AAPG2023	INHERSEED		JCJC
Coordinated by:	Marie SIMONIN	48 Months	357 566€
Axe A.3 : Biology of animals, photosynthetic organisms and microorganisms - CE20			

Plant microbial INHERitance across generations through SEEDs: impact of this primary inoculum on plant fitness and microbiota assembly

Summary table of persons involved in the project:

Partner	Name	First name	Current position	Role & responsibilities in the project (4 lines max)	Involvement (person.month) throughout the project's total duration
INRAE / IRHS	*SIMONIN*	*Marie*	*Researcher*	*Project Coordinator, WP1-WP2-WP3 leader Plant microbiome interactions & Synthetic ecology*	*24p.month*
INRAE / IRHS	*BARRET*	*Matthieu*	*Researcher*	*Bacteriology & Community ecology (WP1-WP2)*	*3p.month*
INRAE / IRHS	*GUSCHINSKAYA*	*Natalia*	*Associate Prof*	*Fungal culturomics & Plant-fungi interactions (WP3)*	*3p.month*
INRAE / IRHS	*SARNIGUET*	*Alain*	*Researcher*	*Microbial competitions & Bacteria-fungi interactions (WP1-WP2)*	*3p.month*
INRAE / IRHS	*VERDIER*	*Jérôme*	*Researcher*	*Seed Molecular Physiology (WP1)*	*3p.month*
INRAE / IRHS	*GARDET*	*Remi*	*Research Engineer*	*Plant production & Phenotyping. Responsible of PHENOTIC platform (WP1-WP2)*	*3p.month*
INRAE / IRHS	*PORTIER*	*Perrine*	*Research Engineer*	*Bacterial taxonomy and curator of CFBP bacterial collection for strain conservation (WP3)*	*3p.month*
INRAE / IRHS	*MARAIS*	*Coralie*	*Research Technician*	*Microbiology – Culturomics & amplicon sequencing on ANAN sequencing platform (WP1-WP2-WP3)*	*12p.month*
INRAE / IRHS	*To be recruited*		*Postdoctoral contract*	*Plant microbiome analysis & Plant phenotyping & Bioinformatics (WP2 & WP3)*	*36p.month*
INRAE / IRHS	*To be recruited*		*Research Technician*	*Plant production & phenotyping (WP1)*	*18p.month*
INRAE / IRHS	*To be recruited*		*Master 2 student*	*Plant production & Plant microbiome analysis (WP1)*	*6p.month*

I declare no evolution of the current proposal relative to the pre-submission.
The budget was revised to take into consideration the revaluation of salaries (post-doc and technician) and the increase of Administrative Management & Structure costs (13% to 13.5%) in 2023. The budget was initially of 341 021€ and is now of 357 566 € (+ 4.6 %).

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I. Proposal's context, positioning and objective(s)

a. Objectives and research hypothesis

All plants harbor a diverse microbiota contributing to their extended phenotypes to adapt to fluctuating environmental conditions and biotic stressors (Cantó *et al.* 2020). Thus, plants are now regarded as a holobiont comprising the host plant and its microbiota (Vandenkoornhuyse *et al.* 2015). The holobiont theory proposes that some core microbial taxa are functionally significant for the plant and form long-lasting interactions. One pathway to maintain these interactions across generations is vertical transmission through seeds. Hence, **seeds represent a cornerstone of the plant holobiont theory to characterize potential transgenerational associations between the host and its microbiota**.

Currently, no information is available on the origin of seed taxa, whether they are vertically (parental plant) or horizontally transmitted (e.g. soil, air, water, pollinators) to seeds and on their potential impacts on plant fitness. In the literature, there is an assumption that seed microbiota is composed of members that are mainly vertically transmitted from the parental plant but very few studies actually aimed to demonstrate it (Fort *et al.* 2021; Chesneau *et al.* 2022). To better understand plant holobiont assembly and stability, studies are needed to truly **quantify the contribution of vertically transmitted taxa to the assembly of seed and plant microbiota during its entire life cycle (Figure 1)**.

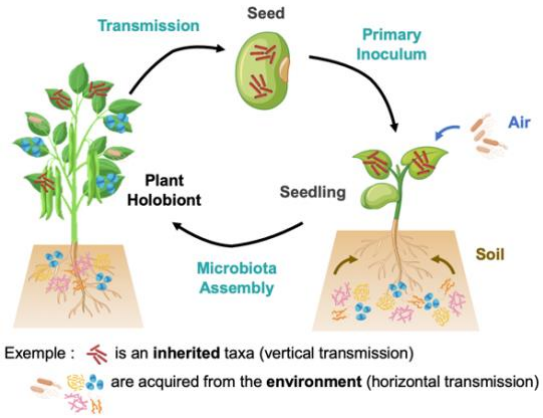

Figure 1: Plant microbial inheritance through seeds

In our recent meta-analysis on seed microbiota (Simonin *et al.* 2022), we identified around 30 bacterial and fungal taxa that constitute the core microbiota of seeds across 50 plant species. These core taxa present in most seed samples are likely crucial for the functions of these microbiota and for hosts' health. However, the core taxa represent only 0.5% of the microbial diversity observed on seeds. The majority of seed-borne taxa (60%) are detected in one plant species suggesting also the presence of specific plant-microbial interactions across the plant phylogeny. These observations encourage to investigate further **potential co-evolutions between plants and seed microbiota by assessing phylosymbiosis** (defined as microbial community relationships that recapitulate the phylogeny of their host, Lim & Bordenstein 2020).

The INHERSEED project aims to characterize microbial inheritance from parental plants to the progeny via seeds on 50 plant species spanning 12 orders across the Angiosperm phylogeny. We will determine the contribution of the vertically transmitted taxa (core or not) to plant microbiota assembly and plant fitness. This approach will enable to assess phylosymbiosis in plants and variations in the prevalence of transgenerational associations across the Angiosperms. The **main research questions addressed in the project are**:

- **Q1:** Do we detect phylosymbiosis in seed microbiota assembly and especially in the inherited fraction?
- **Q2**: Which fraction of seed microbiota is transmitted to seedlings (i.e plant primary inoculum) and what is the proportion of vertically inherited taxa?
- **Q3**: Do inherited taxa have an effect on plant fitness (i.e germination, plant growth and seed production)?
- **Q4**: What are the effects of inherited taxa on plant microbiota assembly (seed to seed)?

Given our expertise on the topic of seed microbiota assembly and transmission, we formulate the **following central hypotheses**:
H1: Few seed taxa are inherited from the parental plant. The diversity and contribution of inherited taxa to seed microbiota varies between plant species.
H2: Inherited taxa have a higher transmission success from seed to seedling than horizontally acquired taxa.
H3: Inherited taxa influence plant phenotypes (seed development, germination, growth) and microbiota assembly.

To address these research questions, we will mobilize the complementary expertise of the team in plant microbiome analysis, seed ecology and physiology, as well as plant phenomics. We expect that the INHERSEED project will generate fundamental new knowledge on plant holobiont assembly and transmission across generations for a large diversity of plant species.

b. Position of the project as it relates to the state of the art

Phylosymbiosis defines a link between host phylogeny and microbiome diversity that is quantifiable. This link or “phylosymbiotic signal” has been detected across the tree of life, starting with insects, mammals or corals (Lim & Bordenstein 2020). However, **very few studies assessed phylosymbiosis in plants** and the research available focused on the belowground compartments (root and rhizosphere, Yeoh et al. 2017; Fitzpatrick et al. 2018; Van Bel et al. 2021) or plant endosphere of “adult plants” (Vincent et al. 2016; Abdelfattah et al. 2022). The seed being a vector of microorganisms between generations, it represents a very relevant plant compartment to assess the relationships between host evolutionary history and microbiota composition.

