INTERNSHIP PROPOSAL M2 RESEARCH 2026-2027

Title: Role of the apoplastic compartment of brassica leaves in controlling infection by the plant-pathogenic fungus *Leptosphaeria maculans*

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INTRODUCTION, SCIENTIFIC CONTEXT

The apoplast, the extracellular compartment of plant tissues, plays a central role in the interactions between plants, their environment, and associated microorganisms. It is involved in water and nutrient transport, cell wall biosynthesis, and immune responses to both biotic and abiotic stresses (Faverdin et al., 2020). This dynamic compartment contains a wide range of proteins, metabolites, and microorganisms, making it a key interface for plant–pathogen interactions.

Apoplastic fungal pathogens, particularly hemibiotrophs such as *Leptosphaeria maculans*, exploit this compartment from the earliest stages of infection. The causal agent of phoma stem canker in oilseed rape (*Brassica napus*), *L. maculans*, enters the plant through stomata and colonizes the apoplast during an asymptomatic phase before causing visible symptoms approximately nine days after infection (Borhan et al., 2022). During this phase, the fungus secretes successive waves of effector proteins (Gay et al., 2021). These effectors suppress plant immune responses and facilitate infection, but may also interact with the resident apoplastic microbiota, highlighting the apoplast as a critical environment for the establishment of infection.

Genetic resistance is the main strategy used to control *L. maculans*. Resistance (*Rlm*) genes from oilseed rape encode membrane-associated receptors that recognize apoplastic avirulence effectors and prevent systemic colonization by the pathogen. Recent studies further suggest that these resistance genes can influence the composition of the oilseed rape microbiota (Gorse et al., 2024). Moreover, in the closely related non-host species *Brassica carinata*, *L. maculans* spores germinate and penetrate through stomata, but fungal development is arrested in the substomatal cavity by a hypersensitive response (Noah et al., 2024).

These observations support the hypothesis that the apoplastic environment of resistant plants, including *B. napus* cultivars carrying *Rlm* genes and the non-host species *B. carinata*, differs from that of susceptible plants. Differences in apoplastic proteins, metabolites, and microbial communities may contribute to restricting fungal colonization. Therefore, the *L. maculans–Brassica* pathosystem provides an excellent model for investigating the molecular and ecological mechanisms that determine the success or failure of apoplastic fungal infections.

The objective of the Master 2 internship is to identify apoplastic compounds and microorganisms capable of inhibiting or promoting the progression of *L. maculans* in the leaves of various *Brassica* species during the course of infection. Thus, we will compare the apoplastic fluid wash (AWF) of three *Brassica* genotypes during the first 7 days of infection by *L. maculans*: a susceptible *B. napus* cultivar (Darmor), a resistant *B. napus* cultivar (Darmor Rlm11), and a non-host *Brassica* species (*B. carinata*).

RESEARCH PROPOSAL

The main objective of this internship is to investigate the role of the apoplast in the interaction between *Brassica* species and *Leptosphaeria maculans*, with a particular focus on protein/metabolic and microbial determinants of plant resistance. By comparing apoplastic fluids collected from a susceptible and a resistant *B. napus* cultivar carrying a *Rlm* resistance gene, as well as from the non-host species *B. carinata* at different stages of infection, temporal changes in apoplastic composition will be assessed.

The internship will involve complementary approaches:

- First, AFW will be evaluated by assessing their effects on L. maculans spore germination and early hyphal growth *in vitro*. These assays will determine whether apoplastic environments from resistant plants or specific infection stages inhibit fungal development.
- Second, the culturable microbial communities inhabiting the apoplast will be explored by isolating bacteria and fungi from the different apoplastic samples. The diversity and composition of these microbial communities will be compared among *Brassica* genotypes and infection stages to identify microorganisms potentially involved in plant protection or pathogen establishment

Third, the metabolite and/or protein composition of the apoplastic fluids will be characterized. Comparative analyses of the most contrasting stages of the inoculation time course will reveal metabolites whose abundance is altered during infection or varies between host genotypes

METHODOLOGIES

- Phytopathology (inoculation with *L. maculans* on *B. napus* and phenotypic assessment)
- Microbiology (*in vitro L. maculans* spore production, nephelometry to assess the effect of AWF on *L. maculans* germination);
- Enzymology: control of AFW quality (NADPH activity measurement),
- Protein and metabolite analysis: western blot and metabolomics data analysis (if available)
- Data and statistics analysis

REFERENCES

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