CHITOSAN TREATMENT TO MANAGE GRAPEVINE DOWNY MILDEW

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Abstract:

Context and purpose of the study

Downy mildew is one of the most important grapevine diseases, caused by the Oomycete *Plasmopara viticola*. The management of the disease in organic agriculture can require up to 15 copper applications per year. However, copper accumulates in the soil, is phytotoxic and is toxic for organisms living in the soil, its use has been restricted in European Union to maximum 28 kg in 7 years. Therefore, testing of alternatives with equal effectiveness is desirable. Among those, the natural biopolymer chitosan, obtained from crab shells, proved to be effective toward downy mildew in plot experiments. The aim of our trials was to extend chitosan applications in large scale experiments in different years, cultivars and environmental conditions.

Material and methods

Trials were carried out in 3 commercial organic vineyards of Marche Region, Central-Eastern Italy, along 3 seasons (since 2019 to 2021). Treatments were applied on cv Verdicchio of wineries Moncaro (in Castelplanio, AN) and Belisario (in Matelica, MC), and on 'Montepulciano' of winery Moncaro, in Angeli di Varano (AN). Chitosan was applied at 0.5% along the season, in the second half of the season following copper, and combined at half rate (0.25%) with half copper rate. Untreated and copper treated plots were used as a control. Downy mildew infections were recorded along the season, and disease incidence, severity and McKinney index were calculated.

Results

The weather conditions were variables among the years and the vineyards. Chitosan treatments reduced McKinney Index of downy mildew, compared to untreated control, on bunches of cv. Verdicchio by 41.7%, 85.5%, and 43.9%, and on cv. Montepulciano by 17.1%, 68.9%, 45.2%, respectively, in 2019, 2020 and 2021. Similar results were obtained on leaves. In 2021, combination of chitosan with reduced copper rates protected leaves and bunches from downy mildew at the same level of copper. This investigation demonstrates that chitosan can be introduced in downy mildew management strategies and can integrate or even replace copper with similar degree of protection and with lower amounts of residues on the grapes.

Keywords: Chitosan, Copper, Downy mildew, Grapevine, *Plasmopara viticola*, *Vitis vinifera*.

1. Introduction

Plasmopara viticola (Berk. & M. A. Curtis) Berl. & De Toni in Sacc. is a widely known polycyclic plant pathogen that causes grapevine downy mildew (GDM), which is among the most important disease that affect grapevines (Gessler et al., 2011). This disease can cause extensive damage to production, both qualitatively and quantitatively. Nowadays, chemical treatments still represent the only effective means to limit GDM infections. In integrated viticulture, and especially in organic viticulture, copper-based products are the most widely used fungicides 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). Copper is a heavy metal, and its repeated use over time results in negative environmental effects, due to its bioaccumulation, phytotoxicity and toxicity toward soil organisms (Hobbelen et al., 2016). Therefore, the European Union established severe restriction to cupric inputs in agriculture, and in Italy the use of copper is limited up to a maximum of 4 kg/ha per year. Through the 'Farm to Fork' strategy, the European Commission planned some objectives to make the food system fair, healthy and respectful of the environment, including to extend the areas under organic farming, and to halve the use of pesticides, including copper, within 2030. 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 that have exhibited effectiveness against *P. viticola*, chitosan has been shown good results in plot trials (Mancini et al., 2018; Romanazzi et al., 2016, 2021). Chitosan is a natural biopolymer, obtained by deacetylation of chitin, which is the second most important polysaccharide in nature after cellulose, and is present in the exoskeleton structure of marine invertebrates, insects, as well as fungi, algae, and yeast. 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 (Marchand et al., 2021; Romanazzi et al. 2022). This biopolymer can elicit host defenses, have antimicrobial activity toward fungi, and produce a semipermeable film on a treated surface (Romanazzi et al., 2018). Our study, developed within the Marche region were involved in the PSR Marche project "Vitinnova", aimed to evaluate the effectiveness of innovative GDM protection strategies on large scale, based on the use of chitosan alone, alternated or in combination with copper to control GDM.

