SFR 4207
Angers
Qualité et Santé du Végétal
Quasav

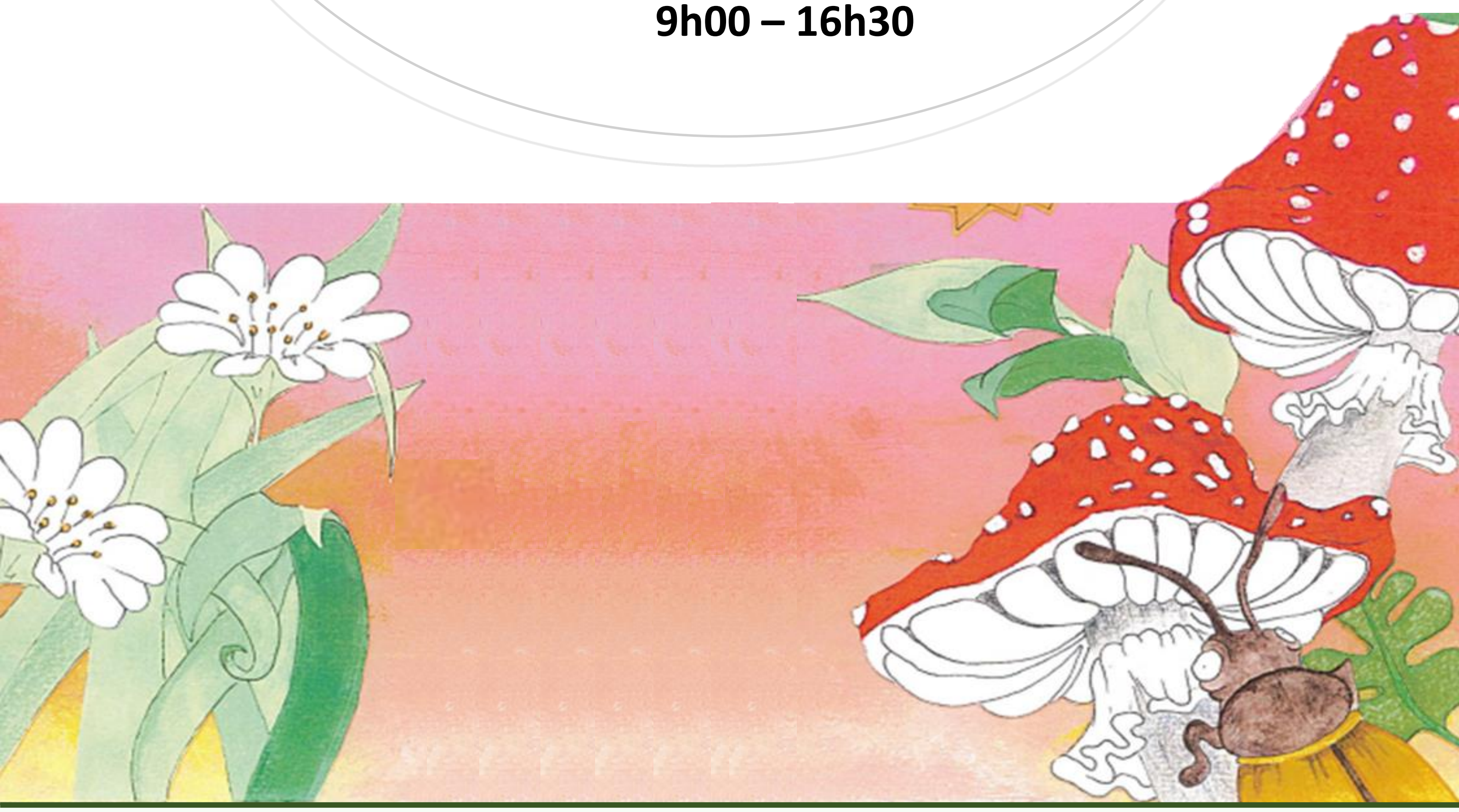
SFR QUASAV

PhD Students Day

16TH EDITION

PhD students work presentation seminar

Thursday, November 23rd
UFR Sciences – Amphitheater D
9h00 – 16h30



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

PLANNING OF THE SFR QUASAV PHD STUDENTS DAY

9h00 Opening of the doctoral students day and introduction

FIRST SESSION

9h10 Benjamin HUBERT – Identification of defence mechanisms in dormant seeds.

