) individuals account for approximately one fourth in F1 population. In this case, a single QTL scan with the non-parametric and two-part model were performed by R/qtl in R version 3.2.3.

By non-parametric model, major QTLs were detected on female linkage group (LG) 3 and 4, and on male LG3, minor QTLs were detected on female LG1 and male LG1 and 6. By two-part model, we showed that the major QTL on LG3 might control the presence of prickle, whereas major QTL on LG4 and minor QTLs on LG1 and 6 might control prickle density in the individuals with prickle.

By histological analysis, we demonstrated that in roses, prickles originated as a deformation of trichomes in combination with cells from the cortex. We looked for homologues of candidate genes controlling trichome initiation and development. Some candidate genes colocalized with previous detected QTLs. These data will be presented, and the role of this genes in trichome development will be presented as well. Part of the results were published in nature plant<sup>[1]</sup>.

We have identified interesting candidate-genes for prickle initiation. The molecular basis of prickle initiation will be explored in more details by transcriptomic approach.

**Keywords:** Prickle, QTL, Rose

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## miPEPs: new tools to study and control *Orobanche cumana*

Sabine TOURNEUR<sup>1</sup>, Jean-Philippe COMBIER<sup>2</sup>, Stéphane MUNOS<sup>3</sup>, Thomas LAURENT<sup>4</sup>, Philippe DELAVault<sup>1</sup>

[sabine.tourneur@univ-nantes.fr](mailto:sabine.tourneur@univ-nantes.fr); [combier@lrsv.ups-tlse.fr](mailto:combier@lrsv.ups-tlse.fr);  
[stephane.munos@inra.fr](mailto:stephane.munos@inra.fr); [thomas@micro-pep.com](mailto:thomas@micro-pep.com); [philippe.Delavault@univ-nantes.fr](mailto:philippe.Delavault@univ-nantes.fr)



<sup>1</sup>Laboratory of Plant Biology and Pathology, University of Nantes, France; <sup>2</sup>Laboratory on Plant Sciences, UMR 5546 UPS-CNRS, Toulouse; <sup>3</sup>Laboratory of Plant-Microbe Interactions, UMR CNRS-INRA 2594/441, Toulouse; <sup>4</sup>MicroPEP Technologies, Toulouse

The broomrape species *Orobanche cumana* causes important losses to the production of sunflower in countries surrounding the Black Sea, in Southern Europe and in growing area of France. Unfortunately, no efficient and sustainable methods to control this parasitic weed are currently available. The objectives of the miPEPiTO project are to develop new molecular tools to investigate the biology of the parasite, and to develop an innovative and sustainable biocontrol technology for management of this pest. One partner of the project has recently discovered a new class of regulatory peptides, the miPEPs (miRNA-encoded peptides), which are encoded by primary transcripts of miRNAs. Each miPEP stimulates the transcription of its own encoding transcript, leading to the production of higher amount of the corresponding miRNA and consequently to a downregulation of specific target genes. This natural molecular regulation of gene expression can be obtained with synthetic miPEPs, so that specific stages of plant development can be perturbed temporally by exogenous treatment with appropriate miPEPs.

We aim to decipher the roles of miRNAs in the biology of the pathosystem *O. cumana* – sunflower, by using exogenous treatments of sunflower and/or *O. cumana* miPEPs; and, to test the hypothesis that some identified miPEPs may negatively affect this pathosystem by decreasing broomrape growth and infection, and by improving sunflower resistance.

To date, thanks to the availability of the *O. cumana* genome, the miRNA repertoire of this parasitic species has been identified. It consists of 42 members grouped in 16 families. Every target gene sequence (43 in total) of these miR genes were identified and their expression analyzed during a period of 48 hours following a GR24 treatment of the seeds. Forty-two miPEPs were also identified and produced, among which 23 inhibit *O. cumana* seed germination. Interestingly, the member a of the miPEP<sub>UN1</sub> showed the strongest inhibiting effect while its member b did not. Two out of the four corresponding miR<sub>UN1</sub> target genes exhibited an up regulation following a GR24 treatment, which was impaired by a miPEP<sub>UN1a</sub> treatment. This down regulation of expression by miPEP<sub>UN1a</sub> is associated to increase of the relevant pri-miR<sub>UN1a</sub> expression. Analysis of miPEPs activities on haustorium development is in progress.