2. Material and methods

Experimental vineyards - Trials were conducted in three different vineyards located in the main production areas of the Marche region, Italy. The experimental field trials were conducted during 2019, 2020 and 2021 in commercial vineyards established with different wine grape varieties of the winery "Terre Cortesi Moncaro Soc. Coop. Agr." in Angeli di Varano (cv. Montepulciano) and Castelplanio (cv. Verdicchio) (Ancona, Italy) and "Belisario s.a.c.", located in Matelica (cv. Verdicchio) (Macerata, Italy).

Treatments - 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. For three years, four different GDM protection strategies were investigated, and they were compared to an untreated control each year:

1) Chitosan alone throughout the whole season
2) Alternating treatments: copper until flowering then chitosan
3) Combined treatments: copper and chitosan mixed at half doses (in 2021 only)
4) Conventional farm application of copper throughout the whole season

These investigations have been carried out under different environmental and operating conditions, both during years with medium-high (2019 and 2020) or low (2021) disease pressure. Chitosan treatments were performed at a concentration of 0.5% of active ingredient when it was used alone or alternated with cooper, while in the case of combination with cooper the concentration of chitosan was 0.25%. Copper-based fungicides were distributed with the conventional rates 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.

Disease assessment - 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.

Statistical analysis - The data were subjected to analysis of variance according to a randomized block de-sign. Statistical analyses were performed using the software SPSS (Statistical Package for Social Science, version 20, IBM, Armonk, NY, USA). The data were first tested for normality and homogeneity of variance using the Levene's test. Upon confirmation, a one-way analysis of variance (ANOVA) was performed to determine any difference and the means for treatments were separated using Fischer LSD (Least Significant Difference), with $P \leq 0.05$. When the homogeneity of variance was not confirmed, Welch's ANOVA was per-formed to determine any differences in treatments effects, and the treatment means were separated using the Games-Howell post hoc test ($P \leq 0.05$).

3. Results and discussion

In 2019, infections started in Castelplanio vineyard on cv. Verdicchio around middle June. On bunches all the applied strategies significantly reduced disease as compared to the untreated control. In detail, McKinney Index resulted in a significant reduction by the application of chitosan, copper□chitosan and copper by 41.7%, 88.5% and 94.6% as compared to the control. No significant difference was found in McKinney Index of GDM between copper□chitosan and copper. On cv. Montepulciano McKinney Index was reduced on bunces sprayed with chitosan alone by 17.1%, as compared to the control. Chitosan effectiveness in plant protection has 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). In an assessment conducted on 8 September 2020, in Matelica on cv. Verdicchio, all the strategies significantly limited *P. viticola* infections on bunches compared to untreated plants and they provided the same protection level than conventional copper treatments (Fig. 1). In the assessment on grape bunches conducted on 27 July 2020, on cv. Verdicchio in Castelplanio (AN), all the strategies under investigation significantly reduced the disease McKinney Index disease amount compared to the untreated bunches (Fig. 2). No significant differences emerged between the copper control and the innovative strategies in this case. The 2021 was not favorable to GDM development, due to the scarcity of rainfall that characterized the whole season. In Angeli di Varano, on cv. Montepulciano an assessment was conducted on bunches, and it has emerged that strategies of combination (chitosan + copper) and farm application (copper control) significantly reduced the McKinney Index by 92.7% and 69.9% respectively if compared to the untreated. 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). Individual application of chitosan has also been shown to be effective in the long term under both conditions of high and low disease pressure (Romanazzi et al., 2016, 2021). Good prospects are offered for the next future by alternating and combined strategies, since the use of copper for the first half of the season followed by chitosan application appears to be useful to limit GDM symptoms even under conditions of high disease pressure (Romanazzi et al., 2021; Soares et al., 2023). 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.

4. Conclusions

This study also highlighted the problems related to compatibility between compounds, to the cost of innovative molecules, and to set up a protocol for innovative approaches diffusion, focusing attention on the possible side effects that can occur passing from experimental scale to commercial scale. According to our results, chitosan can be introduced in downy mildew management strategies and can integrate or even replace copper with similar degree of protection and with lower amounts of residues on the grapes.