Still, seed microbiota remain understudied compared to rhizosphere or phyllosphere microbiota, despite its central position as both the endpoint and beginning of plant microbiota assembly for the next generation (Shade *et al.* 2017). Hence, the **origins and functional roles of seed-borne microorganisms are generally unknown,** at the exception of a few seed-transmitted mutualistic (e.g. *Epichloë* sp. fungi) or pathogenic taxa (e.g *Xanthomonas* sp bacteria, Darsonval *et al.* 2009). The available studies that aimed to characterize the vertical or horizontal transmission of microorganisms mainly focused on a single taxon of a given plant species and just a few pioneer studies aimed to characterize microbial inheritance at the microbiome level (Escobar-Rodríguez *et al.* 2019; Chesneau *et al.* 2022). Results from our team suggest a low contribution of vertical transmission to the assembly of bean and radish seed microbiota (Chesneau *et al.* 2022). Recent work on *Poaceae* describe vertical transmission of few bacterial endophytes (rice, genera *Pantoea* and *Xanthomonas,* Zhang *et al.* 2022) but of a larger proportion of fungi with 90% of seed-borne taxa detected in the stems of parental plants (wheat, barley, Sharon *et al.* 2023). These pioneer studies suggest that vertical transmission largely varies between the microbial groups considered (bacteria, fungi) and plant species. Still, **no study is available on the characterization of plant microbial inheritance across a large plant diversity** with contrasted reproduction types (e.g. allogamous & autogamous pollination) and seed traits (e.g. albuminous, exalbuminous & perisperm seeds).

Seed-borne taxa represent a potential **primary inoculum** for plants that could modulate plant microbiota assembly. However, few studies attempted to characterize this primary inoculum by analyzing the transmission of seed-borne taxa to seedlings and then during plant growth. The available research observed that only a fraction of seed-borne taxa are able to colonize seedlings after germination and emergence (Rochefort *et al.* 2021, Walsh et al. 2021). These first results suggest that the different phenological transitions from parental plant to seeds and from seeds to seedlings represent important bottlenecks in transgenerational transmission. A better description of these **transmission bottlenecks between different plant species will offer key knowledge to determine the effective influence of seed-borne taxa on plant microbiota assembly**.

Research in the animal and human microbiota have demonstrated that inherited taxa from the mother are crucial for survival and host health even after several years of life (Funkhouser and Bordenstein 2013; Bokulich *et al.* 2016). A similar analysis of the **role of inherited seed-borne taxa for plant fitness** (e.g germination, emergence, plant growth) remain to be conducted at a large scale. Recent studies show that seed microbiota can promote seed germination, seedling formation and protect plants from phytopathogens (Rochefort *et al.* 2019; Pal *et al.* 2022). This primary inoculum can influence the establishment of fungal symbiosis in wheat through priority effects (Ridout *et al.* 2019). These studies offer promising insights in **the role of seed-borne microorganisms for plant health.** Still, further research is needed to determine if these patterns are prevalent across the plant phylogeny and to assess the role of vertically and horizontally transmitted taxa in the effects observed.

To better describe the role of seed-borne taxa in plant fitness, **experimental validation through direct manipulation of the seed microbiota is needed to go beyond correlative analyses**. This can be achieved using synthetic ecology approaches that enable to study the dynamics of a community of a known composition and its impact on the host phenotype in a controlled manner (Vorholt *et al.* 2017). This approach aims to establish causality between the plant and its microbiota via reconstructions of synthetic microbial communities (SynComs).

Preliminary work of the team used the **reconstruction of SynComs representative of seed microbiota of different crops** (radish, bean) to study their transmission to the seedling and impact on plant phenotypes (**Figure 2**, Simonin *et al.* 2023). The seed constitutes an ideal model for the establishment of SynComs, due to the low microbial diversity present on each seed (median on a single bean seed = 8 ASVs, Chesneau *et al*. 2022), but also for the ease and speed of measuring phenotypes linked to germination-emergence (a few days, high-throughput phenotyping using imagery, thousands of seeds tracked, Wagner *et al.* 2011).

Our first results indicate that it is possible to reconstruct SynComs representing a gradient of bacterial diversity on radish seeds (**Figure 2A**) and to follow the relative abundance of each strain from seed to seedling using amplicon sequencing of the *gyrB* gene (bacteria, **Figure 2B**). Additionally, the inoculation of single strains or SynComs has been used to assess the effects of seed-borne taxa on the emergence of bean seedlings in soil (**Figure 2C, 2D**, Arnault et al. unpublished). These results show that seed taxa in isolation or as communities can modulate positively or negatively bean emergence but also other phenotypes not presented here (e.g. growth rate, seedling anatomy). This approach represents a unique tool to better **understand seed microbiota transmission and identify specific microbiota-associated phenotypes** (Oyserman et al. 2018).

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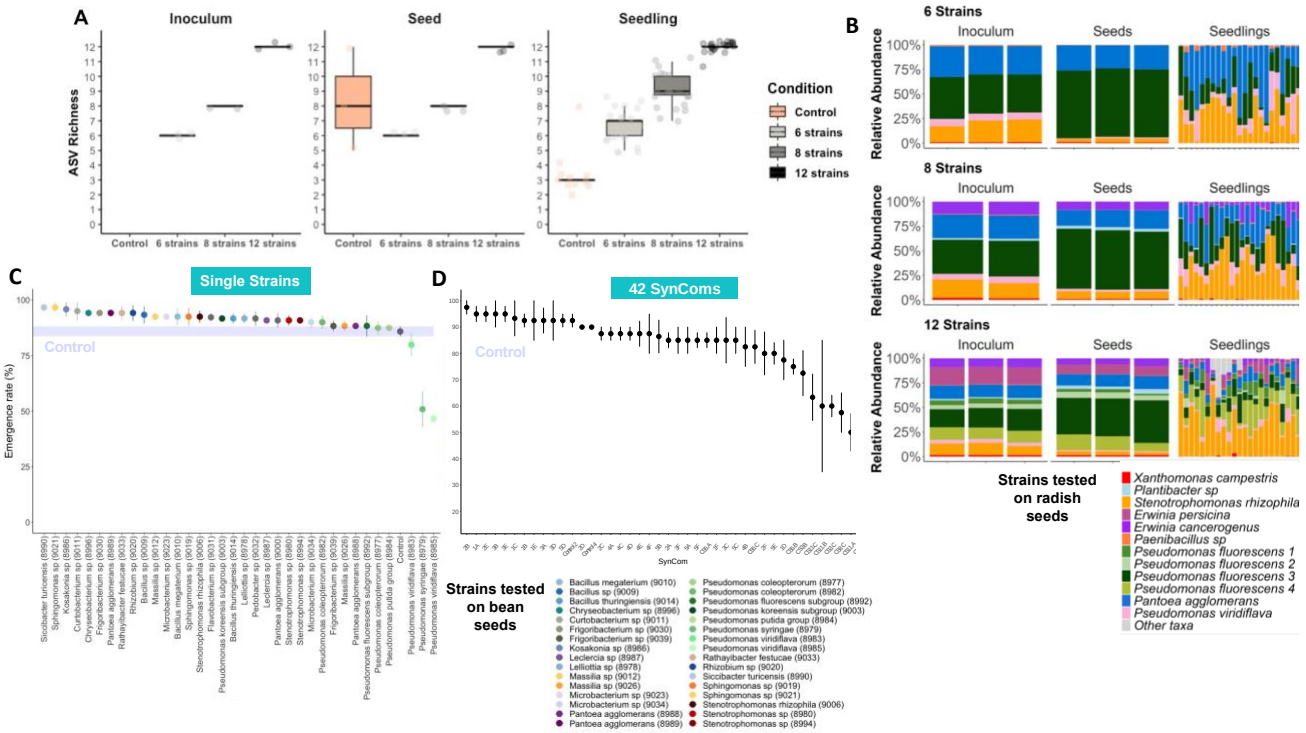

Figure 2: First experiment of inoculation of radish seeds with bacterial synthetic communities (SynComs) under *in vitro* conditions: A) Bacterial taxa richness (ASV number) of the inocula, radish seeds and seedlings inoculated with three SynComs of increasing diversity (6, 8 and 12 strains). B) Taxonomic profiles of the inocula, radish seeds and seedlings in the different replicates. Second experiment of inoculation of single seed-borne bacterial strains or of 42 different SynComs (3 to 11 strains) on bean seeds grown in soil in a climatic chamber: C) Effect of the inoculation of 33 seed-borne bacterial strains on the emergence rate of bean seedlings. D) Effect of the inoculation of 42 different bacterial SynComs on the emergence of bean seedlings.