These initial results are promising and indicate that miPEPs should allow use to increase our knowledge on key molecular mechanisms underlying a complex parasitic interaction and that they should provide a new phytosanitary method to control broomrape parasitism with highly specific and biodegradable natural substances.

**Keywords:** miPEP, miRNA, Germination, Haustorium, Resistance

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## Soybean based intercrops effects on soil mineral nitrogen pool for the following crop.



T. Cheriére<sup>1\*</sup>, M. Lorin<sup>1</sup>, N. Cassagne<sup>1</sup> and G. Corre-Hellou<sup>1</sup>

\*e-mail: t.cheriere@groupe-esa.com

<sup>1</sup>USC LEVA, INRA, Ecole Supérieure d'Agricultures, SFR 4207 QUASAV, 55 rue Rabelais, 49007, Angers Cedex, France

A recent report from the FAO on the State of the world's biodiversity for food and agriculture highlighted the importance of biodiversity for food production as providing more resilience and reducing need for external inputs [1]. European cropping systems tend to be simplified, for example an entire French region shifted from mixed farming systems to simple cropping systems with only few crops [2]. Diversification of cropping systems seems necessary to reduce the environmental impact of crop production but many barriers exist including lack of knowledge on the crop or fear of low competitiveness of leguminous crops against weeds [3]. To help overcome these barriers, we propose to use intercropping as a facilitator for the introduction of a new crop in cropping systems. Indeed, mixing a second crop to the crop to be introduced should improve stability of crop production and increase resilience to stresses. Also, intercropping can facilitate weed control and provide other services to the main crop and enhance global production per unit of area [4].

A common interrogation concerning the introduction of a new crop is the impact of that crop on the following one. It is recognized that leguminous crops, such as soybean, may have a positive impact on the following cereal with N pre-crop effect leading to a reduction of the N fertilizer use [5]. This effect may come from the lower C:N ratios of legume residues leading to a reduced immobilization of soil mineral N during mineralization [5].

Nonetheless, the impact of intercropping on subsequent soil mineral N pool is still poorly understood with regard to the species intercropped with the legume.

An experiment has been set up near Angers, France, to study the pre-crop effect of various soybean based intercrops (IC) on soil mineral N pool for the following wheat. Soybean was intercropped with sunflower, sorghum, lentil and buckwheat in a substitutive design (50:50), in alternate rows. The five crops were sown in sole crops (SC) as control treatments. Each treatment was replicated in three completely randomized blocks. Dry matter (DM) sampling was done at maturity to assess grain and straw yields. Four weeks later, wheat was sown on every plot. Total soil mineral nitrogen content was determined on the 0-90cm soil layer, after intercrop harvest, before wheat sowing and before and after winter.

At soybean harvest, soil mineral N in the 0-90cm layer ranged from 12.72 ( $\pm 5.58$ ) kg/ha for soybean-sunflower IC, to 25.90 ( $\pm 5.92$ ) kg/ha for soybean SC. Nevertheless, no significant difference was found between treatments suggesting that all crops were able to catch most of mineral N available in the soil during their growth.

At wheat sowing, lowest value of soil mineral N was for sorghum SC as pre-crop with 16.23 ( $\pm 3.34$ ) kg/ha and the highest was for soybean SC with 52.44 ( $\pm 2.88$ ) kg/ha. Soil mineral N after soybean-buckwheat IC and soybean-sorghum IC was significantly lower than after soybean SC.

When looking at the effect of soybean both in SC and IC on soil mineral N at wheat sowing, a linear relationship linking soybean straw DM at harvest and total mineral N in the 0-90 soil layer at wheat sowing was highlighted ( $R^2=0.71$ ;  $p<0.001$ ). This suggested that early mineral N availability for the next crop was directly linked to soybean's growth and DM accumulation in the field.

Results of soil mineral N at beginning and end of winter will both be available to bring more insight on the evolution of soil mineral N pool after soybeans IC.

