Innovative approaches to grapevine downy mildew management on large and commercial scale

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1 Introduction

The application of chemical fungicides is still today the only effective mean to prevent *Plasmopara viticola* infections on grapevines. This biotrophic oomycete causes the grapevine downy mildew (GDM), that is one of the main diseases that affect vines worldwide and it can be responsible of significant yield losses for farmers (Massi *et al*., 2021).

Nowadays, phytopathologists in the viticultural sector are focused on identifying innovative and sustainable strategies to protect vines from this disease, since the massive use of some chemical products considered dangerous, but still widely used, is no longer in line with the new principles launched by the European Community. Within the European Green Deal, it has been developed the "Farm to Fork" Strategy, which has several objectives, including reduction of 50% the use of dangerous pesticides and increase of organic farming at 25% of the production by 2030.

Copper-based compounds have always played a central role in plant diseases management, especially in viticulture. Even today, fungicides based on copper are the most used to control GDM (Miotto *et al*., 2014). They are particularly used by farmers because their many positive characteristics, including its low cost, broad spectrum of action, multisite mechanism of action, and effectiveness even against bacteria (La Torre *et al*., 2018).

Although copper can be used in organic farming, it remains a heavy metal; it is therefore subjected to bioaccumulation in the trophic chains, and it shows toxicity for aquatic and soil organisms. Accumulation of this heavy metal in vineyards soils cause quantitative and qualitative changes in the composition of soil micro and macro-organisms (Paoletti *et al*., 1998; Rusjan *et al*., 2007; García-Esparza *et al*., 2006; Duca *et al*., 2016). Moreover, soils contaminated with high concentration of copper can be responsible of phytotoxicity in vines. Furthermore, presence of copper on berries, results in risk for human health and can compromise the quality of the wine, since it is able to catalyse oxidative reactions and it can negatively affect the amino acid composition of musts (García-Esparza *et al*., 2006; Miotto *et al*., 2014; Garde-Cerdán *et al*., 2017; Gutiérrez-Gamboa *et al*., 2019).

Considering these issues, the European Union set increasingly stringent limits on the use of copper in agriculture, up to arrive at the current situation, where an average 4 kg/ha of copper per year is allowed (EU Regulation 1981/2018), with the possibility that products containing this heavy metal will be no longer available after 2025, since they have been included in the list of candidates for replacement.

In addition, it must be considered that GDM management required a high number of treatments throughout the year, especially in organic viticulture, where synthetic fungicides are forbidden, and copper, that acts only preventively, is the only active ingredients with proved effectiveness. Furthermore, several European countries have already banned copper (La Torre *et al*., 2018). European organic farmers of the main wine-growing areas are the most worried by this situation, since they currently do not have concrete and available alternatives to protect their vineyards, and therefore their yields, from *P. viticola* attacks.

In this context, the search for alternative solutions with lower environmental impact is strongly promoted (Bortolotti *et al*., 2006; Sancassani *et al*., 2006; Dongiovanni *et al*., 2010; D'Arcangelo *et al*., 2018). Among natural compounds, basic substances are getting increasing interests for crop protection (Marchand *et al*., 2021), and one of them, the biopolymer chitosan, obtained from crab shells, proved to be effective toward *P. viticola* in plot trials (Mancini *et al*., 2018; Romanazzi *et al*., 2016, 2021). Chitosan is a natural biopolymer obtained from chitin deacetylation, industrially extracted from crustaceans. This biopolymer is used in various fields of applications, from food industry to medical scopes and it was the first basic substance approved by the European Community (chitosan hydrochloride, EU Regulation 2014/563). In contact with plant tissues, this biopolymer shows a triple activity: eliciting, antimicrobial and film-forming (Romanazzi *et al*., 2018). In light of these previous results, two of the main wineries of the Marche region were involved in the *PSR Marche* project "Vitinnova", to carry out a three-year full-field investigation, with the double objective of confirming the effectiveness of chitosan hydrochloride also on a commercial scale (in terms of surfaces treated, distribution methods, volumes and intervention times) and to validate application protocols for the development of innovative GDM managements strategies based on the use of this biopolymer, applying it alone or in association with copper.