9h35 Loup TRAN VAN CANH – Exploiting the diversity of signaling peptides in Solanaceae in response to stress.

10h00 Pierre BOUILLON – Deciphering the genetic architecture of red-flesh trait in Apple.

10h25 **Pause**

SECOND SESSION

10h55 Dorine CANONNE – Impacts of water restriction on the development of urban trees and their associated cli-mate services.

11h20 Gontran ARNAULT – Effective seedling microbiota engineering using Synthetic Community (SynCom) inoculation on seeds.

11h45 Thomas LERENARD – Do wild Brassicaceae defense compounds enhance the antagonistic effect of seed-borne fungi against Alternaria brassicicola ?

12h10 **Pause**

THIRD SESSION

13h40 Julien THIERRY – A study of the climatic benefits of street trees under warm and dry conditions based on both reduced scale and in-situ monitoring approaches.

14h05 Christopher GIHAUT – XopI, an effector of Xanthomonas modulating sugar metabolism in bean plants.

14h30 Claire VIEILLARD – Reusing wastes and by-products from desealing operations to develop fertile soils for greening cities.

14h55 Julien AFFHOLDER – Study of the OcKAI2 receptors, germination stimulants of the parasitic plant *Orobanche cumana*.

DELIBERATIONS

15h20 Start of deliberations

15h50 Announcements of the deliberations and end of the PhD students day

VIDEO ABSTRACTS

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

WEEK 1 : https://youtu.be/Ac_56HC_5io

- **Benjamin HUBERT (**SEEDS, IRHS) – Identification of defence mechanisms in dormant seeds.
- **Loup TRAN VAN CANH** (BiDeFi, IRHS) – Exploiting the diversity of signaling peptides in Solanaceae in response to stress
- **Pierre BOUILLON** (VALEMA, IRHS) – Deciphering the genetic architecture of red-flesh trait in Apple.
- **Dorine CANONNE** (STRAGENE, IRHS & EPHor, Institut Agro) – Impacts of water restriction on the development of urban trees and their associated climate services.

WEEK 2 : https://youtu.be/U8sIWpBdFvM

- **Gontran ARNAULT** (EMERSYS, IRHS) – Effective seedling microbiota engineering using Synthetic Community (SynCom) inoculation on seeds.
- **Thomas LERENARD** (FungiSem, IRHS) – Do wild Brassicaceae defense compounds enhance the antagonistic effect of seed-borne fungi against Alternaria brassicicola ?
- **Julien THIERRY** (EPHor, Institut Agro) – A study of the climatic benefits of street trees under warm and dry conditions based on both reduced scale and in-situ monitoring approaches.
- **Christopher GIHAUT** (EMERSYS, IRHS) – XopI, an effector of Xanthomonas modulating sugar metabolism in bean plants.

Identification of defence mechanisms in dormant seeds

Benjamin Hubert[1], Muriel Marchi[2], Joseph Ly-Vu[3], Abdulsatar Mohammad[4], Olivier Leprince[5], Julia Buitink[6]

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Dormancy is an adaptive strategy that allows seeds to persist in the soil facing (a)biotic stresses and waiting favourable conditions to germination and dispersal of the species. Our team's work has shown that imbibition of dormant *Medicago truncatula* seeds leads to the activation of a defence response (Bolingue *et al*., 2010). However, the defence pathways and their regulation during dormancy remain poorly understood. Here, we set out to identify the molecular pathways underlying defense activation in dormant tomato seeds (*Solanum lycopersicon*). As a measure of seed defence, a method was developed that determines the antimicrobial activity in exudate from imbibing seeds against *Alternaria brassicicola* using nephelometry. Exudates from imbibing seeds that are dormant show antimicrobial activity. In contrast, during imbibition of germinating seeds, this activity is not detectable and only becomes evident at the seedling stage. Using the accessions of the tomato MAGIC population, we identified a large variation in the level of antimicrobial activity in the dormant seed exudates and this activity appears to be tissue specific. Current research focusses on dissecting the molecular pathways in dormant tomato seeds using transcriptomic and metabolomic analyses. These results will contribute to a better understanding how seed defends themselves and will serve to develop new strategies of seed-borne pathogens management and plant breeding.

