

PhD School on Agriculture, Environment and Bioenergy

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(XXXIX cycle, 2023-26)

Project draft

1. Field of interest

AGR/12 Plant pathology

2. Project title

Fungicide resistance in grapevine downy mildew management: from resistance mechanisms to fitness of zoxamide-resistant strains

3. Tutor (membro del Collegio dei Docenti)

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- Eventually: co-tutor/s

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4. Relevance of the topic and state of the art

Viticulture records the strongest use of plant protection products¹. In particular, the control of downy mildew (caused by the oomycete *Plasmopara viticola*) is mainly based on the intensive use of fungicides, predominantly single sites, which can lead to the onset of fungicide resistance in the pathogen population². The application of anti-resistance management is mandatory to preserve the efficacy of the available classes of fungicides. To this purpose, the characterization of the resistance mechanism and the evaluation of the effectiveness of field management is crucial. Zoxamide is an inhibitor of β -tubulin with a low-medium risk for selecting resistant strains³. Since the active substance is widely employed in vineyard, accurate studies are needed to evaluate the sensitivity status of *P. viticola* populations. The purpose of this research is to: i) perform field trials to evaluate the effectiveness of anti-resistance strategies in reducing the selection pressure towards zoxamide-resistant strains; ii) monitoring zoxamide sensitivity in Italian vineyards; iii) characterizing the sensitivity to zoxamide and fitness of pathogen isolates; iv) evaluating the presence of target-site mutations related to resistance; v) evaluating the resistance mechanism inheritance, in case resistant strains are isolated.

5. Layout of the project (draft)

5.1. Materials & Methods:

WP1 – Field trials. One or two vineyards will be selected according to the history of the downy mildew epidemics (high-medium-low) and sensitivity status. A first plot consisting of four replicates of 20 plants will not be treated with fungicides. A second and a third plot (1 ha each) will be treated with antiresistance strategies including a different number of zoxamide treatments (low-high selection pressure). During grapevine growing season, disease intensity will be evaluated in the three plots and infected leaves will be sampled for fungicide resistance assessment (WP3).

WP2 – Monitoring activity. Sensitivity of *P. viticola* populations sampled in commercial vineyards located in different Italian regions will be assessed in WP3. 20 vineyards will be sampled per year, taking into consideration to follow the evolution of resistance for three consecutive years in selected cases.

WP3 – Sensitivity assays. *P. viticola* sensitivity tests will be carried out at increasing concentrations of zoxamide (0, 0.01, 0.1, 1, 10, 100 and 200 mg/L). The assays will be performed both on oospores and sporangia. Sensitive and resistant *P. viticola* strains will be isolated and used in WP4 and 5.

WP4 – Molecular assays. DNA will be extracted from *P. viticola* isolates from WP3 and the β -tubulin gene will be sequenced to assess the presence of point mutations in the target gene that can be associated with zoxamide resistance.

WP5 – The inheritance of resistance mechanisms will be assessed by crossing zoxamide sensitive and resistant strains and assessing the inheritance patterns in F1 populations. The achieved isolates will be characterized for sensitivity as described in WP3 and sequenced as in WP4. The outcome of WP5 is to define if zoxamide sensitivity is a dominant or recessive character. The fitness of resistant strains will be compared to that of sensitive strains in terms of infectivity and sporangia production.

WP6 - Data analysis, scientific paper(s) and thesis writing. An extensive literature search will be needed for the set-up of the experiments and data analysis. A review-literature paper is expected at the beginning of the project.

5.2. Schedule and major steps (3 years):

Activities	First year	Second year	Third year
WP1	Disease intensity and sampling	Disease intensity and sampling	Disease intensity and sampling
WP2	Sampling	Sampling	Sampling
WP3	Sensitivity assay and strain isolation	Sensitivity assay and strain isolation	Sensitivity assay and strain isolation
WP4		DNA extraction and sequencing	DNA extraction and sequencing
WP5			Crossing, biological, and molecular characterization
WP6	Literature review and protocol set-up	Data analysis	Data analysis and thesis writing

6. Available funds

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7. Literature

- ¹ Commission E. *The use of plant protection products in the European Union Data 1992-2003 2007 edition*. 2007.
- ² Massi F, Torriani SFF, Borghi L, Toffolatti SL. Fungicide resistance evolution and detection in plant pathogens: *Plasmopara viticola* as a case study. *Microorganisms* 2021; **9**: 119.

³ FRAC Code List 2022. <https://www.frac.info/>