2 Materials and methods

During 2019, 2020, and 2021, full-field investigations were conducted to study chitosan in innovative GDM management strategies, involving important winery of the area, that are "Terre Cortesi Moncaro Soc. Coop. Agr."and "Belisario s.a.c. ". Trials were conducted in the Marche region, in commercial vineyards located in Angeli di Varano (AN) (cv. Montepulciano), Castelplanio (AN) and Matelica (MC) (both with cv. Verdicchio), managed according to the principles of organic farming.

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Effectiveness of chitosan on commercial scale has been studied under different condition of disease pressure, under different environmental and applicative conditions.

Vineyards were divided in parcels, each one formed by several rows treated in the same way throughout the season to control *P. viticola*. The strategies were distributed according to a randomized block design with three repetitions. Five strategies were investigated per each vineyard:

1. Untreated control: plots were not treated for GDM, but just for other diseases.
2. Treated control: plots have been treated with conventional application of copper-based fungicides from the beginning to the end of the season.
3. Chitosan: chitosan was the only active substance applied against *P. viticola* for all the season.
4. Alternate: here cupric fungicides were used for the first half of the season, followed by applications of chitosan for the second half.
5. Combined: where a mix of chitosan and copper was sprayed for all the season.

The strategy number five, combined, was used just for the last two years (2020 and 2021).

Chitosan treatments were performed at a concentration of 0.50% of active ingredient when it was used alone or alternated with cooper, while in the case of combined treatments the concentrations were halved. Copper-based fungicides were distributed with the conventional dosages used in company protocols, except in the case of combined treatments, where the halved full label dose was adopted.

Treatments have been performed by the companies, according to their protocols and with regular timelines (generally from the end of April to the end of July, depending on the meteorological trend). Conventional applications of copper were carried out with the spraying volumes normally adopted by the companies (that ranged between 150 and 400 L/ha, depending on plants size). The spraying volumes adopted in the treatments containing chitosan were the standard for companies in 2019, then increased in the following years until reaching the standard reference volume for treatments on vines, that is 1000 L/ha.

Throughout the period that involved the experimental trials, vineyards have been constantly monitored to identify the appearance of first symptoms and to see the evolution of GDM, in the experimental plots. Starting from the appearance of first symptoms on the untreated control, assessments have been constantly conducted.

The level of disease present in the experimental parcels was quantified for all the strategies at each assessment, adopting two empirical scales for symptoms severity: one for the leaves (from 0, healthy leaf to 10, infected leaf surface equal to 100%) and one for the bunches (0, healthy to 7, infected surface > 75%). These empirical scales were used to calculate the parameters of incidence, severity (Romanazzi *et al.*, 2016), and weighted average intensity (or McKinney Index) of the disease. Subsequently, a statistical analysis of results was carried out using ANOVA (Analysis of Variance) and applying the Fisher LSD (Least Significant Difference) test according to the probability level of $P \leq 0.05$. The software used for statistical analysis is SPSS20 (Statistical Package for Social Science).

3 Results

Results obtained from three assessments are reported. On cv. ‘Verdicchio’ leaves in Castelplanio on September 23rd, 2019, an assessment showed that all the strategies significantly reduced the McKinney Index, in comparation to the untreated control. In detail, chitosan associated with low quantity of copper ensured reductions similarly to those obtained with conventional application of copper, even if statistical differences emerged. Chitosan distributed alone throughout the entire season inhibited *P. viticola* with significance if compared to the untreated control. Nevertheless, this year in this vineyard, the effectiveness was lower than copper, both if applied alone and if associated with chitosan.

On July 27th, 2020, disease evaluation was run in the vineyard of Castelplanio, on the leaves and grapes of cv. ‘Verdicchio’. The disease pressure in this vineyard was low. The McKinney Index of the GDM was significantly reduced compared to the control for all the strategies adopted. Furthermore, chitosan alone recorded the lowest value of McKinney Index on leaves and grapes (Figure 1).

Assessment conducted in Matelica on leaves on September 14th, 2021, it was highlighted that all treatments reduced the McKinney Index compared to the untreated control. Furthermore, no differences emerged between copper applied as usual for farmers and low dosages of copper, sustained with chitosan (either alternating or combining the two compounds).