Exploiting the diversity of signaling peptides in Solanaceae in response to stress

Loup Tran Van Canh[1], Sandra Pelletier[1], Patricial Mallegol[1], Sylvain Hanteville[1], Masoud Ahmadi-Afzadi[2], Jean-Marc Celton[1], Jean-Pierre Renou[1], Sébastien Aubourg[1]

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In the context of sustainable agriculture and phytosanitary product restrictions, this study aims to take advantage of the diversity and specificity of plant small signaling peptides to manage weed plants by interfering with their Reactive Oxygen Species (ROS) regulation networks. The Black Nightshade species (*Solanum nigrum*) is a major but understudied weed species, for which palliative control practices are the only alternatives to herbicide use. Therefore, we conducted an investigation to identify *S. nigrum* specific candidate secreted peptides, that could impede its development through a transcriptomic screening and profiling approach. We performed a *de novo* reference transcriptome assembly using Trinity and EvidentialGene, combining long and short read sequencing technologies onto samples from multiple organs, developmental stages, and oxidative stress-inducing conditions (H_2O_2, Methylviologen, Atrazine, *Clavibacter michiganensis* and *Xanthomonas euvesicatoria* infections). The proteins deduced from transcript contigs were annotated using InterProScan and BLASTP against the proteomes of *S. lycopersicum* and *A. thaliana*. Transcripts encoding potential small secreted peptide precursors (shorter than 200 amino acids) were identified using SignalP and DeepLoc and/or by similarity to known secreted peptides. Subsequently, a differential RNA-seq analysis was conducted with previously described samples, resulting in a curated subset of 100 candidate peptide precursors, overexpressed in at least one of the applied stress conditions. This subset comprises previously unreported putative Solanaceae-specific secreted proteins, and a group of candidates has been selected for experimental validations. For each of them, the conserved motif predicted from sequence comparisons with homologs has been synthesized to evaluate their exogenous effects on *S. nigrum* seedling growth. This step is currently in progress and further studies are still required to demonstrate the potential of signaling peptides as selective bioherbicides for inhibiting weed growth in the field.

Keywords: Black Nightshade, Weed management, Phytocytokines, Reactive Oxygen Species, Transcriptomic

Deciphering the genetic architecture of red-flesh trait in Apple

Pierre Bouillon [a,b], Etienne Belin [a], Anne-Laure Fanciullino [a],Sylvain Hanteville [a], Béatrice Bonnet [a], Sandrine Balzergue [a],Frédéric Bernard [b], Jean-Marc Celton [a]

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Red-fleshed apple varieties are becoming increasingly popular among fruit consumers. This trait is associated with anthocyanin accumulation, which is known for its beneficial effects on human health. The genetic basis of colour development has been widely characterised, however, current models do not explain the observed variations in red pigmentation intensity and distribution. New molecular markers are currently needed to increase the selection efficiency of this complex trait.

We developed a multifactorial approach to gain knowledge on the control of the red flesh pigmentation in apple. This methodology was applied to investigate the phenotypic diversity in five hybrid F1 families segregating for this trait (450 genotypes) by combining image-based phenotyping with the determination of several biochemical traits that influence colour expression: phenolic compound contents, dry matter contents, and pH values. We used image-based phenotyping to evaluate intensity and distribution of red colour in progenies using colour parameters. We also defined morphometric descriptors to assess variation of colour patterns in the dataset and to study the inheritance of the colour distribution. Important variations in phenolic profiles among hybrid families were identified. Ten of the most discriminating phenolic compounds involved in the expression and stability of red flesh were selected. Targeted analyses were performed to quantify these compounds by UPLC-DAD over two years of fruit harvest (2021 and 2022). As a result, we propose a model including pH and phenolic compound contents to describe red flesh colour development.

The genetic determinism of red-flesh was then investigated. Individuals were genotyped via the Illumina Infinium 20K array and pedigree-based QTL analyses were conducted with FlexQTL™ software. We identified new loci involved in red-flesh trait and anthocyanin accumulation. This study will lead to the development of molecular markers applicable in MAS (Molecular marker Assisted Selection).

Keywords: *Malus domestica, red-flesh trait*

Impacts of water restriction on the development of urban trees and their associated cli-mate services.

