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Laboratoire de Biologie et
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RAPPORT de STAGE EFFECTUE

par

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au

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Végétale

en vue de l'obtention du

Master Biologie Végétale

Parcours : Gestion de la Santé des Plantes

(Responsable de stage :
Lucie POULIN et
Jean-Bernard POUVREAU,
Maîtres de conférences)

Sujet :

An Overview of *Phelipanche ramosa* seeds: Sensitivity to germination stimulants and microbiome profile

Soutenance devant le jury d'examen le vendredi 14 Juin 2019

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THANKS

This report is the result of more than only my six-month internship. It would not have been made without the help of my two internship supervisors and the intercession of several people.

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Table des illustrations

Table des tableaux

Glossary

Alpha diversity:	The mean species diversity within one habitat
Amplicon Single Variant (ASV):	A sequence obtained through PCR and sequencing process; only a single nucleotide polymorphism is needed to detect two different ASV
Beta diversity:	The mean species diversity among different habitats
EC50	Concentration of stimulants for which 50% of the maximum seed germination percentage is reached
Gamma-diversity	The total species diversity in a landscape The gamma-diversity is determined by the alpha and the beta diversity
Mock community:	An artificially composed bacterial community with known bacterial strain that then undergoes the same amplification, sequencing and data processes than other samples
Rarefaction:	For each sample, reads are randomly chosen between the ones present in that sample and according to their relative abundance until the number of read per sample reach an arbitrary threshold, chosen according to the rarefaction curve

Abbreviations

ACN: AcetoNitrile

ASV: Amplicon Single Variant

EC50: Eliciting Concentration 50

GS: Germination Stimulants

HIF: Haustorium Inducing Factors

IRHS: Horticulture and Seeds Research Institute

MTT: MethylThiazolyldiphenyl-Tetrazolium bromide

PPM: Plant Preservative Mixture™

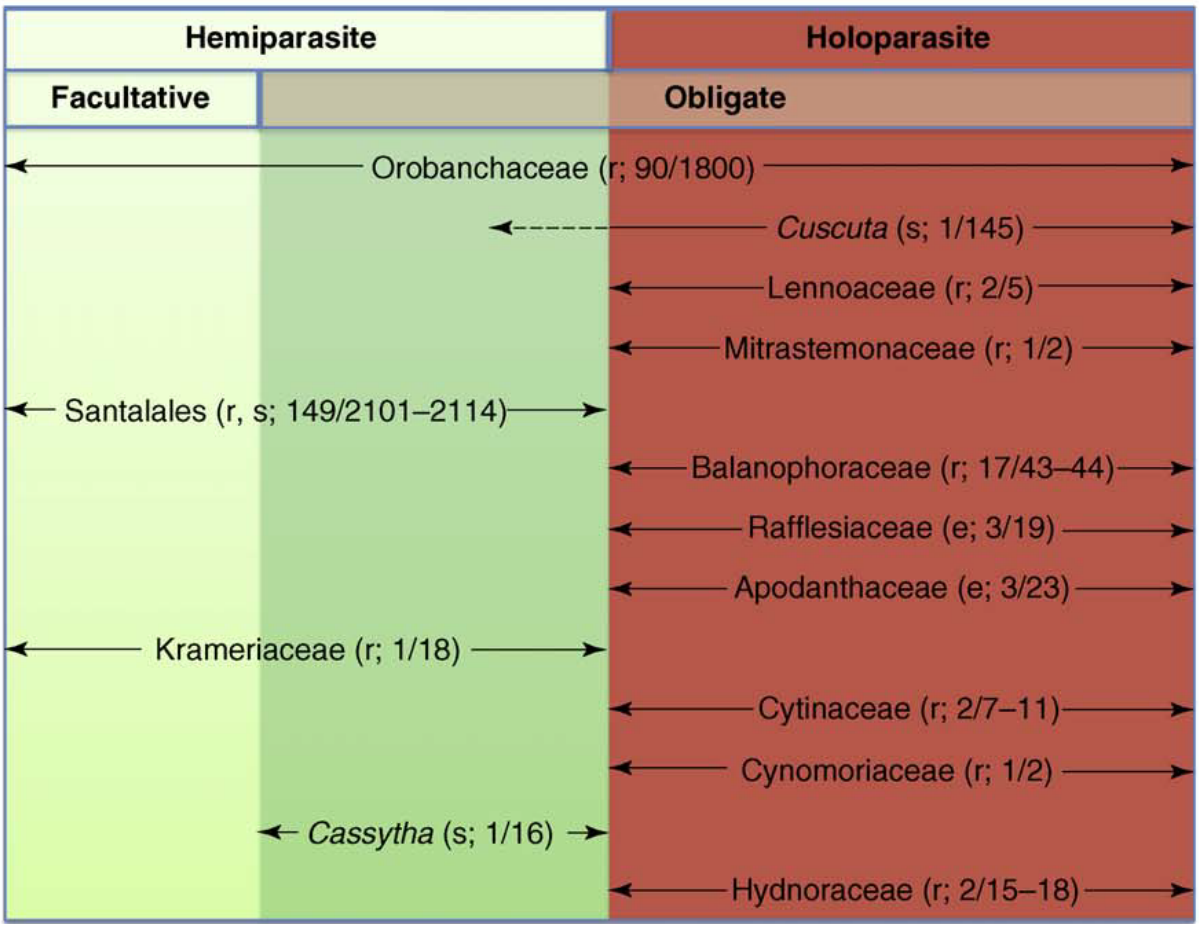


Figure 1: The diversity of parasitic plant. r: epirhize ; s: epiphyte ; e: endophyte ; x/y: number of genera/species. From Westwood *et al.*, 2010

INTRODUCTION

1. Parasitic Plants

1.1. Definition

Autotrophy is the ability of an organism to produce its own organic matter from mineral matter. Most of the autotroph organisms use light energy to assimilate carbon thanks to photosynthesis such as plants. But some plants have lost their photosynthesis capacity during evolution (Heide-Jørgensen, 2013). Instead, these plants use a parasitic nutritional mode. Parasitism is defined as a life trait of an organism which lives at the expense of another organism. Indeed, parasitic plants use a modified root appendix termed haustorium (Candolle, 1813) to penetrate host tissue and connect to the host vascular tissues to obtain water and nutrients.

Among the 4500 different species of parasitic plants, representing 1% of the angiosperm species, only 10% are heterotrophs, also known as holoparasites. In contrast, 90% of parasitic plants can still generate photosynthetic products: these are called hemiparasites (Westwood et al., 2010). This dichotomy is one out of three used to classify parasitic plant (Figure 1). The second dichotomy is based on the degree to which the parasite relies on its host. Indeed, among the hemiparasites, some are facultative parasites, others are obligatory parasites. All the holoparasites are obligatory parasites. The third distinction used to discriminate parasitic plant relates to the parasitic plant fixation site. The epiphytes parasites invade the shoots, while the epirhizes parasites colonize the roots.

Among the twenty described families of parasitic plants, the *Orobanchaceae* family is the only one that includes every type of parasitism. This family is also the largest of parasitic plants with 105 genera and 2070 species (Heide-Jørgensen, 2008). *Orobanchaceae* family also holds a more disastrous record because it contains three of the most devastating parasitic plant genera: *Orobanche*, *Phelipanche* and *Striga* (Parker, 2009, 2012).

1.2. Economic impact

Orobanche, *Phelipanche* and *Striga* have been detected on every continent, except Antarctic, and cause important yield losses worldwide. Africa is the most severely affected continent. Sub-Saharan Africa is mostly affected by *Striga hermonthica* which impairs cereals and legumes crops, mainly maize and cowpea, reducing crop yields by 10% to 90% (Gressel *et al.*, 2004). In Western Africa, 64% of the cereal crops are infested representing 17 million hectares. All in all, *Striga* species have infested from 44 to 50 million hectares, impacted 300 million farmers and costing $ US 7 billion yield losses (Parker, 2009). In Mediterranean and Asian countries, *Orobanche* and *Phelipanche* are more spread, covering 16 million hectares (Parker, 2009). In North Africa, *Orobanche crenata* infests mostly faba bean fields and it is still spreading, while *Phelipanche ramosa* infests mostly *Solanaceae* and *Brassicaceae* crops such as oilseed rape, tomato or potatoes (Gressel *et al.*, 2004).

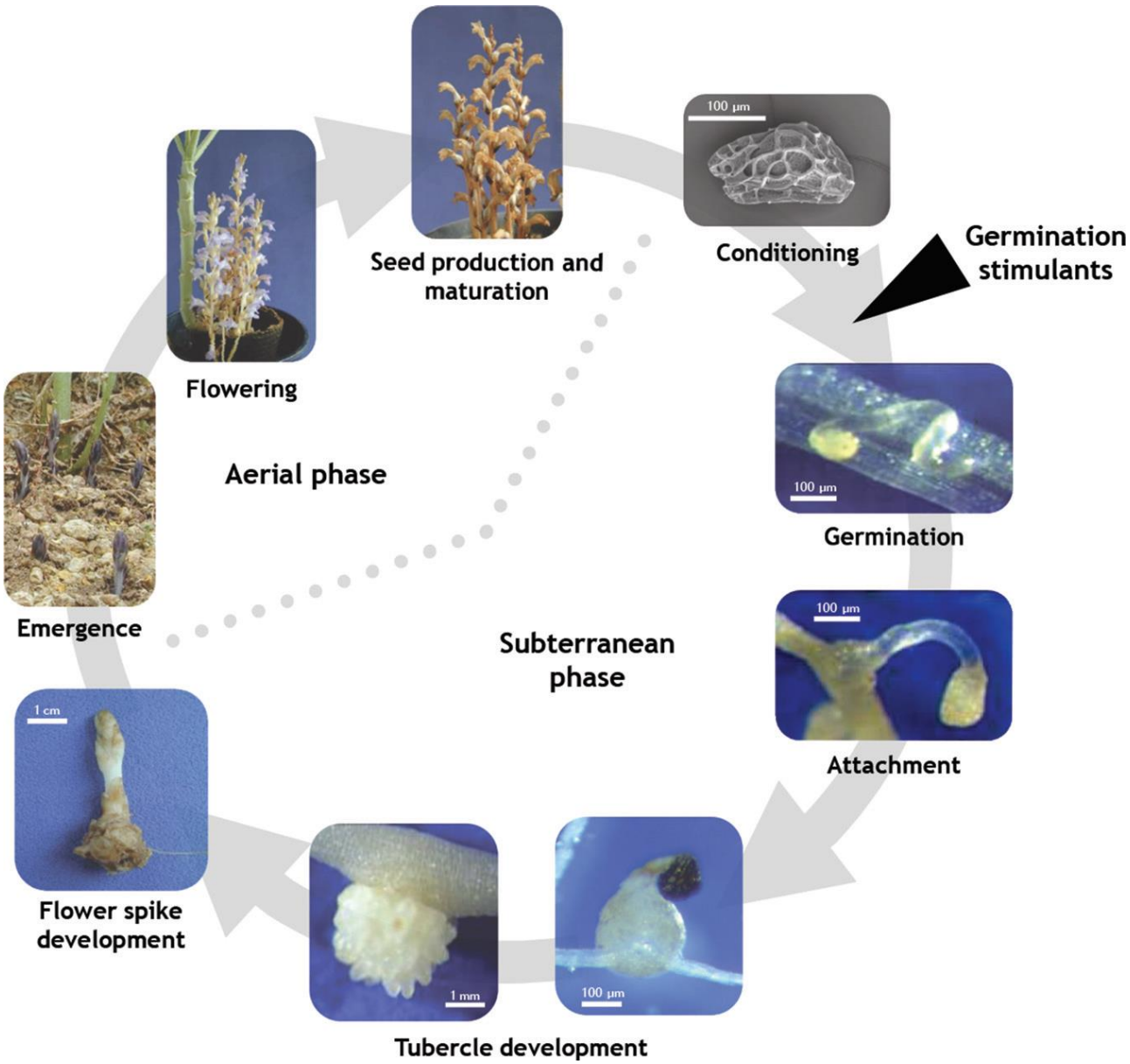


Figure 2: Life cycle of *Phelipanche ramosa*. From Delavault, 2015

Table 1: The three *P. ramosa* genotype and their preferential host

Genotype	*Preferential host*
Genotype 1	Oilseed rape, tobacco
Genotype 2a	Hemp, tobacco, tomato
Genotype 2b	Tobacco, oilseed rape

In France, the most problematic parasitic weed is *Phelipanche ramosa*. It infests oilseed rape, hemp, tobacco, melon and tomato crops, inter alia. This parasitic plant is currently expanding and now threaten the oilseed rape cultivation in the Western France.

1.3. Phelipanche ramosa

P. ramosa, also known as *Orobanche ramosa*, is an epirhize holoparasite. In other words, this parasitic plant attaches to its host roots and it doesn't have any photosynthetic capacity. Therefore, it relies only on its host to grow and complete its life cycle. Its seeds are abundant (25 000 to 100 000 seeds per floral scape) and very tiny (200 µm on average) and therefore spread very easily. They have a long lifespan and can remain viable up to 20 years in the soil. Moreover, these seeds do not germinate freely upon optimal conditions as other seeds do. Conversely, to germinate, these seeds have two requirements: an initial conditioning period (four to five days in laboratory) and a subsequent perception of host secreted germination stimulants (GS) (Figure 2). These GS are present in the host root exudates (Bouwmeester *et al.*, 2007). They allow the seed to germinate and the emergent radicle shifts itself towards the host root. Once the radicle is near enough the host roots, it can perceive Haustorium Inducing Factors (HIF) to initiate the formation of its haustorium. The haustorium is a real sucking organ that allows the parasitic plant to attach to its host roots and to penetrate them. Once the haustorium is fixed to the host root, it connects directly to the host vascular tissues. Therefore, the parasitic phase begins and *P. ramosa* absorb water, minerals and other organic compounds from its host. Afterwards, *P. ramosa* develops a storage tuber outside the host root and starts to grow adventitious roots. Some weeks later, shoots start growing from a tuber axillary bud and finally emerge from the soil. From two to four weeks after the emergence, blooming happens. The bisexual flowers are gathered in racemes and the leaves are flaky downsized.

All along the parasitic phase, representing the most part of its life cycle, *P. ramosa* retrieve water, nutrient and organic compounds from the host plant. Thus, its impact on the host plant growth is major. Moreover, the diagnosis can only be precisely made after the emergence of the parasitic plant aboveground giving the parasite from 6 to 10 weeks to suck out its host nutrients before being detected. And even after the emergence, the infestation cannot be easily contained. Another problematic characteristic of *P. ramosa* is its seeds. Indeed, because of their miniature size making them easily spread, their huge number per floral scape and their extreme viability, it is hard to perceive them and get rid of them. Moreover, there are no efficient control methods against broomrape whether relying on host genetic resistance or chemicals. Theoretically, once a field is infested, it can indefinitely renew the soil seed bank.

Within the *P. ramosa* species, we can distinguish three different genotypes (Stojanova *et al.*, 2019). These three genotypes have been discriminated using 20 microsatellites markers. This genetic differentiation among the species is also linked to different preferential host (Table I). Indeed, the genotype 1 preferentially infests winter oilseed rape and not hemp while genotype 2a infests more hemp, tobacco and tomato. The third genotype (genotype 2b) is more generalist although it is well represented in tobacco fields.

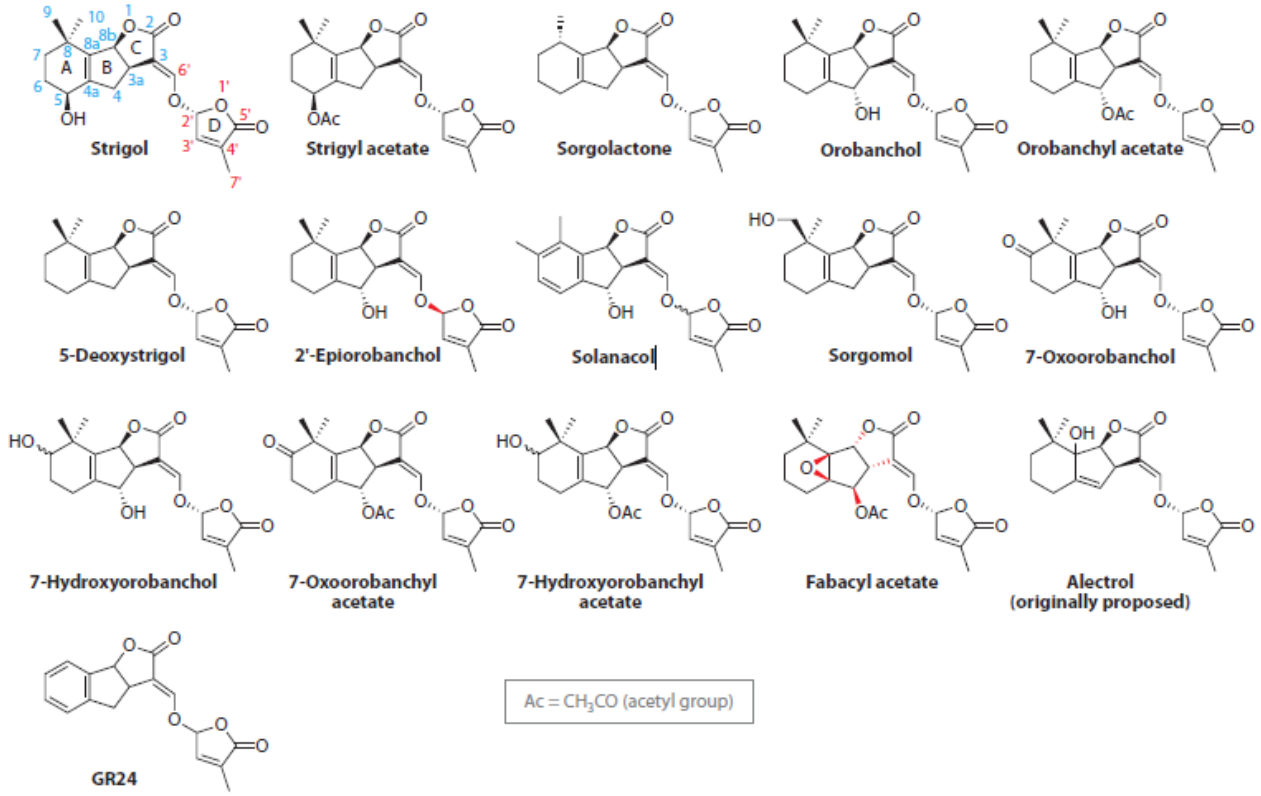


Figure 3: Structure of natural strigolactones and the synthetic analog GR24. (Xie *et al.*, 2010)

2. Rhizospheric interactions

2.1. The parasitic plant – host plant interaction

2.1.1. Germination stimulants (GS)

In contrast with other flowering plants, most of the parasitic seeds need germination stimulants (GS) in order to germinate. The first GS that has been identified is the strigol from cotton exudate which is able to induce the germination of *Striga hermonthica* seeds (Cook *et al.*, 1966). Strigol belongs to the sesquiterpene lactone family and more precisely to the strigolactones (Figure 3). Strigolactones were first described as chemical compounds synthetized by host plant and promoting the germination of parasitic plants (Butler, 1995). Most of the germination stimulants identified so far are strigolactones and derivatives (Yoneyama *et al.*, 2013). Strigolactones also stimulates mycorrhizal fungus ramification by promoting the mitochondrial activity (Besserer *et al.*, 2006). However, we don't know yet how fungi perceive the strigolactones. Strigolactones also play a role in plant architecture and growth by influencing developmental steps such as shoot and root branching and tillering and are therefore considered as phytohormones (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008; Brewer *et al.*, 2013; Ruyter-Spira *et al.*, 2013; Rasmussen *et al.*, 2013). It is now unanimously accepted that strigolactones are phytohormones (Al-Babili & Bouwmeester, 2015).

To study the strigolactone impact on parasitic plants, synthetic analogues have been designed such as GR24 (Johnson *et al.*, 1976; Wigchert *et al.*, 1999). GR24 has the same structure than the strigolactone and it has been validated that the synthetic and the natural molecules have the same activity. However, GR24 have several enantiomers that doesn't have the same activity upon parasitic plants.(Thuring *et al.*, 1997). These different activities could be explained by the stereospecificity of strigolactone receptors (Flematti *et al.*, 2016).

Another germination stimulant of *P. ramosa* is the isothiocyanate (ITC) (Auger *et al.*, 2012). ITC is a product of the glucosinolate degradation. Glucosinolates are plant chemicals that have been widely studied. They are produced mainly by *Brassicaceae* such as oilseed rape, cabbages and *Arabidopsis thaliana* (Fahey *et al.*, 2001; van Dam *et al.*, 2009). These compounds influence the quality of the harvestable part of *Brassicaceae* crops but also play a role in defense against pathogens. Oilseed rape not only accumulates glucosinolate in its shoot but also exudate glucosinolate in its rhizosphere. The degradation of glucosinolate into ITC in the rhizosphere is suspected to be performed by micro-organisms (Albaser *et al.*, 2016; Tian *et al.*, 2018). Moreover, glucosinolates exudate by *Brassicaceae* significantly impact the root and rhizosphere microbial communities (Bressan *et al.*, 2009).

