PhD School on Agriculture, Environment and Bioenergy

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(XXXIV cycle, 2018-20)

Project draft

1.Field of interest

AGR/12 Plant Pathology

2.Project title

Fungicide resistance in grapevine downy mildew management: phenotypic and genotypic characterization of *Plasmopara viticola* populations for resistance to fungicides

3.Tutor Dr Silvia Laura Toffolatti

Co-tutor Prof. Piero Attilio Bianco

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

Fungal plant pathogens are highly flexible to adapt to different environmental conditions, to host defence mechanisms and to fungicide selection [1,2]. Resistance to single-site fungicides is among the major threats for modern agriculture, because it potentially leads to a reduction of disease control in the field (practical resistance). As a consequence, proper disease control strategies have to be implemented to mitigate resistance evolution by reducing the selection pressure associated to the fungicide use. The implementation of sound anti-resistance strategies is based on the risk of a particular fungicide class to evolve resistance, the pathogen risk, the agronomic risk associated to a specific area and the results obtained in sensitivity monitoring activities, that characterize fungicide sensitivity of pathogen strains or populations through bio tests and molecular diagnostic tools [3]. In general, to avoid the increase of the resistant sub-population, single-site fungicides are applied in mixture and/or alternation with partners possessing a different mode of action and with a limited number of sprays within a season.
Downy mildew, caused by *Plasmopara viticola*, is one of the most devastating diseases of the grapevine species cultivated worldwide, *Vitis vinifera* [4]. The disease management is mainly based on the frequent application of fungicides and resistance to individual fungicide classes has been reported. However, little or no information is available on the effects of the application of anti-resistance strategies on the pathogen population.
Aims of the project are: a) to evaluate the sensitivity profile of *P. viticola* isolates to different fungicide classes following the application of different disease management strategies in open field; b) to characterize resistant strains for the mechanism of resistance, fitness and pathogenicity; c) to characterize *P. viticola* populations for genetic variability.

5.Layout of the project (draft)

The project will be articulated in five main work packages (WP).
WP1. Sampling and biological assays of oospore samples. Vineyards will be selected and leaves showing downy mildew symptoms will be sampled to collect the oospores. Sensitivity assays will be carried out on both oospore populations and on *P. viticola* strains isolated from the germinating oospores.
WP2. Sampling and biological assays of sporangia samples. Symptomatic leaves will be sampled in the same vineyard to collect *P. viticola* sporangia. Populations and single isolates will be characterized for sensitivity to fungicides and fitness.

WP3. Molecular assays. The samples will be characterized for the mutations associated to fungicide resistance.
WP4. Genetic variability. All *P. viticola* isolates will be genotyped for 32 microsatellite markers and selected isolates will be more deeply analyzed through whole genome sequencing.
WP5. Data analysis, scientific paper(s) and thesis writing.

5.1.Materials & Methods:

5.1.1 Sampling

Vineyards treated with different anti-resistance strategies will be selected. Untreated plots of experimental vineyards will be used to establish a baseline sensitivity to the fungicides. Sampling will be carried out in two different periods: 1) Autumn, before leaf fall, to collect the sexual spores of the pathogen (oospores); 2) early Summer (bunch closure), to collect the asexual spores.

5.1.2 Biological assays

The pathogen populations will be characterized for sensitivity to different resistance classes (*e.g.* Carboxylic Acid Amides, phenylamides, zoxamide) and *P. viticola* strains will be isolated from the population to be more deeply characterized. Biological assays will be carried out by oospore germinability and by leaf discs' assays [4]. EC_{50}, i.e. the median fungicide concentration inhibiting the oospore germination or *P. viticola* sporulation on leaf discs, will be calculated by probit analysis (SPSS software version, IBM Analytics Italia). Fitness of the strains will be estimated from the germination percentage and infectivity of the oospores and on the sporangia infection [5].

5.1.3 Molecular assay

DNA will be extracted from oospores/sporangia [4]. Where the protocol is available [4,6], allele-specific real-time PCR will be carried out to quantify the percentage of resistant/sensitivity alleles in the samples. Where no information is available, sequencing of the genes coding for the fungicide target will be performed to establish if point mutations are associated to resistance.

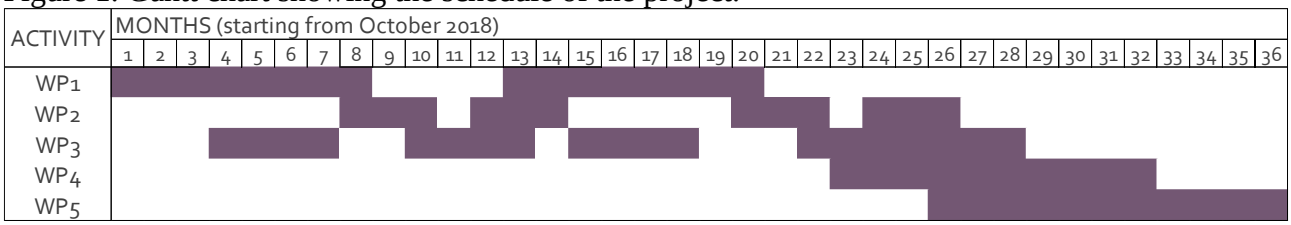
5.1.4 Genetic variability

The genetic diversity of *P. viticola* populations collected from different vineyards will be investigated by microsatellite analysis [7] and/or whole genome sequencing in order to evaluate the effect of fungicide treatments on the genetic structure of the pathogen. Whole genome sequencing could be also used to determine unknown fungicide resistance mechanisms [8].

5.2.Schedule and major steps (3 years):

The schedule of the project, starting from October 2018, is presented in Figure 1.
WP1. Sampling will be carried out in Autumn of year 1 (month 1-4) and 2 (month 13-16). The sensitivity assays of oospores, isolation and sensitivity assays of *P. viticola* strains will be carried out by spring of year 1 (month 5-8) and 2 (month 17-20).
WP2. Sampling and biological assays of sporangia populations and isolates will be carried out in spring of year 1 (month 8-14) and 2 (month 20-26).
WP3. Molecular assays to evaluate the point mutations associated to resistance will be carried out between month 4-7, 10-13, 15-18, 22-28.
WP4. Genetic analysis of variability will be carried out at the end of the project (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, CTE_NAZPR17STOFF_02, Monitoraggio della sostenibilità a Zoxamide in popolazione di *Plasmopara viticola* in Nord Italia, € 30.000.
- Bianco Piero Attilio, RV_ATT_COM16PBIAN_M, Avanzi ex CoFi - 14 -9 - Spese connesse ad attività di ricerca consulenza e formazione ex CoFi – 015615, € 150.000.

6. Literature:

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4. Toffolatti SL, Russo G, Campia P, Bianco PA, Borsa P, Coatti M, Torriani SFF, Sierotzki H, A time-course investigation of resistance to the Carboxylic Acid Amides mandipropamid in field populations of *Plasmopara viticola* treated with anti-resistance strategies. *Pest Management Science* (Accepted for publication, 2018).
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7. Maddalena G, Phenotypic characterization of the interaction between *Plasmopara viticola* and *Vitis vinifera*, PhD Thesis, University of Milan, pp. 170 (2017).
8. Mohd-Assaad N, McDonald BA, Croll D, Multi-locus resistance evolution to azole fungicides in fungal plant pathogen populations. *Molecular Ecology* **25**: 6124-6142 (2016).