



INRAE

Provence-Alpes-Côte d'Azur



RhizosphARE
Interdisciplinary meeting

**Understanding the role of rhizodeposits in
communication between plants and soil
organisms.**

12-14 novembre 2025

Avignon

Programme

Mercredi 12 novembre

13h30 - 14h00 **Accueil café**

14h00 - 14h10 **Ouverture**

14h10 – 15h20 **Session 1**

Chair : Agnès Attard

14h10 – 14h30 **Louis-Valentin METEIGNIER (PHIM, Montpellier).**
Genome-wide association study of neighbor effects identifies candidate genes putatively involved in root secretions.

14h35 – 14h55 **Baptiste BARBAULT (GLYCOME, Rouen).**
Contrasting effects of root exudates on biofilm formation of two plant growth-promoting rhizobacteria.

15h00 - 15h20 **Claire Benezech (PHIM, Montpellier).**
Wheat genotype impacts plant responses to biosolutions.

15h20 – 15h50 **Pause**

15h50 – 17h00 **Session 2**

Chair : Eric Grenier

15h50 – 16h10 **Annette Bérard (EMMAH, Avignon).**
How to use roots and their rhizosphere to modulate the impact of water deficits and improve water management in agrosystems?

16h15 – 16h35 **Josselin Bodilis (GLYCOME, Rouen).**
When roots talk, bacteria reply: transcriptomic signatures of Plant Growth-Promoting Rhizobacteria growing on root exudates.

16h40 – 17h00 **Virginie Lauvergeat (EGFV, Bordeaux).**
Understanding the mechanisms of interaction between grapevine rootstocks and soil microbiota.

17h00 – 18h00 **Introduction aux ateliers du jeudi 13**

19h00 – **Apéritif et Dîner**

RDV à partir de 19h00 au restaurant les Deux J & Cie

1171 route de l'Aérodrome Zone d'activité Agroparc, 84140 Avignon.

Pour ceux qui sont logés à Avignon, il faudra partir vers 21h50 pour prendre le dernier bus C3 en direction D'Avignon.

Jeudi 13 novembre

8h50 – 09h00

Accueil

9h00 – 09h40

Conférencier invité

Naoise Nunan

(CNRS, Institut d'Ecologie et des Sciences de l'Environnement – Paris)

Carbon processing in the soil pore network

09h40 – 10h25

Session 3

Chair : Frédéric Rees

09h40 – 10h00

Andrea CASTALDI (IJPB, Paris-Saclay).

Innovative methodological and analytical developments in the study of plant-soil fauna interactions.

10h05 - 10h25

Laure Boeglin (ISA, Sophia-antipolis).

Metabolomic characterization of Arabidopsis root exudates involved in oomycete attraction during the early stages of attraction.

10h25 – 11h00

Pause

11h00 – 12h10

Session 4

Chair : Jean-Benoît Peltier

11h00 – 11h20

Alexia Gaudry (GLYCOMEV, Rouen).

Characterization and Role of Polymers in Root Extracellular Trap Structuring in Two Fabaceae Species.

11h25 – 11h45

Jeanne Couderc (IGEPP, Rennes).

Investigating defense mechanisms associated with pea resistance to *Aphanomyces euteiches*.

11h50 – 12h10

Samuel LE GALL (IBG3-Agrosphere, Allemagne).

Effect of phylogeny on root mucilage.

12h10 – 13h45

Déjeuner et café sur place

13h45 – 14h00

Photo à de groupe

14h00 – 15h30

Ateliers de réflexion 1

« Construction de nouveaux projets »

15h30 – 15 h45

Pause

15h45 – 17h15

Ateliers de réflexion 2 & 3

« Impact du changement climatique sur le dialogue rhizosphérique entre les organismes. »

« Evaluation d'un outil d'aide au choix des méthode de récolte et d'analyse des exsudats. »

17h15 – 19h00

Temps libre, visite d'Avignon

Comptez 30-40 min pour vous rendre à Avignon en bus.

19h00 – 21h30

Apéritif et

RDV à 19h00 au restaurant le Carré du Palais.

1, Place du Palais des Papes, 84000 Avignon.

