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Applications of chitosan alone, alternated or combined with copper for grapevine downy mildew management in large scale trials

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ABSTRACT

The persistent use of copper-based fungicides to control grapevine downy mildew (GDM) still represents risks to both human health and the environment, especially in organic production. This heavy metal can affect wine quality due to residues' presence on grape bunches. Implementing sustainable viticultural practices to control diseases is crucial for the wine industry. This work aimed to evaluate chitosan effectiveness toward GDM on a large scale, over a period of three years (2019–2021), and in three distinct vineyards with different environmental conditions. Three GDM management strategies were tested: application of 0.5% chitosan individually; in alternation with copper; and during the last year, in combination with low rate of copper, using conventional strategies and untreated plants as controls. Over three consecutive seasons, chitosan treatments effectively reduced GDM McKinney Index on leaves and bunches under different environmental conditions. On bunches, chitosan alone was less effective than copper, when applied after copper had the same effectiveness, and combined with copper was more effective than copper standard. In 2020 and 2021, the analysis of cooper amount detected in berries of two cultivars showed that all strategies allowed to decrease the amount of copper residue in berries. The application of chitosan alone or in alternation with copper was able to reduce this amount as untreated control. This large scale investigation suggests chitosan as alternative that can integrate and eventually replace copper application against GDM. Further studies to validate these strategies in different environments, disease pressure conditions and cultivar are fundamental towards achieving green and sustainable solutions for GDM management strategies.

1. Introduction

In recent years, there has been a growing trend for society and farmers to pay more attention to sustainable production methods that minimize the use of chemical inputs, conserve natural resources, and maintain the health and biodiversity of the ecosystems. Nowadays, there is an increased consumer awareness of pesticide residues in food and wine, which has motivated the wine industry to search for natural and environmentally friendly alternative strategies to control diseases (Schäufele and Hamm, 2017). The implementation of sustainable practices in viticulture has thus become an important issue for the wine industry (Barbosa et al., 2018). Among the practices, disease management represents the major challenge, since it is one of the most impactful practices and it is crucial to ensure the long-term productivity and profitability of the vineyard (Saint-Ges and Bélis-Bergouignan, 2009).

The diseases with the highest economic impact for grapevines are downy mildew (GDM) and powdery mildew (GPM), caused by *Plasmopara viticola* and *Erysiphe necator* respectively (Delière et al., 2015). In areas characterized by warm, humid, and rainy climates, such as some areas of the Mediterranean basin, GDM can lead to serious losses of production, that can reach 100% if no chemical treatments are performed to protect vines (Gessler et al., 2011). *P. viticola* can infect the green organs of plants, reducing their photosynthetic efficiency and thus the ability to store reserve substances for the following season (Jermini et al., 2012).

Copper-based fungicides have consistently played a fundamental

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role in the protection of grapevines from GDM, especially in organic viticulture, where the use of synthetic fungicides is not allowed (La Torre et al., 2018). Copper is classified as a microelement for plant nutrition, but high concentrations can cause phytotoxic responses in vines (Romanazzi et al., 2016). This heavy metal is subject to bioaccumulation (Wang et al., 2023; Xie et al., 2023), and its repeated use results in several negative consequences for the environment and soil fertility, as well as in risks for human health (Hobbelen et al., 2006; Komárek et al., 2010). Although low-impact techniques are available for the remediation of heavy metal-contaminated soils and water, their applicability in agroecosystems should be explored (Mackie et al., 2012; Wang et al., 2023; Xie et al., 2023) and the search for alternative GDM protection strategies remains fundamental for reducing copper pollution. The presence of copper can also cause problems during the winemaking process, affecting the wine quality and its shelf life (Provenzano et al., 2010). Considering all these issues, the use of copper-based products in agriculture has been limited by the European Commission. The Regulation (EU) 2018/1981 of December 13, 2018 fixed a renewal period for copper compounds at 7 years, until December 31, 2025, and the quantity allowed is restricted to 28 kg/ha, on average 4 kg copper/ha per year.

Copper is still today the only effective weapon to prevent *P. viticola* infections in organic vineyards, necessitating up to 15–17 treatments during the season (Dagostin et al., 2011). In this context, a key aim of organic farming is to find alternatives to copper to protect plants from diseases, especially GDM. These alternatives need to be equally effective against GDM while being less dangerous for the environment and humans compared to copper. Several studies have reported the effectiveness of natural substances to control GDM, such as chitosan (Romanazzi et al., 2021), laminarin (Aziz et al., 2007), microorganisms (Puopolo et al., 2014), biostimulants (Gutiérrez-Gamboa et al., 2019). Among these, chitosan has been gaining interest because in field trials it showed promising protection levels from GDM (Romanazzi et al., 2016, 2021). Chitosan is a natural biopolymer obtained from chitin deacetylation and, when applied on plant surfaces, it can perform a threefold activity, having film-forming, antimicrobial and eliciting properties (Romanazzi et al., 2018). Since chitosan is widely used in other fields of application, such as medicine and food industry, it is considered non-toxic, and it was one of the first basic substances approved in the European Union (Regulation EU, 2014/563) for plant protection (Marchand et al., 2021). It is also classified as GRAS (Generally Recognized as Safe) in the United States by the Food and Drugs Administration (Romanazzi et al., 2022). The objectives of this investigation were: (i) evaluate the effectiveness of chitosan against GDM on a commercial scale to reduce pollution caused by chemical treatments, and (ii) quantify the residues of copper on grape bunches collected from plots subjected to different GDM management strategies.

2. Materials and methods

2.1. Experimental vineyards

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 and Castelplanio (Ancona, Italy) and "Belisario s. a.c.", located in Matelica (Macerata, Italy). These investigations were carried out under different environmental and operating conditions, both during years with medium-high (2019 and 2020) or low (2021) disease pressure. The vineyards are in the main wine growing areas of the region: one of cv 'Montepulciano' is located close to the Adriatic coast, in the production area of "Rosso Conero", while the others of cv 'Verdicchio Bianco' were more distant from the sea, where "Verdicchio dei Castelli di Jesi" and "Verdicchio di Matelica" are produced. Details of the vineyards are summarized in Table S1 of supplementary materials.

2.2. Treatments

The effectiveness of chitosan to contain GDM alone or associated with reduced copper rates was studied for three years on two different cultivars, under different environmental and applicative conditions. Four strategies were distributed in all the vineyards according to three randomized blocks experimental design: (i) standard copper-based fungicides during the whole season; (ii) 0.5% chitosan during the whole season; (iii) alternating copper→chitosan, with copper-based fungicides used until mid June (BBCH 60), followed by 0.5% chitosan application for the second half of the season. In 2019 and 2020, these three strategies were tested, while in 2021 a fourth strategy was introduced: (iv) the combination of 0.25% chitosan with copper oxychloride at a reduced rate (half of the full label dose). Strategies were compared to an untreated control, where plants were not treated for GDM.