Originality – Novelty of the present proposal

The originality and novelty of the present proposal relies on:

1. **The assessment of seed microbiota from a large diversity of Angiosperms produced in the same environmental conditions** (**WP1**, common garden experiment performed in a greenhouse). Previous studies focused on 1 to 10 plant species from seeds often produced at multiples sites leading to confounding effects of the influence of production conditions and plant genotypes (Barret et al. 2015; Wassermann et al. 2019).

2. **The first evaluation of phylosymbiosis on seed and seedling microbiota** (**WP1 & WP2**). Few studies assessed phylosymbiosis in plants and none on seeds. The available research assessed phylosymbiosis on root or rhizosphere compartments, (Yeoh et al. 2017; Fitzpatrick et al. 2018; Van Bel et al. 2021) and on the plant endosphere of adult trees (Vincent et al. 2016; Abdelfattah et al. 2022).

3. **The characterization of vertical and horizontal microbial transmission from parental plants to seeds (WP1) and then from seeds to seedlings (WP2) across 50 plant species by considering both bacteria and fungi**. Previous work characterized the vertical transmission of single microbial species or of the microbiome of single plant species (Truyens et al. 2015; Kim et al. 2022; Sharon et al. 2023).

4. **The synthetic ecology approach used to establish causality between seed-borne taxa (inherited or not) and plant phenotypes using SynComs inoculations on parental plants or seeds** (**WP3**). Few studies investigated the phenotypic effects of seed microbiota on plant fitness using SynComs (Figueiredo dos Santos et al. 2021; Simonin et al. 2023).

c. Methodology and risk management

To address the objectives and questions presented, the project will be structured in three work-packages (WP): the **WP1** will characterize microbial inheritance from parental plants to seeds, the **WP2** will characterize the transmission of seed microbiota to seedlings, and the **WP3** will investigate the effects of inherited microorganisms on plant fitness and microbiota assembly during the entire plant life cycle.

We have selected 50 plant species that cover a large part of the Angiosperm phylogeny (12 orders, Apiales, Asterales, Brassicales, Caryophyllales, Cucurbitales, Ericales, Fabales, Lamiales, Poales, Ranunculales, Saxifragales, Solanales, **Figure 3**). To address the goals of the project in a time frame of four years, we selected only annual or biannual plants that can produce seeds from an initial seed in one year in a greenhouse. These plant species are in majority used in agriculture for food, ornamental and biofuel production and some species are model plants in biology (e.g. *Arabidopsis thaliana*, *Nicotiana benthamiana*). The species selection covers a diversity of plant life cycles, pollination and seed traits (e.g size, exalbuminous, albuminous, perisperm).

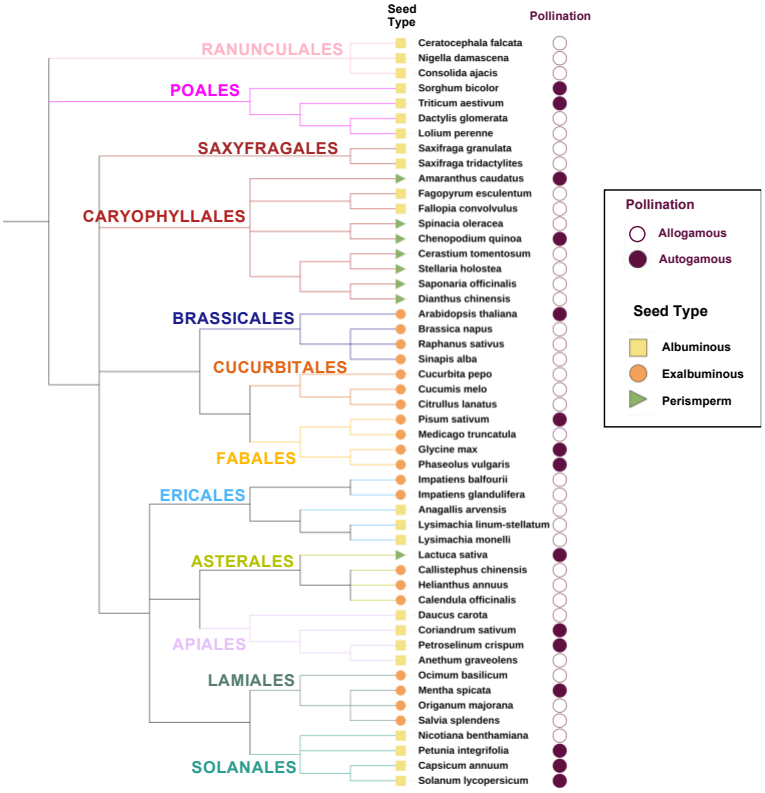

Figure 3: Phylogenetic tree of the 50 plant species selected for the INHERSEED project (made with phyloT based on NCBI taxonomy). Symbols indicate the seed and pollination types of the species.

Variations in these traits are expected to influence microbial inheritance and the stability of the plant holobiont across generations (Vandenkornuyse *et al.* 2015). To address the aims of the project, we will rely on **the skills available** in the EmerSys team (IRHS) on **plant microbiota characterization**, **high-throughput plant phenotyping**, **culturomics** of seed-borne taxa (bacteria & fungi) and an expertise in **plant inoculation at the reproductive stage or on seeds to study microbial transmission**.

WP1: Characterization of microbial inheritance through seeds across 50 plant species

(Months 1 to 18) ***Dedicated ANR-funded staff: 1 Technician & 1 Master 2 student***

Seeds of the 50 plant species will be produced in a large temperate greenhouse (800 m^2) of our in-house plant production facility (PHENOTIC platform) during the first year of the project (**Figure 4**). This WP will be supervised by the scientific coordinator with the assistance of a research technician and a Master 2 student recruited on the project with the help and training of two permanent research technicians and engineers of the PHENOTIC (Rémi Gardet) or ANAN platforms (Coralie Marais). Most of the species selected are biannual or annual plants cultivated in the Loire Valley region, which is an important seed production area in France. The

initial seeds (Generation 0 or G0 hereafter) will be obtained from our internal plant seed collection and from the MATREF collection (GEVES). A minimum of 20 parental plants will be grown per plant species (~ 1000 plants total). The genotypes selected are routinely cultivated in our facility and will be cultivated according to their phenological requirements (e.g. vernalization). For allogamous plants (dioecious or monoecious), pollination will be performed manually or in insect proof cages using bumble bees. This approach will permit to obtain seeds produced in the same soil and the same greenhouse environment, in order to isolate the effect of plant host taxonomy and traits on seed microbiota (**H1** and **Q1)**. For the requirements of the project, we plan to obtain a minimum of 500 seeds for the plant species with a low seed production (e.g. bean) or a minimum of 500 mg for the small-seeded species (e.g. lettuce). During the entire plant life cycles, we will monitor the phenotypes of the parental plants, including plant growth rates, date of phenological stages and seed traits (number, mass, size, water content).