Pour ceux qui sont logés près de l'INRAE - Saint Paul, nous partirons du restaurant à 21h35 pour prendre le dernier bus C3.

Vendredi 14 novembre

009h – 10h05

Session 5

Chair : Sylvain Chéreau

09h00 - 09h20

Jean-Benoît Peltier (IPSIM, Montpellier).

Titre à venir.

09h25 – 09h45

Eric Grenier (IGEPP, Rennes).

Analysis of orientation in phytoparasitic nematodes using innovative tools combining novel attraction test devices and digital trajectory measurement.

09h45 – 10h05

Agnès Attard (ISA, Sophia-antipolis).

Unravelling the spatiotemporal dynamics of the early dialogue between host plants and pathogenic Oomycetes.

10h05 – 10h35

Pause

10h35 – 11h35

Restitution des ateliers, échanges et conclusion

12h00

Clôture

Résumés

Genome-wide association study of neighbor-modulated disease susceptibility in hexaploid wheat identifies candidate genes involved in root secretions

Sylvain POUPONNOT¹, Laura MATHIEU¹, Myriam BEN AMAR¹, Elsa BALLINI¹, Jean-Benoit MOREL¹, Louis-Valentin METEIGNIER¹

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Key-words (max 5): hexaploid wheat, neighbor effects, disease susceptibility, root-root interaction

In intraspecific wheat mixtures, neighbor-modulated susceptibility (NMS) alters disease outcomes when specific genotypes grow side by side. Unexpectedly, we consistently observed that a vast majority of neighbors decreased leaf rust disease in companion cultivars, even after eliminating aerial cues and suppressing microbial communication in the soil, indicating a mechanism beyond competition. We applied a neighbor-aware GWAS in hexaploid wheat to identify the neighbor signals involved in NMS. This identified robust SNPs whose nearest genes are strongly expressed in young wheat roots. Notably, the Arabidopsis orthologs participate in the conventional secretion pathway and in root directional growth under stress. Together, these results point to a root-localized, secretory process mediating NMS and underlying disease suppression in mixtures. I will present the experimental results and candidate loci, and discuss future experiments to functionally validate the role of these candidate genes in plant-plant interactions.

Contrasting effects of root exudates on biofilm formation of two plant growth-promoting rhizobacteria

Baptiste BARBAULT¹, Eulalie FOURNEAU¹, Barbara PAWLAK¹, Josselin BODILIS¹

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Key-words (max 5) : PGPR, root exudates, biofilm, microfluidic

The rhizosphere microbiota, *i.e.* microorganisms living in the soil under the influence of plant roots, includes various plant growth-promoting rhizobacteria (PGPR) that play an important role in plant life by stimulating growth and/or protecting plants against biotic and abiotic stresses (Qu *et al.*, 2020). It has been shown that the establishment of microorganisms in the rhizosphere is linked to root exudates, which modulate chemotaxis, growth, and biofilm formation (Fourneau *et al.*, 2024)

Our objective is to quantify the biofilm formation of two PGPR, *Pseudomonas fluorescens* ATCC 17400 and *Bacillus subtilis* ATCC 6633, in response to root exudates from two plants, *Arabidopsis thaliana* and *Pisum sativum* (var. Astronaute).

In a first part, the study was conducted on polystyrene microplate which allowed the quantification of biofilm-forming bacteria after crystal violet staining. However, in this environment, these two bacteria do not form the same type of biofilm. Indeed, *P. fluorescens* colonizes the edge and bottom of the well (O'Toole and Kolter, 1998), whereas *B. subtilis* forms a biofilm at the air-liquid interface, known as a pellicle (Beauregard *et al.*, 2013). Interestingly, we found that a concentrated solution of root exudates (10 mg/L of carbon) inhibits *P. fluorescens* biofilm formation while the same concentration both increases and modifies the biofilm structure of *B. subtilis*.

In a second part, we are currently developing a microfluidic device capable of maintaining the plant alive during the experimentation. This will allow us to observe both bacterial chemotaxis and biofilm formation *in vivo* and in real time.

A better understanding of the role of root exudate in root-bacteria interactions would allow to modulate the rhizosphere microbiota and reduce the use of chemical inputs, thereby contributing to more sustainable agriculture.

