Fungicide resistance in phytopathogenic fungi: A critical point of successful and sustainable viticulture (case of *Botrytis cinerea* Pers.)

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Abstract: *Botrytis cinerea* Pers. is the causal agent of grey mould, which is a worldwide disease that causes serious losses in more than 200 host species. Grey mould (also called *Botrytis* bunch rot) is one of the most important and destructive disease of grapevines. Effective control of grey mould in vineyard is usually based on preventive repeated fungicide applications during the season. Disease management relies on the application of 1-4 treatments with fungicides possessing a single-site mode of action. However, this fungus has been categorized by FRAC as a high-risk pathogen for fungicide resistance development. Another problem is related with the diversity of fungicides available to growers, which according with the current European legislation on pesticides and the European Green Deal, will be reduced by 50% by 2030. Despite the negative impact of the pathogen on grape production, no information is available on the composition of *B. cinerea* population in our country as well as the fungicide resistance in *B. cinerea* populations from Montenegrin vineyards. Furthermore, understanding fungicide resistance of *B. cinerea* populations and their prevalence could help to develop sustainable and effective control programs. In this study the molecular profiles of *B. cinerea* strains isolated from Montenegrin vineyards and their potential fungicide resistance will be determined to obtain a useful information for the sustainable management of the disease.

Keywords: *Botrytis cinerea*, disease, fungicides resistance, sustainable, control

1. Problem definition and background

Botrytis cinerea Pers. causes serious disease in vineyards and is responsible for significant economic losses worldwide [1]. The pathogen affects all vine organs, especially clusters, and thereby reduces both the quantity and quality of the produced and harvested grape berries. Crop losses result from damage to inflorescences before flowering, to flowers during flowering, to young berries at fruit set, and to berries during ripening, as well as during grapes storage. Quality is reduced because rotted berries cause an alteration of the chemical composition leading to the undesirable flavors in wine [2].

The control of the disease is difficult because of number of factors: large numbers of conidia on multiple inoculum sources, susceptibility of grapevine at multiple growth stages, different infection pathways and a wide range of environmental conditions for infection development. Although some cultural practices, such as leaf removal to increase the canopy airflow play important roles in disease management, control of bunch rot mostly depends on the fungicide's application. However, the routine fungicide scheduling may result in

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unnecessary sprays, which is unacceptable because of environmental and public health concerns [3], high cost of antibotrytics products and the increased risk of fungicide resistance [4].

The site-specific fungicides that have been used for the *Botrytis* control provide highly efficient control of fungal diseases. However, it has been discovered that plant pathogenic fungi can adapt to fungicide treatments by mutations leading to resistance and loss of fungicide efficacy. The gray mold fungus *Botrytis cinerea*, a major cause of pre- and post-harvest losses in fruit and vegetable production, is characterized as a 'high risk' organism for rapid resistance development. In the past 10 years, increasing frequencies of *Botrytis* strains with fungicide resistance have been reported in different parts of the world from wine and table grapes [5,6].

There is therefore the need to improve *B. cinerea* control in vineyards by applying fungicides only when necessary and by following sustainable anti-resistance strategies. The goal of our research is to monitor the presence of *Botrytis* strains resistant to the pesticides found in field populations of *B. cinerea* in Montenegrin vineyards in which those pesticides had been applied frequently in previous 10-15, and more years.

2. Research methodology and our progress

Botrytis infected plant material is collected during the season 2022 (August, September). Grape clusters with bunch rot disease symptoms were sampled from grape cultivars commonly grown in Montenegro such as autochthonous grapevine varieties Vranac, Kratošija, Krstač and internationally grown varieties: Shardonnay, Sauvignon, etc. In the Phytopathology laboratory at Biotechnical faculty, *Botrytis* strains will be isolated from infected plant tissue by streaking out conidia onto potato dextrose agar (PDA) and followed by methodology for the pathogen cultivation.

Fungicide sensitivity assays will be performed by analyzing spore germination and growth of conidia on agar plates containing discriminatory fungicide concentrations of synthetic site-specific botryticides which are frequently used in Montenegrin vineyards for *Botrytis* control such as: pyrimethanil (PYRUS 400 SC), pyraclostrobin + boscalid (Signum), cyprodinl + fludioxonil (SWITCH 62,5 WG), fenhexamid (Teldor), isofetamid (Zenby). Furthermore, *Botrytis* DNA extraction and sequencing, *Botrytis* identification, phylogenetic trees construction and detection of mutations will be performed respectively. For the statistical analysis ANOVA test will be performed.

3. Future works and conclusion

Globally, there has been a significant shift in emphasis from synthetic fungicides to more integrated management of grey mould caused by *Botrytis cinerea* in the last two decades. This change has been driven by several factors, including the problems associated with the rapid emergence of resistance to commonly used synthetic fungicides, increasing consumer demand for low pesticide residues in food crops, increasing demand for food production that

does not negatively affect human and environmental health; the continued growth of organic production systems; and the greater availability of bio fungicides.

In the context of increasing fungicide resistance, public pressure to reduce fungicide uses, commercial constraints, etc., research on sustainable disease management decisions are imperative. Innovative strategies for management of diseases caused by fungi *Botrytis cinerea* that include together with the justifiably reduced use of synthetic fungicides the application of biocontrol agents and other innovative tools (DSS, prediction models) will be a strategy in our research for increasing sustainability that takes in consideration the needs of all involved actors in the chain from growers to the consumers.

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