In the vineyard of Angeli di Varano, the areas dedicated to the trials were around 1.0 ha, 0.6 ha, and 1.5 ha in 2019, 2020 and 2021 respectively. In Castelplanio, strategies were distributed on about 0.7 ha for all the three years, while in the vineyard of Matelica, the experimental area was around 1.5 ha in 2019 and 2021, and it was about 0.6 ha in 2020.

Details about products used in each vineyard to control GDM are listed in Table 1. The differences between the vineyards among the three years in terms of application volumes, number of treatments, concentrations of chitosan and quantity of copper are summarized in Table S2. Standard copper applications were applied with the usual volumes used by the companies.

Treatments were generally carried out from the end of April/beginning of May to late July/beginning of August and started in different periods according to the company schedule. The intervals between treatments ranged between 7 and 12 days, depending on the weather conditions of the season for each area. Products were distributed with three different machines: in Angeli di Varano treatments were made with a Vma articulated atomizer, model "Power 55"; a towed sprayer with rear suction was used in Castelplanio ("Turbmatic Defender MK2" model, produced by "Società Agricola Estense (S.A.E.)") and Matelica ("Poli" model from "Agricolmeccanica" society). The machines were thoroughly cleaned between the application of different products to eliminate carry over.

2.3. Meteorological data

The weather parameters for temperatures (weekly mean; minimum, maximum) humidity and rainfall (weekly mean) were obtained from the Bollettino Agrometeorologico published by *Agenzia Marche Agricoltura Pesca* (AMAP). These relating to the AMAP weather stations located close to the experimental fields, in Camerano (AN), Castelplanio (AN) and Matelica (MC).

2.4. Disease assessment

For each vineyard, all the management strategies were monitored for GDM throughout the season. Experimental vineyards were divided into three blocks. For each treatment, three rows for assessments were chosen by discarding the border lines between plots to eliminate the edge and the drift effects. GDM assessments were carried out by using empirical scales to calculate disease incidence, severity, and McKinney Index (Romanazzi et al., 2016), both for leaves and bunches. For canopy assessments, a fixed number of 100 leaves per plant were assessed, with eleven classes of severity (from 0 to 10), while bunches were counted on each plant and eight classes of severity were adopted (0–7). These parameters and the percentage reductions were calculated based on the formulae listed in the supplementary material.

The GDM evaluations were conducted starting when symptoms caused by the natural inoculum appeared, until September before harvest. Due to the scarcity of rainfall that characterized the Marche region

Table 1
Details of products applied in the different strategies during the three-years investigation in the commercial vineyards to contain grapevine downy mildew infections.

Strategy	Active components (concentration)	Commercial product (distributor)	Angeli di Varano			Castelplanio			Matelica		
			2019	2020	2021	2019	2020	2021	2019	2020	2021
Farm application[a]	Bordeaux mixture (124 g/L)	Bordoflow new (Manica S.p.A., Italy)	×	×	×	×	×	×			
	Copper oxide (75 g/100g)	Cobre Nordox 75 WG (Commercial quimica Massò S.A., Spain)	×	×	×	×	×	×			
	Bordeaux mixture (20 g/100g)	Poltiglia disperss (UPL Italia S.r.l, Italy)	×			×					
	Copper neutralized sulfate (20 g/100g)	Siaram 20WG (Sumitomo Chemical S.r.l., Italy)		×			×				
	Copper neutralized sulfate (124 g/L)	Biocupro (Corteva S.r.l., Italy)			×			×			
	Copper tribasic sulfate (180 g/L) + Copper hydroxide (120 g/L)	Kop-Twin (Chimiberg S.p.A., Italy)	×	×		×	×				
	Copper tribasic sulfate (193 g/L)	Idrorame flow (Chimiberg S.p.A., Italy)	×			×					
	Copper hydroxide (22 g/100g)	Mexiram HI bio (Adama Italia S.r.l., Italy)	×	×		×	×				
	Copper tribasic sulfate (190 g/L)	Tri-Base (Sumitomo Chemical S.r.l., Italy)							×	×	×
	Copper hydroxide (30 g/100g)	Kocide opti (Certis Europe, Italy)							×	×	
	Copper hydroxide (20 g/100g) + Copper oxychloride (10 g/100g)	Airone Extra (Gowan Italia S.r.l., Italy)									×
Chitosan alone	Chitosan hydrochloride (100%)	Chitosano (Agrilaete S.r.l., Italy)	×			×			×		
	Chitosan hydrochloride (50%)	Chitosano denso (Agrilaete S.r.l., Italy)		×	×		×	×		×	×
Copper→ chitosan	Farm applications[a] → Chitosan hydrochloride (100%)	Several copper-based products → Chitosano	×			×					
	Farm applications[a] → Chitosan hydrochloride (100%)	Kocide opti, Tri-Base → Chitosano							×		
	Farm applications[a] → Chitosan hydrochloride (50%)	Several copper-based products → Chitosano denso		×	×		×	×			
	Farm applications[a] → Chitosan hydrochloride (50%)	Kocide opti, Tri-Base, Airone Extra → Chitosano denso								×	×
Chitosan + copper	Copper oxychloride (377.5 g/L) + Chitosan hydrochloride (50%)	Pasta Siapa F Blu (Sumitomo Chemical S.r.l., Italy) + Chitosano denso	-[b]	–	×	–	–	×	–	–	×

[a] Applying copper-based products throughout the whole season.
[b] (–) Not tested.

during spring and summer 2021, the season was not conducive to GDM infections in July, while symptoms of grapevine powdery mildew (GPM) were observed on fruits, and some assessments were carried out to evaluate eventual collateral effects of the innovative GDM management strategies against GPM.

2.5. Analysis of copper residues on berries

Before harvest, in 2020 in Castelplanio and in 2021 in Angeli di Varano and Matelica, three samples of grapes were collected from each strategy studied (1.5 kg/strategy), to determine the residue of copper in grape juice. The analysis was conducted by Cooperativa Terre Cortesi Moncaro using the Hyperlab Smart Automatic Analyzer (Steroglass, San Martino in Campo, Perugia, Italy).

2.6. Statistical analysis

The data were subjected to analysis of variance according to a randomized block design. 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 performed 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

3.1. Meteorological data

The meteorological trends recorded from April to September of each year in the areas under experimentation are reported in Fig. S1. With a regional average temperature of 14.6 °C, 2019 was the warmest year for the Marche region since 1961. Rainfall was also higher than normal, especially in spring with a peak in May. Lower temperatures were recorded in 2020 than in 2019. However, despite a rather wet spring, fewer mm of water fell this year compared to the 1981–2010 reference average. Significantly impacting this figure was the scarcity of winter precipitation. The year 2021 also registered above-normal temperatures. There was a notable lack of precipitation during spring and summer months in this case. Spring 2021 was the second driest spring for the Marche region since 1961 (www.amap.marche.it).

