PhD School on Agriculture, Environment and Bioenergy

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(XXXVI cycle, 2020-23)

Project draft

1.Field of interest

AGR/12 Plant Pathology

2. Project title

Novel perspectives in the control of plant pathogenic Oomycetes

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- Eventually: co-tutor/s Prof. Piero Attilio Bianco, Prof.ssa Simona Masiero

4. Relevance of the topic and state of the art:

Plant pathogenic Oomycetes are a threat for agricultural production worldwide [1,2]. The Irish famine, dating back to 1845, caused by the potato late blight pathogen *Phytophthora infestans*, is one of the most famous examples of the dramatic consequences caused by these fungal-like organisms. Oomycete plant pathogens exhibit biotrophic, necrotrophic, or hemibiotrophic lifestyles. Many biotrophic oomycetes completely rely on host tissues (obligate biotrophy). This is a feature of the grapevine downy mildew *Plasmopara viticola*, one of the most economically damaging pathogens in viticulture. Obligate biotrophs, such as *P. viticola*, are not cultivable on synthetic media but must be propagated on susceptible host tissues. This is one of the main limitations in the study of the pathogen biology and in the screening for fungicide efficacy. On the contrary, necrotrophs, such as *Pythium ultimum*, and hemibiotrophs, that switch from biotropy to necrotrophy such as *Phytophthora* spp., can be grown in axenic cultures [3]. The control of the disease caused by oomycetes is based on fungicide treatments that negatively affect human health and the environment. The road to sustainability passes through the development of low impact fungicides through a target-based approach and, for this reason, the characteristics of the target pathogen must be dissected [4] and unbiased, high throughput methods for fungicide efficacy screening are needed. The aims of this project are: i) developing a method to cultivate in axenic culture the obligate parasite *P. viticola* to identify new fungicide targets; ii) developing fast and unbiased methods for estimating the efficacy of low-impact fungicides; iii) evaluating the biological activity of low-impact fungicides.

5. Layout of the project (draft)

The project will be articulated in five main work packages (WP).

WP1. Culture collection: a collection of oomycete species with different characteristics will be established and maintained for the whole duration of the project.

WP2. Set up of a protocol for the axenic cultivation of the obligate biotroph *P. viticola*: this challenging task aims at first establishing a protocol for the growth of the zoospores until the differentiation of the first haustorium and then a protocol for the growth and sporulation of the pathogen on a synthetic medium.

WP3. Design and application of phenotyping tools to assess disease severity at the lab level: this task aims at obtaining an early detection of pathogen infection in leaf tissues

WP 4. Assessment of the bioactivity of new fungicides through phenotyping tools and microscopy.

WP5. Data analysis, scientific paper(s) and thesis writing.

5.1.Materials & Methods:

5.1.1 Culture collection. Strains of *P. viticola, P. infestans, Phytophthora capsici* and *P. ultimum* will be isolated from field samples or purchased from fungal culture collections. The most suitable media for the *in vitro* growth of cultivable pathogens will be selected based on the assessment of mycelial growth and sporulation. Protocols for the propagation of obligate parasites and fungicide sensitivity assays *in vivo* and *in vitro* will be tested and improved. Strains will be routinely propagated to maintain the collection.

5.1.2. Set up of a protocol for the *in vitro* cultivation of the obligate parasite *P. viticola*. An extensive literature search will be needed to establish which are the requisites for the pathogen growth and particular attention will be given to the protocols established for the cultivation of other obligate parasites of plants and animals [5].

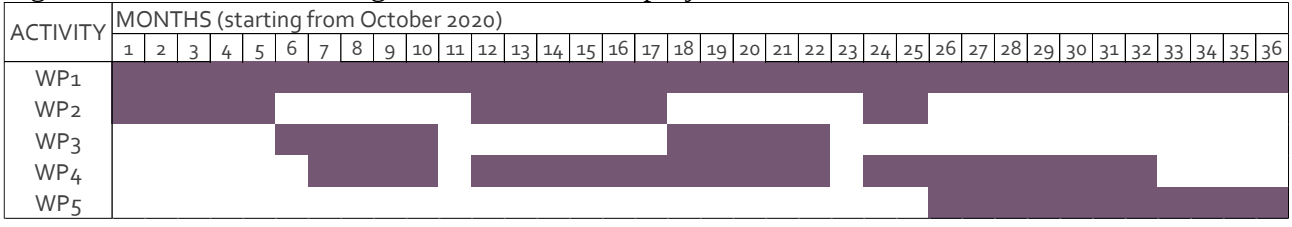
5.1.3 Design and application of phenotyping tools to assess disease severity at the lab level. Machine vision thermography and hyperspectral imaging, and machine learning algorithms will be designed and applied for assessing plant pathogen infection at the laboratory level [6].

5.1.4 Assessment of the bioactivity of new fungicides. Low-impact fungicides will be selected among peptide aptamers and natural compounds able to interfere with the pathogen [7,8]. Protocols developed in WP1 and in WP3 will be used to assess the efficacy of the fungicides in the inhibition of mycelial growth and infection of plant tissues. Using the 'No-limits' imaging platform, the histological and ultrastructural alterations induced by the best performing fungicides to the pathogen will be investigated.

5.2.Schedule and major steps (3 years):

The schedule of the project, starting from October 2020, is visible in Figure 1.
WP1. The establishment of the culture collection will be carried out in Autumn of year 1 and will continue for the whole duration of the project. The set up of inoculation protocols will be carried out from month 1 to month 6.
WP2. Literature search and the set up of the experiments for the *in vitro* growth of *P. viticola* will be carried out in Autumn-Winter of the three years, indicatively between month 1-5, 12-17 and 24-25.
WP3. The experiments on the phenotypic tools for disease severity will be carried out between in Spring Summer of the first and second year, indicatively between month 6-10 and 18-22.
WP4. Efficacy assays will be routinely carried out during the project until indicatively at month 7-10, 12-22, 24-27, microscopy analysis will be carried out at the third year, at months 28-32. (month 23-32).
WP5. Data analysis, scientific paper(s) and thesis writing will start from month 26.

Figure 1. Gantt chart showing the schedule of the project.



6. Available funds (source and amount)

- Silvia Laura Toffolatti, 20343, CTE_NAZPR17STOFF_02 - Monitoraggio della sostenibilità a zoxamide in popolazione di *Plasmopara viticola* in Nord Italia, € 15.005,47

6. Literature:

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