Field evaluation of grapevines resistant to downy and powdery mildews

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1 Introduction

Downy (*Plasmopara viticola*; DM) and powdery (*Erisiphe necator*; PM) mildews are important diseases of grapevine worldwide. Under favourable weather conditions, they cause relevant yield and quality losses, affecting both leaves and clusters.

Disease control in vineyard is generally achieved by using chemical fungicides, copper, and sulphur, which have negative effects on vineyard environment and biodiversity, and human health (La Torre et al., 2018; Lamichhane et al., 2018). Therefore, there is a need for their alternation or substitution (as required by the Directive 2009/128/EC). The use of mathematical models and Decision Support Systems for better scheduling the application of plant protection products, as well as the use of biocontrol agents, botanicals, and resistance inducers (Rossi et al., 2019; Pertot et al., 2017; Caffi et al., 2009) are sustainable alternatives for an Integrated Pest Management (IPM) strategy. Partially resistant varieties are also very promising (Lu, 1997), because their use is simple, inexpensive for farmers, compatible with other management options, and does not have negative environmental impacts.

Resistance to DM and PM has been one of the major concerns of breeding programs (Miclot et al., 2019; Delame et al., 2019; Zini et al., 2019). The sources of resistance to diseases can be found in wild American *Vitis* species as well as in some varieties of *V. vinifera* from Central Asia (Gessler et al., 2011). Nowadays, more than 30 Quantitative Trait Loci (QTL) associated with resistance response to downy and powdery mildews have been identified and used in breeding programs (Di Gaspero and Cattonaro, 2010), which confer partial resistance by modifying some resistance components like infection frequency, latency period, lesion size, spore production, infectious period, infectivity (Bove and Rossi, 2020).

The development of resistant varieties requires screening methods in environmentally controlled conditions and at field level. Although several leaf disc bioassays on resistant grapevines have been performed (Bove and Rossi, 2020; Possamai et al, 2020, Eisenmann et al, 2019; Vezzulli et al, 2018), little research has been focused on the assessment of the resistance level under field conditions on leaves and, particularly, on bunches.

This work focuses on the evaluation of resistance to downy mildew and powdery mildews on both leaves and bunches of grapevine varieties carrying resistance genes under field conditions.

2 Plant materials and disease assessment

The research was performed in 4 years (2017 to 2019, and 2021) in an experimental vineyard located in the campus of Università Cattolica del Sacro Cuore in Piacenza, Northern Italy (45°02′05″N, 9°43′46″E), planted with 16 grape varieties, most of them carrying one or more loci conferring resistance to *P. viticola* (Rpv) and *E. necator* (Ren). The *V. vinifera* variety 'Merlot', which is known to be highly susceptible to downy mildew and powdery mildew, served as positive control. The 15 resistant varieties used in the research were: 'Bronner', 'Johanniter', 'Solaris', 'Calardis Blanc', 'Felicia', 'Villaris', 'Calandro', 'Regent', 'Reberger', 'Merlot Khorus', 'Merlot Kanthus', 'Cabernet Volos', 'Fleurtai', 'Rkatsitelii', and 'Palava'. 'Bronner', 'Johanniter' and 'Solaris' resulted from breeding programs performed at the Institute of Viticulture and Enology in Freiburg (Germany). The Julius Kühn Institut (JKI) in Geilweilerhof, Siebeldingen (Germany) carried out the hybridization of 'Calardis Blanc', 'Felicia', 'Villaris', 'Calandro', 'Regent', and 'Reberger'. The 'Merlot Khorus', 'Merlot Kanthus', 'Cabernet Volos', and 'Fleurtai' varieties were developed at the University of Udine and Institute of Applied Genetics (IGA) in Italy. Varieties were arranged in a complete randomized block design, with four replicate plots, each consisting of four plants. When the research started, the vines were 5-year old and were managed with a single Guyot training system, with 1.2 m in the row and 2.0 m between rows. Fungicides were not applied for the entire duration of the experiment. Air temperature (in °C), relative humidity (in %), rainfall (in mm), and leaf wetness (in hours) were recorded by a standard meteorological station located in the experimental vineyard.

Disease assessments were carried out from inflorescences fully developed (BBCH 57; Lorenz et al., 1995; at mid-May) to berries developing colours (BBCH 83; in the last decade of August). DM and PM were assessed on 33 random leaves and bunches per plot, by using the standard diagrams provided by the European Plant Protection Organization (EPPO), which define the disease severity by classes from 1 to 7 based on the area occupied by the pathogen lesions (EPPO, 2000). Disease severity data were then used to calculate the area under the disease progress curve (AUDPC) of leaves and bunches.