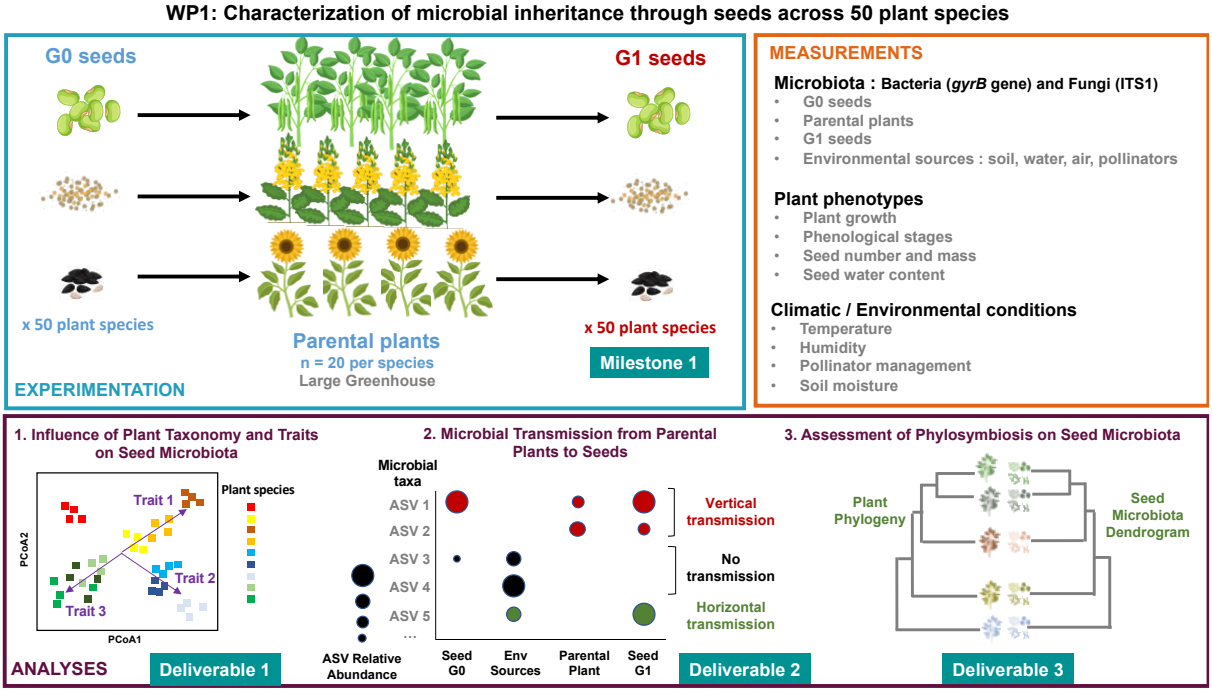

Figure 4: Description of experimentations, measurements and analyses of WP1

Microbiota of the initial seeds (G0, n= 50 x 4 rep = 200), parental plants (n=200), produced seeds (G1, n=200) and all potential sources of microorganisms (water, soil, air, pollinators, n=80) will be characterized using amplicon sequencing (total of 680 samples: 2 runs). We will target the bacterial marker *gyrB* (Barret *et al.* 2015) and fungal ITS1. Sequencing will be done on an in-house Illumina MiSeq (ANAN sequencing platform). We routinely use the *gyrB* marker instead of the 16S rRNA gene markers because it has a better taxonomic resolution on bacteria, going as low as sub-species level that will facilitate microbial transmission detection (Simonin et al. 2023). The amplicon sequencing data will be analyzed in R using *DADA2* (Callahan *et al.* 2016) for denoising to obtain a taxon resolution at the Amplicon Sequencing Variant level (ASV). The *vegan* or *microbiome* packages will be used for the alpha and beta diversity analyses (Oksanen *et al.* 2007; Lahti et al. 2017).

The influence of plant species and traits (size, seed types, pollination) on seed microbiota structure and diversity will be assessed using a combination of multivariate (e.g constrained ordinations, permanova) and univariate analyses (**Figure 4**). We will identify for each plant

species the vertically transmitted taxa that will be detected in G0 seeds, parental microbiota and G1 seeds, but also the origins of the horizontally transmitted taxa (all environmental sources) and the potential reservoirs of vertically transmitted taxa if they are detected in multiple sources. The determination of phylosymbiosis will be done on the total seed microbiota or only the inherited fraction, following Lim and Bordenstein 2020 guidelines. Briefly, a significant association between host phylogeny and seed microbiota similarity will be evaluated through three independent methods: (i) topological congruency tests, (ii) topological comparison with the matching cluster metric and (iii) similarities between host-derived and microbial-derived distance matrices.

WP1 Risk Assessment

One risk associated with this WP is to have an insufficient seed production. This risk will be mitigated through the use of plant genotypes routinely multiplied in our plant production facility and the cultivation of a high number of parental plants per species. Another risk is that no detectable vertical transmission will be observed on the 50 plant species tested. While we think this would be an interesting result in itself, we believe that it is unlikely given that some of the selected plant species had previous reports of bacterial or fungal inheritance (e.g. wheat, bean, watermelon).

The WP1 will yield 4 main milestones and deliverables:

M1. Production of seeds of a large plant diversity in controlled conditions,
D1. Analysis of the influence of plant taxonomy and traits on seed microbiota.
D2. Characterization of inherited microbial taxa across all plants and specific to plant families or seed types.
D3. Assessment of phylosymbiosis on seed microbiota.

WP2 Transmission of seed microbiota to seedling: contribution of inherited taxa to the primary inoculum of plants (M18 to M30) *Dedicated ANR-funded staff: 1 Post-doc*

The objective of **WP2** is to study the transmission of seed microbiota to seedlings (i.e primary inoculum, **Q2** and **H2**) using the G1 seeds (**WP1)** of the 50 plant species (**Figure 5**). This WP will be supervised by the scientific coordinator with the assistance of the postdoctoral associate recruited on the project and permanent research technicians of the PHENOTIC or ANAN platforms. For each plant species, 100 seeds will be sown in soil in a climatic chamber equipped with RGB depth cameras. The seedlings of the different species will be harvested at a similar growth stage (BBCH scale 1) and phenotypic traits will be gathered from imaging (germination rate, seedling growth rate & length) and biomass measurements (aboveground and belowground). The microbiota of seedlings (n=15 per species) and all environmental sources (soil, water, air, n=10 by compartment) will be characterized as in **WP1** (n=780: 2 amplicon sequencing runs).

AAPG2023	INHERSEED		JCJC
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Axe A.3 : Biology of animals, photosynthetic organisms and microorganisms - CE20			

WP2: Transmission of seed microbiota to seedling: contribution of inherited taxa to the primary inoculum of plants