**Keywords:** Soybean intercrops, Nitrogen, Pre-crop effect

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# Study of genotype by environment interaction for carrot root quality control

Wilfried Chevalier <sup>a\*</sup>, Didier Peltier <sup>a</sup>, Emmanuel Geoffriau <sup>a</sup>

Wilfried.chevalier@inra.fr



<sup>a\*</sup> UMR 1345 Institut de Recherche en Horticulture et Semences (IRHS), 42 rue Georges Morel, 49070 Beaucouzé cedex, France

## Abstract

Conditioned both by environment, genotype and the interaction of the genotype itself with the environment, some phenotypic plasticity lies between individuals. This phenomenon allows an adaptive potential of individuals, and may depend on various mechanisms as epistasis, pleiotropy and epigenetics. Depending on studied characters, some genotypes or varieties may be more and less plastic and, in some cases, more and less stable. Thus, study of genotype by environment interaction represents a prevalent tool for breeding, by focusing on stability and adaptability notions of genotypes (Ceccarelli et al., 1994; Kolmodin et al., 2002). Considered as powerful anti-oxidants (El-Agamey et al., 2004), carotenoids have large applications in medical fields as cancer preventive compounds and precursors of vitamin A (Bendich and Oslon, 1989) : it appears interesting to reach a guaranteed carotenoids content in crops. Even if carotenoid accumulation seems to depend on a high genetic component for climacteric fruits as tomato, there is a large effect of GxE interaction overall (Rosello et al., 2010). Thus, the genetic determinism alone does not explain carotenoid accumulation amongst most vegetables. Carrot is depicted as a healthy vegetable, thanks to its ability to accumulate carotenoids at high level in roots. However a large variation is observed. In spite of a well-known carotenoid biosynthesis pathway (Just et al., 2007) and recent progress on the genetic control, carotenoid accumulation in an underground reserve tissue remains unclear. Regulation can occur at several scales and can result from environmental influences. This presentation deals with the implication of genotype by environment interaction by focusing on carotenoids accumulation in carrots and its study for breeding.

**Keywords:** environment effect, specialized metabolites, quality, plasticity, carrot root

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# Genetic and Epigenetic Studies of Skin Color Variations in Gala Apple Mutants

Wuqian Wang<sup>1</sup>, Nicolas Daccord<sup>1</sup>, François Laurens<sup>1</sup>, Gerhard Buck-Sorlin<sup>1</sup>, David Rousseau<sup>1</sup>, Sandrine Balzergue<sup>1</sup>, Etienne Bucher<sup>1, 2</sup>.

1 : IRHS, INRA, AGROCAMPUS-Ouest, Université d'Angers, SFR 4207 QUASAV, 42 rue Georges Morel, 49071 Beaucozoué cedex, France

2 : Agroscope, Research Division Plant Breeding, 8820 Wädenswil, Switzerland



Breeding new colored apples is an important issue for breeders. In Europe, novel apple varieties have to undergo long and expensive distinctness, uniformity and stability (DUS) testing compared to other crop sectors. For apple, numerous novel varieties are “mutants” or “sports” of existing varieties and even though these plants are all clones, phenotypic changes can be observed. Here, in order to distinguish different clonal varieties using molecular tools, we want to develop molecular markers to help us to differentiate different mutants. We have chosen seven Gala mutants showing different fruit pigmentation colors and patterns. These observed phenotypic changes might be the result of genetic and/or epigenetic changes that have occurred during clonal propagation. Here, we want to identify which genetic and epigenetic (DNA methylation) changes are responsible for the different color patterns.

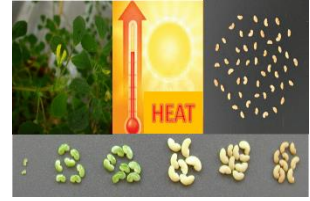
To identify genetic changes we have applied a Whole Genome Sequencing (WGS) method (HiSeqX) with a Paired ends reads length of 150bp. We used bioinformatics software to compare their genome with a reference Gala genome. We found a very high number of SNPs that we need to analyze more precisely. We extracted from WGS data a list of copy number variations and novel transposable element insertions and compared them to the Gala standard genome. To assess potential epigenetic causes, we carried out Whole Genome Bisulfite Sequencing (WGBS) to compare the seven Gala mutants with the reference Gala genome. We used our in-house bioinformatics pipeline to identify Differentially Methylated Region (DMR) and established epigenome maps of each Gala mutant. Also, to find Differentially Expressed Genes correlated or not with DMRs, we perform RNAseq analysis of the fruit peel of Gala mutants. In parallel, we are currently studying how to appreciate the texture of stripe and non-stripe apple mutants using different phenotyping analysis methods.