3 Relationships between disease progress on leaves and bunches

The relationships between the AUDPC on leaves and bunches for the two diseases were investigated by using the Pearson's correlation coefficient. The correlation between the AUDPC on leaves and bunches was significant ($P<0.001$) for both the diseases, with $r = 0.89$ for DM and $r = 0.786$ for PM.

To explore the AUDPC data, scatter plots were drawn representing the 4-year averages and standard errors of AUDPCs on leaves (vertical axis) and bunches (horizontal axis); the plot area was then divided into 4 four quadrants based on the overall averages, which corresponded to the following: (i) high disease on both leaves and bunches; (ii) high disease on leaves and low disease on bunches; (iii) low

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disease on leaves and high disease on bunches; (iv) low disease on both leaves and bunches.

For DM, the four quadrants of the scatter plot were divided by the values 0.92 and 0.87, which were the average AUDPC values on leaves and bunches, respectively (Fig. 1). The sensitive control 'Merlot' showed high values of AUDPC on both leaves and clusters. 'Palava' and 'Rkatsitelii', which do not carry Rpvs, and 'Calandro', which carries the locus Rpv3[1], showed high disease on leaves and low disease on bunches. 'Reberger', which do not carry Rpvs, showed low disease on leaves but high disease on bunches. All others varieties showed low disease on both leaves and bunches.

For PM, the four quadrants were divided by the values 2.09 (leaves) and 1.33 (bunches) (Fig. 2). 'Merlot', 'Rkatsitelii', 'Palava', and 'Cabernet volos', which do not carry Ren *loci*, expressed high sensitivity on both leaves and bunches. 'Merlot Khorus' showed high and low disease on leaves and bunches, respectively. Low sensitivity was observed on all the other varieties.

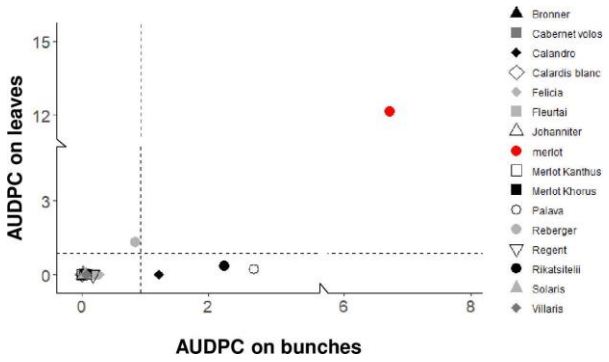

Figure 1: Scatter plots the AUDPC values for *Plasmopara viticola* on leaves and bunches. Dots are average AUDPC values for each variety, and wishers are standard errors. Dotted lines show the mean of AUDPCs on leaves and bunches.

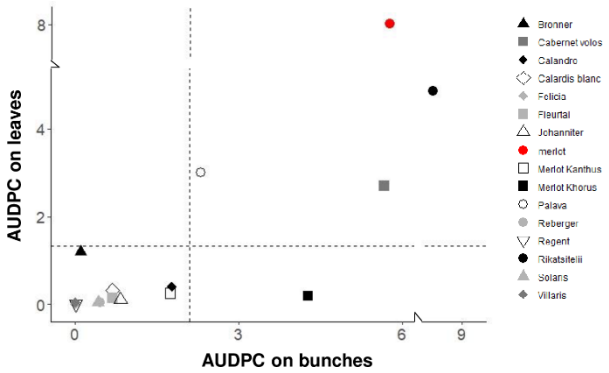

Figure 2: Scatter plots the AUDPC values for *Erisiphe necator* on leaves and bunches. Dots are average AUDPC values for each variety, and wishers are standard errors. Dotted lines show the mean of AUDPCs on leaves and bunches.

4 Grouping the varieties based on resistance level

To group the grape varieties based on the AUDPC values, a multivariate hierarchical cluster analysis was used. Clustering was conducted on data standardized by using the z-scores, based on the within-group similarities and between-group differences in the average values of AUDPC values on leaves and bunches; clusters were finally identified based on the intermediate distance.