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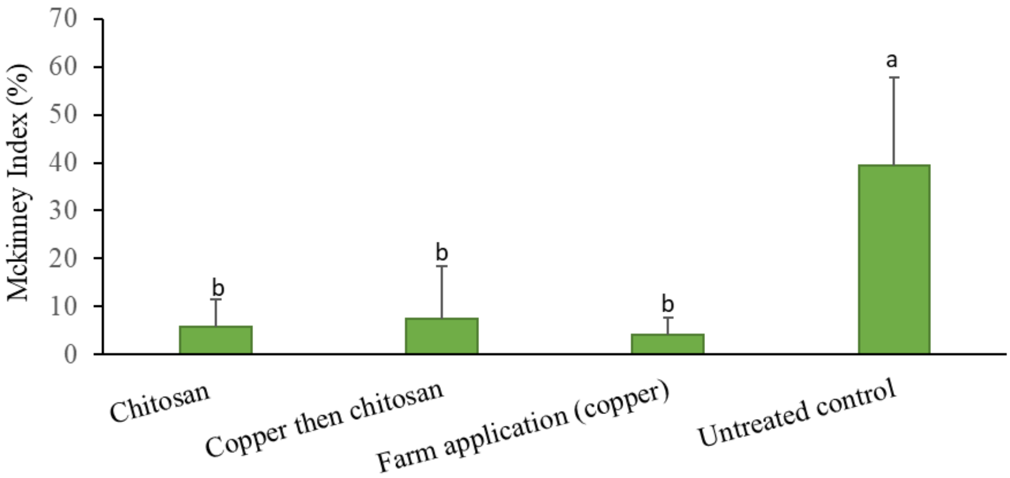

Figure 1: McKinney Index of grapevine downy mildew on grape bunches cv. Verdicchio in Matelica on 8 September 2020, after three management strategies. Histograms followed by different letters are considered statistically different.

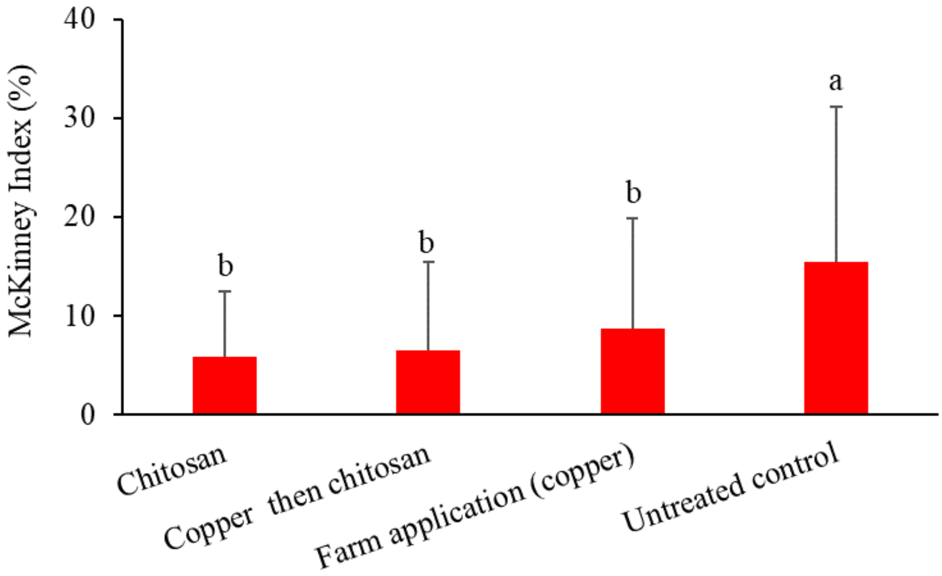

Figure 2: McKinney Index of grapevine downy mildew on bunches cv. Verdicchio in Castelplanio on 27 July 2020, after three management strategies. Histograms followed by different letters are considered statistically different.