Four M2 internships at MycSA – 5 to 6 months in 2022.

TITLE : Involvement of carotenoids in maize resistance to *Fusarium graminearum* and the accumulation of the deoxynivalenol mycotoxin in kernels.

CONTEXT: Gibberella ear rot, caused by the fungal pathogen *Fusarium graminearum*, is a major threat for worldwide maize production. In addition to induce significant yield losses, *F. graminearum* is responsible for the contamination of grains with the deoxynivalenol/DON mycotoxin, which is highly harmful for humans and livestock. Plant breeding strategies are among the most promising and performing approaches to fight against the mycotoxin problem in maize. However, despite significant progress in the understanding of the genetic bases of resistance to *Fusarium*, knowledge remains partial, and selection for GER resistance is still challenging. A large set of metabolites, pre-formed, constitutive as well as inducible defense metabolites have been reported to play a pivotal role in the resistance of maize to *F. graminearum*. While numerous studies have focused on phenylpropanoids compounds, the ecological role of carotenoids, a group of tetraterpenoids, in plant defense mechanisms has been scarcely studied (Picot et al., 2013). Yet these compounds possess strong antioxidant capacities and are capable of quenching various ROS and notably hydroperoxides. These hydroperoxides and notably the 9S-HPODE and the 13S-HPODE yielded by linoleic acid oxidation have been reported as potent modulators of the production of mycotoxins: 9S-HPODE has been shown to promote mycotoxin production by some fungal species whereas 13S-HPODE can exhibit a significant inhibitor effect (Gauthier et al., 2015).

OBJECTIVES : The goal of this internship is to provide new insights supporting the potential role of carotenoids in maize resistance to *F. graminearum* and DON accumulation. The study will particularly focus on the way maize carotenoids could interfere with hydroperoxides and therefore indirectly affect the production of DON.

METHODS : The project will include three steps:
1- Analysis of the composition in lipoxygenase/LOX (total activity measured by polarography and isoform composition characterized through the analysis of linoleic acid oxidation products using LC-DAD) in maize kernels from resistant and susceptible genotypes
2- Effect of carotenoid supplementation on the composition of linoleic oxidation products (9S/13S HPODE ratios) catalyzed y by maize LOX extracts (LC-DAD)
3- Analysis of the DON biosynthesis modulation induced by linoleic oxidation products catalyzed y by maize LOX extracts in presence and absence of carotenoids (fungal cultures, DON extraction and analysis by LC-DAD)

PREREQUISITES :

Background knowledge in plant secondary metabolites and oxidative enzymes. Skills in liquid chromatography, data treatment and statistics will be appreciated.

REFERENCES (2 or 3) :

Gauthier et al., 2015. Metabolomics to Decipher the Chemical Defense of Cereals against *Fusarium graminearum* and Deoxynivalenol Accumulation. Int. J. Mol. Sci., 16, 24839-24872
Picot et al., 2013. Maize Kernel Antioxidants and Their Potential Involvement in Fusarium Ear Rot Resistance. J. Agric. Food Chem., 61, 3389−3395.
KEYWORDS (5) : Lipoxygenases, carotenoids, maize, Fusarium graminearum, Deoxynivalenol

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TITLE : Comparative analysis of the antifungal and anti-mycotoxin activity of a peptide treatment applied to a panel of Fusarium species infecting cereal crops

CONTEXT:

Fusarium head blight (FHB) is one of the most devastating fungal disease affecting cereal crops worldwide. In addition to *Fusarium graminearum*, acknowledged as the major causal agent of FHB, other *Fusarium* species including *Fusarium culmorum*, *Fusarium poae*, *Fusarium langsethiae*, *Fusarium avenaceum* and *Fusarium tricinctum* are involved in the disease. All the previous *Fusarium* species are also responsible for the contamination of cereal grains with mycotoxins which are harmful for livestock and humans. We previously showed that a treatment with a reduced form of the tick defensin DefMT3, referred to as TickCore3 (TC3), decreases *F. graminearum* growth and abrogates the production of type B trichothecenes mycotoxins. First data (which require to be underpinned) suggest that the efficiency of TC3 to reduce fungal growth and mycotoxin production was different according to the considered *Fusarium* species. Factors and mechanisms explaining these differences are not yet known