Bibliography :

Beauregard, P.B. *et al.* (2013) Available at : <https://doi.org/10.1073/pnas.1218984110>.

Fourneau, E. *et al.* (2024) Available at : <https://doi.org/10.3389/fmicb.2024.1473099>.

O'Toole, G.A. and Kolter, R. (1998) Available at : <https://doi.org/10.1046/j.1365-2958.1998.00797.x>.

Qu, Q. *et al.* (2020) Available at : <https://doi.org/10.1021/acs.jafc.0c00073>.

Wheat genotype impacts plant responses to biosolutions

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Key-words (max 5): biosolutions, physiological and molecular responses, genotype-dependent effects, biotic and abiotic stresses

With growing ecological and economic pressures, there is increasing interest in finding alternative ways to boost plant production. One promising approach is the use of biostimulant products, which help plants better cope with abiotic stresses and improve nutrient assimilation. These biostimulants come from a wide range of sources natural, synthetic, or mixed including amino acids, enzymes, algal extracts, plant hormones, beneficial microbes, plant extracts, minerals, and nutrients. While their potential is clear, the exact mechanisms behind their effects are still not fully understood. This requires better characterization of the molecular responses they trigger in order to promote their safe use by farmers, with respect for both human and environmental health.

In our work, we aim to bridge this knowledge gap by characterizing the responses triggered by different biostimulants in durum wheat. These biostimulants activate various pathways that can enhance nutrient assimilation, improve drought tolerance, and boost seed quality. To achieve this, we combine molecular, metabolomic, and physiological approaches across four durum wheat varieties.

Our analyses highlight that molecular, metabolomic, and physiological responses are strongly shaped first by the genotype, and then by the applied biosolutions. We were able to show that specific molecular pathways are activated depending on both the biostimulant used and the plant's genetic background. We also identified correlations between transcriptomic, metabolomic, and physiological traits.

To really push the "lab-to-field" transition forward, we are testing how biosolutions perform under a wide range of stresses from drought and nutrient shortages to temperature fluctuations and biotic challenges. By taking this broader view, we are uncovering key mechanisms underlying biostimulant activity and demonstrating their real potential to enhance plant resilience and productivity in the field.

When roots talk, bacteria reply: transcriptomic signatures of Plant Growth-Promoting Rhizobacteria growing on root exudates

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Key-words: rhizosphere, root exudates, PGPR, transcriptomic

The rhizosphere is the narrow zone of soil surrounding plant roots and directly influenced by root exudates. This complex mixture of molecules enables plants to recruit their rhizosphere microbiota, which supports growth and resistance to biotic and abiotic stresses (Tian *et al.*, 2020). In the context of sustainable agriculture, understanding the molecular dialogue between roots and their microbiota could facilitate the establishment of beneficial microorganisms such as plant growth-promoting rhizobacteria (PGPR) in the rhizosphere. In a previous study, the ability of root exudates from rapeseed (*Brassica napus*), pea (*Pisum sativum*), and ryegrass (*Lolium perenne*) to attract and feed three PGPR strains (*Bacillus subtilis* ATCC 6633, *Pseudomonas fluorescens* ATCC 17400, and *Azospirillum brasilense* Sp245) was compared using a new indicator, the “love match” score (Fourneau *et al.*, 2024). Rapeseed exudates were the most attractive and induced the fastest growth, pea exudates supported the highest bacterial biomass, while ryegrass exudates were least effective. Concerning the PGPRs, *P. fluorescens* and *A. brasilense* responded more efficiently to exudates than *B. subtilis*.

In this work, transcriptomic analysis was used to investigate PGPR gene expression during growth on root exudates. *B. subtilis* and *A. brasilense* upregulated many genes involved in amino acid and carbohydrate metabolism, whereas *P. fluorescens* constitutively expressed most of the genes required for these processes. Beyond a shared core transcriptome, each plant species modulated specific bacterial genes: rapeseed exudates upregulated additional amino acid metabolism genes in all PGPR, while pea exudates upregulated defense-related genes (e.g., resistance and stress proteins in *A. brasilense* and *B. subtilis*) and downregulated motility genes.

Overall, these findings highlight the specific and selective influence of each plant on PGPR through root exudates and may guide the identification of exudate components that best promote bioinoculant establishment in the rhizosphere.