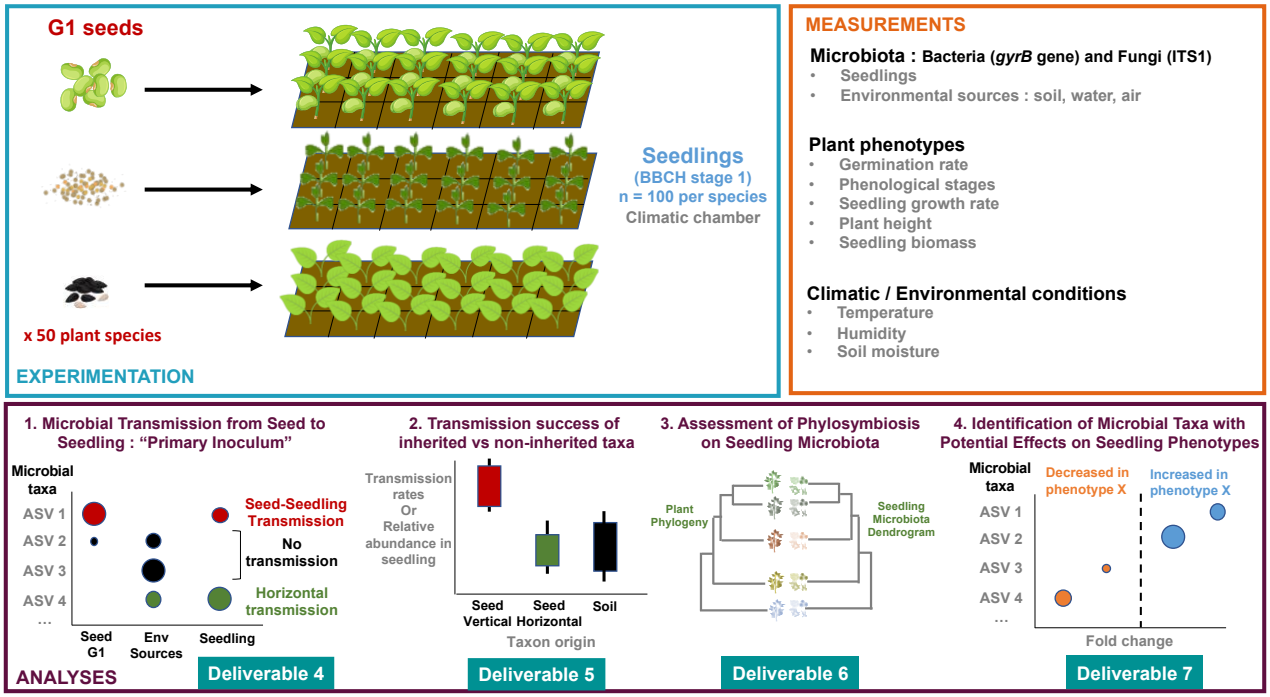

Figure 5: Description of experimentations, measurements and analyses of WP2

We will assess the transmission of seed-borne taxa and especially of inherited taxa to seedlings in function of seed types, plant traits and phylogeny. In particular, we will determine the proportion of seed-borne taxa contributing to the seedling microbiota diversity and relative abundance. For each microbial taxon (ASV), the transmission success will be calculated per plant species and compared across the multiple plant species to identify potential core taxa for seedling microbiota assembly. Phylosymbiosis analyses will be performed on seedling microbiota and will be compared to the signals observed on seeds (**WP1**). We expect the phylosymbiotic signal to be weaker than in seeds due to the important contribution of soil microorganisms to seedling microbiota assembly. Relationships between seed-borne taxa abundances and seedling phenotypes will be explored to identify potential (inherited) taxa modulating seedling phenotypes using TITAN2 (Baker and King 2010) and differential abundance testing (DAtest, Russel *et al.* 2018).

WP2 Risk Assessment

A risk of this WP is that some G1 seeds will not be viable and/or will not germinate in our climatic chambers. This risk will be mitigated by preliminary germination tests to optimize the growth conditions and also thanks to the expertise of the research technician of the PHENOTIC platform that routinely cultivate the majority of plant species of the project.

The WP2 will yield 4 main deliverables:

D4. Contribution of seed microbiota to seedling microbiota assembly for all plants: identification of the "primary inoculum".

D5. Comparison of transmission rates of seed-borne taxa to seedlings between inherited and non-inherited taxa for each plant species,

D6. Assessment of phylosymbiosis on seedling microbiota,

D7. Identification of taxa with potential effects on seedling phenotypes.

AAPG2023	INHERSEED		JCJC
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WP3 Effects of inherited microorganisms on plant fitness and microbiota assembly across the entire plant life cycle (M24 to M48) *Dedicated ANR-funded staff: 1 Post-doc*

To establish causal relations between inherited taxa and plant phenotypes or microbiota assembly (**Q3**, **Q4** & **H3**), we will use microbial inoculation experiments at different plant life stages (reproductive stage or seeds, **Figure 6**). This WP will be supervised by the scientific coordinator with the assistance of the postdoctoral associate recruited on the project and permanent research technicians of the PHENOTIC or ANAN platforms.

WP3: Effects of inherited microorganisms on plant fitness and microbiota assembly across the entire plant life cycle

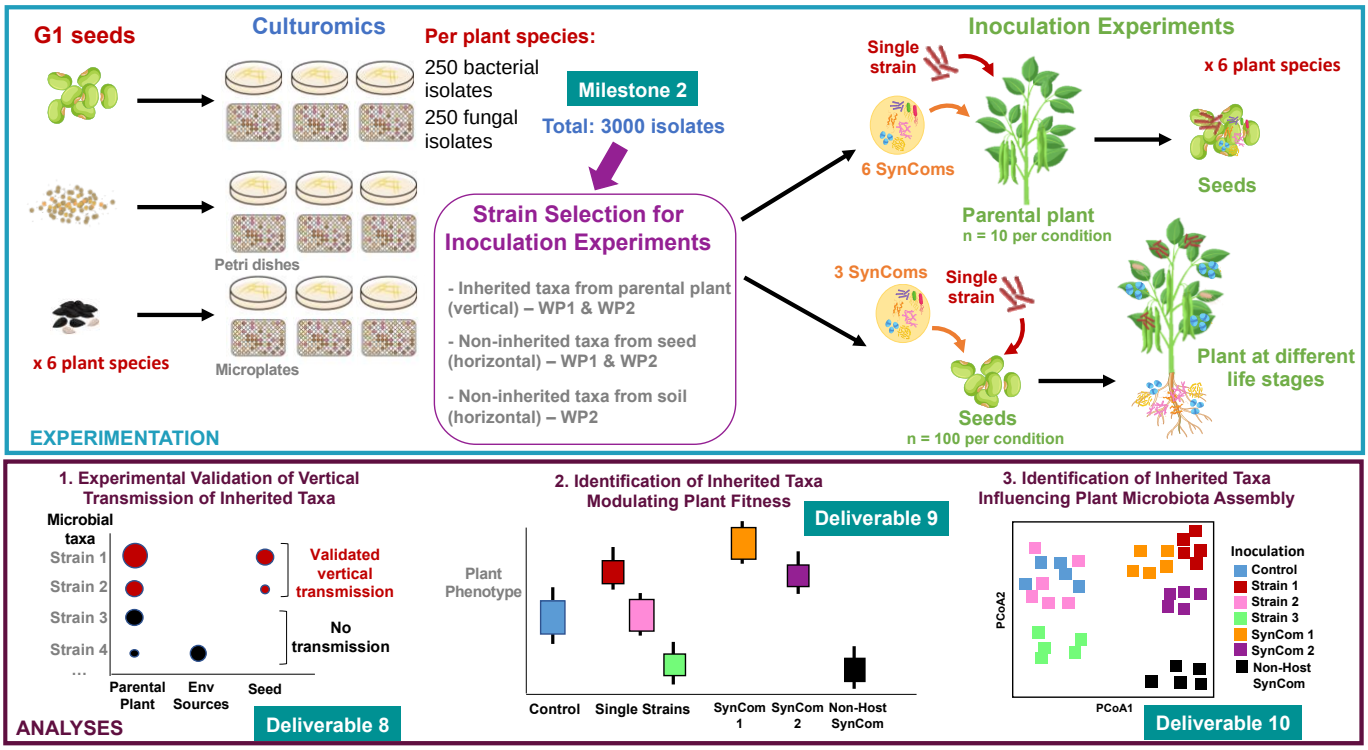

Figure 6: Description of experimentations, measurements and analyses of WP3

To constitute the microbial strain pool for inoculation experiments, high-throughput microbial culture (culturomics) will be used to isolate bacteria, yeast and filamentous fungi from G1 seeds. Culturing and then testing a large collection of microorganisms from all 50 plants would provide a powerful dataset to compare the transmission and effects on respective plant phenotypes. However, in the timeframe of the project this will represent an unachievable and cumbersome task. Therefore, the microbial collection will be obtained from 6 plant species. The selection of the 6 plant species will be based on the deliverables of the **WP1** and **WP2,** to integrate plant species with vertically-transmitted microorganisms to seeds and seedlings, different seed types (e.g exalbuminous, albuminous) and a reliable growth in our greenhouse conditions.

We aim to isolate a total of 3000 strains (500 by plant species) with different media routinely used for seed samples in our lab (e.g. TSA10%, R2A, malt agar, PDA). These culturomics techniques are efficient to isolate a large diversity of seed-borne taxa and generally represent ~75% of the relative abundance of the initial microbiota (Chesneau et al. 2022). All bacterial strains will be conserved in the CIRM-CFBP, an international collection of plant-associated bacteria hosted in the team and the fungal strains will be preserved in the local COMIC strain

collection. The genotyping of the 3,000 isolates will be performed by amplicon sequencing of the *gyrB* bacterial and ITS fungal markers. The sequences obtained will be compared to the amplicon sequencing results of the G1 seeds and seedlings (**WP1 & WP2**) to identify the inherited and non-inherited taxa and to **WP2** results for taxa with potential phenotypic effects.

A selection of strains (bacteria or fungi) will then be inoculated alone or as synthetic communities (SynComs), composed of only inherited or non-inherited strains (5 SynComs per plant species) in a greenhouse. An additional SynCom composed of inherited strains isolated from another host plant species (i.e microbiota transplant experiment) will be tested to experimentally validate the potential phylosymbiotic signals detected in **WP1** & **WP2**. We will expect a reduced microbial inheritance and host performance/fitness upon host-microbiota mismatches. These observations could further validate the functional role of naturally inherited taxa for plant fitness and the likely co-evolution of hosts and inherited taxa.