OBJECTIVES :

The purpose of this internship is to clarify the antifungal and anti-mycotoxin activity of the TC3 peptide towards the different *Fusarium* species involved in FHB disease. Fungal biomass and mycotoxins yields will be compared in control and TC3-supplemented cultures inoculated by different strains of the aforementioned *Fusarium* species. This first screening step will be completed by targeted transcriptomic studies. The TC3-induced modulation of the expression of key genes (involved in

mycotoxin biosynthetic pathways or that have been identified through a RNAseq study applied to *F. graminearum*) will be quantified and compared within the set of considered fungal species.

METHODS : Microbiology (fungal cultures), RNA extraction**,** RT-qPCR, biochemical analysis of fungal secondary metabolites using HPLC/DAD or HPLC/MS

PREREQUISITES : Background knowledge in fungal physiology. Skills in molecular biology, biochemistry, data treatment and statistics will be appreciated.

REFERENCES (2 or 3) :

Tonk M, Cabezas-Cruz A, Valdés JJ, Rego RO, Grubhoffer L, Estrada-Peña A, Vilcinskas A, Kotsyfakis M, Rahnamaeian M. Ixodes ricinus defensins attack distantly-related pathogens. Dev Comp Immunol. 2015 Dec;53(2):358-65. doi: 10.1016/j.dci.2015.08.001. Epub 2015 Aug 5. PMID: 26255244.
Leannec-Rialland, V., Cabezas-Cruz, A., Atanasova, V. *et al.* Tick defensin γ-core reduces *Fusarium graminearum* growth and abrogates mycotoxins p supplementroduction with high efficiency. *Sci Rep* **11,** 7962 (2021). https://doi.org/10.1038/s41598-021-86904-w

KEYWORDS (5) : Mycotoxin, Fusarium species, cereals, antifungal Peptide, transcriptional control

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TITRE : Nature et rôle des métabolites secondaires impliqués dans la communication chimique mise en place lors de l'interaction entre la bactérie S-104 et deux espèces de Fusarium : *F. graminearum* et *F. tricinctum*, toutes deux productrices de mycotoxines.

CONTEXTE: Ce stage s'inscrit dans le cadre de l'étude de l'influence d'un nouvel agent bactérien, récemment isolé au laboratoire à partir d'une souche de *Fusarium tricinctum*, dans la régulation du métabolisme primaire (croissance) et secondaire (sporulation, production de mycotoxines) de champignons phytopathogènes impliqués dans la fusariose des épis de céréales à paille (« Fusarium Head Blight »). Ce stage vise à mieux comprendre les mécanismes à l'origine de l'efficacité de la bactérie S-104 pour inhiber le développement fongique et la production de mycotoxines de deux espèces impliquées dans la fusariose.

OBJECTIFS : L'objectif du stage est d'étudier la communication chimique mise en place lors de l'interaction entre la bactérie S-104 et les espèces fongiques *F. graminearum* et *F. tricinctum*, par la détermination de la nature et du rôle des métabolites secondaires sécrétés. Il s'attachera (1) à identifier les métabolites secondaires sécrétés par les deux partenaires, bactérien et fongique, lors de leur confrontation : en particulier les molécules bactériennes de type PKs, NRPs et/ou lipopeptides et les métabolites secondaires fongiques, potentiellement à visée antibactérienne, comme la bikaverine ou les mycotoxines de type trichothécènes ainsi que les enniatines et la beauvericine ; (2) à préciser comment

ces métabolites fongiques et bactériens participent au dialogue moléculaire et (3) comment ils sont susceptibles de modifier le métabolisme et la croissance des partenaires en interaction.

METHODES : (Approches Microbiologiques, Biologie Moléculaire, Biochimie, Métabolomique)

- Co-cultures en milieux liquides avec différentes séquences d'introduction des deux partenaires : Fusarium spp. puis bactérie, bactérie puis Fusarium spp. et les deux simultanément. Dans ces cultures, seront analysés les croissances fongiques et bactériennes (qPCR), les teneurs en mycotoxines ainsi que les profils chromatographiques des métabolites secrétés. Ces différents paramètres seront comparés entre les différentes conditions de culture ainsi qu'avec les conditions témoins (cultures des souches seules).