D. Canonne[1,2], S. Demotes-Mainard[2], M. Saudreau[3], J. Thierry[1], B. Dubuc[2], L. Ledroit[2], D. Cesbron[2], C. Lebras[2], L. Brialix[1], D. Lemesle[1], S. Herpin[1], P.E. Bournet[1]

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Street trees can improve thermal comfort of city dwellers through the cast shadow and the transpiration they provide. These two mechanisms are linked to the crown light interception capacity which depends on tree architecture and leaf traits. These two characteristics can be themselves impacted by water availability. Yet, little is known on urban tree functioning regarding climate services they render in the context of global change and enhanced water scarcity.

The aim of this work is to study the effects of contrasted water supplies on tree development and associated climate services. In this prospect, a 1/5th scale outdoor canyon street, North-South oriented, with two central tree alignments consisting each in five ornamental apple trees, was used in Angers, France. Each alignment was equipped with meteorological and soil sensors with continuous data acquisition from May 2022 to September 2022. Architectural and foliar measurements were also carried out on four specific sub-periods. Before July 2022, all trees of both alignments were well-watered. Then, from the beginning of July 2022 and during two months, a moderate water restriction was applied to one of the tree alignments, while the other was kept well-watered. In the water-restricted alignment, irrigation was adjusted according to the analysis of daily micrometric variations of the trunk diameter and soil water content data. The target was to ensure that soil moisture remained above the wilting point, but in the non-readily available water content so that water remained difficult to extract by the roots.

Both tree transpiration and architecture differences between the two treatments were observed and highlight the impact of the water deficit on tree functioning in an urban environment. For instance, at the end of August 2022, the leaf surface area of the well-watered trees was on average almost twice as high as that of the water-restricted trees. The tree leaf surface area was deeply analyzed in order to appreciate architectural traits as a whole. It was also discussed regarding the tree climatic contribution, characterized by the reduction of the air temperature and Universal Thermal Comfort Index (UTCI). For the purpose of guiding the choice of tree species to improve human thermal comfort, these results support the need to consider tree architectural and hydric functional traits.

Keywords: *Urban environment, Water constraint, Tree architecture, Transpiration, Thermal comfort*

Effective seedling microbiota engineering using Synthetic Community (SynCom) inoculation on seeds

Gontran Arnault, Coralie Marais, Anne Préveaux, Martial Briand, Anne-Sophie Poisson, Alain Sarniguet, Matthieu Barret and Marie Simonin

Microbiota engineering through inoculation of synthetic communities (SynComs) is gaining attention as a potential way to improve plant growth and health. Seeds represent a vector of dissemination of plant-associated microorganisms. Hence, inoculation of SynComs on seeds could be a relevant way to improve plant protection and yield using a limited amount of inoculum. However, many questions are to be answered before a possible application of SynComs on seeds: (1) What is the influence of SynCom concentration and composition on its stability and ability to colonize seeds and seedlings ? (2) And, what is the impact of the SynCom on seedlings phenotype?

To answer these questions, a collection of 1250 bacterial strains isolated from bean seeds and seedlings has been obtained. A total of 43 SynComs were designed and tested to study the impact of inoculum concentration and composition. A metabarcoding approach was performed to track our bacterial strains in the inocula, seeds and seedlings. On average, the SynCom is representing 97% of the relative abundance of the seed microbiota and 80% of the seedling microbiota, even in a coalescence context with the natural microbiota of the potting soil. (1) We showed that mass effect, strain identity and biotic interactions were the main drivers of strains and SynComs ability to colonize seeds and seedlings. (2) Also, the 43 SynComs showed significant and contrasted effects on seedlings phenotype related to seed vigor (emergence and abnormal seedling rates). Three SynComs with beneficial effects were further investigated for their impact on plant metabolome and microbiome assembly dynamics.

To conclude, our SynCom inoculation approach on bean seeds is a promising method to manipulate plant microbiota and phenotypes for future agricultural applications.

Do wild Brassicaceae defense compounds enhance the antagonistic effect of seed-borne fungi against *Alternaria brassicicola*?