3.2. Evaluation of grapevine downy mildew infections: 2019

In 2019, GDM symptoms on cv. Montepulciano appeared on the vegetation in mid June. On the leaf assessment of July 15, 2019, GDM incidence was higher in the untreated control and the alternating treatments (copper→chitosan), while it was lower for chitosan alone, and for farm application, that reduced incidence compared to the untreated control by 7.70% and 41.01% respectively. Disease severity was significantly lower in plots treated with chitosan than in the others, while farm application did not reduce the severity of symptoms and copper→chitosan showed values significantly higher than the untreated control. McKinney Index in plots treated with copper→chitosan was similar to the control, while it was reduced in plots treated with chitosan and copper by 14.67% and 36.84%, respectively (Table 2).

GDM incidence on bunches in untreated plots was very high (96.06%). All the strategies showed significant reductions of disease incidence compared to the control. Disease severity was higher in plots untreated and treated with chitosan, with significant reductions in plots treated with copper→chitosan. The McKinney Index was significantly reduced on plants sprayed with chitosan alone, copper→chitosan, and copper by 17.14%, 48.60%, and 76.70%, respectively, as compared to the control (Table 2).

In cv. Verdicchio Bianco, infections started in Castelplanio vineyard around the middle of June on leaves. On 23 September, all the strategies significantly reduced GDM incidence and severity on leaves, compared to untreated plants. The same was observed for McKinney Index which was significantly reduced by 25.83%, 88.45% and 96.70% by chitosan, copper→chitosan and copper, respectively (Table 3).

On bunches all the applied strategies significantly reduced the three disease parameters as compared to the untreated control. In detail, chitosan, copper→chitosan and copper reduced disease incidence of 37.35%, 71.79% and 77.42%, respectively. The same treatments reduced disease severity by 8.70%, 67.65% and 77.01%, respectively. McKinney Index resulted in a significant reduction by the application of chitosan, copper→chitosan and copper by 41.69%, 88.45% and 94.64% as compared to the control. No significant difference was found in McKinney Index of GDM between copper→chitosan and copper (Table 3).

In 2019 the results in the vineyard of Matelica (cv. Verdicchio Bianco) were unclear due to the low disease pressure: GDM McKinney Index in the untreated control was 0.02% on leaves and 0.82% on bunches.

3.3. Evaluation of grapevine downy mildew infections: 2020

The first GDM infections appeared on leaves and bunches of Montepulciano in late June. In Angeli di Varano, GDM incidence, severity, and McKinney Index on leaves, were reduced significantly in comparison with the untreated control for all the strategies applied (Table 4). Compared to untreated control the McKinney Index recorded respective reductions of 46.67%, 79.42% in chitosan and copper→chitosan. On bunches, GDM incidence was significantly reduced compared to the control only with alternating treatments, by 53.70%. Chitosan and copper→chitosan reduced, with significance, the severity of symptoms compared to both the controls (untreated and farm application) and McKinney Index compared to the untreated control, by 68.87% and 74.53%, respectively. It must be highlighted that in this case, the conventional application of copper did not significantly reduce the parameters measured.

In 2020, the vineyard in Castelplanio had a high disease pressure on leaves, with over 50% GDM incidence in untreated plants. GDM symptoms appeared at the beginning of July. During the assessment on leaves and bunches the two strategies significantly reduced the disease amount compared to the untreated. Chitosan showed the highest reductions for all the parameters at the same level of farm application. In details, it was observed that on leaves GDM McKinney Index recorded significant reductions with chitosan, copper→chitosan and copper by 83.94%, 71.95% and 83.90%, respectively. On grape bunches, the same strategies significantly reduced the McKinney Index by 62.01%, 58.06%, and 43.43%, respectively (Table 5).

During the season 2020, symptoms of GDM on cv. Verdicchio Bianco in Matelica appeared in June. On the assessment on leaves and bunches, significant reductions in GDM incidence, severity and McKinney Index were recorded with all the strategies in comparison with the untreated control for both leaves and bunches. Chitosan, copper→chitosan and conventional farm applications of copper reduced the disease weighted medium intensity on leaves by 70.12%, 78.26% and 92.79% respectively. On bunches, the same strategies reduced McKinney Index by 85.48%, 81.36%, and 89.65%. No differences with significance emerged between copper control and chitosan alone or alternated with copper in terms of fruits protection from GDM (Table 6).

3.4. Evaluation of grapevine downy mildew infections: 2021

The 2021 season was not favorable to GDM development, due to the scarcity of rainfall that characterized the whole season. In Angeli di Varano, on cv. Montepulciano (Table 7), the first GDM symptoms appeared on bunches in the untreated plot on 6 July. An assessment was conducted on bunches ten days later than the appearance of symptoms and it emerged that chitosan combined with copper was the only strategy that significantly reduced GDM incidence from the untreated control, by 88.56%. McKinney Index was reduced significantly by the combined treatments at the same level of copper. While the other strategies (chitosan and copper→chitosan) did not differ significantly from the control.

Some important rainfalls were recorded at the end of the summer, and they favored the development of GDM, especially on leaves. The disease was well controlled on leaves with chitosan, copper→chitosan, chitosan + copper and conventional farm application of copper: these strategies significantly inhibited *P. viticola*, reducing incidence by 27.40%, 71.39%, 71.78%, and 53.15% respectively. No statistical differences emerged between copper control and chitosan associated with

Table 2
Details of the GDM infections recorded on grapevine leaves and bunches cv. Montepulciano in Angeli di Varano during 2019 after three management strategies.

Treatments	Leaves (July 15, 2019)			Bunches (July 31, 2019)		
	Incidence (%)	Severity (1–10)	McKinney Index (%)	Incidence (%)	Severity (1–7)	McKinney Index (%)
Chitosan	56.20 ±17.41 b[b]	2.64 ±0.52 c	15.47 ±7.34 b	85.12 ±20.65 b	4.35 ±1.29 a	53.13 ±21.14 b
Copper→chitosan	58.91 ±24.77 ab	3.23 ±0.73 a	19.87 ±10.52 a	77.06 ±31.08 c	2.99 ±1.39 b	32.96 ±20.46 c
Farm application[a]	35.92 ±9.50 c	3.11 ±0.74 ab	11.45 ±4.68 c	31.33 ±19.69 d	3.30 ±1.84 b	14.94 ±13.32 d
Untreated control	60.89 ±16.49 a	3.01 ±0.58 b	18.13 ±5.47 a	96.06 ±11.05 a	4.69 ±1.51 a	64.12 ±21.78 a

[a] Applying copper-based products during the season.
[b] Different letters in the same column indicate significant differences according to Fisher LSD test and Games-Howell post hoc test (with P ≤ 0.05).

Table 3
Details of the GDM infections recorded on grapevine leaves and bunches cv. Verdicchio Bianco in Castelplanio on September 23, 2019 after three management strategies.