2.1.2. Haustorium induction factor (HIF) and parasitic transition

After germination, the radicle grows towards the host plant roots using chemotropism. To establish a connection with its host, the parasitic plant needs to develop an haustorium i.e. the host attachment organ (Kuijt, 1969). To that purpose, the parasitic plant perceives host derived compounds termed HIF for Haustorium Initation Factors. HIF triggers the swelling of the root tip and the rise of papillae anchoring the early haustorial structures (EHSs) to the host root (Joel & Losner-Goshen, 1994; Goyet *et al.*, 2017). Most of the HIF are phenolic compounds (Albrecht *et al.*, 1999) such as benzoquinone which induces haustorial development in *Striga asiatica* (Keyes *et al.*, 2000). Phytohormones

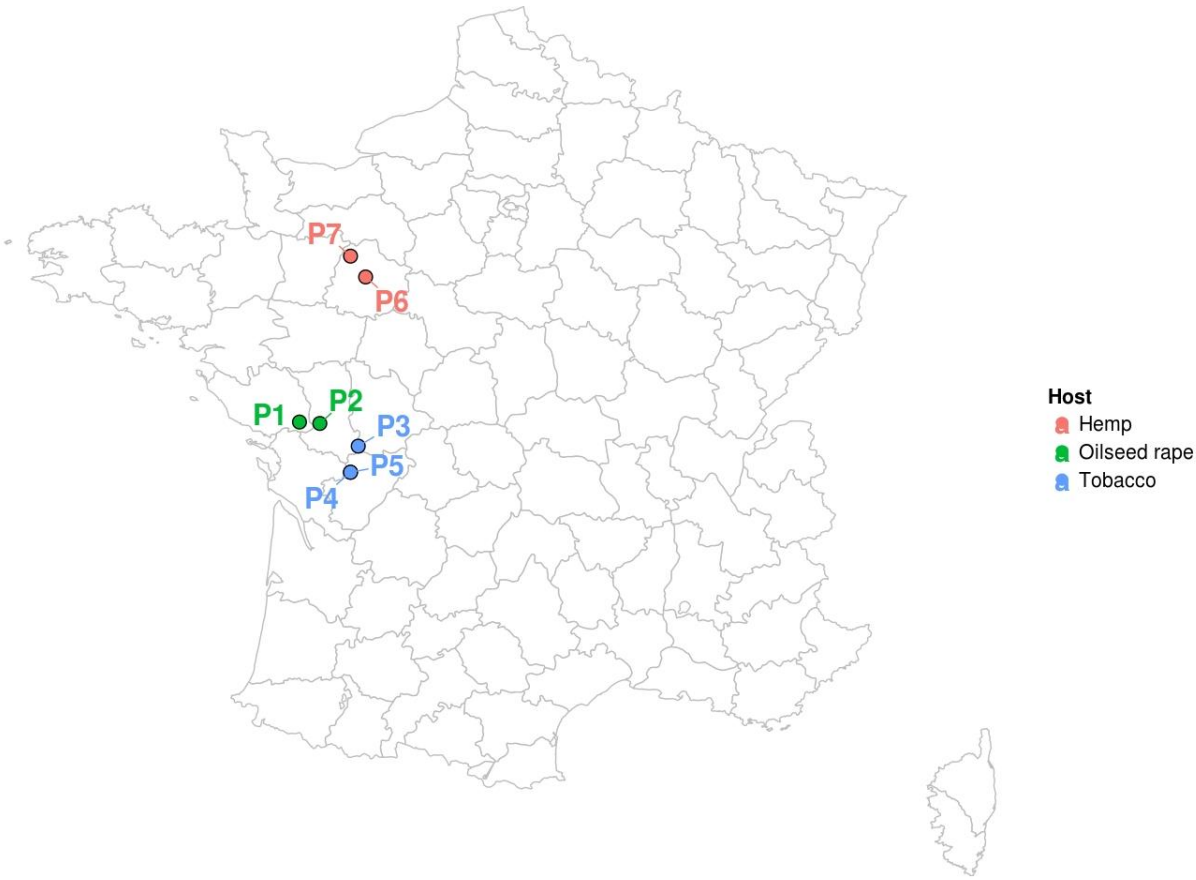


Figure 4 : Map of the seven plots of the MOrdOr project sampling campaign. P4 and P5 are confounded because nearby.

and mycotoxins can also induce EHS (Tomilov *et al.*, 2005; Fernández-Aparicio *et al.*, 2016). Within the phytohormones, cytokinin are now well described as HIF for *P. ramosa* (Goyet *et al.*, 2017).

2.2. A third partner?

Given that the interaction between an epirhize parasite and its host occurs in the soil, other partners, present in the soil, can interact with the two main partners. Besides, parasitic plants exploit chemical signals (e.g. strigolactones and ITC) exudate by the host plant for other partners such as microorganisms. In that respect, soil and seed microbiota seems to play an important role as third partners in the host plant – parasitic plant interaction.

First, it is admitted that seed and soil microbiota largely influence the growth and phenotype of a plant. Regarding non-parasitic plant, the soil microbial activity drives biomass accumulation of *Arabidopsis thaliana* (Sugiyama *et al.*, 2012). The appropriate soil microbial community can also lead to enhance the plant tolerance to drought (Lau & Lennon, 2012). Different soil microbiota can also lead to a better disease resistance (Mendes *et al.*, 2011; Santhanam *et al.*, 2015).

Secondly, micro-organisms can also be sensitive to strigolactones (Besserer *et al.*, 2006) and can interact with glucosinolates by degrading them into ITC (Albaser *et al.*, 2016; Tian *et al.*, 2018). Some bacteria also produce and secrete cytokinins, influencing the surrounding plants (Kudoyarova *et al.*, 2014; Großkinsky *et al.*, 2016). Moreover a fungal species, *Fusarium oxysporum*, is already known to allow the biocontrol of *P. ramosa* in controlled conditions (Kohlschmid *et al.*, 2009).

Increasingly, new scientific evidences support the role of the microbiota within the rhizosphere interactions. Moreover, scientific community suspects tripartite dialogue involving interaction between the host plant, the parasitic plant and the surrounding microbial community.

3. Project aims and internship goals

The Plant Biology and Pathology Laboratory (LBPV, Nantes, France), where I made this master thesis, focuses its research efforts on the parasitic plants and more particularly on broomrape species. My master thesis falls within the MOrdOr project (Seed-carried Microbiota from Orobanche during the early parasitic (Orobanche) cycle). The major aim of this project is to characterize the seed associated microbiota of *P. ramosa*. This project walks into the emphasis put by the unit a couple of years ago, on the exploration of the rhizospheric interactions between the parasitic plant and its host. In that respect, the team centered its research on host root exudates, the germination stimulants diversity, their parasite receptors and the implicated genes. The MOrdOr project addresses the lack of understanding of the role and activity of belowground microbiota in this rhizospheric interaction.

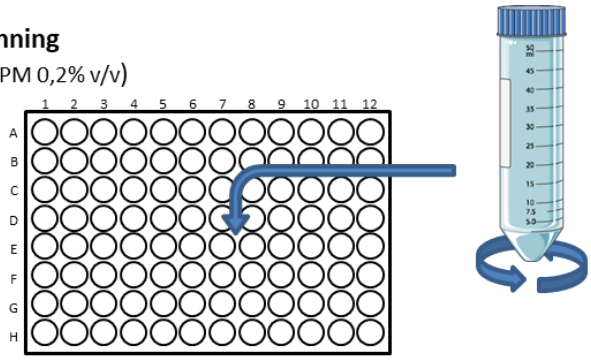
My internship falls within this project and attempts to answer three main questions. What are composed of the microbial communities associated with *P. ramosa* seeds, in France? Which factors influence their composition? Is *P. ramosa* seed microbiota related to phenotype and/or genotype?

1

Seed sterilization, ditribution and conditionning

(10 g L^{-1} w/v, phosphate buffer 1 mmol L^{-1}, pH 7.5, PPM 0,2% v/v)

Distribution under stirring of seeds suspension
- 50 µL/well; end of 100 µL tips cut

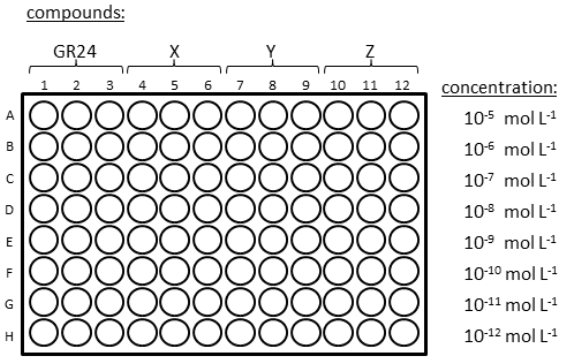


Conditionning
- 7 days, sealed plate, in the dark, 21°C

2

Seed germination, stimulation and incubation

Dilution plate of tested compounds
- 90 µL/well of water:acetone (99:1; v/v)
- solubilization of tested compounds at 10^{-2} mol L^{-1} in acetone
- dilution at 10^{-4} mol L^{-1} in water
- dilution 10x in plate, triplicate, from 10^{-5} to 10^{-12} mol L^{-1} (from line A to H) in acetone 1 %, transfer 10 µL from a line to the next, then homogenize



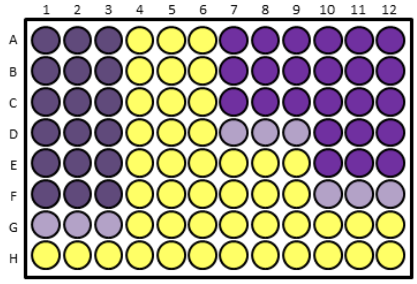
Stimulation of germination
- add 40 µL/well of water in conditionned seeds plate
- add 10 µL/well of compounds 10x from dilution plate

Incubation
- 3 days, sealed plate, in the dark, 21°C

3

MTT reduction and control under binocular microscope

- add 10 µL/well of MTT solution
- 24 h incubate, in the dark, not sealed plate, 21°C
- determination of maximum and minimum percentage of germination under a binocular microscope (6 wells)



4

Solubilization of formazan salts and absorbance reading

- add 100 µL/well of solubilization buffer
- 24h of solubilization, in the dark, sealed plate 30°C, 150rpm
- absorbance reading (570 and 630 nm: $A_{570-630nm}$)

5

Extrapolation for germination rate determination

-dose-response analysis and EC_{50} determination

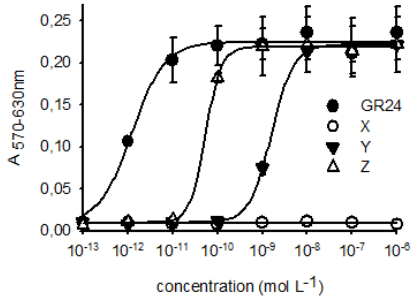


Figure 5:Experimental design for the seed germination assays (adapted from Pouvreau *et al.*, 2013)

The master thesis presented here is based upon 26 seed lots and their surrounding soil samples. These samples were obtained in 2017 during a sampling campaign performed in western France in oilseed rape, hemp and tobacco infested fields (Figure 4). The seed samples were then genotyped using twenty microsatellites markers (Stojanova *et al.*, 2019). The internship was divided into two axes: i) phenotyping the seed samples for their behavior in response to germination stimulants, and ii) profiling the seed microbiota. Afterwards, the seed sensitivity to GS and the microbiota profiles will be examined in regard of the seed genotypes and their relative hosts.

Materials & Methods

1. Seed lots

All the biological material was available for use before my training period. Seed and soil lots used in this thesis were obtained from a sampling campaign in 2017. Seed and soil lots were sampled in seven different fields in the western France (Figure 4). To collect the seeds, floral scape of broomrape plants were collected at different points in each field on aeras of a few square meters. The surrounding soil was also collected for each broomrape.

The collected seed samples contained also dusts and plant debris. To get rid of those, raw samples were sieved at different grain sizes. The seeds used in this study were obtained by keeping what goes through a 250 µm sieve and what stay in a 180 µm sieve. Indeed, the size of the *P. ramosa* seeds are about 200 µm diameter on average. After sieving, the seed lots were conserved in glass jars in a dry culture chamber at 25°C and kept in darkness.

2. Germination assay

2.1. Disinfection and conditioning

Before proceeding to germination assays (Figure 5), the seeds were disinfected. To that aim, they were soaked five minutes into bleach (2,4%) and then wash out seven times with sterilized water to prevent bleach from killing seeds (adapted from Lechat *et al.*, 2012). Afterwards, seeds were incubated in 50 mL Falcon tubes with 15 mL of incubation liquid medium containing Hepes buffer (1 mM), to stabilize the pH at 7,5, and 0,1% of PPM (Plant Preservative Mixture) to prevent contaminations. Then Falcon tubes, with a seed density of 10 mg per mL, were stocked in a climate chamber at 21°C and keep in darkness for seven days (Pouvreau *et al.*, 2013).

2.2. Seed 96 well plate filling and germination stimulant application

After seven days of incubation, the incubation medium was renewed by washing out seeds with sterilized water three times and adding a new incubation medium with the same composition plus agarose (0,05% in final) to prevent the seeds sedimentation.

Then, each plate well was filled with 50 µL of seed suspension, 40 µL of sterilized water and 10 µL of germination stimulant. The germination stimulants tested were: (+)-GR24, (-)-GR24, (+)eGR24, (-)eGR24, 2-PEITC (2-phényléthyl isothiocyanate), and racGR24 (racemic mix of (+)-GR24 and (-)-GR24). A dilution series was performed for the

stimulants using water with ACN (0.1%) as a diluent for homogenizing ACN background proportions and avoid solvent bias. The serial dilutions of stimulants were then pipetted into wells at concentrations ranging from 10^{-6} to 10^{-13}.

2.3. MTT, lysis buffer and absorbance measurement

Three days after the GS addition, wells with germinated seeds were determined under a binocular microscope. Then 10 µL of tetrazolium salt (MTT) was added in each well. The plate was incubated back in the growth chamber in dark overnight. The next day, i.e. four days after adding the GS, 100 µL of lysis buffer were added and the plate was stocked back in the growth chamber in dark. The next day, i.e. five days after adding the GS, the absorbance was measured with a POLARstar® Omega microplate reader. The absorbance raw data were then used to determine the dose-response profile.

2.4. Bio-informatic analysis

Drc R package was used to model the dose-response curve and to determine the EC50 (eliciting concentration 50), which is the concentration of stimulants for which 50% of the maximum seed germination percentage is reached, the standard deviation (Sd) from the model and the maximum germination rate.

These three numbers were then used to compare samples between each other. Box plot of the EC50 of the seed lots in response to the six tested molecules were plotted. An ANOVA with a Tukey post-hoc was carried out to specify statistically significant differences between samples (p-value < 0.05).

3. Microbiota profiling

3.1. Amplicon library construction and MiSeq sequencing

The first step was to obtain a seed macerate. To that purpose, 400mg of seeds were macerated in 25 mL of PBS 1% and Tween 0,05% in a 50 mL falcon tube. The falcon tubes were maintained under agitation (400 rpm) for 2h30. Then the macerate was filtered with 100 µm sterile filters to remove seeds. The filtered macerate was then dropped in new falcon tubes. A centrifugation at 8,000 rpm at 4°C during 15 min was carried out. The pellet was resuspended in 2 mL of supernatant to obtain the microbial suspension. Eight hundred µL of the microbial suspension was mix with 800 µL of glycerol (50%) and conserved at -80°C. Two hundred µL of the microbial suspension was used to extract DNA. The rest of the microbial suspension was conserved at -80°C.

To obtain DNA from stimulated seeds, four samples representing the genotypic diversity were used: P2S2, P3S2, P4S1 and P6S1. For each sample, 50mg of seeds were conditioned in 5 mL of buffered distilled water (hepes 1µM, pH=7,5) in flasks for 5 days at 21°C and in the dark. Then, exudates were applied at a 10^{-3} fold dilution and the samples were stocked at 21°C in darkness for 4 days. Afterwards, DNA extraction was processed and a visual assessment of germination was done.

For the soil samples, the DNA was extracted directly from 300 mg of soil.

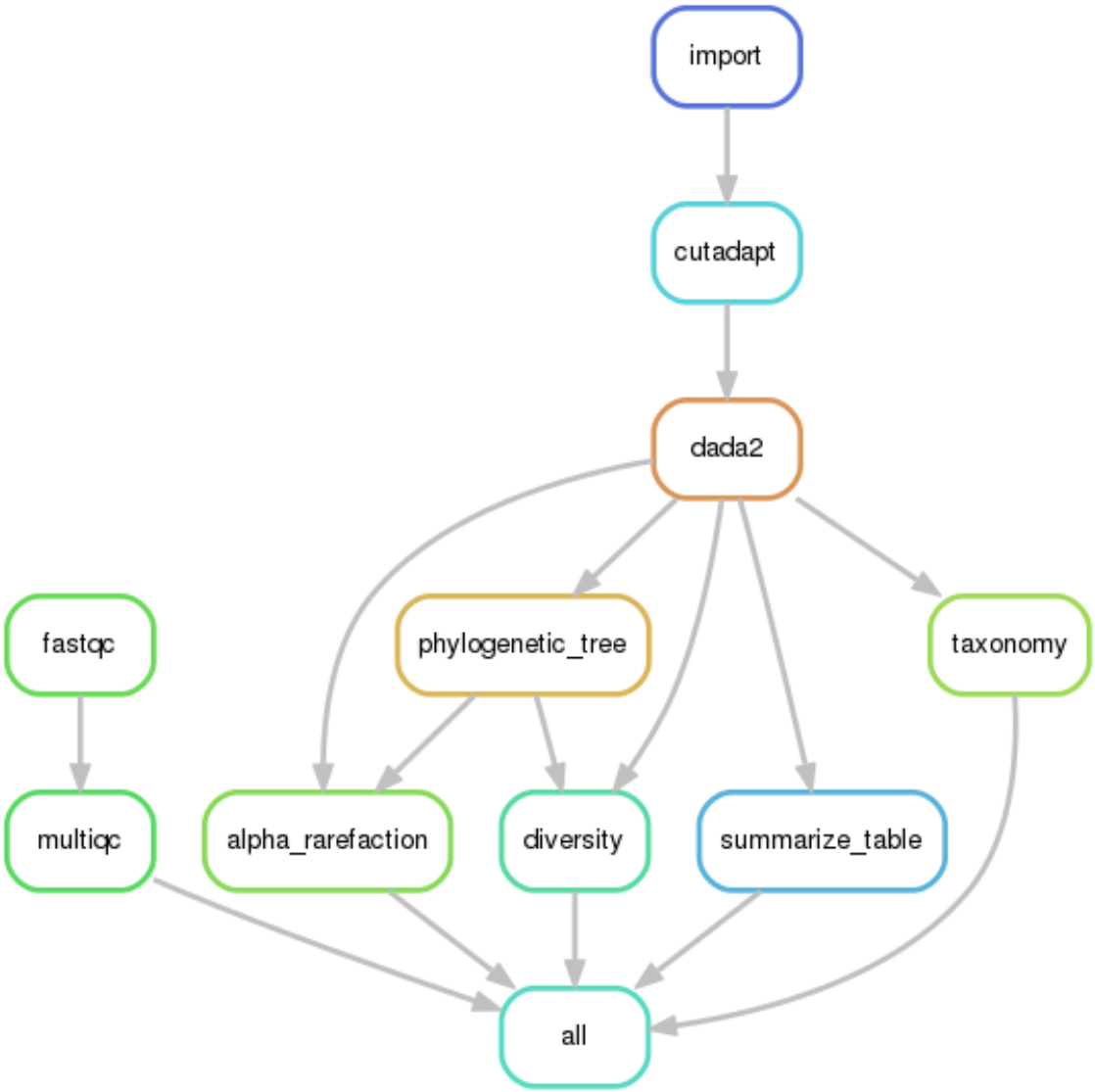


Figure 6: Qiime2 workflow (by Erwan DELAGE)

The DNA extraction was carried out with the NucleoSpin ® Soil kit (Macherey-Nagel) for all the samples i.e. seed, soil and stimulated seed samples giving a total of 246 samples, including triplicates and controls.