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Antagonistic fungi can be source of biological control agents (BCAs) and may play a significant role in managing plant diseases. However, how the host plant might have an impact on their ability to control seed-borne plant pathogens has rarely been considered. Currently, there is no alternative methods to pesticides to fight *Alternaria brassicicola*, a seed-borne causal agent of the black leaf spot disease on many Brassica crops. In order to devise new management strategies, investigating natural species of crop relatives that exhibit resistance traits could be source of novel solutions. Indeed, resistant wild Brassicaceae species host antagonistic fungi and synthetize defense compounds that may impact the interactions among seed-borne fungi. While some fungi can metabolize phytoalexins such as camalexin, and phytoanticipins like glucosinolates, other fungi including *A. brassicicola* are inhibited by these plant defense compounds and their breakdown products such as isothiocyanates (ITCs). Therefore, one of the objectives of my thesis is to explore the role of camalexin and ITCs on the potential seed-borne fungal antagonists and their impact on *A. brassicicola*. As a first step, fungal antagonists to *A. brassicicola* isolated from resistant wild Brassicaceae, were identified by confrontation assays on agar media. Then, in liquid media (by using nephelometry) enriched or not with camalexin and ITCs, the effect of these fungi on the growth of *A. brassicicola* as well as their response to the plant secondary metabolites were measured. Indolic compounds such as camalexin and indole ITCs showed a lower impact on some of the antagonistic fungi than on *A. brassicicola*. Furthermore, defense compounds seemed to enhance the antagonistic effect of some fungal isolates on *A. brassicicola*, with an additive and synergistic effect. These results indicate that potential biological control agents against *A. brassicicola* might be stimulated by the host plant. However, the underlying mechanisms need more investigation.

Keywords: *Brassicaceae, Alternaria, camalexin, glucosinolates, seed-borne antagonistic fungi*

A study of the climatic benefits of street trees under warm and dry conditions based on both reduced scale and in-situ monitoring approaches.

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Global warming comes with a multiplication of extreme events such as heatwaves. In cities, this phenomenon is amplified by the urban configuration and the nature of the materials whose optical and thermal properties enhance overheating. Vegetation, especially street trees, may partially counteract overheating by means of two processes, cast shadows and evapotranspiration, which may depend on the morphological and physiological characteristics of the considered tree species. At the same time, the quantity and the distribution of rainfalls are getting modified. The ability of street trees to thrive and maintain significant cooling benefits under heat and drought conditions is therefore of high interest. In this prospect, a research methodology based on two experimental devices has been developed. On the one hand, a 1/5 scale canyon street, partially planted with drip-irrigated potted Malus Coccinella® 'Courtarou' and equipped with soil moisture and meteorological sensors was built in Angers in order to quantify the human thermal comfort improvement provided by street trees experiencing different water supplies. Results showed that trees exposed to a water stress, although they significantly reduced their transpiration rates, were able to maintain a level of benefits close to that of the well-watered trees, because the radiation interception by their foliage was still important. On the other hand, the green spaces department of the city of Paris monitored the microclimate inside the crowns of 36 street trees, representing 9 different species in 5 sites in Paris from 2019 to 2022. First analyses showed that the variability of climatic benefits provided by the different tree species was low even during dry periods. Further analyses will rely on the complementarity of both the reduced scale and in-situ experimental facilities and on the assessment of some morphological and physiological variables of the trees to provide stakeholders with data helping them select the most adapted species regarding both the resistance to drought and the heat mitigation.

Keywords: *Urban climate, Water balance, Transpiration, Radiation balance, Human Thermal Stress*

XopI, an effector of Xanthomonas modulating sugar metabolism in bean plants.

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Xanthomonas citri pv. *fuscans* (*Xcf*) and *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) are the bacterial strains responsible for the Common Bacterial Blight of Bean. Their pathogenicity is mainly based on the injection of Type III Effector (T3E) proteins into the plant cell via a Type III Secretion System. The genome sequencing of the *Xcf* CFBP4885 strain led to the identification of 29 T3Es, among which XopI. How XopI participates in the strain pathogenicity is still unknown. Therefore, the aim is to study the functions of XopI.

The gene encoding XopI was cloned into binary vectors, enabling Agrobacterium-mediated transient expression. The response of susceptible and resistant bean cultivars to the transient expression of *xopI* was compared to that of the gus reporter. Based on the sequenced genome of Phaseolus vulgaris G19833, a total of 66 differentially expressed genes (DEGs) were identified in response to the transient expression XopI. These differentially expressed genes are related to the maintenance of function and structure of chloroplasts and mitochondria. This raises the question whether XopI may impact the plant sugar metabolism.

In order to assess the impact of XopI on plant sugar metabolism, the activity of the main enzymes involved in sugar metabolism were quantified on leaf tissues transiently expressing *xopI*. The activity of three enzymes involved in the glycolysis (G6PDH, PFK and PGI) were significantly decreased in the susceptible plants, while no changes in the activity of those enzymes could be observed in the resistant plants.