We will start with vascular inoculations on parental plants (n = 10 per condition) at the reproduction stage (Darsonval *et al.* 2009) to validate experimentally the strains' and SynComs' ability to be vertically transmitted to seeds (720 samples, 2 sequencing runs). This experiment will also permit to assess the effect of inherited taxa on seed production (number, mass) and germination rates but also on seed microbiota assembly compared to non-inoculated plants or non-inherited strains. In a second set of experiments, the vertically-transmitted strains or 3 SynComs will be inoculated on surface-sterilized seeds (n = 100 per condition) following Simonin et al. (2023) to assess their effects on plant growth until senescence. We will monitor the fate and effect of the inoculated strains on the assembly of plant microbiota at 2 phenological stages (vegetative, reproductive) on multiple plant compartments (aboveground, belowground, seeds, 384 samples, 1 sequencing run). The methodology used to characterize the microbiota and plant phenotypes will be the same as the ones described in **WP1** and **WP2** and all plant assays will be performed in non-sterilized soil.

We will track the fate of each inoculated strain with their unique *gyrB* or ITS ASVs at the different phenological stages to validate their vertical transmission and determine the tissues that they preferentially colonize (aboveground or belowground). The effects of single strains and SynComs on plant performance (germination & growth rates, biomass) and fitness (number of fruits & seeds, seed mass) will be statistically assessed using generalized linear models in R. Alpha and beta diversity analyses will be conducted to determine the effects of inherited taxa on plant microbiota assembly. In particular, we will determine if inherited taxa act as "keystone" members that favor the recruitment of specific microorganisms (e.g. other mutualistic taxa) and increase plant microbiota diversity.

WP3 Risk Assessment

A risk is that we will not be able to isolate some inherited taxa from seeds using culturomics. Though, our strong experience in seed microbiota culturomics demonstrated that abundant taxa associated with seeds are efficiently isolated with our protocols (Chesneau *et al.* 2022) for both bacterial (73% of microbiota relative abundance) and fungal taxa (77%). In case, a strain could not be isolated, we will rely on existing microbial collections like the CIRM-CFBP hosted in our team (> 9000 plant-associated bacterial strains).

A second risk associated with this WP is that vertical transmission will not be reproducible through vascular inoculation on the parental plant. While these observations represent interesting results in itself, we will focus in priority on plant species presenting stable vertical

transmission across replicates in **WP1**. Moreover, if vascular inoculations are not conclusive, we will use a floral inoculation protocol developed in the team.

The WP3 will yield 4 main milestones and deliverables:
M2. Microbial collection of seed-borne taxa from 6 plant species.
D8. Experimental validation of vertical transmission of inherited taxa identified in WP1
D9. Identification of inherited taxa modulating plant fitness.
D10. Identification of inherited taxa influencing plant microbiota assembly.

Table 1: Gantt diagram of the INHERSEED project (M: Milestones, D: Deliverables, S: Semesters, P: Publications)

	2024	2025		2026		2027		2028
TASKS	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**
WP1		**M1**	**D1 D2 D3**			**P1**		
WP2				**D4 D5**	**D6 D7**			**P2**
WP3					**M2**	**D8**	**D9 D10**	**P3**
PROGRESS REPORT		**X**		**X**		**X**		
FINAL REPORT								**X**

Data management and open science practices

The project supervisor will be in charge of designing the Data Management Plan of the project and will act as the manager in charge of the quality of data management and documentation. The Data Management Plan will be implemented during the first 6 months of the project following the ANR DMP template. We will set up an internal tracking number for each sample developed with the PHENOTIC platform.

To ensure the standardization and re-usability of the microbiome data generated, we will follow the amplicon sequencing protocol of the Earth Microbiome project. All sequencing data and associated metadata generated will be deposited in the European Nucleotide Archive (ENA). The seed microbiota data generated in **WP1** will be integrated to the Seed Microbiota database that is maintained by the team (Simonin et al. 2023) for future meta-analysis and queries of the scientific community. We will use open-source software to conduct bioinformatic and statistical analyses (R) and all the scripts will be accessible on GitHub to ensure reproducibility. Plant trait data will be deposited in the TRY Plant Trait Database. All bacterial and fungal strains isolated in the project will be deposited in public microbial collections (CIRM-CFBP and COMIC).

II. Organisation and implementation of the project

a. Scientific coordinator and its consortium / its team

Marie Simonin is a microbial ecologist interested in deciphering how plants and microbiota interact, especially to promote stable interactions under abiotic or biotic disturbances. She obtained her PhD in 2015 and then held postdoctoral positions in the USA (Duke University) and France (IRD). She has been recruitment as an INRAE researcher in 2019 in the team "Emergence and systematics of plant-associated bacteria" of the Research Institute in Horticulture and Seeds (IRHS). She developed her research project on the role of seed microbiota for plant fitness on various crops (e.g. bean, radish, rapeseed, tomato, wheat). She recently performed a global meta-analysis on seed microbiota (Simonin et al. 2022) and developed novel approaches using synthetic microbiota inoculation combined with high-throughput plant phenotyping to better establish causality between microbiota and plant phenotypes (Simonin et al. 2023). Over the last 3 years, she was involved in different projects dealing with plant microbiota and holobiont theory. She led an INRAE/Region Pays de la Loire project in 2020-2022, co-led 3 other projects (2020-2022: Angers University; 2020-2022: INRAE Metaprogramme Holoflux, 2022-2024: CNRS EC2CO) and is a work-package leader on seed microbiome engineering of the SUCSEED project (ANR PPR-CPA). In addition to these projects, she supervised 13 students (5 DUT/BTS, 3 M1, 5 M2), and three PhD students (B. Perrotta, 2016-2019; A-S Masson, 2018-2021; G. Arnault 2021-2024). She has authored 38 peer-reviewed articles (h = 22; 1606 citations) and also several science communication articles to reach a broader audience in the society (The Conversation, Planet-Vie, NatureVolve).

The colleagues who will collaborate with M. Simonin through this project include scientists from the IRHS: with their complementary skills, **Matthieu Barret** (Researcher-INRAE, bacteriology & community ecology), **Alain Sarniguet** (Researcher-INRAE, microbial competitions & bacteria-fungi interactions), **Perrine Portier** (Research engineer-INRAE, bacterial taxonomy and curator of CFBP strain collection), **Coralie Marais** (Research technician-INRAE, microbiology, culturomics & amplicon sequencing), **Natalia Guschinskaya** (Assistant Prof, fungal culturomics & plant-fungi interactions), **Rémi Gardet** (Research engineer-IA, plant production & phenotyping) and **Jérôme Verdier** (Researcher-INRAE, seed molecular physiology) will constitute the core team. This team will be completed by two recognized experts from other research institutes in France: **Amélie Cantarel** (Assistant Prof, LEM, Lyon, plant ecology & traits) and **Manuel Blouin** (Professor, Agroecology, Dijon, holobiont theory & experimental evolution).

The coordinator will dedicate at least 50% of her research time (24p.months) to the project and will recruit a Master 2 student (6 months), a technician (18 months) and a postdoctoral associate (3 years) to complete this team. This funding will represent the first large-scale project for the scientific coordinator that will be pivotal to develop her own research theme and independence. This innovative project will strengthen Marie Simonin's collaborative network by gathering a group of experts from different fields (plant ecology, microbial ecology, seed physiology, plant-microbe interactions) locally but also with two other research institutes (LEM and Agroecology). This project will provide a springboard that will elevate Dr. Simonin into one of the major players in the plant holobiont research field in France and internationally. The results obtained during the INHERSEED project will be important for future applications to ERC Consolidator or ANR PRC grants.