Fourneau, E. *et al.* (2024) ‘A “love match” score to compare root exudate attraction and feeding of the plant growth-promoting rhizobacteria *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azospirillum brasilense*’, *Frontiers in Microbiology*, 15, p. 1473099.

Tian T. *et al.* (2020) The role of rhizodeposits in shaping rhizomicrobiome. *Environ. Microbiol. Rep.*, 12(2): 160-172.

Innovative methodological and analytical developments in the study of plant-soil fauna interactions

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Key-words (max 5): *Arabidopsis thaliana*, *Protaphorura fimata*, Metabolomic, GC-HRMS, LC-HRMS

Plant-soil interactions are modified by both biotic and abiotic factors, and understanding these dynamics is essential to predict ecosystem responses to climate change. Root exudates play a central role in rhizosphere dynamics through the release of primary and specialized metabolites, which are involved in defense mechanisms, competition, and symbiosis. However, the influence of soil fauna on root exudation remains poorly understood. To date, studying root exudation in soil matrices has been technically challenging, and the most commonly used systems hydroponic or hybrid fail to reproduce realistic interactions with soil fauna. Previously, in *Poa annua*, we employed the EcoRoots system – rhizoboxes with nitrocellulose traps at the root apex – to collect exudates *in situ* for GC-HRMS profiling. We showed that living soil fauna restructured exudate profiles, with earthworms exerting the strongest effect, underscoring the need for soil-realistic sampling [1-2].

Presently, we use a pot-based soil-hydroponic hybrid system in which plants remain in soil while a subset of roots is externally bathed in solution for exudate collection. We test how the springtail *Protaphorura fimata* modulates the quantity and composition of *Arabidopsis thaliana* exudates between the wild-type *Col-0* ecotype and the GSL-deficient *gk0* mutant.

We hypothesize that *P. fimata* reprograms root-shoot signaling to increase export of defensive specialized metabolites, especially glucosinolates (GLS). Accordingly, a GLS-deficient *gk0* mutant should display compensatory salicylic-acid/jasmonic-acid (SA/JA) signatures and stronger root morphological perturbations.

Our multiblock metabolomic approach couples hybrid in-soil sampling with complementary analytical techniques: (i) polymeric sorbent beads (Amberlite XAD7HP) to enrich GLS and other semipolars for LC-/HRMS; (ii) aqueous fractions for primary metabolites (e.g., sugars) by GC-HRMS after derivatization; and (iii) volatiles captured on sorptive tubes (Sorbstar®) and analyzed by thermal-desorption GC-HRMS. Preliminary collection tests confirm selective GLS enrichment and complementary coverage of aqueous sugars and volatiles.

This methodology will provide a robust multiblock approach for the investigation of root exudation in other plant species with allelopathic potential or agronomic relevance.

[1] V. Bohm et al., mai 2025, doi: 10.1016/j.jcoa.2025.100205.

[2] A. Levasseur et al., juin 2025, doi: 10.1016/j.soilbio.2025.109771.

Metabolomic characterization of *Arabidopsis* root exudates involved in oomycete attraction during the early stages of attraction

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Key-words : Root exudates; Metabolomics; UHPLC-MS/MS; *Arabidopsis thaliana*; *Phytophthora parasitica*

In the soil environment, root exudates shape the rhizosphere by mediating chemical communication between plants and microorganisms (Zhalnina *et al.*, 2018.). These secreted metabolites influence microbial communities, nutrient acquisition, and plant health. Oomycetes of the *Phytophthora* genus are destructive soil-borne pathogens whose biflagellate zoospores swim through the soil water film to locate and infect plant roots. The earliest stage of infection, known as the attraction phase, seems to be governed by root-derived chemical cues, yet the identity and mode of action of these signals remain largely unknown (Bassani *et al.*, 2020).

