

INTERNSHIP PROPOSAL M2 RESEARCH 2026-2027

Title: Identification and characterization of determinants of sexual reproduction in *Leptosphaeria maculans*, a pathogenic fungus of rapeseed.

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INTRODUCTION, SCIENTIFIC CONTEXT

The filamentous fungus *Leptosphaeria maculans* is responsible for one of the major diseases of rapeseed (*Brassica napus*): crown necrosis. To limit the impact of this phytopathogen on crops, a genetic control strategy is used in the field. This strategy involves growing rapeseed varieties selected to express a resistance gene (*R*) that confers specific resistance to strains of *L. maculans* expressing the corresponding avirulence gene (*AvrLm*). However, the fungus is capable of rapidly overcoming these resistance mechanisms¹. The emergence of new virulent strains capable of attacking resistant varieties occurs following sexual reproduction on crop residues. This process takes place throughout the fall and results in the production of ascospores that, on the one hand, arise from genetic mixing and, on the other hand, constitute the primary inoculum. However, despite its fundamental role in infection and the evasion of varietal resistance, this stage of sexual reproduction has received little attention to date in *L. maculans*, whether *in vitro* or *in natura*.

RESEARCH PROPOSAL

One of the obstacles to studying sexual reproduction in the laboratory is the frequent development of sterile strains in axenic culture. The primary objective of this internship will therefore be to determine the (epi)genetic causes of this sterility phenotype through whole-genome sequencing and transcriptomic analyses. A second objective will be to determine the role in the sexual reproduction of *L. maculans* of genes known to be key players in other fungi. Thus, part of the internship will focus on the RID protein, which plays an essential role in sexual reproduction, particularly in *Podospora anserina*² but also in the RIP (Repeat-Induced Point Mutation) mechanism—known notably in *Neurospora crassa*³—to defend the genome against invasion by repetitive elements. The RID homolog in *L. maculans* will be studied for its involvement in development, pathogenesis, and sexual reproduction (through inactivation and overexpression) and for its expression and localization during sexual reproduction (using a fusion with a fluorophore). Finally, to obtain a more detailed description of the developmental kinetics of sexual fruiting bodies, RT-qPCR analysis will be performed on candidate genes encoding proteins known to be involved in various stages of sexual reproduction in other filamentous fungi⁴.

METHODOLOGIES

- Molecular biology: DNA extraction, RNA extraction, RT-qPCR, molecular cloning (PCR, ligation, bacterial transformation)
- Microbiology: fungal transformation, selection of transformants, spores production, sexual reproduction
- Microscopic observations
- Phytopathology (inoculation with *L. maculans* on *B. napus* and phenotypic assessment)
- Data analysis: whole-genome sequencing results, RNAseq results

REFERENCES

¹Rouxel T, Balesdent MH (2021) L'intégration des connaissances sur les résistances variétales par les acteurs de la sélection: un gage d'efficacité et de durabilité ? L'exemple du phoma du colza. In : Lannou C, Roby D, Ravigné V, Hannachi M, Moury B (Eds.). L'immunité des plantes. Pour des cultures résistantes aux maladies. Editions Quae, Versailles, France, 392 pp.

²Grognet P, Timpano H, Carlier F, Aït-Benkhalil J, Berteaux-Lecellier V, Debuchy R, Bidard F, Malagnac F. A RID-like putative cytosine methyltransferase homologue controls sexual development in the fungus *Podospora anserina*. PLoS Genet. 2019 Aug 14;15(8):e1008086. doi: 10.1371/journal.pgen.1008086. PMID: 31412020; PMCID: PMC6709928.

³Freitag M, Williams RL, Kothe GO, Selker EU. A cytosine methyltransferase homologue is essential for repeat-induced point mutation in *Neurospora crassa*. Proc Natl Acad Sci U S A. 2002 Jun 25;99(13):8802-7. doi: 10.1073/pnas.132212899. Epub 2002 Jun 18. PMID: 12072568; PMCID: PMC124379.

⁴ Bidard F, Grognet P, Lelandais G, Imbeaud S, Mucchielli-Giorg M.-H, Debuchy R, Berteaux-Lecellier V, Malagnac F, Sexual reproduction is controlled by successive transcriptomic waves in *Podospora anserina*. Peer Community Journal, Volume 5 (2025), article no. e68. <https://doi.org/10.24072/pcjournal.574>.