AAPG2023	INHERSEED		JCJC
Coordinated by:	Marie SIMONIN	48 Months	357 566€
Axe A.3 : Biology of animals, photosynthetic organisms and microorganisms - CE20			

Implication of the scientific coordinator in on-going project(s)

Name of the researcher	Person.month	Call, funding agency, grant allocated	Project's title	Name of the scientific coordinator	Start - End
Marie SIMONIN	18p.month	Projet Prioritaire de Recherche – Cultiver et protéger autrement, ANR, 3M€	SUCSEED: Stopping the use of Cides on SEEDs	Matthieu Barret (IRHS)	2021-2027
Marie SIMONIN	5p.month	EC2CO Microbiome, CNRS-INSU, 50k€	MiRe-A : Microbiote et Renouées du Japon : de l'initiation au contrôle du cycle de l'Azote.	Amélie Cantarel (LEM)	2022-2024
Marie SIMONIN	4p.month	Projet Précompétitifs, Plant Alliance & Consortium Biocontrôle, 150k€	SeedBioProtect : Protection des semences par des solutions de biocontrôle	Loïc Rajjou (IJPB)	2023-2025

b. Implemented and requested resources to reach the objectives

Partner 1: Research Institute in Horticulture and Seeds (IRHS - UMR 1345)

The required funds are estimated to be **357 566€**, distributed as follow:

Staff expenses: **217 516€**

- 36-month salary for a postdoctoral researcher (**163 394 €**; WP2/WP3 experimentations and analyses).
- 18 months for a technician (**50 726 €** - WP1 experimentations – plant production)
- 6-months Master 2 internship (**3 396 €** - WP1 experimentations – seed microbiota analysis**)**
- *Permanent staff (not funded by ANR): 381 667€ - 57 person.month*

Instruments and material costs: **76 427 €**

- 30 013 € for greenhouse and climatic chambers rental on the PHENOTIC platform

- Rental of a very large greenhouse (800 m^2) for 1 year for the seed production on 50 plant species (WP1) = 25 711€
- Rental of a climatic chamber for 4 months for the seed to seedling experiments of WP2 and WP3 = 1904 €
- Rental of medium size greenhouse (150 m^2) for 6 months for the inoculations on parental plants in WP3 = 2397 €

- **1000 € for plant production material** (pots, trays, soil, tutors, bumble bee hive rental)

- **39 962 € for microbiota analyses** using amplicon sequencing for **2564 samples** distributed as follows:

WP1: 200 G0 seeds (50 species x 4 rep), 200 parental plant samples, 20 soils, 20 air, 20 water, 20 insects, 200 G1 seeds = 680 samples

WP2: 750 seedlings (50 species x 15 rep), 10 soil, 10 water, 10 air = 780 samples

WP3: Exp1 on vertical transmission of single strains inoculated on parental plants (36 conditions x 2 plant stages x 5 rep = 360 samples); Exp 2 on SynCom inoculation on parental plants (6 plant species x 6 SynComs x 2 plant stages x 5 rep = 360 samples); Exp 3 on inoculation on seeds (6 plant species x 4 strain/SynCom x 2 plant stages x 2 compartments x 4 rep = 384 samples).

The itemized microbiota analyses costs are:

- DNA extraction kits for seeds, plants, soil, water, insects = 5789 €

- Library preparation for *gyrB* gene and ITS1 region amplicon sequencing (Taq, primers, purification, Picogreen quantification, qPCR KAPA) = 28 573€

- Plastic consumables (tips, plates, films) = 5 600 €

- **3 452 € for isolate taxonomic identification** using amplicon sequencing for **3000 strains**

- Library preparation for gyrB gene and ITS1 region amplicon sequencing (Taq, primers, purification, Picogreen quantification, qPCR KAPA) = 2 952€

- Consumables (tips, plates, films) = 500 €

- **2000 € for computer/supply equipment** for the Postdoctoral associate.

Building and ground costs – no cost

Outsourcing / subcontracting : **17 093 €**

- **13 093 €** for ANAN sequencing platform (Angers University)

- Illumina MiSeq reagent kit V3 600-cycle and machine costs for 7 sequencing runs for microbiota analyses = 11 701 €

- Illumina MiSeq reagent kit V2 500-cycle and machine costs for 1 sequencing run for isolate taxonomic identification = 1392 €

- 4000 € for publication costs in open-access journal

Overheads costs : **4000€**

- 4 000 € for participation to 2 international conferences for the coordinator & postdoctoral associate

Administrative Management & Structure costs (13.5%): **42 530€**

Requested means by item of expenditure and by partner*

	Partner *IRHS*
Staff expenses	217 516€
Instruments and material costs	76 427 €
Building and ground costs	none
Outsourcing / subcontracting	17 093 €
Overheads costs (including missions expenses, general and administrative costs & other operating expenses	4000 €
Administrative management & structure costs**	42 530 €
Sub-total	**357 566€**
Requested funding	**357 566€**

III. Impact and benefits of the project

Scientific impacts

The INHERSEED project will generate **key results to (in)validate the plant holobiont theory along three main lines of evidence**: 1) We will provide for the first time an **extensive characterization of microbial inheritance in flowering plants**; 2) We will determine if **phylosymbiosis between host phylogeny and seed or seedling microbiota** exists within or across 12 Angiosperm plant orders, suggesting intimate host–microbiota associations over evolutionary time. 3) We will validate the **functional role of inherited taxa on plant fitness and plant microbiota assembly** to determine if microbial inheritance is associated with positive fitness outcomes. This project will greatly advance knowledge on the role of seed taxa as a primary inoculum of plants and its significance for plant survival. More globally these results will represent a foundation to further explore the evolutionary selective pressure leading to the presence or absence of phylosymbiosis in plants.

We anticipate to publish at least 3 research papers:

P1: Assessment of vertical transmission and phylosymbiosis on 50 plant species (**WP1 & 2**)

P2: Effects of seed-borne taxa on plant microbiota assembly across its entire life cycle (**WP2** & **WP3**)

P3: Effects of vertically-transmitted seed microorganisms on plant fitness (**WP3**)

In addition to generating new knowledge and publications, **the project will produce original datasets and biological resources useful for the scientific community**. In particular, the unique dataset of seed microbiota characterization across 50 plant species (**WP1**) will be included in the Seed Microbiota Database curated by the scientific coordinator (Simonin et al. 2022), to facilitate future meta-analysis and access of the scientific community. A large diversity of bacterial and fungal strains isolated from 6 diverse plant species will be extensively characterized to become available to the community in microbial collections.

The beneficial taxa identified in the project could be considered as candidates for biostimulation or biocontrol applications. After uncovering the mechanisms responsible for the beneficial effects, these strains could be considered for future inoculations on seeds in agriculture or for crop breeding strategies considering plant-microbiota interactions and microbial inheritance.

Overall, the project will yield main scientific breakthroughs in plant-microbiota interactions, seed ecology and sustainable agriculture.

Economic and societal impacts

Gaining a better understanding of the **role of native microbiota for seed germination and plant growth is particularly timely as the seed industry is preparing for a major revolution in seed treatments**. The EU recently banned pesticides that are applied for seed protection (fungicides, insecticides). This is part of a larger dynamic in response to a strong societal demand to reduce the use of synthetic fertilizers and pesticides in agriculture. One avenue to replace the use of synthetic chemicals on seeds is to harness the power of seed microbiota by changing practices to favor the presence of beneficial microorganisms or by developing microbially-based treatments on seed (inoculation of microbial strains or

metabolites). Thus, promoting scientific research on seed microbiota can contribute to the development of natural and environmentally-friendly alternatives to pesticides and fertilizers.

As the plant species studied in the project are used for food, ornamental, or biofuel production, the transfer potential of this proposal to the Agro-Tech industry is high. The project results will be presented at conferences promoting academia-industry interactions such as miCROPe and Phytobiomes. Moreover, the team already has ongoing collaborations with the seed and biocontrol associations (UFS, FNAMS, IBMA, AFAIA) that will facilitate the knowledge transfer at the local and national level.

The results of the INHERSEED project will generate new knowledge to harness and engineer crop microbiomes in line with the farm to fork strategy of the European Green Deal and Sustainable Goal Development 2 (Zero Hunger) and 12 (Responsible Consumption and Production).

Project dissemination

The project supervisor and team are committed to a more open science that will be central to the INHERSEED project dissemination strategy.