In this work, we developed a reproducible workflow for the collection and metabolomic profiling of *Arabidopsis thaliana* root exudates to identify compounds involved in the attraction of *Phytophthora parasitica* zoospores. Preliminary work highlighted that inhibition of pump activity reduces zoospore attraction towards the root. We thus use a comparative approach of the composition of root exudates between untreated and treated roots with cation pump inhibitors. Roots were cultivated hydroponically under controlled conditions, and exudates were collected in sterile water. Samples were analyzed by non-targeted UHPLC-MS/MS, and metabolite annotation was performed using mzmine 4.7.3 and Sirius 6.1.0. As a biological reference, we use a comparative approach between untreated roots and roots pre-treated with cation pump inhibitors, since our preliminary work highlighted that inhibition of pump activity has been shown to reduce zoospore aggregation on the root surface. This strategy aims to feature changes in the composition of root exudates that may underlie the release of attractant signals.

Current analyses focus on data processing and metabolite identification to build a foundation for correlating metabolite composition with zoospore attraction, and for pinpointing the metabolites and plant functions that drive the earliest steps of oomycete infection. Ultimately, this work will contribute to deciphering the molecular dialogue between roots and soilborne oomycetes at the earliest steps of infection.

Bassani *et al.*, 2020. *Microorganisms*. doi: 10.3390/microorganisms8071012

Zhalnina *et al.*, 2018. *Nature Microbiology* <https://doi.org/10.1038/s41564-018-0129-3>.

Characterization and Role of Polymers in Root Extracellular Trap Structuring in Two Fabaceae Species

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Key-words (max 5): root extracellular trap ; mucilage ; enzymatic treatment ; pectin ; xyloglucan

The root extracellular trap (RET) is a network formed by root associated cap-derived cells (AC-DCs) and their mucilaginous secretions. The RET is located at the root tip where it establishes protective interface against abiotic and biotic stresses. The mucilage is mainly composed of complex cell wall polysaccharides (including pectin and xyloglucan) and glycoproteins, that are entangled to each other within the network. Despite our knowledge of the composition of RET mucilage, the structural organization of the RET remains poorly understood. Here, we have investigated the role of pectin and xyloglucan polysaccharides in RET structuring in two *Fabaceae* species, namely *Pisum sativum* and *Glycine max*. We first characterized the composition of the RET using immunocytochemistry and gas chromatography techniques, and then assessed the effect of treatments with hydrolytic enzymes targeting pectin and xyloglucan on the RET and its isolated mucilage. The effects of the enzyme treatments were determined by microscopical analysis of the RET surface and its physico-chemical properties using the high-pressure size-exclusion chromatography. The data show that RET of two species is enriched in pectin and xyloglucan, and it is quite recalcitrant to enzymatic hydrolysis as compared to other root tissues. In contrast, we found that enzymatic treatments were much more effective on isolated mucilage and only at low concentration. Taken together, our findings show that RET displays a remarkable resistance to hydrolytic enzymes highlighting the complexity of its structure, probably resulting from specific arrangements of its macromolecules necessary for its function.

Investigating defense mechanisms associated with pea resistance to *Aphanomyces euteiches*

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Key-words : *Pisum sativum*, *Aphanomyces* root rot, oospores, zoospores, metabolites

Root rot caused by pathogens such as the oomycete *Aphanomyces euteiches*, can drastically reduce pea crop yields. Some pea lines exhibit partial resistance, which is conferred by QTL regions spread across their genome (Hamon et al., 2013; Leprévost et al, 2023). A major-effect QTL, *Ae-Ps 7.6*, reduces root mycelium colonization and delays symptoms in pea roots (Lavaud et al., 2016). It is not known whether this QTL conferring partial resistance or multiple QTL conferring high resistance in pea have an effect on the earlier stages of pea interaction with *A. euteiches* in the rhizosphere. Defense mechanisms associated with these resistance QTL are also unknown.

This study aims to investigate the effect of rhizodeposits from different resistant to susceptible pea genotypes, on the early phases of the *A. euteiches* life cycle in the rhizosphere. It also aimed to identify metabolites produced in the roots and rhizodeposits of pea genotypes that showed contrasting effects on the pathogen at the early stages of the interaction.

A hydroponic device for growing plants and collecting rhizodeposits has been elaborated. Methods for testing zoospore attractiveness using a microfluidic device and oospore germination assays of *A. euteiches* in response to pea exudates are currently being developed. Metabolites extracted from root samples of pea near-isogenic lines differing only at the major effect QTL have been analysed using HPLC-Orbitrap and the results are being interpreted.