From the extracted DNA, the following steps were carried out at the ANAN (Nucleic Acid ANalysis) facility of the IRHS (Horticulture and Seed Research Institute) in Angers. The first PCR was carried out with AccuPrimeTM Taq DNA Polymerase High Fidelity (REF 12346-086, Invitrogen by Thermo Fisher Scientific). Two taxonomic markers were amplified, 16S and ITS, using respectively primers 16S_515f and 16S_806r (Caporaso *et al.*, 2011) and ITS1_f and ITS2 (Buée *et al.*, 2009). Afterwards, amplicons were purified using Sera-Mag™ Magnetic carboxylate modified particles (GE Healthcare, 24152105050250). Then, a second PCR amplification was performed to incorporate Illumina adapters and barcodes using GoTaq® G2 DNA Polymerase (REF M7845, Promega). Subsequently, amplicons were purified as for the first PCR. Thereafter, amplicons were quantified using Quant-iT™ PicoGreen™ dsDNA Assay kit (Invitrogen P11496) in order to prepare an equimolar pool containing all the samples. The equimolar pool was then precisely dosed by qPCR using KAPA Library Quant Illumina kit (Roche, 07960140001) and diluted to 4 nM of DNA. The DNA pool was denatured with caustic soda. Then soda was neutralized with HT1 buffer, diluting the pool to 20 pM and 5% of denatured PhiX page at 20 pM was added. The [pool + PhiX] mix was finally diluted to 12 pM and 600 µL was added in the sequencer cartridge (MiSeq Reagent Kit v3 MS-102-3003).

3.2. Bio-informatic analysis

3.2.1. Raw data filtering

Raw reads were processed using a workflow (Figure 6) implement on Qiime2 (Bolyen *et al.*, 2018). Reads quality was checked using Fastqc (Andrews, 2010) and Multiqc (Ewels *et al.*, 2016). Reads were then filtered and trimmed using Cutadapt (Martin, 2011). Denoising was implemented using Dada2 (Callahan *et al.*, 2016) which computes Amplicon Sequence Variants (ASV) (Callahan *et al.*, 2017), detects chimeric sequences and removes them from the data set. Taxonomy was assigned based on the 16S rRNA gene Silva database (Quast *et al.*, 2013). Once the abundance table and the taxonomic table were obtained, subsequent analyses were implemented on R studio using Phyloseq package (1.24.2).

As microbiota studies can be biased by reagent contaminants (Salter *et al.*, 2014), we identified contaminant ASV using iscontaminant function of the Decontam package (1.1.2) with the “either” method and a threshold at 0.1. Then, we removed ASV identified as contaminant from the dataset.

3.2.2. Microbial diversity estimation

Different ecological indexes were used to study the structure and composition of the seed microbiota (Hill, 1973): i) the observed richness that correspond to the number of detected ASVs and ii) the Shannon and iii) inverse Simpson’s index reflexing the alpha diversity. The alpha diversity represents the species diversity in one habitat or in one condition. For both these indices, the higher they are, the higher the diversity between species is. At the same time, these indices are affected by differences between sample sizes. Therefore, we had to homogenize the sample sizes by rarefying at a

Table 2: Datasets overview. Sample count contains sample triplicate.

Dataset	***Sample count***	***16S ASV count***	***ITS ASV count***	***Rarefaction threshold (16S / ITS)***
Raw dataset	236	5,215	2,849	-
Cleaned dataset	216	4,819	2,552	1500 / 1500
Seed samples	78	642	359	1,500 / 2,000
Soil samples	66	3,706	2,064	2,500 / 2,000
Stimulated seed samples	72	1002	576	2,000 / 1,500

certain number of reads (Table 2). Differences in richness and alpha-diversity were evaluated as whole by an ANOVA test with post hoc Tuckey test between each variable.

Beta diversity represents the species diversity among different habitats or conditions. It was investigated by Bray-Curtis ordination (Bray & Curtis, 1957; Beals, 1984). Bray-Curtis index is based on abundance unlike Jaccard index which is based on presence/absence matrix (Whittaker, 1972). Hence, Bray-Curtis distances were calculated on normalized ASV abundance i.e. ASV counts were divided by the number of reads per sample and multiply par 10^6. Afterwards, distance matrices were ordinated using a Principal Coordinate Analysis (PCoA). To assess the weight of each variable on the dissimilarity, a principal coordinate analysis was performed with the capscale function and a post hoc permutated multivariate analysis of variance (PERMANOVA; Anderson, 2001) was implemented with the adonis function running 9,999 permutations. Both functions are part of the vegan package (2.5-4) and allow us to extract determination coefficient for each variable. This determination coefficient is then divided by the number of factor levels of the concerned variable.

3.2.3. Microbial taxonomy composition analysis

The taxonomic composition of a community refers to the abundance and prevalence of each species or Phylum compared to the others. The abundance of an ASV is the number of reads detected for this ASV. The relative abundance is the percentage of the reads detected for one ASV compared with all the reads. The prevalence of an ASV is the number of samples is detected in. The relative prevalence is the percentage of the whole samples is detected in. In this study, the taxonomic composition is conveyed by the plot composition function of the Phyloseq package and the upset plot function of the UpSetR package (1.3.3).

ASV with a relative prevalence of 100% were considered as part of the core microbiome. At the opposite, differential presence estimation was assessed across originating host: ASV found only in samples of one originating host were considered unique to this host. No relative prevalence threshold was defined to this analysis. Thus, if one ASV was found in only one sample triplicate, it was counted as specific to the originating host of this sample triplicate. Conversely, if one ASV was found in every sample triplicate coming from one originating host and was also present in only one triplicate of another originating host, this ASV was not counted as specific to the first host.

The differential abundance was assessed with the edgeR package (3.24.3). only ASV with a variance higher than 10^{-5} were conserved for this analysis. ASV considered as significantly differentially abundant among tested samples as a FDR (Benjamini and Hochberg's False Discovery Rate) lower than 0.001 and a logFC (Fold Change logarithm) lower than -2 or higher than 2.

4. Genotyping analysis and hierarchical clustering forming dendrograms

4.1. Genotyping analysis

Before the beginning of my internship, seed lot were genotyped using 20 microsatellites markers and following the protocol developed and refined in collaboration with the LBPV (Stojanova *et al.*, 2019).

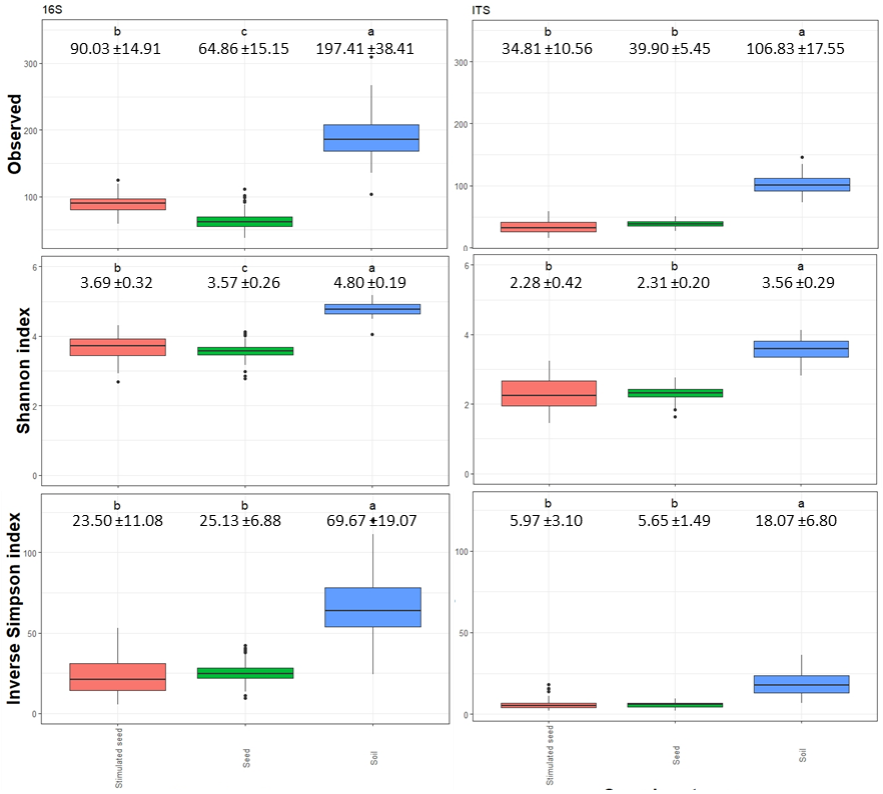


Figure 7: Alpha diversity measures for each sample category with the observed diversity (ASV count per sample), the Shannon index and the inverse Simpson index. Measures for bacterial community (16S) and fungi community (ITS) in cleaned datasets. Alpha diversity was assessed with the number of ASV rarefied at 1,500 reads per sample. Each color corresponds to a sample category. Letters represent statistically different groups (ANOVA and post-hoc Tukey, p-value < 0.05); Figures under letters correspond to mean and standard deviation.

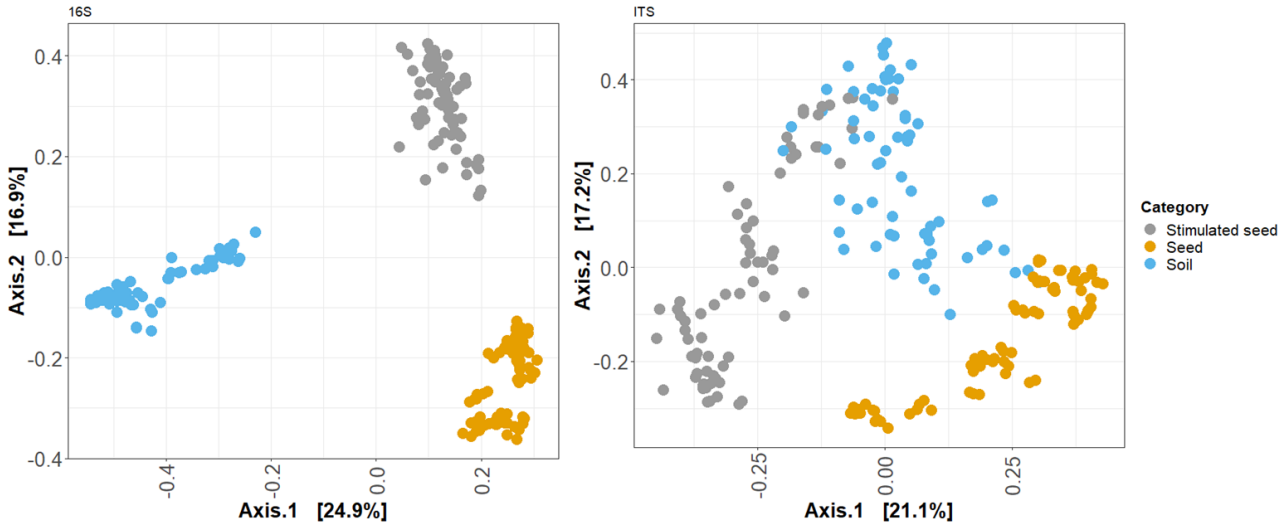


Figure 8: Bray-Curtis ordination according to the sample category (colors) for the cleaned dataset. Distances were calculated on normalized abundance.

4.2. Hierarchical clustering

Four dendrograms were built using the same method: one with the SSR data, one with the EC50 obtained with germination assays, one with the bacterial community abundance table and one with the fungal community abundance table. First, hierarchical clustering was implement with the pvclust function of the pvclust package (2.0-0). This function calculated Euclidean distance and then clustered using UPGMA method while bootstrapping one hundred times. The obtained trees were exported to newick format. Afterwards, trees were visualized and annotated using iTOL (Letunic & Bork, 2019).

RESULTS

Herein, we present first the results obtained regarding the microbial community analysis. Afterwards, we present the results obtained for germination assays as a phenotype analysis. Finally, we present the result obtained by genotyping analysis and compared it to the microbiota profile and the phenotype profile.

1. Seed microbial community diversity and core microbiota

1.1. Seed microbiota is distinct from soil and stimulated seed microbiota

1.1.1. Dataset display

The sequencing output contains 20,431,764 reads of which 79.4% of the sequenced nucleotides had a quality greater than or equal to Q30. Before proceeding to the diversity analysis, contaminant ASV, control samples and chloroplast ASV were removed from the raw dataset. Within the cleaned dataset, the number of read per sample ranged from 7,170 to 16,384 reads for the 16S dataset and from 1,222 to 29,509 for the ITS dataset. The sample size median was at 10,352 with a standard deviation of 1,749.66 for 16S and 7,958 ± 2,677.55 for ITS.

The raw dataset contained 236 samples with 5,215 ASV and 2,849 ASV for the 16S gene marker and the ITS gene marker respectively (Table 2). The cleaned dataset contained 216 samples with 4,819 ASV and 2,552 ASV for 16S and ITS respectively. ASV abundance ranged very drastically from 2 to 145,310 reads and from 2 to 286,852 reads for 16S and ITS respectively. The 16S ASV abundance median was 37 with a standard deviation of 3,263.06. The ITS ASV abundance median was 15 with a standard deviation of 7,794.78.

These numbers reveal that sequencing depth was better for 16S than ITS gene marker although it was satisfactory in both datasets. These numbers also outline the high variability of sample size and ASV abundance.

1.1.2. Alpha diversity was higher in soil

Regarding the sensitivity of alpha-diversity indexes to sample size heterogeneity, samples were rarefied at different thresholds chosen according to rarefaction curves (Table 2). Overall, the highest diversity was observed within soil samples comparing to seed samples, stimulated or dry (Figure 7). Indeed, the three alpha diversity indexes were two to three times higher in soil samples than in other samples both in the bacterial and fungi communities. Concerning seed samples, stimulated seed and dry seed samples didn't show statistical differences except for observed diversity and

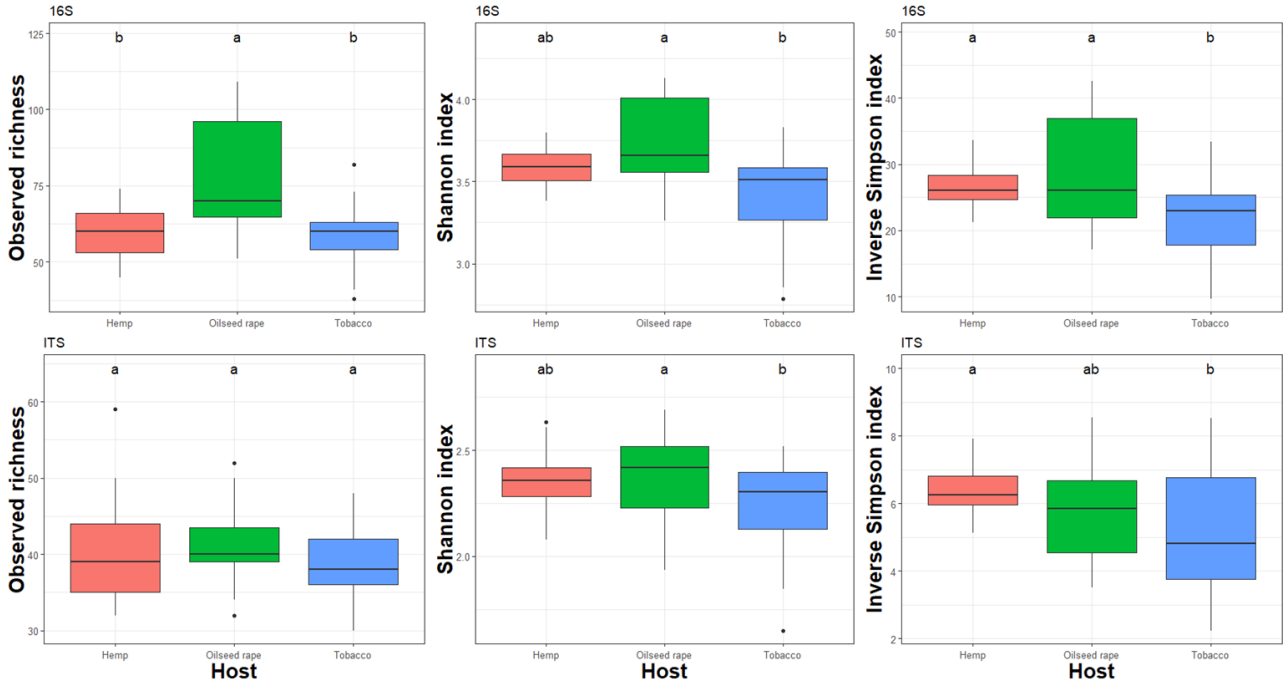


Figure 9: Alpha diversity measures for each originating host sample with the observed diversity (ASV count per sample), the Shannon index and the inverse Simpson index. Measures for bacterial community (16S) and fungi community (ITS)within seed samples. Alpha diversity was assessed with the number of ASV rarefied at 1,500 reads per sample for 16S and 2,000 reads per sample for ITS. Each color corresponds to a sample category. Letters represent statistically different groups (ANOVA and post-hoc Tuckey, p-value < 0.05); Figures under letters correspond to mean and standard deviation.

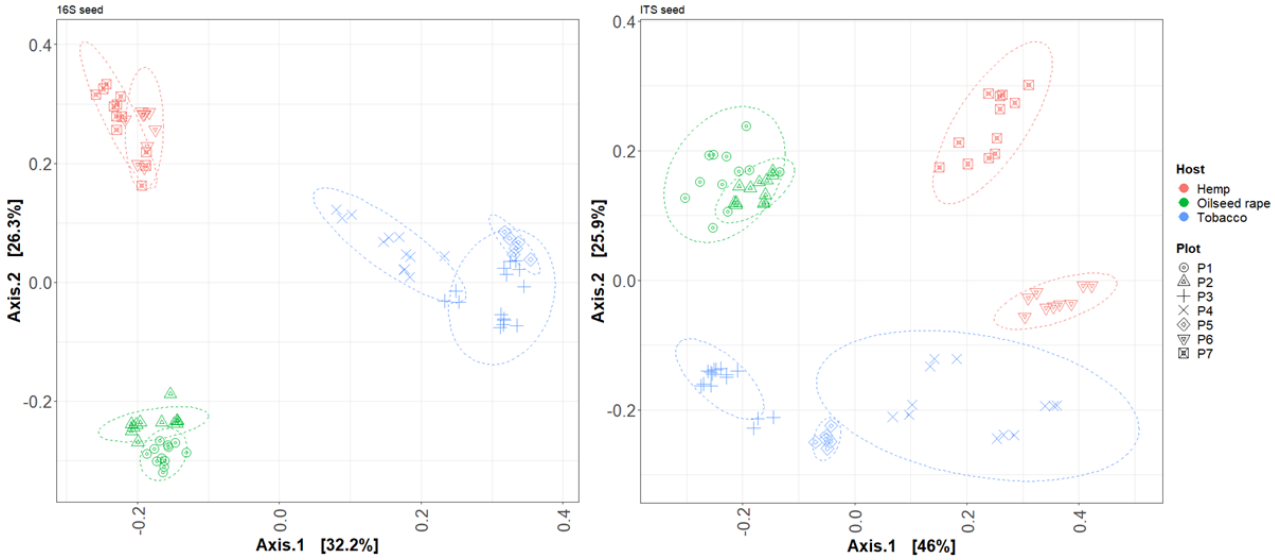


Figure 10: Bray-Curtis ordination according to the originating host (colors) and field (shape) for the seed sample dataset. Distances were calculated on normalized abundance.

Shannon index of bacterial community. Moreover, diversity was higher in bacterial communities than in fungal communities (Figure 7).

1.1.3. Beta diversity clustered seed samples

We used a principal coordinate analysis based on a Bray-Curtis distance matrix (Figure 8) to determine whether seed samples were clustered apart from other sample categories (stimulated seed and soil).

For the 16S gene marker, the first and second dimensions explained 24.9% and 16.9% of the data dispersion respectively. Seed and soil samples were clustered toward each other mainly within the first dimension while stimulated seed samples were clustered toward the seed samples within the second dimension.

For the ITS gene marker, the first and second dimensions explained 21.1% and 17.2% of the data dispersion respectively. Stimulated seed and soil samples overlapped greatly. However, seed samples clustered toward others within both dimensions.

These PCoA underlines that seed microbiota was distinct from the soil microbiota and the stimulated seed microbiota, allowing us to explore alone the seed microbiota.

1.2. Seed microbiota diversity was different regarding the originating host

1.2.1. Alpha diversity was homogenous across originating host

Among seed samples, bacterial alpha diversity was homogenous except for five samples out of 26 samples. Three samples had a higher richness within the three indexes than other samples. These three samples were coming from the first field where oilseed rape was cultivated. Two samples had a lower richness with the three indexes than other samples. These two samples came from two different field where tobacco was cultivated.

Therefore, when assessing alpha diversity by host (Figure 9), statistical differences for the three indexes used were observed only between oilseed rape and tobacco host within the bacterial community: parasitic seeds coming from oilseed rape host seemed to have a higher bacterial alpha diversity than parasitic seeds coming from tobacco host. However, these statistical differences were clearly supported by the five outlier samples.

Moreover, when assessing alpha diversity for the fungal microbiota, no statistical differences were observed between originating host. Thus, alpha diversity can be considered as homogenous across originating host.

1.2.2. Beta diversity was mostly driven by originating host and field

We used a principal coordinate analysis based on a Bray-Curtis distance matrix (Figure 10) to determine how samples were clustered apart from each other.

Regarding bacterial community, the first and second dimensions explained 32.2% and 26.3% of the data dispersion respectively. Tobacco samples were clustered toward other samples within the first dimension while oilseed rape and hemp samples were clustered toward each other within the second dimension. Inside the host clusters, we can distinguish sub-clusters for each field.