As detailed in the previous *Data management and open science practices* section, all data (raw sequences reads, plant traits, metadata) will be deposited in public databases (e.g. European Nucleotide Archive, TRY) following FAIR guidelines. All scripts for data analyses will be shared on GitHub.

We will increase the accessibility to the publications generated by the project by using preprint servers (BioRxiv), article repositories of post-prints (HAL-INRAE archive), and publishing in open-access journals owned by scientific associations (e.g., New Phytologist, ASM journals). We have increased the requested resources for publication in order to pay the open-access processing charges. At least one publication will be submitted to a Peer Community In journal (Microbiology or Ecology) that promote a more accessible and transparent publishing process. All publications and datasets will be published with the most open CC-BY license.

In addition, results will be disseminated through communications in international scientific conferences (e.g. ISME, Phytobiomes) and in local networking meetings organized by the competitiveness cluster Vegepolys. Moreover, we will disseminate the knowledge of the project during teaching at Universities (Angers, Nantes) and in Engineering schools (Agrocampus Ouest, VetAgroSup) at the Master level (~15h per year).

Science and Society

At the beginning of the project, a website and dedicated Twitter account will be created to promote the results and announcements of the project. These media will also permit to the project to reach a broader audience in the industry, science journalists and general public. Additionally, the scientific coordinator is listed on the national website “Les Expertes” promoting the representation of female experts in the media for interviews and press articles. She is regularly contacted by national/international media to provide a point of view on topics related to microbiome science and sustainable agriculture. We plan to also write a “*The Conversation*” article on the results of the project to increase science literacy on seed microbiota and its role in plant health.

IV. References related to the project (blue references are from the team)

Abdelfattah A, Tack AJM, Wasserman B et al. Evidence for host–microbiome co-evolution in apple. New Phytol 2022;234:2088–100.

Baker ME, King RS. A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods Ecol Evol 2010;1:25–37.

Barret M, Briand M, Bonneau S et al. Emergence Shapes the Structure of the Seed Microbiota. Appl Environ Microbiol 2015;81:1257–66.

Bokulich NA, Chung J, Battaglia T et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med 2016;8:343ra82-343ra82.

Callahan BJ, McMurdie PJ, Rosen MJ et al. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581–3.

Cantó C de la F, **Simonin M**, King E et al. An extended root phenotype: the rhizosphere, its formation and impacts on plant fitness. Plant J 2020;103:951–64.

Chesneau G, Laroche B, Préveaux A et al. Single Seed Microbiota: Assembly and Transmission from Parent Plant to Seedling. mBio 2022;0:e01648-22.

Darrasse A, **Barret M**, Cesbron S et al. Niches and routes of transmission of *Xanthomonas citri pv. fuscans* to bean seeds. Plant Soil 2018;422:115–28.

Darsonval A, Darrasse A, Durand K et al. Adhesion and fitness in the bean phyllosphere and transmission to seed of *Xanthomonas fuscans subsp. fuscans*. Mol Plant Microbe Interact 2009;22:747–57.

Figueiredo dos Santos L, Fernandes Souta J, de Paula Soares C et al. Insights into the structure and role of seed-borne bacteriome during maize germination. FEMS Microbiol Ecol 2021;97:fiab024.

Fitzpatrick CR, Copeland J, Wang PW et al. Assembly and ecological function of the root microbiome across angiosperm plant species. Proc Natl Acad Sci 2018;115:E1157–65.

Fort T, Pauvert C, Zanne AE et al. Maternal effects shape the seed mycobiome in *Quercus petraea*. New Phytol 2021;230:1594–608.

Funkhouser LJ, Bordenstein SR. Mom Knows Best: The Universality of Maternal Microbial Transmission. PLOS Biol 2013;11:e1001631.

Kim H, Jeon J, Lee KK et al. Longitudinal transmission of bacterial and fungal communities from seed to seed in rice. Commun Biol 2022;5:1–14.

Lahti L, Shetty S, Blake T. Tools for microbiome analysis in R. Microbiome Package Version 099 2017;88:2012–7.

Lim SJ, Bordenstein SR. An introduction to phylosymbiosis. Proc R Soc B Biol Sci 2020;287:20192900.

Mitter B, Pfaffenbichler N, Flavell R et al. A New Approach to Modify Plant Microbiomes and Traits by Introducing Beneficial Bacteria at Flowering into Progeny Seeds. Front Microbiol 2017;8, DOI: 10.3389/fmicb.2017.00011.

Oksanen J, Kindt R, Legendre P et al. The vegan package. Community Ecol Package 2007.

Oyserman BO, Medema MH, Raaijmakers JM. Road MAPs to engineer host microbiomes. Curr Opin Microbiol 2018;43:46–54.

Pal G, Kumar K, Verma A et al. Seed inhabiting bacterial endophytes of maize promote seedling establishment and provide protection against fungal disease. Microbiol Res 2022;255:126926.

Ridout ME, Schroeder KL, Hunter SS et al. Priority effects of wheat seed endophytes on a rhizosphere symbiosis. Symbiosis 2019;78:19–31.

Rochefort A, Briand M, **Marais C** et al. Influence of Environment and Host Plant Genotype on the Structure and Diversity of the *Brassica napus* Seed Microbiota. Phytobiomes J 2019;3:326–36.

Rochefort A, **Simonin M, Marais C** et al. Transmission of Seed and Soil Microbiota to Seedling. mSystems 2021, DOI: 10.1128/mSystems.00446-21.

Rodríguez CE, Antonielli L, Mitter B et al. Heritability and Functional Importance of the *Setaria viridis* Bacterial Seed Microbiome. Phytobiomes J 2019;4:40–52.

Russel J, Thorsen J, Brejnrod AD et al. DAtest: a framework for choosing differential abundance or expression method. 2018:241802.

Shade A, Jacques M-A, **Barret M**. Ecological patterns of seed microbiome diversity, transmission, and assembly. Curr Opin Microbiol 2017;37:15–22.

Sharon O, Sun X, Ezrati S et al. Transmission mode and assembly of seed fungal endophyte communities in wheat and wheat wild relatives. Phytobiomes J 2023, DOI: 10.1094/PBIOMES-11-22-0084-R.

Simonin M, Briand M, Chesneau G et al. Seed microbiota revealed by a large-scale meta-analysis including 50 plant species. New Phytol 2022;234:1448–63.

Simonin M, Préveaux A, **Marais C** et al. Transmission of synthetic seed bacterial communities to radish seedlings: impact on microbiota assembly and plant phenotype. 2023:2023.02.14.527860.

Truyens S, Weyens N, Cuypers A et al. Bacterial seed endophytes: genera, vertical transmission and interaction with plants. Environ Microbiol Rep 2015;7:40–50.

Van Bel M, Fisher AE, Ball L et al. Phylosymbiosis in the Rhizosphere Microbiome Extends to Nitrogen Cycle Functional Potential. Microorganisms 2021;9:2476.

Vandenkoornhuyse P, Quaiser A, Duhamel M et al. The importance of the microbiome of the plant holobiont. New Phytol 2015;206:1196–206.

Vincent JB, Weiblen GD, May G. Host associations and beta diversity of fungal endophyte communities in New Guinea rainforest trees. Mol Ecol 2016;25:25–41

Vorholt JA, Vogel C, Carlström CI et al. Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. Cell Host Microbe 2017;22:142–55.

Wagner M, Demilly D, Ducournau S, Dürr C, Léchappé J. Computer vision for monitoring seed germination from dry state to young seedlings. Seed Test 2011: 142, 49-51.

Wassermann B, Cernava T, Müller H et al. Seeds of native alpine plants host unique microbial communities embedded in cross-kingdom networks. Microbiome 2019;7:1–12.

Yeoh YK, Dennis PG, Paungfoo-Lonhienne C et al. Evolutionary conservation of a core root microbiome across plant phyla along a tropical soil chronosequence. Nat Commun 2017;8:215.

Zhang X, Ma Y-N, Wang X et al. Dynamics of rice microbiomes reveal core vertically transmitted seed endophytes. Microbiome 2022;10:216