To test whether the observed decrease of enzymatic activities impacts the relative amounts of soluble sugars in leaf tissues, a GC-MS approach was conducted to quantify all small soluble metabolites from Agro-infiltrated leaf tissues. So far, no changes in plant metabolite profile have been identified 36 hours after inoculation. However, a difference in plant metabolite composition has been observed between the susceptible and resistant bean variety. The identification of these specific differentiating metabolites may be of interest, as they could provide further insights into the susceptibility or resistance of bean plants.

Keywords: *Xanthomonas*; T3E; Sugar metabolism; Agro-infiltration; RNAsequencing; Enzyme activity; Metabolite profiling

Reusing wastes and by-products from desealing operations to develop fertile soils for greening cities

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European cities want to limit their urban sprawl but also want to increase their greening which provides ecosystem services. Urban green areas can be implemented through soil desealing. Still minor, desealing operations usually consist in removing the surface pavement without worrying about environmental risks such as contaminant transfer. As part of this study, soil desealing experiments were led to evaluate the capacity of desealed soils to become multi-functional to ensure the development of vegetation.

The objectives were to study the physico-chemical and biological parameters of desealed soils by reusing impervious cover and road subbases materials to limit the use of topsoil. A three-month experiment consisted of cultivation pots monitored under controlled conditions. Fourteen mixes were studied, combining hydrocarbon asphalt (2 formulations), 2 road sub-bases aggregates (depth between 5-40 cm), green waste compost and topsoil. A mixture of topsoil and compost (2:1) was used as a reference to mimic typical soil formulation for urban green space. All treatments were sown with ryegrass. Air temperature, light and moisture in the greenhouse were monitored daily. The vegetation growth was monitored twice a week and biomass was weighted twice during the experiment. Microbiological activity and biomass were evaluated at the beginning and end of the monitoring. Soil fertility, innocuity (metal traces) were assessed at three times in each modality.

The results of the experiment showed that whatever the modality, the risks of contaminant transfer to the soil were low and below the admissible thresholds. Reuse of the sealing material do not lead to contaminant transfers into the water fraction nor to the soil fraction but vegetation development was slightly reduced. Green waste compost is the major factor that improve the fertility and the ryegrass development. Aggregates excavated from a green space pathway allow a great development of ryegrass without any amendment or topsoil addition. Mixes with road materials were functional but needed an organic amendment (compost only or compost and topsoil) to give the same results than the soil typically used for urban green space planning. This study may help have a better understanding of the effect of the soil desealing processes.

Keywords: *desealing, Technosols, urban soils, soil fertility, soil functions, ecosystem services, urban green areas, waste reuse*

Study of the OcKAI2 receptors, germination stimulants of the parasitic plant *Orobanche cumana*

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Orobanche cumana (*O. cumana*) is an obligate root parasite from the *Orobanchacea* family which infests mainly the cultivated sunflower (*Helianthus annuus*) by connecting to the vascular system and uptaking water, sugar and nutrients from the host. This specie, originated from eastern Europe, has progressively spread in middle East, China and Mediterranean countries and can induce severe yield losses up to 100%. The first cases of infestation in France were spotted 15 years ago, affecting today over 300 field plots. In order to specifically identify its host, *O. cumana* is able to recognize particular molecules exudated in the soil by the sunflower, hereafter indicated as germination stimulants (GS). These molecules from the strigolactones and sesquiterpene-lactone families trigger the germination of the seeds, leading to the attachment and therefore parasitism. At the moment, 10 receptors from the α/β hydrolase family, the KARRIKIN INSENSITIVE 2 (KAI2), are considered for the perception of the GS and the initiation of germination pathway. The aim of this project is to understand which KAI2(s) is/are involved in the germination process of *O. cumana* and with which GS. It implies several *in silico*, *in vitro* and *in vivo* approaches such as phenotyping, molecular docking, mutant complementation, enzymatic tests and transcriptomic studies, by considering the diversity between different populations of parasites. The first results show the impact of three natural GS on the germination of O. cumana, the discovery of a new receptor and give hints for the explanation of differences observed between populations. On a long-term perspective, this work will help establishing stable solutions to reduce the impact of the parasite on the crops.

Keywords: *Orobanche cumana*, receptor, strigolactones, sesquiterpene lactone, KAI2, seed germination stimulant