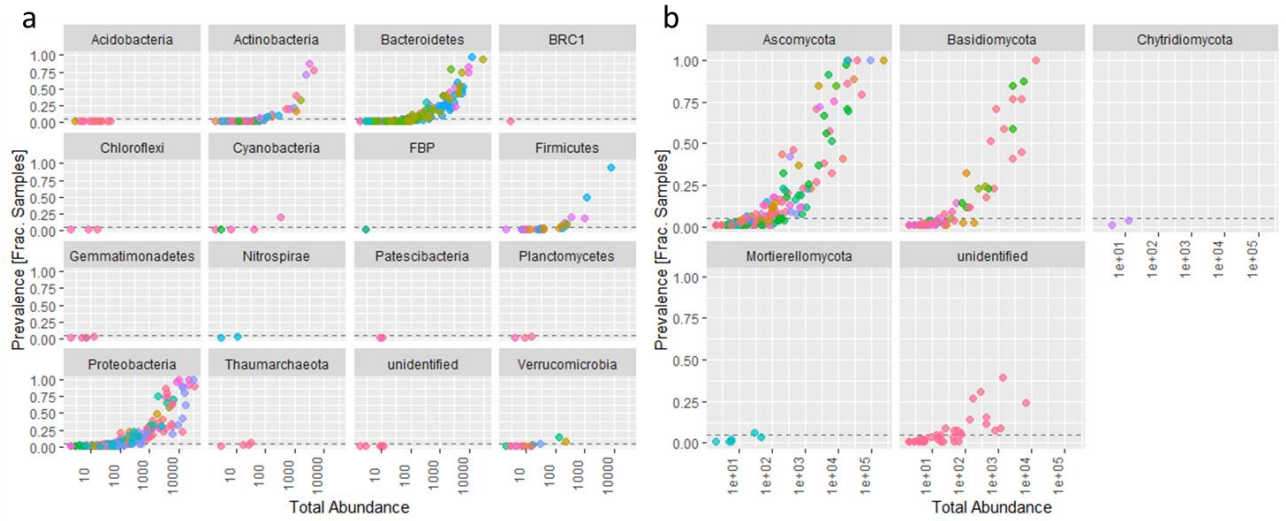


Figure 11: ASV prevalence of each phyla as a function of its raw abundance for the bacterial ASV (a) and the fungal ASV (b). Each color represents a genus.

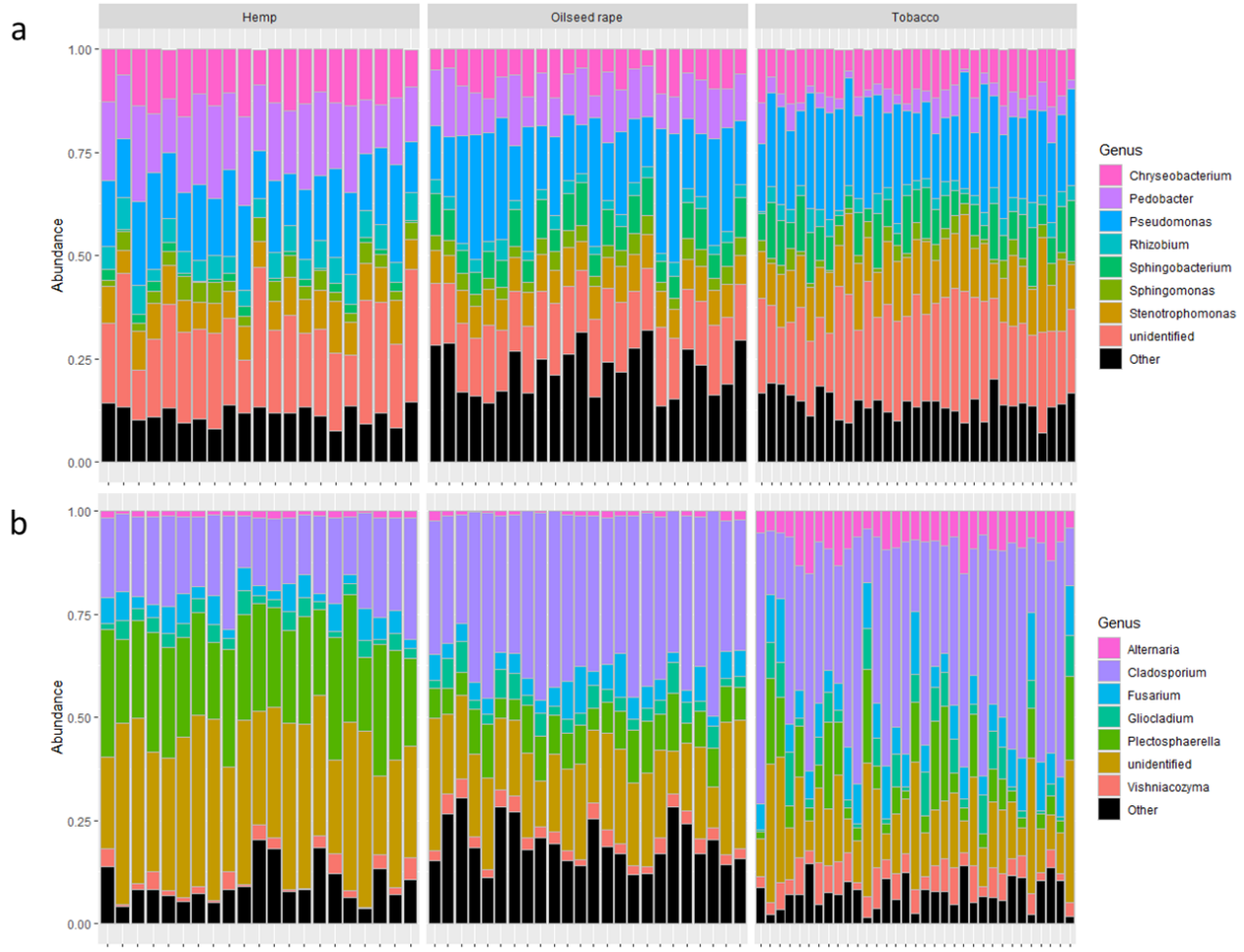


Figure 12: Genus relative abundance regarding the parasitic seed originating host. The x-axis represents the samples (one bar per sample). Colors refer to genera.

Regarding fungal community, the first and second dimensions explained 46% and 25.9% of the data dispersion respectively. However, samples were not as well clustered by originating host than for bacterial community. Indeed, only samples originating from oilseed rape were well clustered toward the others within both dimensions. Samples originating from tobacco and hemp were first clustered by their originating field. This highlights the terroir effect on fungal communities associated to seeds.

Assessing the PCoA variable weight thanks to Adonis function, the determination coefficients of each variable were in the same order of magnitude for both bacterial and fungal communities. For 16S and ITS respectively, each originating host explained 18.52% and 16.93% of the data dispersion while each originating field explained 11.14% and 11.45%. These figures clearly support the clusters observed within ordination.

Regarding the clusters, the seed samples originating from oilseed rape showed the most homogenous microbial communities while the seed samples originating from tobacco were the most heterogenous. Overall, seed microbial communities seemed to be clustered according to their originating host and field.

1.3. Taxonomic composition of the seed microbiota was different regarding the originating host

1.3.1. The most abundant ASV were conserved among seed samples

Regarding bacterial community, 98.6% of the ASV remained to the bacteria kingdom. Four ASV remained to the *Archaea* and 5 ASV were unassigned. Within these kingdoms, four phyla represented 99.76% of the total abundance, 90.5% of ASV and were present in 100% of the sample triplicate (Figure 11): *Proteobacteria* (64.54% of the total abundance and 40.5% of ASV), *Bacteroidetes* (29.64% and 35.82%), *Actinobacteria* (3.37% and 9.97%) and *Firmicutes* (2.21% and 4.21%). At the genus level, 14.3% of the ASV were unidentified representing 19.84% of the total abundance. However, these ASV were numerous and diverse but at low abundance because their median abundance was zero among all samples. Additionally, seven genera (Figure 12) represented 63.47% of the total abundance and gathered 23.05% of the ASV and were present in 100% of the samples triplicate: *Pseudomonas* (19.85% of the total abundance and 3.27% of the ASV), *Pedobacter* (10.45% and 4.98%), *Stenotrophomonas* (10.11% and 1.25%), *Chryseobacterium* (9.82% and 2.65%), *Sphingobacterium* (6.45% and 6.39%), *Rhizobium* (3.58% and 1.71%) and *Sphingomonas* (3.21% and 2.80%).

Regarding fungal community, every ASV belonged to the fungi kingdom. At the phylum level, 13.09% of the ASV were unidentified, representing 1.74% of the total abundance (Figure 11). Unidentified fungi phyla had a median abundance of 60.5 but were not present in every sample triplicate (only in 69 out of 78 samples). Besides these unidentified phyla, two phyla represented 98.24% of the total abundance with 84.68% of the ASV and were present in every sample triplicate: *Ascomycota* (91.75% of the total abundance and 64.62% of ASV) and *Basidiomycota* (6.50% and 20.06%). At the genus level (Figure 12), 40.11% of the ASV were unidentified, representing 22.73% of the total abundance (median abundance at zero). Moreover, six genera were present in every sample triplicate representing 64.43% of the total abundance and 11.98% of the ASV: *Cladosporium* (33.19% of the total abundance and 1.11% of

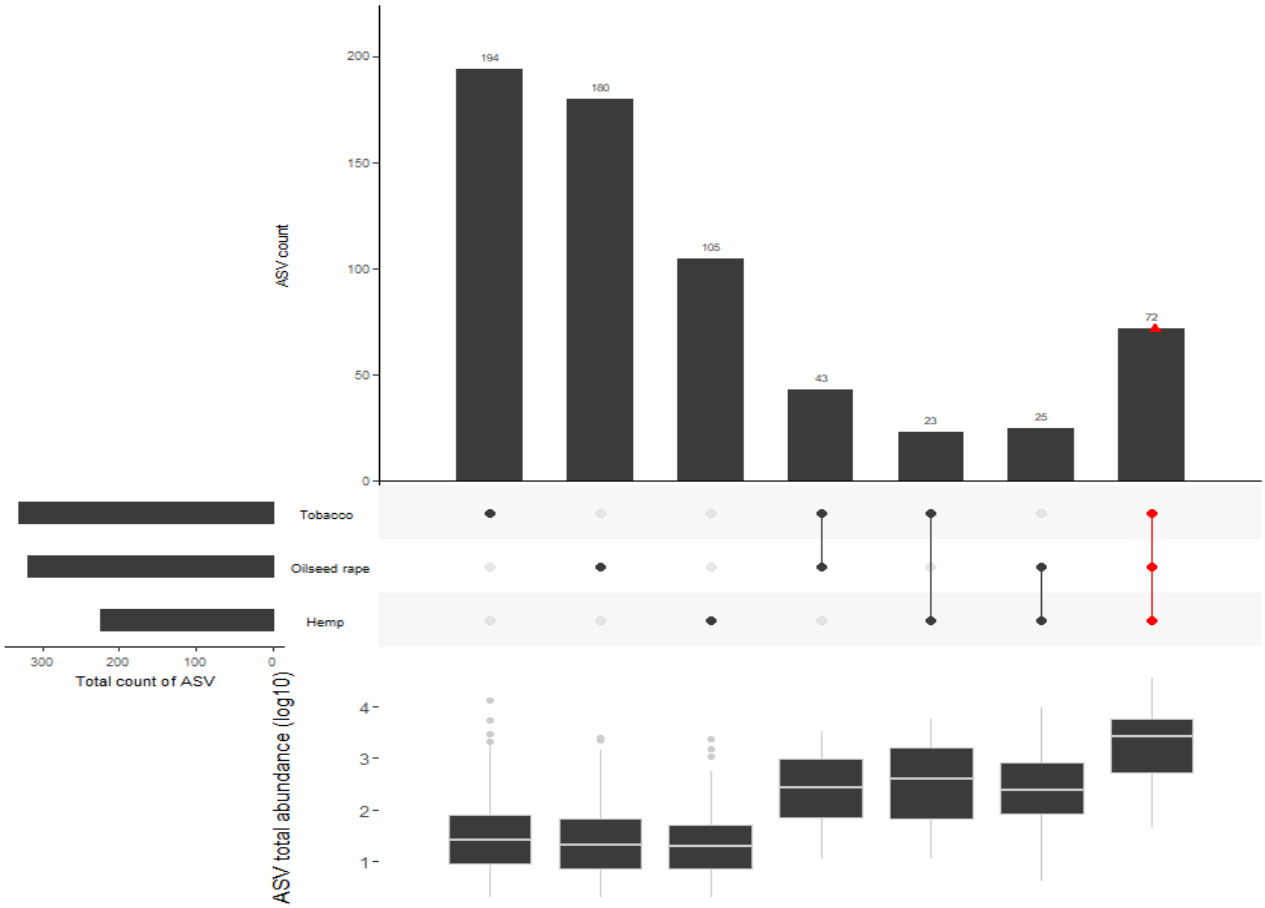


Figure 13:Upset plot is a kind of Venn diagram. Each point represents the intersection between sets (here sets are originating host). Vertical bars represent ASV count for each intersection. Horizontal bars represent ASV count in each set. Boxplots represent ASV abundance for each intersection.

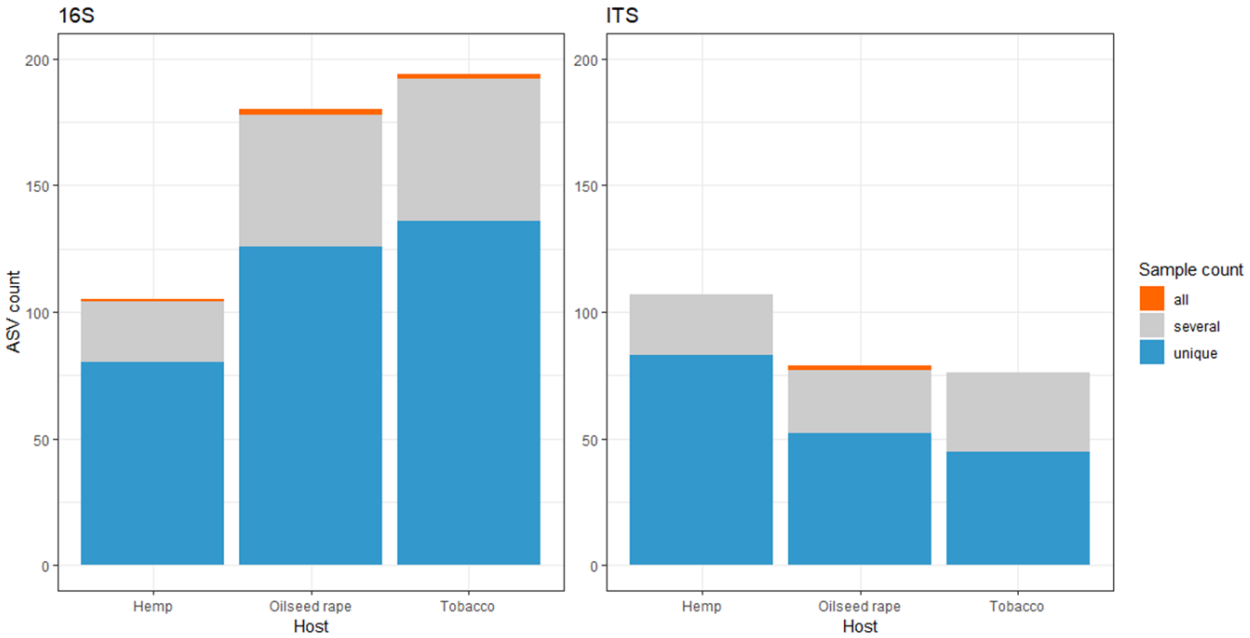


Figure 14: ASV present in only one of the originating hosts. Colors refer to ASV that are present in every samples of one of the originating hosts (all, red); in several samples of one of the originating hosts (several, gray); in only one sample of one of the originating hosts (unique, blue)

the ASV), *Fusarium* (6.81% and 4.46%), *Gliocladium* (4.49% and 1.95%), *Mycosphaerella* (2.67% and 0.28%), *Plectosphaerella* (13.48% and 1.39%) and *Vishniacozyma* (3.78% and 2.78%).

These seven bacterial genera and six fungi genera were thus part of the *P. ramosa* seed core microbiota. To identify more precisely the seed core microbiota, we looked at the ASV present in every sample (Annexes I and II).

Regarding the bacterial community, ten ASV were present in every represented 1.56% of the ASV and 26.46% of the total abundance. Regarding the fungal community, seven ASV were present in every sample represented 1.95% of the ASV and 60.91% of the total abundance.

Within these 17 ASV (Annex III), three were unidentified, nine were identified at the genus level and five were identified at the species level, by reference to the Silva taxonomic database. The ASV sequences were compared to NCBI database (BLAST). The best hits were listed in the annex I. For one ASV that was previously identified at the species level by reference to the Silva taxonomic database, had a different identification by reference to the NCBI database: ASV2639_*Mycosphaerella_tassiana* was identified as *Cladosporium floccosum* in the NCBI database.

1.3.2. Majority of ASV were unique to one of the seed samples

As some ASV were present in every samples, some ASV were only found in some samples. Considering the host specialization of *P. ramosa*, we looked at the ASV present only in one of the originating hosts.

For bacterial community (Figure 13), 180 ASV were present only in samples originating from oilseed rape (representing 28.04% of the ASV and 3.52% of the total abundance), 105 only in samples originating from hemp (16.35% and 1.97%) and 194 only in samples originating from tobacco (30.21% and 9.65%). Thus, 74.6% of the ASV, representing 15.14% of the total abundance, were present only for one of the originating hosts. At the opposite, 72 ASV were present in all the originating host, representing 20.06% of the total ASV and 53.72% of the total abundance.

For fungal community, 79 ASV were present only in samples originating from oilseed rape (representing 22.01% of the ASV and 0.33% of the total abundance), 107 only in samples originating from hemp (29.80% and 0.94%) and 76 only in samples originating from tobacco (21.17% and 0.78%). Thus, 72.98% of the ASV, representing 2.05% of the total abundance, were present only for one originating host. At the opposite, 43 ASV were present in all the originating hosts, representing 11.98% of the total ASV and 92.72% of the total abundance.

However, within these ASV between 59.21% and 77.57% of them were, in fact, present in only one of the samples (Figure 14, Annexes I and II). Within the bacterial community, only two ASV were found in every samples originating from oilseed rape and not in the other samples (ASV668_Sphingobacterium_sp. MIMdw12 & ASV3317_Nannocystis_unidentified); one ASV was found in every samples originating from hemp and not in the other samples (ASV718_Pedobacter_unidentified); two ASV were found in every samples originating from tobacco and not in the other samples (ASV638_Sphingobacterium_sp. 23D10-4-9 & ASV2965_Pseudomonas_unidentified). Within the fungal community, two ASV were found in every samples originating from oilseed rape and not in the other samples (ASV1153_unidentified & ASV1708_Leptosphaeria_maculans). No ASV were found in every samples originating from hemp nor tobacco and not in the other samples.

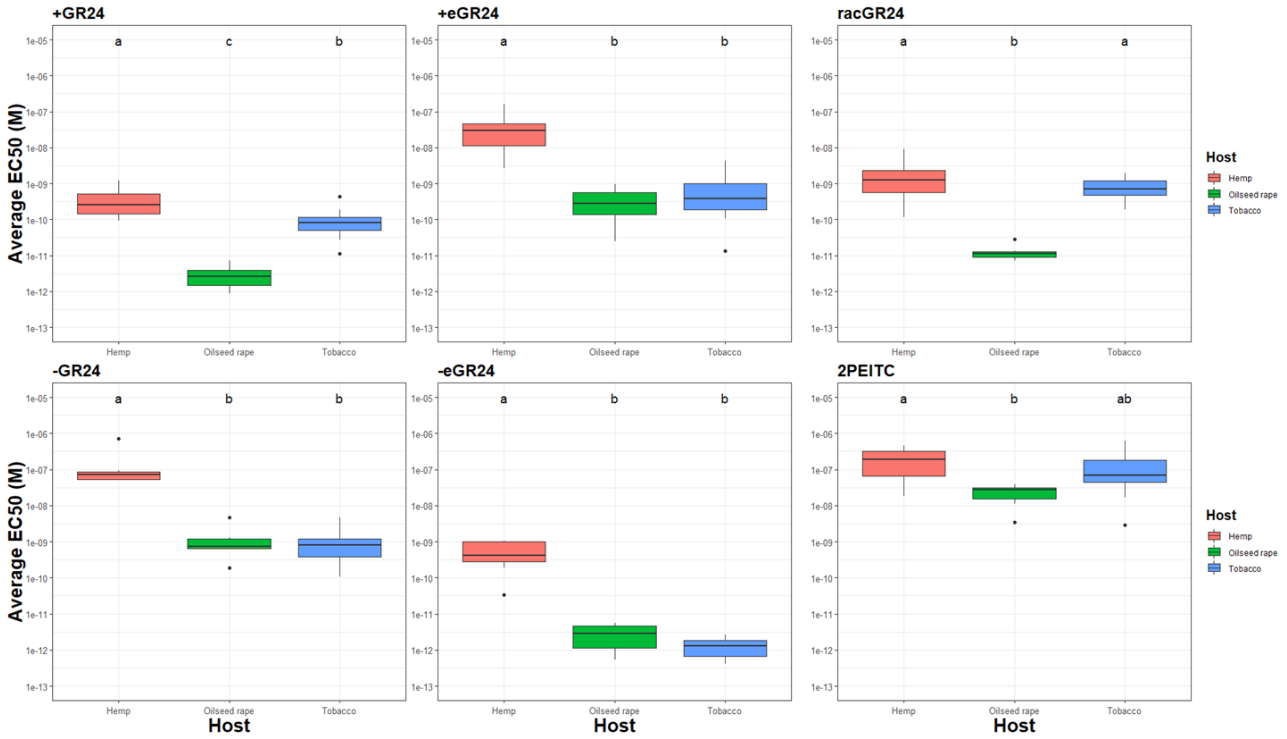


Figure 15:Average EC50 (in molar) according to the originating host and for the four enantiomers of GR24 (+GR24, -GR24, +eGR24 and -eGR24), the racemic mix of GR24 (racGR24) and the 2PEITC (2-phényléthyl isothiocyanate). Colors refer to originating host. Letters represent statistically different groups (ANOVA and post-hoc Tuckey, p-value < 0.05).