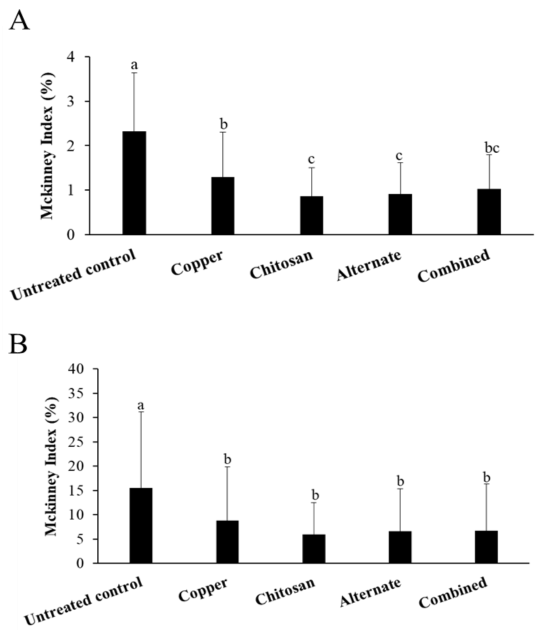

Figure 1: McKinney Index of grapevine downy mildew on cv. ‘Verdicchio’ leaves (A) and grape bunches (B). Results obtained from the assessment conducted on July 27th, 2020,

in the vineyard of Castelplanio. Different letters correspond to significantly different values according to the Fisher LSD (Least Significant Difference) test, $P \leq 0.05$.

4 Discussion

The search for innovative solutions for GDM management is promoted by the limitations that have been placed on the use of copper compounds in agriculture. The EU Regulation 1981/2018 restricts the maximum quantity of copper allowed per year (i.e., an average of 4 kg/ha) and inserts copper in the list of active ingredients candidate to substitution, frightening operators of this sector, especially those who works in the system of organic farming.

This work, developed within the *PSR Marche* project "Vitinnova", aimed to evaluate on a commercial scale and over three consecutive years (2019, 2020 and 2021), the effectiveness of innovative GDM approaches based on the use of chitosan, as an alternative or complementary compound to copper for the protection of vines from GDM.

Chitosan effectiveness in plant protection has already been demonstrated in several studies, including its effectiveness against *P. viticola* (Aziz *et al*., 2006; La Torre *et al*., 2010; Dagostin *et al*., 2011; Romanazzi *et al*., 2016, 2021). Nevertheless, is it also important to transfer knowledges acquired in previous investigations in full field trials, involving companies that are interested to innovative solutions in line with principles launched by the European Community. The effectiveness of this polymer seems to be due to the threefold activity that it can carry out in contact with plant tissues: induction of resistance, direct antimicrobial activity and biofilm formation. Recently, some studies have been carried out to test compatibility between chitosan and widely used pesticides (Romanazzi *et al*., 2020). Those results open the way to a large-scale diffusion of chitosan-based formulations, since compatibility plays a fundamental role to ensure that an innovative compound will be well accepted by companies, that usually performs treatments with more than one product combined simultaneously.

From this multi-year field study, it emerged that chitosan has confirmed its effectiveness against GDM, even on a commercial scale (considering extension of surfaces, distribution systems, volumes, and timing of treatments) and in different cultivar and environmental conditions, such as operating conditions, climatic, and pressure of the disease. Furthermore, the results obtained suggest that the effectiveness of chitosan-based treatments is connected to some factors, such as the concentration of the active ingredient, and the volumes of spraying, that must ensure adequate wetting of the vegetation. The ability of the biopolymer to carry out its threefold activity is closely linked to the distribution systems, in addition to its origin and its intrinsic properties.

5 Conclusions

This study highlighted the problems related to compatibility between compounds, and contributed to set up a protocol for innovative approaches, focusing attention on the possible side effects that can occur passing from experimental scale to commercial scale.

Results obtained confirmed the validity of chitosan in vines protection from GDM and make it useful insights of the new scenarios for viticulture, characterized by increasingly stringent limitation on the use of chemical fungicides and a possible future banning of copper compounds.

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