Treatments	Leaves			Bunches		
	Incidence (%)	Severity (1–10)	McKinney Index (%)	Incidence (%)	Severity (1–7)	McKinney Index (%)
Chitosan	51.26 ±17.18 b[b]	2.66 ±0.74 b	14.38 ±8.74 b	31.60 ±30.44 b	5.56 ±1.77 b	25.67 ±28.36 b
Copper→chitosan	9.72 ±7.48 c	2.51 ±0.82 b	2.24 ±1.39 c	14.23 ±17.73 c	1.97 ±1.64 c	4.78 ±10.66 c
Farm application[a]	3.09 ±4.56 d	1.97 ±0.88 c	0.64 ±1.07 d	11.39 ±18.08 c	1.40 ±0.99 d	2.36 ±6.13 c
Untreated control	60.98 ±10.79 a	3.09 ±0.95 a	19.39 ±8.33 a	50.44 ±37.37 a	6.09 ±1.15 a	44.02 ±33.53 a

[a] Applying copper-based products during the season.
[b] Different letters in the same column indicate significant differences according to Fisher LSD test and Games-Howell post hoc test (with $P \leq 0.05$).

Table 4
Details of the GDM infections recorded on grapevine leaves and bunches cv. Montepulciano in Angeli di Varano during 2020 after three management strategies.

Treatments	Leaves (September 7, 2020)			Bunches (July 23, 2020)		
	Incidence (%)	Severity (1–10)	McKinney Index (%)	Incidence (%)	Severity (1–7)	McKinney Index (%)
Chitosan	10.04 ±5.81 b[b]	1.72 ±0.54 b	1.84 ±1.33 b	4.41 ±6.28 ab	1.05 ±0.23 b	0.66 ±0.96 b
Copper→chitosan	4.42 ±3.40 c	1.47 ±0.65 c	0.71 ±0.67 c	3.82 ±5.17 b	1.00 ±0.00 b	0.54 ±0.74 b
Farm application[a]	3.89 ±2.70 c	1.62 ±0.70 bc	0.66 ±0.56 c	5.50 ±9.00 ab	1.37 ±1.50 a	1.27 ±3.85 ab
Untreated control	15.98 ±9.92 a	2.06 ±0.66 a	3.45 ±2.72 a	8.25 ±9.24 a	1.61 ±1.28 a	2.12 ±3.43 a

[a] Applying copper-based products during the season.
[b] Different letters in the same column indicate significant differences according to Fisher LSD test and Games-Howell post hoc test (with $P \leq 0.05$).

Table 5
Details of the GDM infections recorded on grapevine leaves and bunches cv. Verdicchio Bianco in Castelplanio during 2020 after three management strategies.

Treatments	Leaves (September 7, 2020)			Bunches (July 27, 2020)		
	Incidence (%)	Severity (1–10)	McKinney Index (%)	Incidence (%)	Severity (1–7)	McKinney Index (%)
Chitosan	12.41 ±8.12 c[b]	2.70 ±0.91 b	3.83 ±3.11 c	18.50 ±13.82 b	2.10 ±1.42 b	5.87 ±6.45 b
Copper→chitosan	19.86 ±11.11 b	3.16 ±0.85 b	6.69 ±4.42 b	19.04 ±17.83 b	2.09 ±1.04 b	6.48 ±8.92 b
Farm application[a]	12.46 ±7.25 c	2.96 ±0.66 b	3.84 ±2.74 c	21.62 ±16.67 b	2.46 ±1.30 b	8.74 ±11.15 b
Untreated control	51.27 ±14.90 a	4.71 ±0.63 a	23.85 ±7.22 a	35.38 ±31.67 a	2.93 ±1.09 a	15.45 ±15.74 a

[a] Applying copper-based products during the season.
[b] Different letters in the same column indicate significant differences according to Fisher LSD test and Games-Howell post hoc test (with $P \leq 0.05$).

Table 6
Details of the GDM infections recorded on grapevine leaves and bunches cv. Verdicchio Bianco in Matelica on September 8, 2020 after three management strategies.

Treatments	Leaves			Bunches		
	Incidence (%)	Severity (1–10)	McKinney Index (%)	Incidence (%)	Severity (1–7)	McKinney Index (%)
Chitosan	11.75 ±7.24 b[b]	2.08 ±0.57 b	2.57 ±1.89 b	19.65 ±15.90 b	1.98 ±0.99 b	5.71 ±5.69 b
Copper→chitosan	9.51 ±5.47 b	1.91 ±0.43 b	1.87 ±1.33 b	20.94 ±20.51 b	2.13 ±1.50 b	7.33 ±11.17 b
Farm application[a]	3.38 ±2.10 c	1.89 ±0.91 b	0.62 ±0.44 c	18.32 ±15.36 b	1.62 ±0.94 b	4.07 ±3.66 b
Untreated control	29.82 ±15.43 a	2.89 ±0.86 a	8.60 ±4.45 a	63.90 ±25.41 a	4.34 ±1.47 a	39.32 ±18.43 a

[a] Applying copper-based products during the season.
[b] Different letters in the same column indicate significant differences according to Fisher LSD test and Games-Howell post hoc test (with $P \leq 0.05$).

the heavy metal both for incidence and for the McKinney Index, that was reduced by 64.84%, 68.47%, and 62.40%, respectively with copper→-chitosan, chitosan + copper and farm application, compared to the untreated control.

The first GDM symptoms on cv. Verdichio Bianco appeared in the untreated control of Castelplanio at the beginning of July but the disease

Table 7
Details of the GDM infections recorded on grapevine leaves and bunches cv. Montepulciano in Angeli di Varano during 2021 after four management strategies.

Treatments	Leaves (September 29, 2021)			Bunches (July 16, 2021)		
	Incidence (%)	Severity (1–10)	McKinney Index (%)	Incidence (%)	Severity (1–7)	McKinney Index (%)
Chitosan	26.42 ±23.17 b[b]	3.80 ±1.63 a	12.09 ±12.32 a	4.08 ±9.39 a	1.48 ±0.55a	1.20 ±2.80 ab
Copper→chitosan	10.41 ±12.73 c	3.61 ±2.19 a	5.33 ±8.00 b	8.44 ±11.67 ab	1.62 ±0.79 a	2.15 ±3.61 a
Chitosan + copper	10.27 ±12.73 c	3.71 ±2.03 a	4.78 ±6.76 b	1.10 ±2.70 b	1.00 ±0.00 c	0.16 ±0.39 b
Farm application[a]	17.05 ±12.98 bc	2.39 ±1.63 b	5.70 ±6.95 b	3.57 ±7.14 ab	1.18 ±0.54 b	0.66 ±1.41 b
Untreated control	36.39 ±22.53 a	3.72 ±1.65 a	15.16 ±10.71 a	9.62 ±25.30 a	1.44 ±0.62 a	2.19 ±5.49 a

[a] Applying copper-based products during the season.
[b] Different letters in the same column indicate significant differences according to Fisher LSD test and Games-Howell post hoc test (with $P \leq 0.05$).

pressure remained very low for the whole summer and the treatment efficacity was unclear.