1.3.3. Most abundant ASV were differentially abundant between originating host

As genes can be differentially expressed, bacteria and fungi ASV can be differentially abundant. Therefore, we compared each host samples with the others. In oilseed rape host samples, 14 bacterial and 18 fungal ASV were differentially abundant: seven bacterial and seven fungal ASV were more abundant and seven bacterial and 11 fungal ASV were less abundant compare to other hosts. In hemp host samples, 15 bacterial and 12 fungal ASV were differentially abundant: eight bacterial and four fungal ASV were more abundant, seven bacterial and eight fungal ASV were less abundant than in other host samples. In tobacco host samples, 12 bacterial and 13 fungal ASV were differentially abundant: five bacterial and five fungal ASV were more abundant and seven bacterial and eight fungal ASV were less abundant than in other host samples.

Within these differentially abundant ASV, five were part of the core microbiota (Annex IV). In the bacterial core microbiota, ASV2265_*Stenotrophomonas*_unidentified was more abundant in oilseed rape (2.7 logFC) and tobacco (2.93 logFC) host samples while ASV2876_*Paenibacillus*_unidentified was more abundant in hemp host samples (2.47 logFC). In the fungal core microbiota, ASV1817_unidentified was more abundant in oilseed rape host samples (2.6 logFC) and less abundant in tobacco host samples (-2.01 logFC). ASV2459_unidentified was more abundant in hemp host samples (2.53 logFC) and less abundant in tobacco host samples (-3.82 logFC). ASV2467_*Fusarium*_unidentified was less abundant in hemp host samples than in others (-2.38 logFC).

1.3.4. Ten core ASV were conserved from soil to germinating seeds

For four samples, microbial communities of soil, seeds, conditioned seeds and stimulated seeds were sequenced. Therefore, we looked at the conserved ASV among these four stages: 33 bacterial and 49 fungi ASV were detected in every stage, representing respectively 1.52% and 4.65% of the ASV and 44.26% and 93.77% of the total abundance.

Within these conserved taxa, ten were part of the seed core microbiota (Annex IV). Three of these conserved ASV were part of the bacterial seed core microbiota: ASV2261_*Stenotrophomonas_rhizophila*, ASV2990_*Pseudomonas*_unidentified and ASV710_*Pedobacter*_unidentified. All the ASV of the fungal core microbiota were conserved among the four stage samples.

2. Seed phenotype was different regarding the originating host

Upon the four GR24 enantiomers tested on germination assays, seeds originating from hemp were less sensitive then seeds originating from oilseed rape or tobacco (Figure 15). However, no difference was observed between the seeds originating from oilseed rape and the one originating from tobacco except for the +GR24.

Regarding the racemic mix of GR24, seeds originating from oilseed rape was more sensitive than others. As the racemic mix was composed of (+)-GR24 and (-)-GR24, this reflects the higher sensitivity of seeds from oilseed rape to the (+)-GR24 enantiomer.

Regarding the 2PEITC, seeds from hemp were less sensitive comparing to seeds from oilseed rape while seeds from tobacco showed an intermediate sensitivity. Knowing that i) 2PEITC are mainly found in oilseed rape exudates and ii)

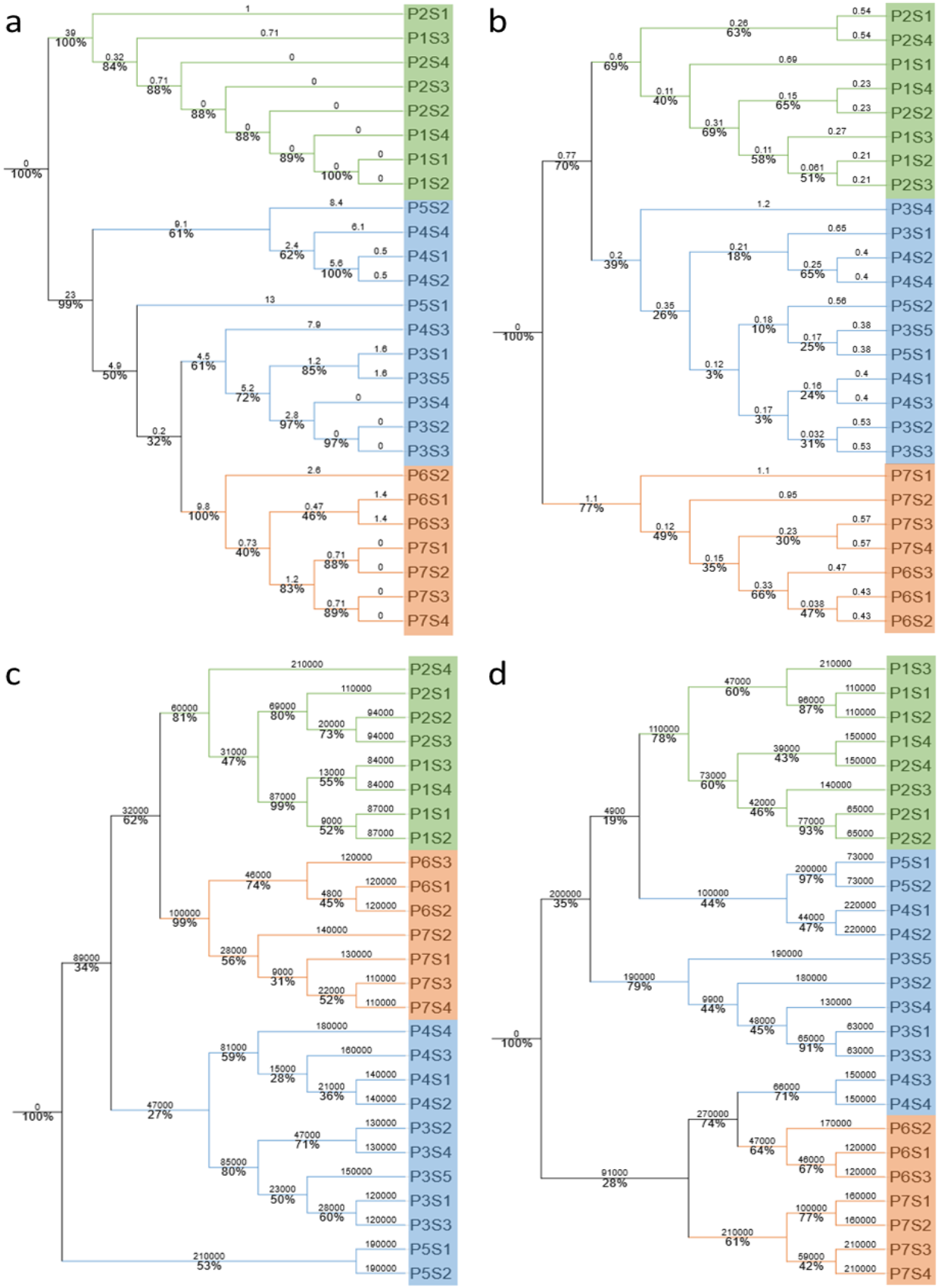


Figure 16:Dendrograms based on Euclidean distance and UPGMA clustering for the SSR genotyping (a), the EC50 phenotype (b), the bacterial community abundance table (c) and the fungal community abundance table (d). Branch length are written above the corresponding branch. Bootstrap values were obtained with one hundred iterations and are written below the branch before the corresponding node.

P. ramosa investing hemp haven't been found investing oilseed rape while the ones found in tobacco can infest both the other hosts, this appears to be one phenotype of the host specialization.

3. Seed genotype, phenotype and microbiota profile clustered according to their originating host

Using the SSR markers sequenced on seed samples, the dendrogram (Figure 16a) revealed that samples originating from oilseed rape were well clustered with a bootstrap of 100% and a branch length of 39. This cluster represents a monophyletic group and seems to correspond to the genotype 1 describe for *P. ramosa* (Stojanova *et al.*, 2019). Seeds originating from hemp also clustered in a monophyletic group and seems to correspond to the genotype 2a of *P. ramosa*, with a 100% bootstrap value of the node but with a shorter branch length of 9.8. Seeds originating from tobacco are separated in two clusters and represents a polyphyletic group.

Applying the same method to seed phenotype (i.e. EC50 of six molecules tested), the dendrogram (Figure 16b) showed three clusters corresponding to the originating host of the parasitic seeds. However, the tobacco and hemp clusters are less closely related than in the genotype dendrogram. Indeed, on the phenotype dendrogram, oilseed rape samples are closer of tobacco samples than hemp samples.

Applying the same method on abundance table of seed microbiota (Figure 16c, d), we drew two dendrograms for the bacterial and the fungal communities respectively. On these two dendrograms, oilseed rape samples formed well supported cluster with respective bootstrap values of 81% and 78%. In the bacterial community dendrogram, oilseed rape samples were closer of hemp samples while in the fungal community dendrogram, they were closer of tobacco samples. Hemp samples formed a well-supported cluster in the bacterial community (bootstrap of 99%) while they formed two clusters in the fungal community. Tobacco samples formed three clusters in both dendrograms.

Overall, considering the four dendrograms, seed samples originating from oilseed rape established well-supported clusters for all parameters, constituting the most stable feature of this study. Then seed samples originating from hemp formed three well-supported clusters out of four dendrograms. Seed samples originating from tobacco showed the more heterogenous feature, constituting not one well-supported cluster out of four dendrograms.

DISCUSSION

1. Distinction between originating host and parasite genotype effects

The sampling strategy did not permit to get seeds from each genotype groups (1, 2a and 2b) that were originating from different hosts and thus we cannot distinguish the genotype effect from the host effect on any of the measured variables (response to GS, microbiota profiles…). Past studies showed that genotype 1 parasitized mostly oilseed rape in western France, genotype 2a was parasitizing only hemp in east and west France and genotype 2b was somewhat intermediate present on tobacco, hemp and sometime oilseed rape and somehow more widespread (Stojanova *et al.*, 2019). To complete the study collection and fully cover the diversity, more fields in the south (tobacco) and eastern

France (Hemp, oilseed rape) could have been involved. Also, to distinguish these effects, further experiments should be carried on. One could consider experimental evolution approaches forcing seeds from different genotypes on several hosts for a study of next generation seeds. Germination assays and microbiota profiling of seeds could be processed as in the present study and microbiota would be evaluated and compared to host endophytes. This would give us further understanding on the microbial transfer and selection, on the possible adaptation of *P. ramosa* seeds and on the overall tripartite evolutions. Another approach in the field would be to cultivate the three hosts of interest in three distinct sampled plots (originally cultivated by oilseed rape, hemp, and tobacco: e.g. P1, P3, P6) and then to estimate each parasite genotype aggressivity and measure infestation rates on each host. Stojanova *et al.* have already implemented cross-infection assays and shown that genotype is more important than originating host to explain parasitic seed phenotype (Stojanova *et al.*, 2019). However, they did not look at microbial communities. In conclusion, in our study, the effect of originating host on microbial community cannot be distinguished from the parasite genotype effect.

Another confounding effect in microbiota profiling is the geographic location of the sample fields. Indeed, as we can see on the Figure 4, each parasite genotype / originating host have been sampled in one region different from the other samples. As reported, seed microbial communities can be influenced by the terroir i.e. the geographic location they are produced in, especially for fungal seed associated communities (Klaedtke *et al.*, 2016). In our study, we have noticed this terroir effect on both bacterial and fungal communities as they tended to be clustered by sampling site. This terroir effect can then hide a part of the genotype / host effect on microbiota profile. To address this bias, cross-infection assays could be implemented in different fields containing each the same composition of several host crops and several parasite genotypes. Then, originating host effect, parasite genotype effect and terroir effect could be estimated on microbiota profile.

2. Studying living organisms implies biases

2.1. Growth chambers restrain biological biases and controls are needed to address technical biases

For field observations, there are always many parameters that can account for the variation of a given measured variable, as there are uncontrolled parameters between samples. Herein this study, there were a number of other parameters that were not studied in detail and that could interfere with the microbiota profiling. For example, the harvesting status, the time of sampling and the maturity of capsules and seeds could have an effect and have to be considered. Indeed, in four fields, crops were already harvested (P1, P2, P4, P5 and P7) while others were still untouched (P3 and P6). The harvesting process may have contaminated *P. ramosa* seeds and may have changed the actual microbial profiling. One possible thing to do would be to compare the proportion of shared communities between soil and seed samples in harvested fields as opposed to unharvested ones. Also, as *P. ramosa* plants do not complete their biological cycle at the same time depending on crop cultivation and growth, sampling was done at different times. In fact, sampling was systematically carried out at advanced fruiting times. Thus, samples on oilseed rape fields were

collected early July and samples from tobacco and hemp fields were collected in September. This seasonal parameter could be of importance and can be address by cross-infections implemented in growth chambers.

Moreover, during the DNA extraction, amplification and purification, technical biases could have occurred. The first one we handled here is the presence of reagent contaminant in the sequenced samples. These reagent contaminants can critically biased microbiota analyses (Salter *et al.*, 2014). To prevent contaminants to interfere with our analysis, we sequenced set as controls several reagents as controls and sequenced DNA in it, as suggested by Salter *et al.* Then, we used bio-informatic tools to remove contaminants *in silico*. However, the efficiency of these three technical steps can also modify the estimated abundance of each ASV.

2.2. ASV identification needs well-resourced taxonomic database

In this study, we used Silva taxonomic database for 16S marker and UNITE taxonomic database for ITS1 marker. As the taxonomic databases can diverge, we compared the taxonomic composition obtained with the Silva database with one obtained with the RDP database. Taxonomic assignation was similar using these two databases. Therefore, we choose to use Silva database as there were more entries in it (Balvočiūtė & Huson, 2017).

Nonetheless, as specified in the results, some ASV remained unidentified: 1.09% and 14.3% 16S ASV at the phylum and genus levels respectively; 13.09% and 40.11% ITS ASV at the phylum and genus levels respectively. These figures underline the need to pursue the taxonomic referencing effort on environmental micro-organisms but above all, pointed out that ASV count for each phylum and genus are certainly underestimated.

3. Functions of the core microbiota

The core microbiome is defined as micro-organisms common across similar habitats and that are presumed to play a key role in functions within these habitats (Turnbaugh *et al.*, 2007; Shade & Handelsman, 2012). In this study, we determined ten bacterial ASV and seven fungal ASV as part of the *P. ramosa* seed core microbiota. The functions of each of these ASV within the seeds still have to be discussed.

Pseudomonas are ubiquitous *Proteobacteria*. *P. viridiflava* and *P. fluorescens* are fluorescent *Pseudomonas*. *P. viridiflava* was first described in 1930 by Burkoldher (Burkholder, 1930) and is known as pathogen for *Arabidopsis thaliana* and numerous and diverse crops (Billing, 1970; Wilkie *et al.*, 1973; Jakob *et al.*, 2002). Contrariwise, *P. fluorescens* has long been known as a plant commensal bacterium that was study for its biocontrol activity against several pathogens (Howell & Stipanovic, 1979; Voisard *et al.*, 1989; Vidhyasekaran & Muthamilan, 1995; Paulsen *et al.*, 2005). However, it was more recently described as a species complex (Garrido-Sanz *et al.*, 2016) which could be a human pathogen with large antimicrobial resistance (Trivedi *et al.*, 2015), compromising its use as biological control agent. Additionally, it was also surveyed for its siderophore production and its applications for soil bioremediation (Ferret, 2012; Luján Adela M. *et al.*, 2015; Bruce *et al.*, 2017). *P. donghuensis* produces siderophores as well (Gao *et al.*, 2015) and also volatile organic compounds (VOCs) with antimicrobial properties (Ossowicki *et al.*, 2017). *P. graminis* has been surveyed for its potential as a biocontrol agent during fresh-cut fruit storage (Behrendt *et al.*, 1999;

Alegre *et al.*, 2013; Plaza *et al.*, 2016). *P. lutea* and *P. rhizosphaerae* were recently described bacteria that solubilize phosphate *in vitro* (Peix *et al.*, 2003, 2004). *P. abietaniphila* is able to degrade polychlorinated biphenyls (PCBs), dehydroabietic acid (DhA) and resin acid in pulp and paper mill effluent (Martin & Mohn, 1999; Muttray *et al.*, 2001; Kimura *et al.*, 2015; Fujihara *et al.*, 2015). *P. koreensis* and *P. baetica* are recently described bacteria (Kwon *et al.*, 2003; López *et al.*, 2012).

Stenotrophomonas are *Proteobacteria* that contribute to nitrogen and sulfur biogeochemical cycles, that have been repeatedly and ubiquitously found throughout the environment and that can be closely associated with plants (Ryan *et al.*, 2009; Berg *et al.*, 2010). *S. rhizophila* and *S. maltophila* are endophytes known as biological control agents and as plant growth promoting rhizobacteria or PGRP (Berg *et al.*, 1994; Dunne *et al.*, 1998; Ryan *et al.*, 2009). First to be described and study, *S. maltophila* however poses a risk for human health, especially for immunosuppressed patients (Berg *et al.*, 2005; Hagemann *et al.*, 2006). *S. rhizophila* was described latter (Wolf *et al.*, 2002) and does not pose human health risk (Hagemann *et al.*, 2006). *S. rhizophila* is increasingly studied, especially as a PGRP for increasing plant tolerance to salinity stress (Schmidt *et al.*, 2012; Alavi *et al.*, 2013; Egamberdieva *et al.*, 2016). *S. chelatiphaga* was also described latter for degrading environmental pollutant (Kaparullina *et al.*, 2009).

Agrobacterium tumefaciens is a well-known phytopathogenic *Proteobacteria* that causes gall in several crops. This bacteria is also known as *Rhizobacterium radiobacter* (Aujoulat *et al.*, 2011).

Pedobacter are part of the *Bacteroidetes* phylum and closely related to *Sphingobacterium* (Margesin & Shivaji, 2015). *Pedobacter terrae*, *P. agri* and *P. humicola* were isolated from soil (Yoon *et al.*, 2007; Roh *et al.*, 2008; Dahal & Kim, 2016). *P. roseus* was isolated from a pond (Hwang *et al.*, 2006). *P. suwonensis* was isolated from the rhizosphere of *Brassica campestris* in China (Kwon *et al.*, 2007).

Sphingomonas are Gram-negative *Proteobacteria*. *S. faeni* was described as psychrotolerant and could be used to prevent food-spoilage at low temperature (Busse *et al.*, 2003; Mageswari *et al.*, 2015). *S. olei* was described as a aliphatic hydrocarbon degrading bacteria (Young *et al.*, 2007; Chaudhary & Kim, 2017).

Paenibacillus are part of the *Firmicutes* phylum. Interest for *Paenibacillus* is growing as many species showed PGRP and biocontrol activities (Priest, 2015; Lal *et al.*, 2016; Grady *et al.*, 2016). *P. illinoisiensis* alone produces chitinases and siderophores, combining antifungal activity and iron nutrition promotion (S *et al.*, 2016; Liu *et al.*, 2017).

Rhodobacter are *Proteobacteria* of which the eleven known species are photosynthetic bacteria that can live in anoxic conditions using hydrogen or sulfur as electron donors (Imhoff, 2015). The most studied species is *R. sphaeroides*.

Cladosporium are endophytic *Ascomycota*. *Cladosporium cladosporioides* and *C. tenuissimum* are known to produces taxol and cladospolides which have antifungal activities but also shown plant growth regulation activities (Scott *et al.*, 1971; Hirota *et al.*, 1985; Fujii *et al.*, 1995; Zhang *et al.*, 2009; Wang *et al.*, 2013).