After identifying variations in germination and attraction responses of spores to rhizodeposits from different genotypes, rhizodeposits and root samples of selected genotypes will be analysed using untargeted metabolomics approach to determine biomarkers and molecular signatures associated with partial resistance.

The results of this study will provide a better understanding of the defense mechanisms associated with pea resistance to *A. euteiches* and identify breeding and biocontrol targets for pea protection against the disease.

Hamon et al. (2013). *BMC Plant Biol* **13**, 45 <https://doi.org/10.1186/1471-2229-13-45>

Lavaud et al. (2016) *BMC Plant Biol* **16**, 166. <https://doi.org/10.1186/s12870-016-0822-4>

Leprévost et al. (2023). *Front. Plant Sci.* **14** 1189289. <https://doi.org/10.3389/fpls.2023.1189289>

Effect of phylogeny on root mucilage

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Key-words (max 5): Root mucilage, phylogeny, angiosperms, water retention, mucilage-induced respiration

The diversity of root metabolite exudated in the rhizosphere, and their function related to the soil hydrology and the microbial communities, is one of the products of plant evolution. The first terrestrial plants (from the Ordovician period) developed rhizoids that secrete simple compounds, such as organic acids, sugars and phenols, to mobilize nutrients and interact with primitive symbiotic fungi. Then, in the Devonian period, true roots appeared that explored the soil more deeply and developed polysaccharide root mucilage, which may have played a protective and hydraulic maintenance role. As plants evolved up to the development of angiosperms, the nature and function of exudates became increasingly complex.

If the chemical composition and functions of exudates are indeed the product of this evolution, phylogenetic similarities should be found between the compositions of root exudates.

To test this hypothesis, we conducted an experiment in which mucilage was collected from the primary roots of germinated seeds grown under controlled conditions using the aeroponic technique. Eight cultivated angiosperm species were selected for study: four dicotyledons (tomato, alfalfa, lupin and rapeseed) and four monocotyledons (wheat, rye, corn and sorghum). MIR measurements were performed on the collected mucilage using a Tensor 27 FTIR spectrometer (Bruker Optics, Wissembourg, France). The samples were scanned between 4000 and 600 cm^{-1} , with each spectrum obtained by averaging 16 successive scans. We also measured the mucilage maximal water retention and total sugar content of the mucilage using the Dubois method. We also performed a MicroResp™ assay to measure the respiration induced by different mucilages in three contrasting soils, which were selected for their distinct microbial biomass, texture, organic carbon content, and water-holding capacity.

Our results confirm that phylogenetic history drives the root mucilage compositions and that these induce different hydro-physical behaviours and affinities for edaphic microbial catabolism.

Analysis of orientation in phytoparasitic nematodes using innovative tools combining novel attraction test devices and digital trajectory measurement.

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Key-words (max 5): Nematode, Attraction, 3D printing devices, Digital vision, Automatic video processing

Abstract (max 350 words): Vertebrates respond mainly to visual, auditory and olfactory signals for communication, whereas in nematodes, chemical stimuli are of paramount importance. Nematodes have well-developed sensory organs for perceiving physical and chemical signals. The perception of these signals will determine whether or not they are attracted to a host plant or sexual partner. Our project proposes to use cyst nematodes and root-knot nematodes to develop miniaturised experimental devices coupled with trajectory measurement tools in order to characterise and quantify as accurately as possible the behaviour and, more specifically, the attraction of these nematodes.

In the initial phase, we checked that 3D-printed devices have no toxic effects on nematodes. Then we attempted to recreate either the “Laloum” devices (Laloum et al. 2020) or the commercial “Ibidi” devices (μ -Slide I Luer from Ibidi) using 3D printing. In some cases, the resulting devices posed visibility challenges and have been changed in favor of a design combining 3D-printed structures with glass lamellae on top, which proved successful. For video analysis, different filming methods were tested, like a camera attached to a binocular loupe or a camcorder. A bench test was designed to fix the devices at a distance from the camera, and optimal lighting conditions were established. We developed automated video processing to remove the background and track nematodes. These advances opened possibilities for obtaining more comprehensive behavioral equations specific to each attractant, surpassing the current simple counting and normalization methods.

Liste des participants

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