And for next step, we need to do WGBS and RNA-seq for second year, and we also need to analysis the sequencing data, and to compare the two years data. Finally, we want to find some genetic and/or epigenetic markers for distinguishing different Gala mutants.

**Keywords:** Gala mutants, epigenetics, genetics, skin color, markers



## Impacts of heat stress on maturation and quality of *Medicago* seeds



ZJ. Chen<sup>1</sup>, B. Ly Vu<sup>1</sup>, J. Ly Vu<sup>1</sup>, J. Buitink<sup>1</sup>, O. Leprince<sup>1</sup>, J. Verdier<sup>1</sup>

Email: zhijuan.chen@inra.fr

<sup>1</sup> Research Institute in Horticulture and Seeds, INRA-Agrocampus-ouest-Universite d'Angers, Angers, France

Legumes are important crop species as they produce seeds for human food and animal feed. *Medicago truncatula* is the model plant of legumes that is one of major crop families. In *Medicago*, the sub-optimal environmental conditions affect seed developmental timing, mainly during the late maturation stage, leading to changes in seed germinative quality [1]. The goal of our project is to decipher the impacts of heat stress on *Medicago* seed maturation and quality.

In our study, we first identified physiological changes of mature seeds under heat stress conditions, then the underlying molecular mechanisms of heat stress response at transcriptomic and epigenetic levels. In parallel, we performed genome-wide association studies using 200 selected *Medicago* HAPMAP accessions to identify genes associated with heat stress adaptability. By combining these analyses results, we will identify candidate genes related to heat stress response or heat stress adaptability and initiate the functional validation to understand the seed stress response. Deciphering these mechanisms will ultimately lead to improvement of germinative qualities of seeds produced under sub-optimal conditions.

**Keywords:** Seed, heat stress, longevity, germination, epigenetics

**Reference:**

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## Thanks to:

### The participants :

Alexandre Bantz	alexandre.bantz@etud.univ-angers.fr
Alexis Porcher	alporcher@etud.univ-angers.fr
Caroline Lacault	Caroline.Lacault@inra.fr
Diana Lopez-Arias	diana.lopez-arias@inra.fr
Elise Bizouerne	elise.bizouerne@agrocampus-ouest.fr
Enora Dupas	enora.dupas@anses.fr
Juliette Benejam	juliette.benejam@inra.fr
Justine Foucher	justine.foucher@inra.fr
Louise Paillat	louise.paillat@agrocampus-ouest.fr
Michela Skopikova	michaela.skopik@gmail.com
Ning-Ning Zhou	ningning.zhou@qq.com
Sabine Tourneur	sabine.tourneur@univ-nantes.fr
Timothée Cherièr	t.cheriere@groupe-esa.com
Wilfried Chevalier	wilfried.chevalier@etud.univ-angers.fr
Zhijuan Chen	Zhijuan.Chen@inra.fr
Wuqian WANG	Wuqian.Wang@inra.fr

### The jury members :

Alain Vian	alain.vian@univ-angers.fr
Ronan Symoneaux	r.symoneaux@groupe-esa.com
Julia Buitink	julia.buitink@inra.fr
Sabine Demotes	sabine.demotes-mainard@inra.fr
Alain Sarniguet	alain.sarniguet@inra.fr
Jean-Bernard Pouvreau	Jean-Bernard.Pouvreau@univ-angers.fr
Joëlle Fustec	j.fustec@groupe-esa.com
Caroline Deshayes	caroline.deshayes@univ-angers.fr
Guillaume Viault	guillaume.viault@univ-angers.fr
Jean-Jacques Helesbeux	jean-jacques.helesbeux@univ-angers.fr
Benoit Remenant	benoit.remenant@anses.fr
Patrice Cannavo	patrice.cannavo@agrocampus-ouest.fr

# **SFR QUASAV**

## **PhD Students' Day**

**12<sup>TH</sup> EDITION**

**PhD students' work presentation seminar**

**Tuesday, the October 8th  
UFR Sciences – Building L - Amphitheater L004  
08h45-17h00**