Plectosphaerella cucumerina is a phytopathogenic *Ascomycota* that is also studied for is potential biocontrol activity against potato cyst nematodes and *Pseudomonas aeruginosa* (Uecker, 1993; Atkins *et al.*, 2003; Pétriacq *et al.*, 2016; Zhou *et al.*, 2017).

Fusarium are *Ascomycota* that harbor well-known phytopathogenic species such as *Fusarium avenaceum, F. acuminatum* and *F. oxysporum* (Michielse & Rep, 2009; Munkvold, 2017). *Fusarium avenaceum* and *F. acuminatum* are part of the same species complex and were found to have an ice nucleation activity (Pouleur *et al.*, 1992; Munkvold, 2017). Some strains of *F. oxysporum* are nonpathogenic and one was found to induce resistance against pathogenic strain in tomato (Fuchs *et al.*, 1997).

Vishniacozyma are a *Basidiomycota* yeast. *V. victoria* were frequently observed in phylloplane and also used as a biocontrol agent against fruit mold during conservation (Kemler *et al.*, 2017; Glushakova & Kachalkin, 2017; Gramisci *et al.*, 2018).

Alternaria is an *Ascomycota* genus that includes both pathogenic and saprophytic species. *A. infectoria* is a species complex closely related to *Alternaria alternata* (Andersen & Thrane, 1996; Andersen *et al.*, 2009). Both were described as phytopathogenic fungi (Mc Roberts & Lennard, 1996; Vergnes *et al.*, 2006; Perelló *et al.*, 2008).

All these species were conserved among the *P. ramosa* seeds and therefore should play a key role in the parasite fitness. Moreover, some of these species were also found in soil and stimulated seeds, emerging the hypothesis that they were selected from soil and conserved among the parasitic life cycle.

4. Dendrogram clusters are based on Euclidean distance

Genetic distance based on microsatellites, phenotypic measures (EC50 here) and microbial diversity data sets were very different. To compare these three datasets altogether, we choose to use the basic clustering method: Euclidean distance calculation and UPGMA clustering. Using the same method for these three datasets allowed us to compare the resulting dendrograms between each other's.

However, Euclidean distance is not the distance calculation method usually apply on these types of data. For microsatellite data, Bruvo's distance calculation is recommended (Bruvo *et al.*, 2004; Stojanova *et al.*, 2019). For microbial diversity, three distance calculations are commonly use: Jaccard index, Bray-Curtis index and UniFrac (Lozupone & Knight, 2005; Anderson *et al.*, 2011; Parks & Beiko, 2012). Jaccard calculation integrate presence versus absence of the taxonomic unit in the samples. Bray-Curtis calculation is based in the taxonomic unit abundance within samples. UniFrac is as well based on abundance but also on phylogenetic distance of the taxonomic units.

Using different distance calculation methods can lead to different clusters, , thus the Euclidean / UPGMA approach was compared to other methods and validated. Indeed, clusters observed for phenotype reproduced the statistic differences observed with ANOVA and post-hoc Tukey. The cluster observed for bacterial and fungal communities were retrieved in the PCoA based on Bray-Curtis distances.

Therefore, clusters were considered comparable among the different methods used. Parasitic seeds originating from oilseed rape showed the most homogenous features. Parasitic seeds originating from tobacco showed the most heterogenous features. Parasitic seeds originating from hemp showed intermediate features. This observation mirrors the host range of *P. ramosa* described genotypes (Stojanova *et al.*, 2019). Indeed, seeds of genotype 1, whose preferential host is winter oilseed rape, have the narrowest host range and the closest genetic distances. Seeds from

genotype 2b, whose preferential host is tobacco, have the largest host range and the highest diversity and heterogeneity. Seeds of genotype 2a, whose preferential host is hemp, have an intermediate host range and intermediate heterogeneity.

CONCLUSIONS

In this study, we used 26 *P. ramosa* seed lots to explore the seed microbiota and to find recurrent pattern linked to the originating host.

By comparing microbial communities present in these lots, we extracted the *P. ramosa* seed core microbiota. This core microbiota is composed of ten bacterial haplotypes belonging to seven genera (*Pseudomonas*, *Stenotrophomonas*, *Sphingomonas*, *Pedobacter*, *Paenibacillus*, *Rhizobium* and *Rhodobacter*) and seven fungal haplotypes belonging to four genera (*Vishniacozyma*, *Alternaria*, *Plectosphaerella*, *Fusarium* and *Cladosporium*). Five of these haplotypes were differentially abundant between originating host and ten were also found in soil and stimulated seeds.

Considering several factors, we implemented PCoA to determine which factor were the most influent on the microbial community composition. We determined that the factor with the greatest weight was the originating host of the seeds, closely followed by the originating field of the seeds. We can therefore conclude that the originating host *P. ramosa* shape the composition of its seed microbial communities. Additionally, within each originating host, the originating field shape the seed microbial communities.

Assessing the seed sensitivity to plant allelochemicals, we determined that germination phenotype was different regarding the originating host of the seeds. Regarding the seed genotype, seeds originating from oilseed rape fields and seeds originating from hemp fields constituted two monophyletic groups, while seeds originating from tobacco fields showed the highest heterogeneity. Therefore, as seed lots came from a sampling campaign, distinction between the genotype effect and the originating host effect may need further genotypic analysis such as the use of multilocus genotypes (MLG). However, we can conclude that the seed originating from oilseed rape fields had the most homogeneous genotype, phenotype and microbial community composition. At the opposite, seed originating from tobacco fields had the most heterogeneous genotype, phenotype and microbial community composition while seeds originating from hemp fields showed intermediate genetic diversity, and microbial community composition and distinct phenotypes.

PERSPECTIVES

1. Further analysis on the obtained dataset

During the sequencing effort, not only seed lots but also soil and stimulated seed microbial communities were sequenced. Therefore, these obtained data will be investigated next. The soil sample analysis could help us determine if the soil microbial communities are also influence by the host. Regarding the literature, it is already established that soil microbiome influence crop health and that crops can also shape the soil microbiome (Mendes *et al.*, 2011; Chaparro *et al.*, 2012; Berendsen *et al.*, 2012; Berg *et al.*, 2014; Hartman *et al.*, 2018).

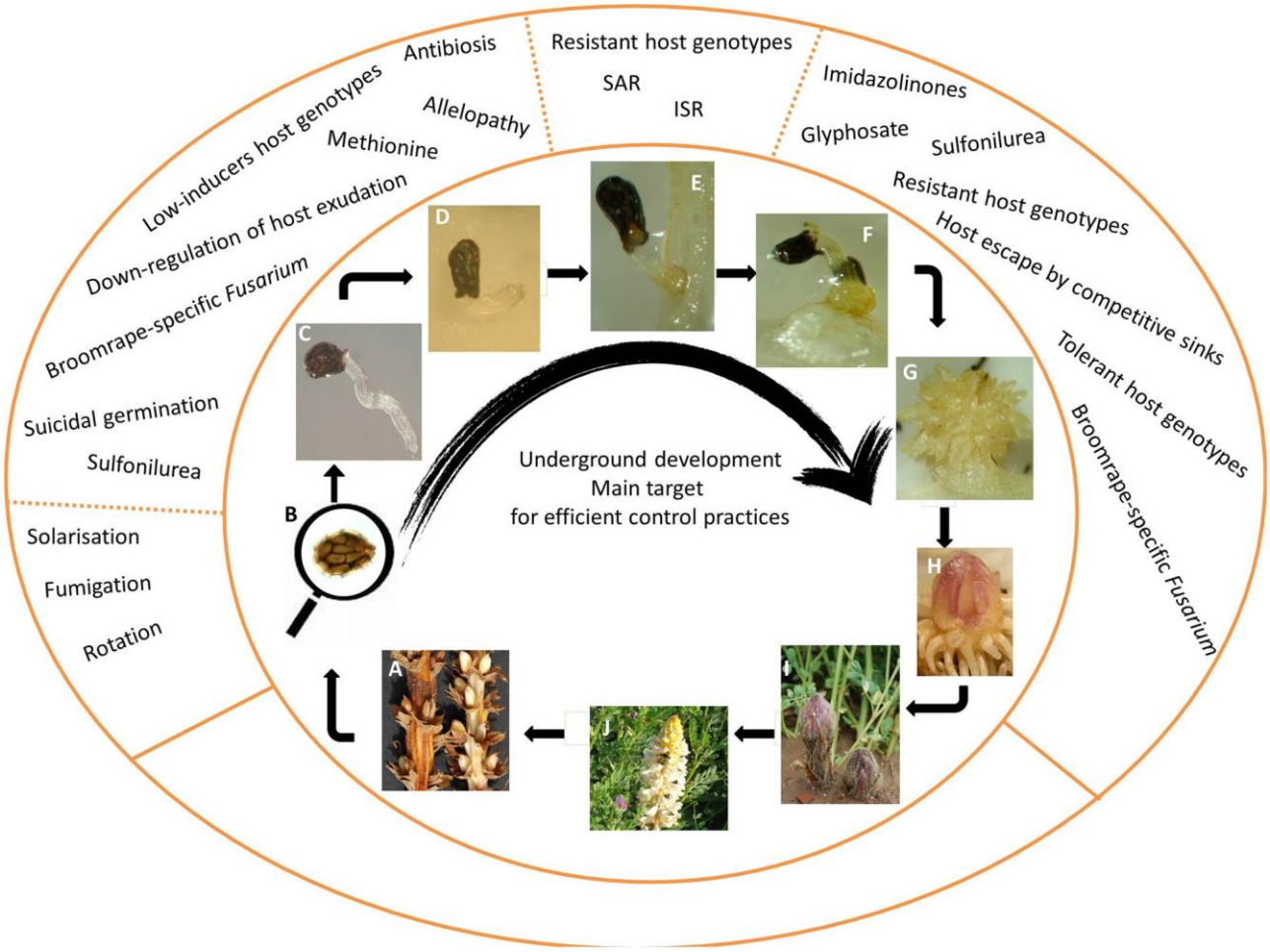


Figure 17: Illustration of broomrape life stages and mechanisms of control. (A) Fructification and dehiscence of capsules containing mature seeds; (B) microscopic view of a seed (size ranging 0.2–2 mm) that undergoes successive dispersal, primary dormancy and annual release of secondary dormancy; (C) broomrape embryo does not develop morphologically identified cotyledons or shoot meristem and upon host-induced germination, only a radicle emerges from the seed with the function of searching and contacting the host root; (D) upon haustorial induction, the radicle stops elongating and a single terminal haustorium is differentiated. The first function of haustorium is as adhesion organ to host root surface mediated by a papillae cell layer; (E) adhesion to the root 3 days after germination induction; (F) upon vascular connection with the host, bloomrape initiates the development of the tubercle, the broomrape storage organ for host-derived nutrients. A swelling of the host root at the penetration point is also observed due the parasitic stimulation of host tissue proliferation; (G) tubercle develops a crown of adventitious roots; (H) tubercle differentiates apical shoot meristem (single shoot meristem for Orobanche species and several shoot meristems for Phelipanche species); (I) the underground shoot eventually emerges through the root surface; (J) flowering and pollination occur. Some broomrape species are out crossers while others are self-pollinating. From Fernández-Aparicio *et al.*, 2016

Considering the stimulated seeds, further analysis will focus on the influence of each germination stimulant on the microbiota composition.

2. Microbiota functional profiling

The genera belonging to the core microbiota may have a primordial function that confers selective advantages in the *P. ramosa* seed habitat. Some of the identified haplotypes are known to promote plant growth. Some are known as pathogens. However, to clearly understand the role and functions of the core haplotypes, a metatranscriptomic approach could be considered. Indeed, metagenomic approaches allow us to identify bacteria and fungi present in the habitat but does not reveal us their functions and roles. At the opposite, an RNA amplicon sequencing approach allows us to determine which genes are effectively expressed and by which organisms (Klappenbach *et al.*, 2000; Kaul *et al.*, 2016). Therefore, RNAseq approaches appears to be the most suitable method to unravel the intricate molecular mechanisms of the tripartite dialogue occurring belowground between the micro-organisms, the parasitic plant ant its host plant.

3. Parasitic plant control methods

As parasitic plant is a long-standing and a recurrent issue in agriculture, lots of methods have been developed trying to reduce the impact of a field infestation on crop yields (Fernández-Aparicio *et al.*, 2016). These methods include cultural practices, soil solarization, chemical control, biological control and host resistance (Figure 17). The control methods target different stage of the broomrape life cycle and therefore try to i) reduce the seed soil bank, ii) inhibit the parasitic seed germination, iii) reduce the parasite seedling attachment to its host plant and iv) reduce the aggressivity of broomrape once it is connected to its host. However, broomrape are still spreading across Europe and Asia, bringing to light the lack of specific control program across countries and the low effectiveness of the already used methods.

Nevertheless, some new and promising biological methods are still under scientific evaluation. Among these new methods, some use micro-organisms to act as biocontrol agent against *P. ramosa*. Indeed, some fungi produce efficient mycoherbicides against broomrape (Müller-Stöver, 2001; Boari & Vurro, 2004; Kohlschmid *et al.*, 2009). Some bacteria induce suicidal germination while others inhibit the radicle growth of different *Orobanchaceae* species (Ahonsi *et al.*, 2003; Dadon *et al.*, 2004; Barghouthi & Salman, 2010). Moreover, some micro-organisms such as the arbuscular mycorrhizal fungi *Rhizobium leguminosarum* or the *Proteobacteria Azospirillum brasilense* interfere in the broomrape capacity to recognize their host by modifying the composition of the host root exudates (Dadon *et al.*, 2004; Mabrouk *et al.*, 2007; Fernández-Aparicio *et al.*, 2010; López-Ráez *et al.*, 2011; Louarn *et al.*, 2012). Therefore, decipher the mechanisms of the tripartite dialogue between the micro-organisms, the parasitic plant ant its host plant could help find new biological control methods against *Phelipanche ramosa*.

Bibliography

Ahonsi MO, Berner DK, Emechebe AM, Lagoke ST, Sanginga N. **2003**. Potential of ethylene-producing pseudomonads in combination with effective N2-fixing bradyrhizobial strains as supplements to legume rotation for Striga hermonthica control. *Biological Control* **28**: 1–10.

Alavi P, Starcher M, Zachow C, Mueller H, Berg G. **2013**. Root-microbe systems: the effect and mode of interaction of Stress Protecting Agent (SPA) Stenotrophomonas rhizophila DSM14405T. *Frontiers in Plant Science* **4**.

Al-Babili S, Bouwmeester HJ. **2015**. Strigolactones, a Novel Carotenoid-Derived Plant Hormone. *Annual Review of Plant Biology* **66**: 161–186.

Albaser A, Kazana E, Bennett MH, Cebeci F, Luang-In V, Spanu PD, Rossiter JT. **2016**. Discovery of a Bacterial Glycoside Hydrolase Family 3 (GH3) β-Glucosidase with Myrosinase Activity from a Citrobacter Strain Isolated from Soil. *Journal of Agricultural and Food Chemistry* **64**: 1520–1527.

Albrecht H, Yoder JI, Phillips DA. **1999**. Flavonoids Promote Haustoria Formation in the Root ParasiteTriphysaria versicolor. *Plant Physiology* **119**: 585–592.

Alegre I, Viñas I, Usall J, Anguera M, Altisent R, Abadias M. **2013**. Antagonistic effect of Pseudomonas graminis CPA-7 against foodborne pathogens in fresh-cut apples under simulated commercial conditions. *Food Microbiology* **33**: 139–148.

Andersen B, Sørensen JL, Nielsen KF, van den Ende BG, de Hoog S. **2009**. A polyphasic approach to the taxonomy of the Alternaria infectoria species–group. *Fungal Genetics and Biology* **46**: 642–656.

Andersen B, Thrane U. **1996**. Differentiation of Altemaria infectoria and Alternaria alternata based on morphology, metabolite profiles, and cultural characteristics. *Canadian Journal of Microbiology* **42**: 685–689.

Anderson MJ, Crist TO, Chase JM, Vellend M, Inouye BD, Freestone AL, Sanders NJ, Cornell HV, Comita LS, Davies KF, *et al.* 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecology Letters* **14**: 19–28.

Andrews S. **2010**. *FastQC: a quality control tool for high throughput sequence data*.

Atkins SD, Clark IM, Sosnowska D, Hirsch PR, Kerry BR. **2003**. Detection and Quantification of Plectosphaerella cucumerina, a Potential Biological Control Agent of Potato Cyst Nematodes, by Using Conventional PCR, Real-Time PCR, Selective Media, and Baiting. *Applied and Environmental Microbiology* **69**: 4788–4793.

Auger B, Pouvreau J-B, Pouponneau K, Yoneyama K, Montiel G, Le Bizec B, Yoneyama K, Delavault P, Delourme R, Simier P. **2012**. Germination Stimulants of Phelipanche ramosa in the Rhizosphere of Brassica napus Are Derived from the Glucosinolate Pathway. *Molecular Plant-Microbe Interactions* **25**: 993–1004.

Aujoulat F, Jumas-Bilak E, Masnou A, Sallé F, Faure D, Segonds C, Marchandin H, Teyssier C. **2011**. Multilocus Sequence-Based Analysis Delineates a Clonal Population of Agrobacterium (Rhizobium) radiobacter (Agrobacterium tumefaciens) of Human Origin. *Journal of Bacteriology* **193**: 2608–2618.

Balvočiūtė M, Huson DH. **2017**. SILVA, RDP, Greengenes, NCBI and OTT — how do these taxonomies compare? *BMC Genomics* **18**: 114.

Barghouthi S, Salman M. **2010**. Bacterial inhibition of Orobanche aegyptiaca and Orobanche cernua radical elongation. *Biocontrol Science and Technology* **20**: 423–435.

Beals EW. **1984**. Bray-Curtis Ordination: An Effective Strategy for Analysis of Multivariate Ecological Data. In: MacFadyen A, Ford ED, eds. Advances in Ecological Research. Academic Press, 1–55.

Behrendt U, Ulrich A, Schumann P, Erler W, Burghardt J, Seyfarth W. **1999**. A taxonomic study of bacteria isolated from grasses: a proposed new species Pseudomonas graminis sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **49**: 297–308.

Berendsen RL, Pieterse CMJ, Bakker PAHM. **2012**. The rhizosphere microbiome and plant health. *Trends in Plant Science* **17**: 478–486.

Berg G, Eberl L, Hartmann A. **2005**. The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environmental Microbiology* **7**: 1673–1685.

Berg G, Egamberdieva D, Lugtenberg B, Hagemann M. **2010**. Symbiotic Plant–Microbe Interactions: Stress Protection, Plant Growth Promotion, and Biocontrol by Stenotrophomonas. In: Seckbach J, Grube M, eds. Cellular Origin, Life in Extreme Habitats and Astrobiology. Symbioses and Stress: Joint Ventures in Biology. Dordrecht: Springer Netherlands, 445–460.

Berg G, Grube M, Schloter M, Smalla K. **2014**. Unraveling the plant microbiome: looking back and future perspectives. *Frontiers in Microbiology* **5**.

Berg G, Knaape C, Ballin G, Seidel D. **1994**. Biological control of Verticillium dahliae kleb. by natural occurring rhizosphere bacteria. *Archives of Phytopathology and Plant Protection* **29**: 249–262.

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Séjalon-Delmas N. **2006**. Strigolactones Stimulate Arbuscular Mycorrhizal Fungi by Activating Mitochondria. *PLOS Biology* **4**: e226.

Billing E. **1970**. Pseudomonas viridiflava (Burkholder, 1930; Clara 1934). *Journal of Applied Bacteriology* **33**: 492–500.

Boari A, Vurro M. **2004**. Evaluation of Fusarium spp. and other fungi as biological control agents of broomrape (Orobanche ramosa). *Biological Control* **30**: 212–219.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, *et al.* 2018. *QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science*. PeerJ Inc.

Bouwmeester HJ, Roux C, Lopez-Raez JA, Bécard G. **2007**. Rhizosphere communication of plants, parasitic plants and AM fungi. *Trends in Plant Science* **12**: 224–230.

Bray JR, Curtis JT. **1957**. An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecological Monographs* **27**: 325–349.

Bressan M, Roncato M-A, Bellvert F, Comte G, Haichar F el Z, Achouak W, Berge O. **2009**. Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. *The ISME Journal* **3**: 1243–1257.

Brewer PB, Koltai H, Beveridge CA. **2013**. Diverse Roles of Strigolactones in Plant Development. *Molecular Plant* **6**: 18–28.

Bruce JB, Cooper GA, Chabas H, West SA, Griffin AS. **2017**. Cheating and resistance to cheating in natural populations of the bacterium Pseudomonas fluorescens. *Evolution* **71**: 2484–2495.

Bruvo R, Michiels NK, D'souza TG, Schulenburg H. **2004**. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology* **13**: 2101–2106.

Buée M, Reich M, Murat C. **2009**. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity - Buée - 2009 - New Phytologist - Wiley Online Library.

Burkholder WH. **1930**. *Bacterial diseases of the bean*. Cornell University, Agricultural Experiment Station; Ithaca, New York.