Symptoms of GDM appeared late in the season in Matelica (cv. Verdicchio Bianco) during 2021. Indeed, the first assessment was conducted on bunches at the end of July, where all the treatments significantly reduced the disease incidence, severity and McKinney Index compared to the untreated control (Table 8). Chitosan, copper→chitosan, chitosan + copper, and farm application reduced GDM incidence on grape bunches by 42.56%, 62.77%, 37.39%, and 77.64%, respectively.

Close to the harvest (14 September), assessments were conducted on leaves. The three disease parameters were significantly reduced by all the strategies, compared to the untreated control. Chitosan alone, copper→chitosan, chitosan + copper reduced GDM McKinney index ex by 65.19%, 95.58%, 97.24% and 97.79%, respectively. Strategies based on the use of a low quantity of copper supported by chitosan reduced all parameters and no significant difference observed between farm application and chitosan + copper (Table 8).

Over three consecutive seasons, individual application of chitosan 0.5% exhibited an average reduction of McKinney Index compared to the untreated control of 42.96% and 50.03% on leaves and bunches, respectively. Copper→chitosan reduced the Index on average by 75.87% on leaves and 62.69% on bunches, while chitosan 0.25% combined with low doses of copper by 82.85% and 67.42% on leaves and bunches respectively. These two strategies had the same and higher efficiency than copper alone, respectively (Fig. 1).

3.5. Collateral effects on grapevine powdery mildew

The year 2021 was not favorable to the development of GDM, due to the meteorological trend that characterized the Marche region from April to late summer. However, during field inspections of July, symptoms of GPM were observed on bunches; consequently, assessments of disease parameters were conducted to evaluate the effectiveness against GPM of the GDM management strategies under investigation (Table 9).

During the assessment conducted in Angeli di Varano, all the strategies except chitosan alone significantly reduced incidence, severity, and McKinney Index of GPM.

In Castelplanio GPM infections were under control in all the plots treated with GDM strategies, with significant reductions of the parameters in comparison with the untreated control.

In Matelica no strategies reduced GPM incidence compared to the untreated plants, while the McKinney Index was significantly reduced in the plot treated with just copper; symptoms severity was higher in bunches treated with chitosan.

3.6. Analysis of copper contamination on berries

At the end of 2020 and 2021 seasons, samples of grapes were collected to quantify the copper contamination of fruits before reaching the winery (Fig. 2). Analysis of 2020 on Verdicchio Bianco samples from Castelplanio showed that the amount of residual copper on berries was 2.2, 2.5, and 2.2 times smaller in untreated grapes, treated with chitosan and in those treated with copper for the first half of the season and then with chitosan, respectively, than the amount found on control grapes treated with conventional farm application of copper. In 2021 this copper detection assessment was repeated, both in Angeli di Varano (cv. Montepulciano) and Matelica (cv. Verdicchio Bianco) vineyards and in both cases all the treatments resulted in significant postharvest reduction of copper amount on berries, if compared to individual application of copper. Furthermore, no statistically significant differences were observed among chitosan alone, copper→chitosan and untreated control in all the analysis performed, while grapes treated with chitosan plus copper combined exhibited intermediate level of contamination.

Table 8
Details of the GDM infections recorded on grapevine leaves and bunches on Verdicchio Bianco in Matelica during 2021 after four management strategies.

Treatments	Leaves (14 September)			Bunches (July 29, 2021)		
	Incidence (%)	Severity (1–10)	McKinney Index (%)	Incidence (%)	Severity (1–7)	McKinney Index (%)
Chitosan	3.46 ±3.69 b[b]	1.75 ±0.49 b	0.63 ±0.74 b	10.66 ±10.63 b	1.66 ±1.07 a	2.50 ±2.93 ab
Copper→chitosan	0.63 ±1.13 c	1.30 ±0.46 d	0.08 ±0.16 c	6.91 ±9.55 bc	1.47 ±0.67 a	1.51 ±2.42 bc
Chitosan + copper	0.27 ±0.81 c	1.61 ±0.49 bc	0.05 ±0.15 c	11.62 ±16.96 b	1.22 ±0.37 a	2.16 ±4.15 bc
Farm application[a]	0.29 ±0.87 c	1.49 ±0.50 c	0.04 ±0.12 c	4.15 ±5.60 c	1.44 ±1.49 a	0.86 ±1.69 c
Untreated control	7.21 ±6.59 a	2.18 ±0.66 a	1.81 ±2.14 a	18.56 ±22.41 a	1.45 ±0.63 a	3.90 ±5.00 a

[a] Applying copper-based products during the season.
[b] Different letters in the same column indicate significant differences according to Fisher LSD test and Games-Howell post hoc test (with $P \leq 0.05$).