For DM, the hierarchical cluster analysis grouped the varieties at different rescaled distances (Figure 3); by applying to the dendrogram a 4-unit dissimilarity cutoff point, four clusters were identified (from CLU1 to CLU4). CLU1 included only the control 'Merlot', which showed low resistance to downy mildew. Medium resistance to downy mildew was observed in CLU2 and CLU3, while high resistance was recorded for CLU4. CLU2 included only 'Reberger', while CLU3 included 'Calandro', 'Palava', and 'Rkatsitelii'. All the other varieties were grouped into CLU4. According to the among-cluster ANOVA and LSD test, clusters were significantly different for the level of resistance to downy mildew ($P < 0.001$), with CLU1 as well as CLU4 significantly different from all other clusters.

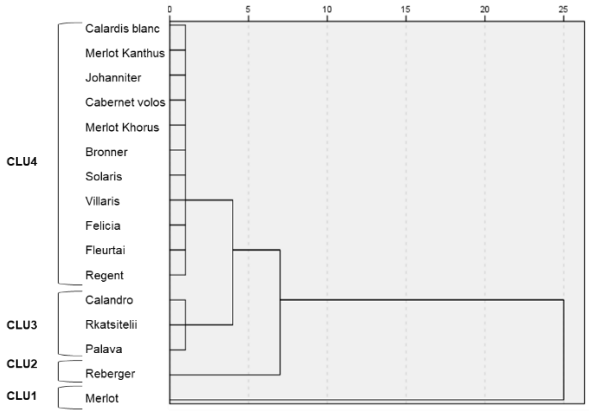

Figure 3: Dendrogram resulting from a hierarchical cluster analysis of the values of area under the disease progress curve (AUDPC) of *Plasmopara viticola* on leaves and bunches of the grapevine varieties, in the four years of the experimental trials. Four clusters were identified in the dendrogram (CLU1 to CLU4).

For PM, the hierarchical cluster analysis grouped the varieties in three clusters (from CLU1 to CLU3, Figure 4) by applying to the dendrogram a 5-unit dissimilarity cutoff point. CLU1 included 'Merlot' and 'Rkatsitelii' that represent low resistance to the disease. In fact, both the positive control 'Merlot' and 'Rkatsitelii' do not carry Rens. Low to medium levels of resistance were recorded for varieties in CLU2, which were 'Palava' (no Rens in its genome), and 'Cabernet volos' and 'Merlot Khorus' (presence of Rens still unknown). High resistance to powdery mildew was observed for all varieties in CLU3. According to the among-cluster ANOVA and LSD test, clusters were significantly different for the level of resistance to powdery mildew ($P = 0.002$), with CLU3 significantly different from CLU1 and CLU2.

5 Conclusions

In this work, a main goal was to assess the extent to which partial resistance is able to control DM and PM under field conditions, and to provide information on the resistance level on both leaves and clusters.

Overall, the majority of the grape varieties included in this study showed high resistance to both diseases, on leaves and,

especially, on bunches.

The varieties were clustered in four and three groups for their different level of resistance to downy and powdery mildew, respectively. These clusters well summarize the overall resistance of the plant, reflecting the main findings from the scatter plots.

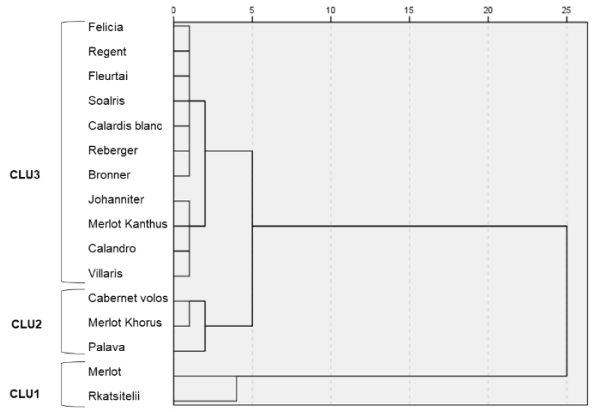

Figure 4: Dendrogram resulting from a hierarchical cluster analysis of the values of area under the disease progress curve (AUDPC) of *Erisiphe necator* on leaves and bunches of the grapevine varieties, in the four years of the experimental trials (from 2017 to 2019, and 2021). Three clusters were identified in the dendrogram (CLU1 to CLU3).

Although leaf disc laboratory screening is economical, time-saving and useful for the selection of resistant genotypes, the observation of natural infections in the vineyard during the growing season allows a reliable and complete assessment of the plant phenotype. This knowledge on the behaviour of resistant grapevine varieties under field condition can support breeding programs.

Furthermore, these findings highlight the importance of the development of disease management strategies accounting for the overall and organ-specific level of resistance of these varieties. The implementation of these strategies would allow farmers to take the best advantage from resistance in the disease control in field, and to also protect these varieties from the risk of erosion of resistance.

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