Busse H-J, Denner EBM, Buczolits S, Salkinoja-Salonen M, Bennasar A, Kämpfer P. **2003**. Sphingomonas aurantiaca sp. nov., Sphingomonas aerolata sp. nov. and Sphingomonas faeni sp. nov., air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria, and emended description of the genus Sphingomonas. *International Journal of Systematic and Evolutionary Microbiology* **53**: 1253–1260.

Butler LG (Purdue U. **1995**. Chemical communication between the parasitic weed Striga and its crop host: a new dimension in allelochemistry.

Callahan BJ, McMurdie PJ, Holmes SP. **2017**. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal* **11**: 2639–2643.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Candolle AP de. **1813**. *Théorie élémentaire de la botanique, ou, Exposition des principes de la classification naturelle et de l'art de décrire et d'étudier les végétaux*. Roret.

Caporaso JG, Lauber C, Walters W. **2011**. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample | PNAS.

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM. **2012**. Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils* **48**: 489–499.

Chaudhary DK, Kim J. **2017**. Sphingomonas olei sp. nov., with the ability to degrade aliphatic hydrocarbons, isolated from oil-contaminated soil. *International Journal of Systematic and Evolutionary Microbiology* **67**: 2731–2738.

Cook CE, Whichard LP, Turner B, Wall ME, Egley GH. **1966**. Germination of Witchweed (Striga lutea Lour.): Isolation and Properties of a Potent Stimulant. *Science* **154**: 1189–1190.

Dadon T, Nun NB, Mayer AM. **2004**. A factor from Azospirillum brasilense inhibits germination and radicle growth of Orobanche aegyptiaca. *Israel Journal of Plant Sciences* **52**: 83–86.

Dahal RH, Kim J. **2016**. Pedobacter humicola sp. nov., a member of the genus Pedobacter isolated from soil. *International Journal of Systematic and Evolutionary Microbiology* **66**: 2205–2211.

van Dam NM, Tytgat TOG, Kirkegaard JA. **2009**. Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems. *Phytochemistry Reviews* **8**: 171–186.

Dunne C, Moënne-Loccoz Y, McCarthy J, Higgins P, Powell J, Dowling DN, O'Gara F. **1998**. Combining proteolytic and phloroglucinol-producing bacteria for improved biocontrol of Pythium-mediated damping-off of sugar beet. *Plant Pathology* **47**: 299–307.

Egamberdieva D, Jabborova D, Berg G. **2016**. Synergistic interactions between Bradyrhizobium japonicum and the endophyte Stenotrophomonas rhizophila and their effects on growth, and nodulation of soybean under salt stress. *Plant and Soil* **405**: 35–45.

Ewels P, Magnusson M, Lundin S, Käller M. **2016**. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* **32**: 3047–3048.

Fahey JW, Zalcmann AT, Talalay P. **2001**. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **56**: 5–51.

Fernández-Aparicio M, García-Garrido JM, Ocampo JA, Rubiales D. **2010**. Colonisation of field pea roots by arbuscular mycorrhizal fungi reduces Orobanche and Phelipanche species seed germination. *Weed Research* **50**: 262–268.

Fernández-Aparicio M, Reboud X, Gibot-Leclerc S. **2016**. Broomrape Weeds. Underground Mechanisms of Parasitism and Associated Strategies for their Control: A Review. *Frontiers in Plant Science* **7**.

Ferret C. **2012**. Rôle des Pseudomonas fluorescents dans la biodisponibilté des métaux contaminant les minéraux du sol: application à la phytoremédiation.

Flematti GR, Scaffidi A, Waters MT, Smith SM. **2016**. Stereospecificity in strigolactone biosynthesis and perception. *Planta* **243**: 1361–1373.

Fuchs J-G, Moënne-Loccoz Y, Défago G. **1997**. Nonpathogenic Fusarium oxysporum Strain Fo47 Induces Resistance to Fusarium Wilt in Tomato. *Plant Disease* **81**: 492–496.

Fujihara H, Yamazoe A, Hosoyama A, Suenaga H, Kimura N, Hirose J, Watanabe T, Futagami T, Goto M, Furukawa K. **2015**. Draft Genome Sequence of Pseudomonas abietaniphila KF701 (NBRC110664), a Polychlorinated Biphenyl-Degrading Bacterium Isolated from Biphenyl-Contaminated Soil. *Genome Announcements* **3**: e00473-15.

Fujii Y, Fukuda A, Hamasaki T, Ichimoto I, Nakajima H. **1995**. Twelve-membered lactones produced by cladosporium tenuissimum and the plant growth retardant activity of cladospolide B. *Phytochemistry* **40**: 1443–1446.

Gao J, Xie G, Peng F, Xie Z. **2015**. Pseudomonas donghuensis sp. nov., exhibiting high-yields of siderophore. *Antonie van Leeuwenhoek* **107**: 83–94.

Garrido-Sanz D, Meier-Kolthoff JP, Göker M, Martín M, Rivilla R, Redondo-Nieto M. **2016**. Genomic and Genetic Diversity within the Pseudomonas fluorescens Complex. *PLOS ONE* **11**: e0150183.

Glushakova AM, Kachalkin AV. **2017**. Endophytic yeasts in leaf galls. *Microbiology* **86**: 250–256.

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, Pillot J-P, Letisse F, Matusova R, Danoun S, Portais J-C, *et al.* 2008. Strigolactone inhibition of shoot branching. *Nature* **455**: 189–194.

Goyet V, Billard E, Pouvreau J-B, Lechat M-M, Pelletier S, Bahut M, Monteau F, Spíchal L, Delavault P, Montiel G, *et al.* 2017. Haustorium initiation in the obligate parasitic plant Phelipanche ramosa involves a host-exudated cytokinin signal. *Journal of Experimental Botany* **68**: 5539–5552.

Grady EN, MacDonald J, Liu L, Richman A, Yuan Z-C. **2016**. Current knowledge and perspectives of Paenibacillus: a review. *Microbial Cell Factories* **15**: 203.

Gramisci BR, Lutz MC, Lopes CA, Sangorrín MP. **2018**. Enhancing the efficacy of yeast biocontrol agents against postharvest pathogens through nutrient profiling and the use of other additives. *Biological Control* **121**: 151–158.

Gressel J, Hanafi A, Head G, Marasas W, Obilana AB, Ochanda J, Souissi T, Tzotzos G. **2004**. Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. *Crop Protection* **23**: 661–689.

Großkinsky DK, Tafner R, Moreno MV, Stenglein SA, García de Salamone IE, Nelson LM, Novák O, Strnad M, van der Graaff E, Roitsch T. **2016**. Cytokinin production by *Pseudomonas fluorescens* G20-18 determines biocontrol activity against *Pseudomonas syringae* in *Arabidopsis*. *Scientific Reports* **6**: 23310.

Hagemann M, Hasse D, Berg G. **2006**. Detection of a Phage Genome Carrying a Zonula Occludens like Toxin Gene (zot) in clinical isolates of Stenotrophomonas maltophilia. *Archives of Microbiology* **185**: 449–458.

Hartman K, van der Heijden MGA, Wittwer RA, Banerjee S, Walser J-C, Schlaeppi K. **2018**. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* **6**: 14.

Heide-Jørgensen H. **2008**. *Parasitic flowering plants*. BRILL.

Heide-Jørgensen HS. **2013**. Introduction: The Parasitic Syndrome in Higher Plants. In: Joel DM, Gressel J, Musselman LJ, eds. Parasitic Orobanchaceae: Parasitic Mechanisms and Control Strategies. Berlin, Heidelberg: Springer Berlin Heidelberg, 1–18.

Hirota A, Sakai H, Isogai A. **1985**. New Plant Growth Regulators, Cladospolide A and B, Macrolides Produced by Cladosporium cladosporioides. *Agricultural and Biological Chemistry* **49**: 731–735.

Howell CR, Stipanovic RD. **1979**. Control of Rhizoctonia solani on cotton seedlings with Pseudomonas fluorescens and with an antibiotic produced by the bacterium. *Phytopathology* **69**: 480–482.

Hwang CY, Choi DH, Cho BC. **2006**. Pedobacter roseus sp. nov., isolated from a hypertrophic pond, and emended description of the genus Pedobacter. *International Journal of Systematic and Evolutionary Microbiology* **56**: 1831–1836.

Imhoff JF. **2015**. Rhodobacter. In: Bergey's Manual of Systematics of Archaea and Bacteria. American Cancer Society, 1–12.

Jakob K, Goss EM, Araki H, Van T, Kreitman M, Bergelson J. **2002**. Pseudomonas viridiflava and P. syringae—Natural Pathogens of Arabidopsis thaliana. *Molecular Plant-Microbe Interactions* **15**: 1195–1203.

Joel DM, Losner-Goshen D. **1994**. The attachment organ of the parasitic angiosperms Orobanche cumana and O. aegyptiaca and its development. *Canadian Journal of Botany* **72**: 564–574.

Johnson AW, Rosebery G, Parker C. **1976**. A novel approach to Striga and Orobanche control using synthetic germination stimulants. *Weed Research* **16**: 223–227.

Kaparullina E, Doronina N, Chistyakova T, Trotsenko Y. **2009**. Stenotrophomonas chelatiphaga sp. nov., a new aerobic EDTA-degrading bacterium. *Systematic and Applied Microbiology* **32**: 157–162.

Kaul S, Sharma T, K. Dhar M. **2016**. "Omics" Tools for Better Understanding the Plant–Endophyte Interactions. *Frontiers in Plant Science* **7**.

Kemler M, Witfeld F, Begerow D, Yurkov A. **2017**. Phylloplane Yeasts in Temperate Climates. In: Buzzini P, Lachance M-A, Yurkov A, eds. Yeasts in Natural Ecosystems: Diversity. Cham: Springer International Publishing, 171–197.

Keyes WJ, O'Malley RC, Kim D, Lynn DG. **2000**. Signaling Organogenesis in Parasitic Angiosperms: Xenognosin Generation, Perception, and Response. *Journal of Plant Growth Regulation* **19**: 217–231.

Kimura N, Yamazoe A, Hosoyama A, Hirose J, Watanabe T, Suenaga H, Fujihara H, Futagami T, Goto M, Furukawa K. **2015**. Draft Genome Sequence of Pseudomonas abietaniphila KF717 (NBRC 110669), Isolated from Biphenyl-Contaminated Soil in Japan. *Genome Announcements* **3**: e00059-15.

Klaedtke S, Jacques M-A, Raggi L, Préveaux A, Bonneau S, Negri V, Chable V, Barret M. **2016**. Terroir is a key driver of seed-associated microbial assemblages. *Environmental Microbiology* **18**: 1792–1804.

Klappenbach JA, Dunbar JM, Schmidt TM. **2000**. rRNA Operon Copy Number Reflects Ecological Strategies of Bacteria. *Applied and Environmental Microbiology* **66**: 1328–1333.

Kohlschmid E, Sauerborn J, Müller-Stöver D. **2009**. Impact of Fusarium oxysporum on the holoparasitic weed Phelipanche ramosa: biocontrol efficacy under field-grown conditions. *Weed Research* **49**: 56–65.

Kudoyarova GR, Melentiev AI, Martynenko EV, Timergalina LN, Arkhipova TN, Shendel GV, Kuz'mina LY, Dodd IC, Veselov SY. **2014**. Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. *Plant Physiology and Biochemistry* **83**: 285–291.

Kuijt J. **1969**. The biology of parasitic flowering plants. University of California Press, Berkeley. *The biology of parasitic flowering plants. University of California Press, Berkeley.*

Kwon S-W, Kim B-Y, Lee K-H, Jang K-Y, Seok S-J, Kwon J-S, Kim W-G, Weon H-Y. **2007**. Pedobacter suwonensis sp. nov., isolated from the rhizosphere of Chinese cabbage (Brassica campestris). *International Journal of Systematic and Evolutionary Microbiology* **57**: 480–484.

Kwon SW, Kim JS, Park IC, Yoon SH, Park DH, Lim CK, Go SJ. **2003**. Pseudomonas koreensis sp. nov., Pseudomonas umsongensis sp. nov. and Pseudomonas jinjuensis sp. nov., novel species from farm soils in Korea. *International Journal of Systematic and Evolutionary Microbiology* **53**: 21–27.

Lal S, Chiarini L, Tabacchioni S. **2016**. New Insights in Plant-Associated Paenibacillus Species: Biocontrol and Plant Growth-Promoting Activity. In: Islam MT, Rahman M, Pandey P, Jha CK, Aeron A, eds. Bacilli and Agrobiotechnology. Cham: Springer International Publishing, 237–279.

Lau JA, Lennon JT. **2012**. Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences*: 201202319.

Lechat M-M, Pouvreau J-B, Péron T, Gauthier M, Montiel G, Véronési C, Todoroki Y, Le Bizec B, Monteau F, Macherel D, *et al.* 2012. PrCYP707A1, an ABA catabolic gene, is a key component of Phelipanche ramosa seed germination in response to the strigolactone analogue GR24. *Journal of Experimental Botany* **63**: 5311–5322.

Letunic I, Bork P. **2019**. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research.*

Liu D, Yang Q, Ge K, Hu X, Qi G, Du B, Liu K, Ding Y. **2017**. Promotion of iron nutrition and growth on peanut by Paenibacillus illinoisensis and Bacillus sp. strains in calcareous soil. *Brazilian Journal of Microbiology* **48**: 656–670.

López JR, Diéguez AL, Doce A, De la Roca E, De la Herran R, Navas JI, Toranzo AE, Romalde JL. **2012**. Pseudomonas baetica sp. nov., a fish pathogen isolated from wedge sole, Dicologlossa cuneata (Moreau). *International Journal of Systematic and Evolutionary Microbiology* **62**: 874–882.

López-Ráez JA, Charnikhova T, Fernández I, Bouwmeester H, Pozo MJ. **2011**. Arbuscular mycorrhizal symbiosis decreases strigolactone production in tomato. *Journal of Plant Physiology* **168**: 294–297.

Louarn J, Carbonne F, Delavault P, Bécard G, Rochange S. **2012**. Reduced Germination of Orobanche cumana Seeds in the Presence of Arbuscular Mycorrhizal Fungi or Their Exudates. *PLOS ONE* **7**: e49273.

Lozupone C, Knight R. **2005**. UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. *Applied and Environmental Microbiology* **71**: 8228–8235.

Luján Adela M., Gómez Pedro, Buckling Angus. **2015**. Siderophore cooperation of the bacterium Pseudomonas fluorescens in soil. *Biology Letters* **11**: 20140934.

Mabrouk Y, Simier P, Arfaoui A, Sifi B, Delavault P, Zourgui L, Belhadj O. **2007**. Induction of Phenolic Compounds in Pea (Pisum sativum L.) Inoculated by Rhizobium leguminosarum and Infected with Orobanche crenata. *Journal of Phytopathology* **155**: 728–734.

Mageswari A, Subramanian P, Srinivasan R, Karthikeyan S, Gothandam KM. **2015**. Astaxanthin from psychrotrophic Sphingomonas faeni exhibits antagonism against food-spoilage bacteria at low temperatures. *Microbiological Research* **179**: 38–44.

Margesin R, Shivaji S. **2015**. Pedobacter. In: Bergey's Manual of Systematics of Archaea and Bacteria. American Cancer Society, 1–17.

Martin M. **2011**. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **17**: 10–12.

Martin VJJ, Mohn WW. **1999**. A Novel Aromatic-Ring-Hydroxylating Dioxygenase from the Diterpenoid-Degrading Bacterium Pseudomonas abietaniphila BKME-9. *Journal of Bacteriology* **181**: 2675–2682.

Mc Roberts N, Lennard JH. **1996**. Pathogen behaviour and plant cell reactions in interactions between Alternaria species and leaves of host and nonhost plants. *Plant Pathology* **45**: 742–752.

Mendes R, Kruijt M, Bruijn I de, Dekkers E, Voort M van der, Schneider JHM, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, *et al.* 2011. Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science* **332**: 1097–1100.

Michielse CB, Rep M. **2009**. Pathogen profile update: Fusarium oxysporum. *Molecular Plant Pathology* **10**: 311–324.

Müller-Stöver D. **2001**. Possibilities of biological control of Orobanche crenata and O. cumana with Ulocladium botrytis and Fusarium oxysporum f. sp. orthoceras. *Agroecology* **3**.

Munkvold GP. **2017**. Fusarium Species and Their Associated Mycotoxins. In: Moretti A, Susca A, eds. Methods in Molecular Biology. Mycotoxigenic Fungi: Methods and Protocols. New York, NY: Springer New York, 51–106.

Muttray AF, Yu Z, Mohn WW. **2001**. Population dynamics and metabolic activity of Pseudomonas abietaniphila BKME-9 within pulp mill wastewater microbial communities assayed by competitive PCR and RT-PCR. *FEMS Microbiology Ecology* **38**: 21–31.

Ossowicki A, Jafra S, Garbeva P. **2017**. The antimicrobial volatile power of the rhizospheric isolate Pseudomonas donghuensis P482. *PLOS ONE* **12**: e0174362.

Parker C. **2009**. Observations on the current status of Orobanche and Striga problems worldwide. *Pest Management Science* **65**: 453–459.

Parker C. **2012**. Parasitic Weeds: A World Challenge. *Weed Science* **60**: 269–276.

Parks DH, Beiko RG. **2012**. Measuring Community Similarity with Phylogenetic Networks. *Molecular Biology and Evolution* **29**: 3947–3958.

Paulsen IT, Press CM, Ravel J, Kobayashi DY, Myers GSA, Mavrodi DV, DeBoy RT, Seshadri R, Ren Q, Madupu R, *et al.* 2005. Complete genome sequence of the plant commensal Pseudomonas fluorescens Pf-5. *Nature Biotechnology* **23**: 873.

Peix A, Rivas R, Mateos PF, Martínez-Molina E, Rodríguez-Barrueco C, Velázquez E. **2003**. Pseudomonas rhizosphaerae sp. nov., a novel species that actively solubilizes phosphate in vitro. *International Journal of Systematic and Evolutionary Microbiology* **53**: 2067–2072.

Peix A, Rivas R, Santa-Regina I, Mateos PF, Martínez-Molina E, Rodríguez-Barrueco C, Velázquez E. **2004**. Pseudomonas lutea sp. nov., a novel phosphate-solubilizing bacterium isolated from the rhizosphere of grasses. *International Journal of Systematic and Evolutionary Microbiology* **54**: 847–850.

Perelló A, Moreno M, Sisterna M. **2008**. Alternaria infectoria species-group associated with black point of wheat in Argentina. *Plant Pathology* **57**: 379–379.

Pétriacq P, Stassen JHM, Ton J. **2016**. Spore Density Determines Infection Strategy by the Plant Pathogenic Fungus Plectosphaerella cucumerina. *Plant Physiology* **170**: 2325–2339.

Plaza L, Altisent R, Alegre I, Viñas I, Abadias M. **2016**. Changes in the quality and antioxidant properties of fresh-cut melon treated with the biopreservative culture Pseudomonas graminis CPA-7 during refrigerated storage. *Postharvest Biology and Technology* **111**: 25–30.

Pouleur S, Richard C, Martin J-G, Antoun H. **1992**. Ice Nucleation Activity in Fusarium acuminatum and Fusarium avenaceum. *Applied and Environmental Microbiology* **58**: 2960–2964.

Pouvreau J-B, Gaudin Z, Auger B, Lechat M-M, Gauthier M, Delavault P, Simier P. **2013**. A high-throughput seed germination assay for root parasitic plants. *Plant Methods* **9**: 32.

Priest FG. **2015**. Paenibacillus. In: Bergey's Manual of Systematics of Archaea and Bacteria. American Cancer Society, 1–40.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. **2013**. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**: D590–D596.

Rasmussen A, Depuydt S, Goormachtig S, Geelen D. **2013**. Strigolactones fine-tune the root system. *Planta* **238**: 615–626.

Roh SW, Quan Z-X, Nam Y-D, Chang H-W, Kim K-H, Kim M-K, Im W-T, Jin L, Kim S-H, Lee S-T, *et al.* 2008. Pedobacter agri sp. nov., from soil. *International Journal of Systematic and Evolutionary Microbiology* **58**: 1640–1643.

Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H. **2013**. The biology of strigolactones. *Trends in Plant Science* **18**: 72–83.

Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Berg G, van der Lelie D, Dow JM. **2009**. The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nature Reviews Microbiology* **7**: 514–525.

S SARN, S KM, H S. **2016**. Characterization of antifungal Paenibacillus illinoisensis strain UKCH21 and its chitinolytic properties. *African Journal of Microbiology Research* **10**: 1380–1387.

Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW. **2014**. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology* **12**: 87.

Santhanam R, Luu VT, Weinhold A, Goldberg J, Oh Y, Baldwin IT. **2015**. Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. *Proceedings of the National Academy of Sciences* **112**: E5013–E5020.

Schmidt CS, Alavi M, Cardinale M, Müller H, Berg G. **2012**. Stenotrophomonas rhizophila DSM14405T promotes plant growth probably by altering fungal communities in the rhizosphere. *Biology and Fertility of Soils* **48**: 947–960.