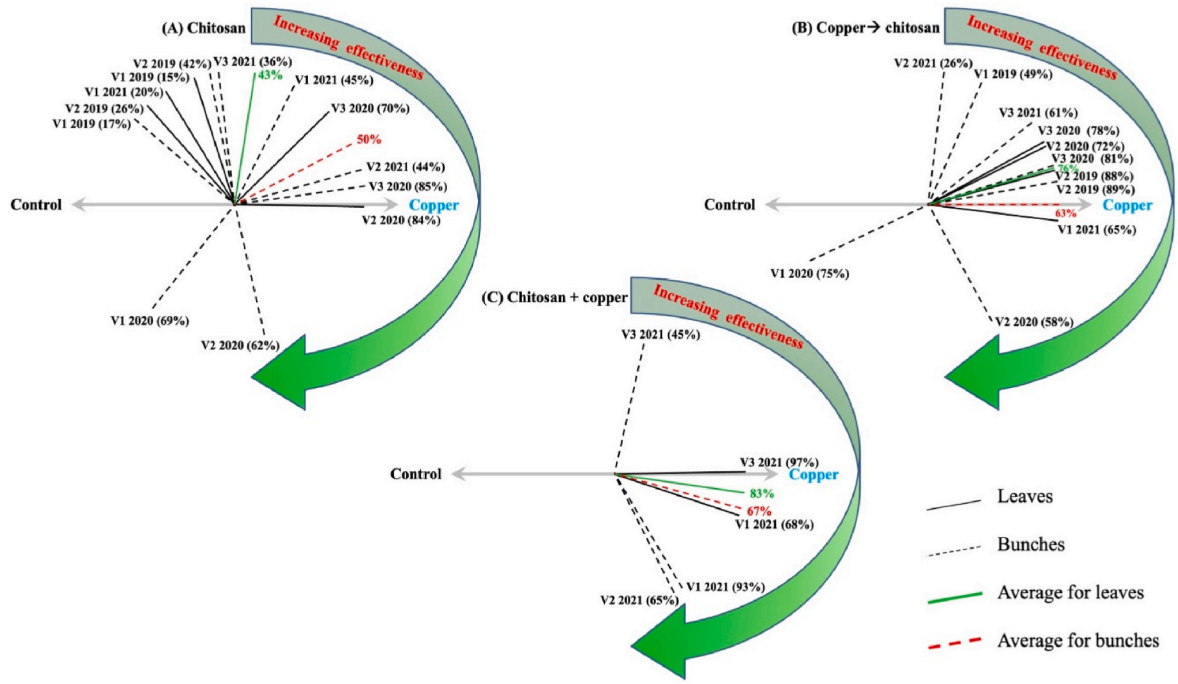

Fig. 1. Representative results of GDM McKinney Index reduction compared to untreated control of the following strategies: (A) individual application of chitosan, (B) alternating treatments and (C) combined treatments during 2019, 2020 and 2021 in three different vineyards. Line positions show the levels of protection compared to copper (angle of 180°). V1 = Angeli di Varano (cv. Montepulciano); V2= Castelplanio (cv. Verdicchio Bianco); V3 = Matelica (cv. Verdicchio Bianco).

Table 9
Details of GPM infections recorded on Montepulciano and Verdicchio Bianco bunches respectively in Angeli di Varano, Castelplanio and Matelica during July 2021.

Cultivar, vineyard	Dates of assessments	Treatments	Disease parameters on bunches		
			Incidence (%)	Severity (1–7)	McKinney Index (%)
Montepulciano, Angeli di Varano (AN)	July 16, 2021	Chitosan	2.81 ±7.18 a[b]	1.52 ±0.77 a	0.62 ±1.75 a
		Copper→chitosan	0.00 b		0.00 b
		Chitosan + copper	0.10 ±0.66 b	1.00 ±0.15 b	0.01 ±0.09 b
		Farm application[a]	0.17 ±1.16 b	1.00 ±0.15 b	0.02 ±0.17 b
		Untreated control	3.15 ±9.75 a	1.32 ±0.52 a	0.70 ±2.46 a
Verdicchio Bianco, Castelplanio (AN)	July 5, 2021	Chitosan	12.58 ±18.72 ab	1.73 ±0.84 b	3.35 ±5.89 b
		Copper→chitosan	9.16 ±14.28 bc	1.46 ±0.53 c	1.91 ±3.33 bc
		Chitosan + copper	5.91 ±12.68 bc	1.08 ±0.19 d	0.91 ±1.92 c
		Farm application[a]	2.20 ±6.51 c	1.60 ±0.68 bc	0.90 ±2.86 c
		Untreated control	16.99 ±21.64 a	2.23 ±1.38 a	6.27 ±10.00 a
Verdicchio Bianco, Matelica (MC)	July 29, 2021	Chitosan	2.70 ±7.84 a	2.20 ±1.30 a	0.91 ±3.06 ab
		Copper→chitosan	2.66 ±6.32 a	1.54 ±1.05 b	0.53 ±1.32 ab
		Chitosan + copper	4.84 ±8.74 a	1.60 ±0.66 b	1.14 ±2.04 ab
		Farm application[a]	2.24 ±5.19 a	1.06 ±0.18 c	0.34 ±0.78 b
		Untreated control	9.71 ±16.24 a	1.70 ±1.25 b	2.14 ±3.83 a

[a] Applying copper-based products during the season.
[b] Different letters in the same column indicate significant differences according to Fisher LSD test and Games-Howell post hoc test (with $P \leq 0.05$).

4. Discussion

The transition toward a sustainable economic model in the EU is steered by the goals of the European Green Deal, launched in December 2019. One of the main elements of this new growth plan is the strategy Farm to Fork, which will guide future agricultural policy choices and Common Agricultural Policy objectives for the period 2023–2027. In this context, crop protection is becoming increasingly complex, due to

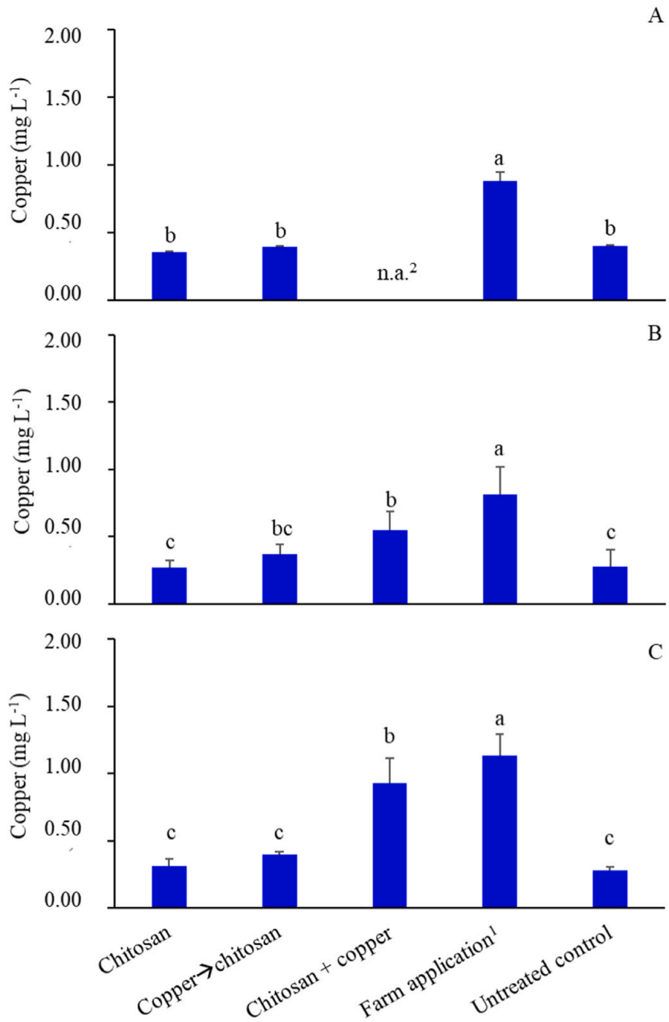

Fig. 2. (A) Quantity of copper detected after the season 2020 on the grapes of Castelplanio vineyard (cv. Verdicchio Bianco). (B) Quantity of copper detected after the season 2021 on the grapes of Angeli di Varano vineyard (cv. Montepulciano). (C) Quantity of copper detected after the season 2021 on the grapes of Matelica vineyard (cv. Verdicchio Bianco). Different letters are significantly different according to Fisher LSD test, with $P \leq 0.05$.
[1]applying copper-based products during the season. [2]N.A.: Data not available.

the continuous limitations on the use of chemical pesticides. New approaches to plant protection are thus needed to manage major diseases. Copper provides high protection levels but relying solely on copper fungicides for GDM management contradicts organic farming principles due to the risks, related to the use of this heavy metal, for the environment, for human health and for wine quality (Provenzano et al., 2010). The search for alternative GDM protection strategies remains fundamental to reduce copper impact, supporting growers toward the establishment of new *P. viticola* management systems. Chitosan effectiveness to contain GDM infections has already been demonstrated in plot trials (Romanazzi et al., 2016, 2021; Farouk et al., 2017), with the purpose of reducing the copper quantity distributed each year in vineyards to protect vines from *P. viticola*, but validation on large scale is still necessary. This promising biopolymer has also been tested for other crop diseases, where it has been associated especially with the improvement of gene expression for defense-related mechanisms (Landi et al., 2017), or in postharvest applications (Rajestary et al., 2021; Romanazzi and Moumni, 2022). To confirm chitosan effectiveness in vineyard applications against GDM and to test it over time with various levels of disease pressure, under different operative and environmental conditions, this three-year investigation extended the trials on large scale, involving two of the main wineries of the Marche region.