- Effet de surnageants bactériens sur la croissance fongique et la toxinogénèse. Les profils métabolomiques de surnageants bactériens et leur capacité à inhiber/stimuler la croissance et la toxinogénèse seront mis en relation. Afin d'identifier les métabolites bactériens jouant un rôle clé dans l'interaction, un fractionnement bioguidé (chromatographie basse pression et HPLC semi-préparative) du surnageant démontré comme le plus actif sera entreprise. Une analyse par spectrométrie de masse permettra d'apporter des éléments sur la nature et l'identification structurale du/des composés bioactifs.

REFERENCES: - (2018) Genome Sequence of the Emerging Mycotoxin-Producing Filamentous Fungus Fusarium tricinctum Strain INRA104. N. Ponts, F. Richard-Forget, H. Zhang, G. Barroso, C. Zhao. Genome Announc. 2018 Jun 21;6(25):e00509-18. doi: 10.1128/genomeA.00509-18.
- (2020) Evolution of Fusarium tricinctum and Fusarium avenaceum mitochondrial genomes is driven by mobility of introns and of a new type of palindromic microsatellite repeats. N. Ponts, C. Gautier, J. Gouzy, L. Pinson-Gadais, M. Foulongne-Oriol, C. Ducos, F. Richard-Forget, JM.l Savoie, C. Zhao , G. Barroso. BMC Genomics. 21(1):358. doi: 10.1186/s12864-020-6770-2.

KEYWORDS: Fusariose de l'épi, Bactérie, Dialogue moléculaire, mycotoxines, Interactions microbiotiques.

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TITLE : Changes in microbiota and mycotoxins during wheat grain storage and consequences on the quality for bread making.

CONTEXT: Mycotoxins in cereals are a threat for economy and health. The internship subject is a contribution to two research projects in progress at MycSA:
- Myco3C 'To Identify and limit mycotoxin risks in cereals produced, stored, processed in short supply chains'. Storage is a hub of these chains in which mycotoxin evolution depends on previous contamination in fields and will affect the transformation of grains into flour and bread.
- SilArchaeBio 'Underground silos for storing grains in pre-industrial period: learning from experimental archaeology and biology'. Storage in underground pits using natural temperature regulation and natural anaerobic conditions was practiced by ancient populations and appears to be an environmentally friendly method. How does these silos work at a biological point of view is of concern for archaeologists and agronomists.

OBJECTIVES :

Using wheat grains from organic agriculture contaminated by mycotoxins before harvest, the objective is to Characterize the putative over-contamination of grain by mycotoxins or metabolization of mycotoxins due to the microbial activities in wheat grains during the storage and their consequences on use of the grains as food.

METHODS :

- Experimental design for simulating the climate in underground pits and events affecting the grain quality in on-farm storage.
- Analysis of mycotoxins and their metabolites (HPLC-MS).
- Detection and identification of microorganisms in grains (Pasteurian microbiology and molecular taxonomy).

PREREQUISITES :

Interest for the agriculture.

REFERENCES (2 or 3) :

Martinez Tuppia C., Atanasova-Penichon V., Chereau S., Ferrer N., Marchegay G., Savoie J.-M., Richard-Forget F. (2017). Yeast and bacteria from ensiled high moisture maize grains as potential mitigation agents of fumonisin B 1. *Journal of the Science of Food and Agriculture*, 97 (8), 2443-2452, https://dx.doi.org/10.1002/jsfa.8058, https://hal.inrae.fr/hal-02156680

Merel D., Savoie J.-M., Mata G., Salmones D., Ortega C., Atanasova V., Chéreau S., Monribot-Villanueva J., Guerrero-Analco J. (2020). Methanolic extracts from cultivated mushrooms affect the production of fumonisins B and fusaric acid by *Fusarium verticillioides*. *Toxins*, 12 (6), 1-19, https://dx.doi.org/10.3390/toxins12060366, https://hal.inrae.fr/hal-02793859

KEYWORDS (5) : mycotoxins, microbiology, biomolecule analyses, experimentation, cereals

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