Scott PM, Walbeek WV, Maclean WM. **1971**. CLADOSPORIN, A NEW ANTIFUNGAL METABOLITE FROM CLADOSPORIUM ClADOSPORIOIDES. *The Journal of Antibiotics* **24**: 747–755.

Shade A, Handelsman J. **2012**. Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology* **14**: 4–12.

Stojanova B, Delourme R, Duffé P, Delavault P, Simier P. **2019**. Genetic differentiation and host preference reveal non-exclusive host races in the generalist parasitic weed *Phelipanche ramosa* (M Vurro, Ed.). *Weed Research* **59**: 107–118.

Sugiyama A, Bakker MG, Badri DV, Manter DK, Vivanco JM. **2012**. Relationships between Arabidopsis genotype-specific biomass accumulation and associated soil microbial communities. *Botany* **91**: 123–126.

Thuring JWJF, Nefkens GHL, Zwanenburg B. **1997**. Asymmetric Synthesis of All Stereoisomers of the Strigol Analogue GR24. Dependence of Absolute Configuration on Stimulatory Activity of Striga hermonthica and Orobanche crenata Seed Germination. *Journal of Agricultural and Food Chemistry* **45**: 2278–2283.

Tian S, Liu X, Lei P, Zhang X, Shan Y. **2018**. Microbiota: a mediator to transform glucosinolate precursors in cruciferous vegetables to the active isothiocyanates. *Journal of the Science of Food and Agriculture* **98**: 1255–1260.

Tomilov AA, Tomilova NB, Abdallah I, Yoder JI. **2005**. Localized Hormone Fluxes and Early Haustorium Development in the Hemiparasitic Plant Triphysaria versicolor. *Plant Physiology* **138**: 1469–1480.

Trivedi M, Patil S, Shettigar H, Gangwar M, Jana S. **2015**. Antimicrobial sensitivity pattern of Pseudomonas fluorescens after biofield treatment. *Infectious Diseases and Therapy* **3**.

Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. **2007**. The Human Microbiome Project. *Nature* **449**: 804–810.

Uecker FA. **1993**. Development and Cytology of Plectosphaerella Cucumerina. *Mycologia* **85**: 470–479.

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, *et al.* 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**: 195–200.

Vergnes DM, Renard M-E, Duveiller E, Maraite H. **2006**. Identification of Alternaria spp. on wheat by pathogenicity assays and sequencing. *Plant Pathology* **55**: 485–493.

Vidhyasekaran P (Tamil NAU, Muthamilan M. **1995**. Development of formulations of Pseudomonas fluorescens for control of chickpea wilt. *Plant disease (USA)*.

Voisard C, Keel C, Haas D, Dèfago G. **1989**. Cyanide production by Pseudomonas fluorescens helps suppress black root rot of tobacco under gnotobiotic conditions. *The EMBO Journal* **8**: 351–358.

Wang X, Radwan MM, Taráwneh AH, Gao J, Wedge DE, Rosa LH, Cutler HG, Cutler SJ. **2013**. Antifungal Activity against Plant Pathogens of Metabolites from the Endophytic Fungus Cladosporium cladosporioides. *Journal of Agricultural and Food Chemistry* **61**: 4551–4555.

Westwood JH, Yoder JI, Timko MP, dePamphilis CW. **2010**. The evolution of parasitism in plants. *Trends in Plant Science* **15**: 227–235.

Whittaker RH. **1972**. Evolution and Measurement of Species Diversity on JSTOR. *Taxon* **21**: 213–251.

Wigchert SCM, Kuiper E, Boelhouwer GJ, Nefkens GHL, Verkleij JAC, Zwanenburg B. **1999**. Dose−Response of Seeds of the Parasitic Weeds Striga and Orobanche toward the Synthetic Germination Stimulants GR 24 and Nijmegen 1. *Journal of Agricultural and Food Chemistry* **47**: 1705–1710.

Wilkie JP, Dye DW, Watson DRW. **1973**. Further hosts of Pseudomonas viridiflava. *New Zealand Journal of Agricultural Research* **16**: 315–323.

Wolf A, Fritze A, Hagemann M, Berg G. **2002**. Stenotrophomonas rhizophila sp. nov., a novel plant-associated bacterium with antifungal properties. *International Journal of Systematic and Evolutionary Microbiology* **52**: 1937–1944.

Yoneyama K, Kisugi T, Xie X, Yoneyama K. **2013**. Chemistry of Strigolactones: Why and How do Plants Produce so Many Strigolactones? In: Molecular Microbial Ecology of the Rhizosphere. John Wiley & Sons, Ltd, 373–379.

Yoon J-H, Kang S-J, Oh T-K. **2007**. Pedobacter terrae sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology* **57**: 2462–2466.

Young CC, Ho M-J, Arun AB, Chen W-M, Lai W-A, Shen F-T, Rekha PD, Yassin AF. **2007**. Sphingobium olei sp. nov., isolated from oil-contaminated soil. *International Journal of Systematic and Evolutionary Microbiology* **57**: 2613–2617.

Zhang P, Zhou P-P, Yu L-J. **2009**. An Endophytic Taxol-Producing Fungus from Taxus media, Cladosporium cladosporioides MD2. *Current Microbiology* **59**: 227.

Zhou J, Bi S, Chen H, Chen T, Yang R, Li M, Fu Y, Jia A-Q. **2017**. Anti-Biofilm and Antivirulence Activities of Metabolites from Plectosphaerella cucumerina against Pseudomonas aeruginosa. *Frontiers in Microbiology* **8**.

Appendix

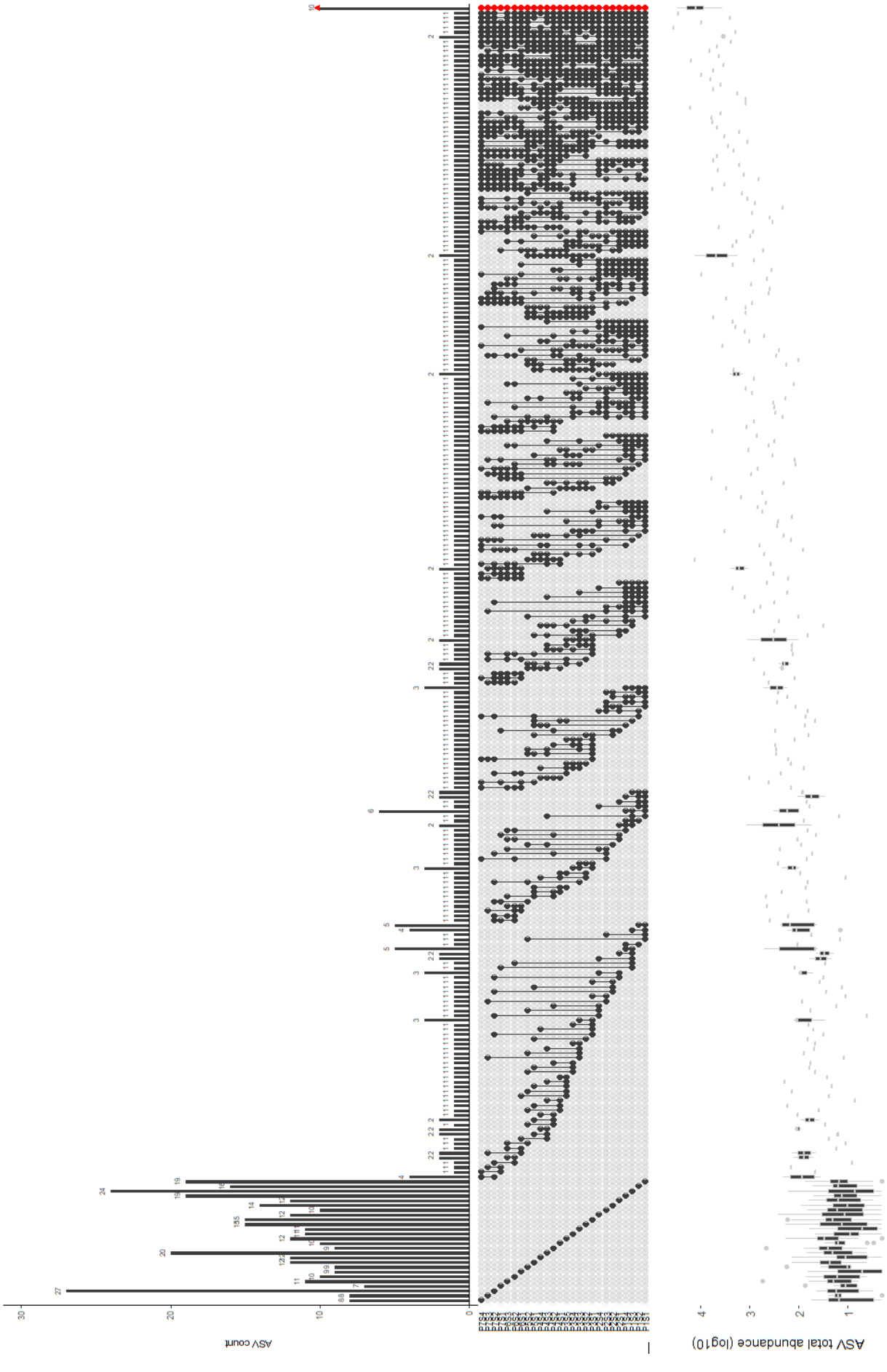
Annex I: Upset plot of the bacterial ASV in seed samples

Annex II: Upset plot of the fungal ASV in seed samples

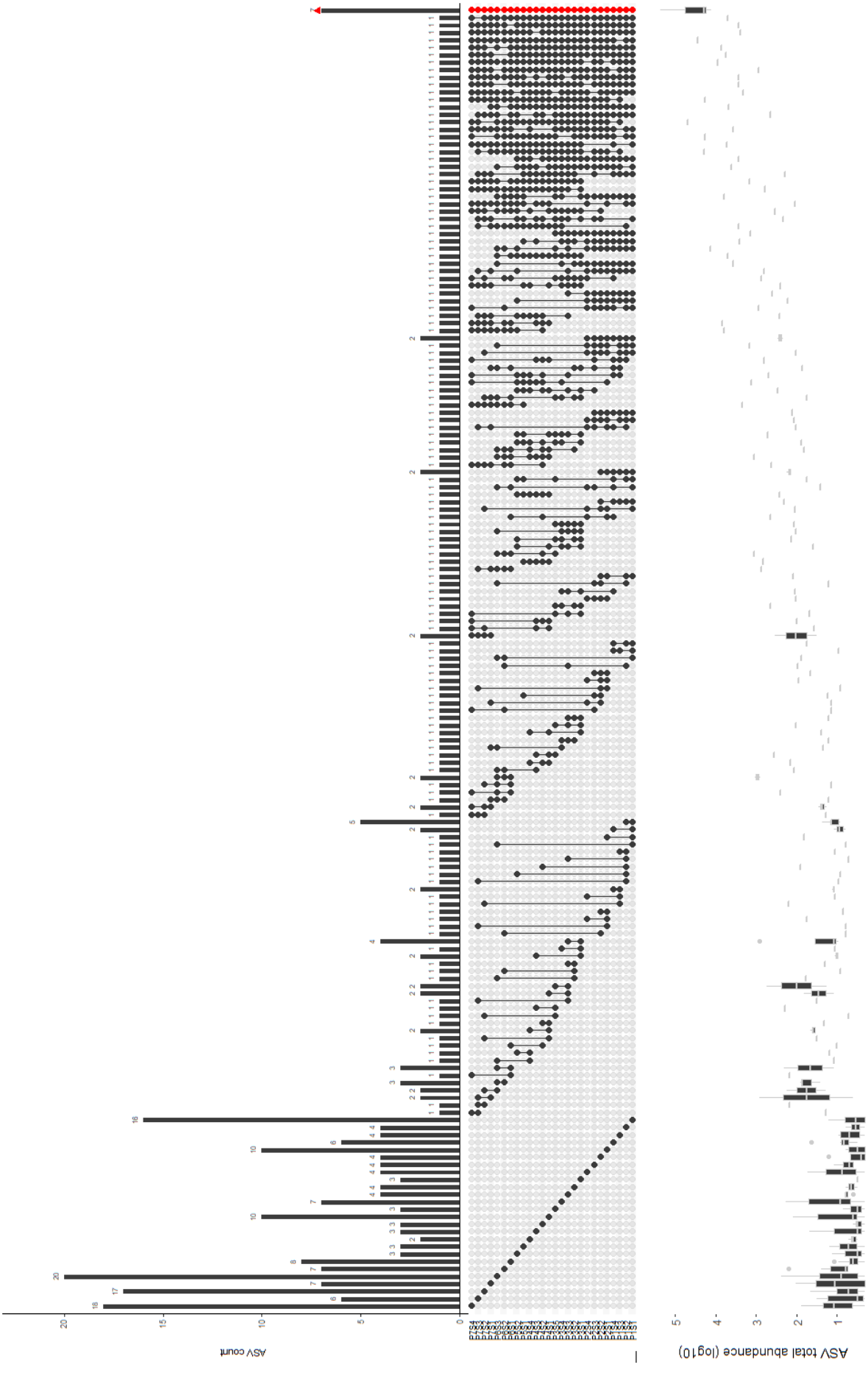
Annex III: Table of the core microbiota ASV

Annex IV: Core microbiota differential abundance and conservation among samples categories

Annex I: Upset plot of the bacterial ASV in seed samples



Annex II: Upset plot of the fungal ASV in seed samples



Annex III: Table of the core microbiota ASV

Gene marker	ASV identifier	Median abundance	Abundance sum (in percent of the total abundance)	Prevalence (in percent of the total sample triplicates)	Top hit BLAST
16S	ASV2990_*Pseudomonas*_unidentified	350.50	5.55	100.00	*Pseudomonas viridiflava* *Pseudomonas rhizosphaerae* *Pseudomonas abietaniphila* *Pseudomonas graminis* *Pseudomonas lutea* *Pseudomonas donghuensis*
	ASV2261_*Stenotrophomonas*_*rhizophila*	289.50	4.07	100.00	
	ASV1971_*Rhizobium*_unidentified	187.50	2.41	91.03	*Rhizobium radiobacter* *Agrobacterium tumefaciens*
	ASV2265_*Stenotrophomonas*_unidentified	159.00	4.09	92.31	*Stenotrophomonas tumulicola* *Stenotrophomonas maltophilia*
	ASV710_*Pedobacter*_unidentified	108.50	2.31	97.44	*Pedobacter roseus* *Pedobacter suwonensis* *Pedobacter agri* *Pedobacter borealis* *Pedobacter humicola* *Pedobacter terrae*
	ASV2956_*Pseudomonas*_unidentified	136.00	2.45	88.46	*Pseudomonas fluorescens* *Pseudomonas koreensis* *Pseudomonas baetica*
	ASV1578_*Sphingomonas*_unidentified	124.00	1.92	100.00	*Sphingomonas faeni* *Sphingomonas olei*
	ASV2268_*Stenotrophomonas*_*chelatiphaga*	102.00	1.52	96.15	
	ASV2876_*Paenibacillus*_unidentified	51.50	1.48	93.59	*Paenibacillus illinoisensis* *Paenibacillus hordei* *Paenibacillus kyungheensis*
	ASV1998_unidentified	48.50	0.66	87.18	*Rhodobacter sp.*
ITS	ASV2644_*Cladosporium*_unidentified	2563.5	32.82	100	*Cladosporium cladosporioides* *Cladosporium tenuissimum*
	ASV1920_*Plectosphaerella*_*cucumerina*	732.5	13.34	100	
	ASV2459_unidentified	284.5	5.18	100	*Fusarium avenaceum* *Fusarium acuminatum*
	ASV2467_*Fusarium*_unidentified	148	2.33	97.44	*Fusarium oxysporum*
	ASV2639_*Mycosphaerella*_*tassiana*	131.5	2.67	100	*Cladosporium floccosum*
	ASV140_*Vishniacozyma*_*victoriae*	123.5	1.85	100	
	ASV1817_unidentified	111	2.72	100	*Alternaria infectoria*

Annex IV: Core microbiota differential abundance and conservation among samples categories

Gene marker	ASV identifier	Differential abundance	Presence in soil and stimulated seeds
16S	ASV2990_*Pseudomonas*_unidentified		Yes
	ASV2261_*Stenotrophomonas_rhizophila*		Yes
	ASV1971_*Rhizobium*_unidentified		
	ASV2265_*Stenotrophomonas*_unidentified	Oilseed rape (2.7 logFC) Tobacco (2.93 logFC)	
	ASV710_*Pedobacter*_unidentified		Yes
	ASV2956_*Pseudomonas*_unidentified		
	ASV1578_*Sphingomonas*_unidentified		
	ASV2268_*Stenotrophomonas_chelatiphaga*		
	ASV2876_*Paenibacillus*_unidentified	Hemp (2.47 logFC)	
	ASV1998_unidentified		
ITS	ASV2644_*Cladosporium*_unidentified		Yes
	ASV1920_*Plectosphaerella_cucumerina*		Yes
	ASV2459_unidentified	Hemp (2.53 logFC) Tobacco (-3.82 logFC)	Yes
	ASV2467_*Fusarium*_unidentified	Hemp (-2.38 logFC)	Yes
	ASV2639_*Mycosphaerella_tassiana*		Yes
	ASV140_*Vishniacozyma_victoriae*		Yes
	ASV1817_unidentified	Oilseed rape (2.6 logFC) Tobacco (-2.01 logFC)	Yes

	Diplôme: Master Mention : Biologie Végétale (BV) Parcours : Gestion de la Santé des Plantes (GSP)

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Titre français :

Un aperçu des graines de *Phelipanche ramosa* : sensibilité aux stimulants de germination et profil microbien

Titre anglais :

An Overview of Phelipanche ramosa seeds: Sensitivity to germination stimulants and microbiome profile

Résumé (1600 caractères maximum) :

Phelipanche ramosa, ou orobanche rameuse, est une plante parasite de la famille des *Orobanchaceae*. Elle s'attaque à de nombreux hôtes en Europe et dans le bassin méditerranéen et provoque d'importantes pertes de rendement comme sur colza, dont elle menace la culture dans l'ouest de la France. L'une des particularités des orobanches est la germination de leurs graines : elles ne peuvent germer que si elles ont perçu un stimulant de germination (GS), en provenance de leur plante hôte. Deux principaux GS ont été identifié : les strigolactones et les isothiocyanates. Ces molécules sont connues pour être exsudées par certaines plantes mais peuvent également être modifiées ou dégradées par des micro-organismes. En outre, trois génotypes distincts ont été répertoriés pour *P. ramosa*, avec chacun un hôte préférentiel : le génotype 1 sur colza, le génotype 2a sur chanvre et le génotype 2b sur tabac. Les échantillons de graines sur lesquels se base notre étude recouvrent bien cette diversité génétique. Dans cette étude, nous observons que les graines ne répondent pas toutes de la même façon aux GS et cela en fonction de l'hôte sur lequel elles ont été prélevées. Parallèlement, nous décrivons ici le microbiote associé aux graines de *P. ramosa*. Nous observons que l'hôte d'origine est le principal facteur influençant la composition du microbiote et notons également un effet terroir. Nous définissons également le core microbiote associé aux graines de *P. ramosa*. Cette étude poursuit donc l'effort de compréhension fait sur la spécialisation d'hôte chez l'orobanche et met en lumière une participation de plus en plus évidente du microbiote dans l'interaction plante-plante parasite.

Abstract (1600 caractères maximum) :

Phelipanche ramosa, a.k.a. broomrape, is a parasitic plant of the *Orobanchaceae* family that infests numerous hosts in Europe and Mediterranean basin. It causes huge yield losses on various crops and especially on oilseed rape which cultivation is threatened in western France. One the special features of broomrape is its seed germination. Indeed, broomrape seeds cannot germinate without perceiving a germination stimulant (GS) exuded by its host. Two mains GS have been identified hitherto: strigolactones and isothiocyanates. These molecules are known to be exuded by several crops but can also be modified or degraded by microorganisms. Additionally, three *P. ramosa* genotypes have been distinguished with specific preferential hosts: oilseed rape for genotype 1, hemp for genotype 2a and tobacco for genotype 2b. This study is based on seed samples that cover this genetic diversity. Herein, we observed that seeds coming from different hosts had different sensitivities to GS. Concomitantly, we described the bacterial and fungal communities associated to *P. ramosa* seeds and observed that the originating host of the parasitic seeds was the most influent factor shaping the seed microbiome. We also noticed a terroir effect, especially on fungal communities. Furthermore, we characterize the *P. ramosa* seed core microbiome. Thus, this study continues the research effort on broomrape host specialization and highlights the growing evidence of the key role of microbiome in host plant – parasitic plant interactions.

Mots-clés : plantes parasites ; *Phelipanche ramosa* ; stimulants de germination ; microbiote ; diversité

Key Words: parasitic plants; *Phelipanche ramosa*; germination stimulants; microbiome; diversity

* *Elément qui permet d'enregistrer les notices auteurs dans le catalogue des bibliothèques universitaires*