In 2019 and 2020 disease pressure resulted high and medium respectively, while 2021 was less favorable to *P. viticola* and the disease pressure was lower. Chitosan treatments effectively reduced GDM McKinney Index on leaves and bunches under different environmental and operational conditions for three consecutive years. Regarding the average of McKinney Index reductions compared to untreated control in these three years, our results showed that when chitosan was alternated or combined with reduced quantities of copper, it provided the same and higher efficacy, respectively, than the conventional application of copper. In addition, comparing with copper effectiveness, chitosan-based strategies demonstrated greater protection on bunches than leaves. This is likely due to the lower susceptibility of the bunches compared to the leaves (Ash et al., 2000), and because unripe fruits are highly reactive to resistance inducer application (Prusky and Romanazzi, 2023).

Replacing copper with chitosan is not straightforward and, at least initially, growers could opt for a coexistence of the two active substances. This would allow to preserve the reliability of copper while respecting the limits imposed by the European Commission and, simultaneously, to transfer into companies knowledge of how to apply this innovative product. In addition, chitosan is currently much expensive than copper and, for the time being, their cooperation is also useful to keep chitosan-based GDM management strategies costs low. The use of copper for the first half of the season followed by chitosan application appears to be useful to limit GDM symptoms under conditions of high disease pressure (Romanazzi et al., 2021), reducing the amount of copper sprayed in the year. Treatments carried out with copper at the beginning of the season, when grapevines are more susceptible to GDM (Ash, 2000), are useful to limit the primary infections of *P. viticola*. The right combination of copper and chitosan formulations can potentially exhibit synergistic effects and simultaneously lead to a drastic reduction of copper distributed per treatment. Applications of chitosan at halved concentration (compared to the standard reference of 0.5% w/v of a. i.) combined with reduced doses of copper provide high levels of protection, while reducing costs of chitosan treatments and satisfying European copper thresholds. On the other hand, it must be considered that doses below the minimum of label are forbidden by law, therefore the legislation needs to be adapted to these technical innovations. Also, combined treatments with current formulations are more complex to manage with untrained personnel. Currently, there are three main critical points that can limit the diffusion of chitosan: its cost, the need for a ready-to-use formulation as conventional pesticides, and the development of precise application protocols, especially for combined treatments. All these aspects can be overcome when chemical industries invest on this substance (once its effectiveness becomes recognized on a commercial scale). Not all molecules can be mixed with chitosan and it has been seen that also the mixing system can affect compatibility between compounds (data not shown). The chelation is the combination mechanism of chitosan and copper (Dev et al., 2020). Chitosan-NH_2 groups may serve as coordination sites for copper ions (Negm et al., 2015). Under our working conditions, the copper oxychloride used in this study formed a sprayable mixture with chitosan.

Promising prospects are also offered by individual application of chitosan 0.5%, that resulted comparable to copper and effective under both conditions of high and low disease pressure in long term plot trials (Romanazzi et al., 2016, 2021) and in this study. Even vineyard applications of chitosan at lower concentration were successful in reducing GDM symptoms severity, about 30%, and enhancing the defense response on Glera cultivar (Mian et al., 2023).

Copper has a broad spectrum of action (Vogelweith and Thiéry, 2018) and, in addition to controlling other diseases, it carries out an indirect effect against GPM, reducing the risk of infection due to a slight phytotoxicity which thickens the plant tissues. Similarly, the threefold activity of chitosan showed itself to be effective even against GPM. This observed collateral effect on GPM is supported by the study of

Ruano-Rosa et al. (2022), which showed the effectiveness of chitosan-based bioactive formulations against GPM, in both *in vitro* and *in vivo* trials.

Our analyses carried out for two years on the harvested grapes, showed that all the innovative strategies tested provided significant reductions of the heavy metal residues on the berries compared to the copper control. No significant differences were observed in the copper levels between untreated grapes and those treated with either chitosan alone or with copper followed by chitosan. Copper→chitosan strategy not only provided effective protection from the disease but also allowed growers to obtain residual copper levels on berries similar to those found where copper was not used at all. In copper→chitosan, treatments with copper were performed until mid June (BBCH 60), while in chitosan + copper, reduced amounts of copper have been applied for the entire season. This suggests that the strategy by which chitosan is applied influences the amount of copper residues on grapes at harvest. The use of chitosan does not lead to negative side effects on the wine, in contrast to copper which instead has several negative implications, for instance on amino acids content, fermentation, organoleptic characteristics and on wine shelf life (Garde-Cerdán et al., 2017). These strategies can therefore also play a crucial role in improving wine quality (Gutiérrez-Gamboa et al., 2019).

Chitosan approvals as GRAS and as basic substance assure on its safety, both towards human health and the environment. Treatments close to harvest are thus allowed, while copper treatments are usually stopped at veraison to avoid residues. Our results demonstrate that chitosan is a low environmental impact and effective alternative to copper for GDM management on commercial scale, both in stand-alone applications and when involved in low-rate copper strategies. The use of chitosan reduces pollution caused by chemical treatments for GDM management. This not only satisfies the demand for sustainable crop protection practices but also offers a potential marketing advantage. Thanks to its potential broad spectrum of action, it can protect vines also from gray mold (Aziz et al., 2006), when bunches are ripening, and their susceptibility to *Botrytis cinerea* increases (Petrasch et al., 2019). Possessing different GDM management strategies available is useful because the pathogen pressure is not the same every year but varies according to the meteorological trend. The inclusion of chitosan in the protection programs will allow vines protection while reducing the quantities of copper sprayed in vineyards, with positive consequences for humans, wine quality, and environment (Duca et al., 2016). Indeed, as Fernández-Calviño et al. (2010) reported, copper's negative effects on soil are related to its concentration.

5. Conclusions

This study proved that chitosan can replace or support low doses of copper in new GDM management strategies. These experiments on large scale conducted for three consecutive years provide reference data and technical support for innovative vineyard protection programs in line with the new rules and principles adopted in Europe. Generally, chitosan-based strategies showed higher effectiveness on bunches than on leaves compared to conventional copper treatments. Alternating treatments exhibited the same protection level as farm application, while combined treatments even higher. Alternating treatments not only provided effective protection from the disease, but also allowed growers to obtain residual copper levels on berries similar to those found where copper was not used at all. The cost of chitosan, the need to perform treatments with high volumes and at least 0.5% of active substance, as well as its complexity of use compared to conventional pesticides, may represent limits to its diffusion. Further investigations on large scale are needed to test different chitosan formulations and concentrations, in different environmental conditions and with other innovative compounds, aiming to complement and eventually replace conventional fungicides for GDM management.

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CRediT authorship contribution statement

Gianfranco Romanazzi: Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization, Validation, Writing – review & editing. **Simone Piancatelli:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Roberto Potentini:** Supervision, Resources, Methodology, Conceptualization. **Giuliano D'Ignazi:** Supervision, Resources, Methodology, Funding acquisition, Conceptualization, Project administration. **Marwa Moumni:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Gianfranco Romanazzi reports financial support was provided by Marche Region. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2024.142131.

References

Ash, G., 2000. Plant Health Instrum. The Plant Health Instr. https://doi.org/10.1094/PHI-I-2000-1112-01.

Aziz, A., Trotel-Aziz, P., Dhuicq, L., Jeandet, P., Couderchet, M., Vernet, G., 2006. Chitosan oligomers and copper sulfate induce grapevine defense reactions and resistance to gray mold and downy mildew. Phytopathology 96, 1188–1194. https://doi.org/10.1094/PHYTO-96-1188.

Aziz, A., Poinssot, B., Daire, X., Adrian, M., Bézier, A., Lambert, B., Joubert, J.-M., Pugin, A., 2007. Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. Mol. Plant Microbe Interact. 16, 1118–1128. https://doi.org/10.1094/MPMI.2003.16.12.1118.

Barbosa, F.S., Scavarda, A.J., Sellitto, M.A., Marques, D.I.L., 2018. Sustainability in the winemaking industry: an analysis of Southern Brazilian companies based on a literature review. J. Clean. Prod. 192, 80–87. https://doi.org/10.1016/j.jclepro.2018.04.253.

Dagostin, S., Schärer, H.-J., Pertot, I., Tamm, L., 2011. Are there alternatives to copper for controlling grapevine downy mildew in organic viticulture? Crop Protect. 30, 776–788. https://doi.org/10.1016/j.cropro.2011.02.031.

Delière, L., Cartolaro, P., Léger, B., Naud, O., 2015. Field evaluation of an expertise-based formal decision system for fungicide management of grapevine downy and powdery mildews. Pest Manag. Sci. 71, 1247–1257. https://doi.org/10.1002/ps.3917.

Dev, V.V., Baburaj, G., Antony, S., Arun, V., Krishnan, K.A., 2020. Zwitterion-chitosan bed for the simultaneous immobilization of Zn (II), Cd (II), Pb (II) and Cu (II) from

multi-metal aqueous systems. J. Clean. Prod. 255, 120309 https://doi.org/10.1016/j.jclepro.2020.120309.
Duca, D., Toscano, G., Pizzi, A., Rossini, G., Fabrizi, S., Lucesoli, G., Servili, A., Mancini, V., Romanazzi, G., Mengarelli, C., 2016. Evaluation of the characteristics of vineyard pruning residues for energy applications: effect of different copper-based treatments. J. Agric. Eng. 47, 22–27. https://doi.org/10.4081/jae.2016.497.
Farouk, S., Belal, B.E.A., El-Sharkawy, H.H.A., 2017. The role of some elicitors on the management of Roumy Ahmar grapevines downy mildew disease and it's related to inducing growth and yield characters. Sci. Hortic. 225, 646–658. https://doi.org/10.1016/j.scienta.2017.07.054.
Fernández-Calviño, D., Soler-Rovira, P., Polo, A., Díaz-Raviña, M., Arias-Estévez, M., Plaza, C., 2010. Enzyme activities in vineyard soils long-term treated with copper-based fungicides. Soil Biol. Biochem. 42, 2119–2127. https://doi.org/10.1016/j.soilbio.2010.08.007.
Garde-Cerdán, T., Mancini, V., Carrasco-Quiroz, M., Servili, A., Gutiérrez-Gamboa, G., Foglia, R., Pérez-Álvarez, E.P., Romanazzi, G., 2017. Chitosan and laminarin as alternatives to copper for *Plasmopara viticola* control: effect on grape amino acid. J. Agric. Food Chem. 65, 7379–7386. https://doi.org/10.1021/acs.jafc.7b02352.
Gessler, C., Pertot, I., Perazzolli, M., 2011. *Plasmopara viticola*: a review of knowledge on downy mildew of grapevine and effective disease management. Phytopathol. Mediterr. 50, 3–44.
Gutiérrez-Gamboa, G., Romanazzi, G., Garde-Cerdán, T., Pérez-Álvarez, E.P., 2019. A review of the use of biostimulants in the vineyard for improved grape and wine quality: effects on prevention of grapevine diseases. J. Sci. Food Agric. 99, 1001–1009. https://doi.org/10.1002/jsfa.9353.
Hobbelen, P.H.F., Koolhaas, J.E., van Gestel, C.A.M., 2006. Bioaccumulation of heavy metals in the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in relation to total and available metal concentrations in field soils. Environ. Pollut. 144, 639–646. https://doi.org/10.1016/j.envpol.2006.01.019.
Jermini, M., Blaise, P., Gessler, C., 2010. Quantitative effect of leaf damage caused by downy mildew (*Plasmopara viticola*) on growth and yield quality of grapevine 'Merlot' (*Vitis Vinifera*). Vitis 49, 77–85.
Komárek, M., Čadková, E., Chrastný, V., Bordas, F., Bollinger, J.-C., 2010. Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. Environ. Int. 36, 138–151. https://doi.org/10.1016/j.envint.2009.10.005.
Landi, L., De Miccolis Angelini, R.M., Pollastro, S., Feliziani, E., Faretra, F., Romanazzi, G., 2017. Global transcriptome analysis and identification of differentially expressed genes in strawberry after preharvest application of benzothiadiazole and chitosan. Front. Plant Sci. 8, 235. https://doi.org/10.3389/fpls.2017.00235.
La Torre, A., Iovino, V., Caradonia, F., 2018. Copper in plant protection: current situation and prospects. Phytopathol. Mediterr. 57, 201–236. https://doi.org/10.14601/Phytopathol_Mediterr-23407.
Mackie, K.A., Müller, T., Kandeler, E., 2012. Remediation of copper in vineyards – a mini review. Environ. Pollut. 167, 16–26. https://doi.org/10.1016/j.envpol.2012.03.023.
Marchand, P.A., Davillerd, Y., Riccioni, L., Sanzani, S.M., Horn, N., Matyjaszczyk, E., et al., 2021. BasicS, an euphresco international network on renewable natural substances for durable crop protection products. Chron. Bioresour. Manag. 5, 77–80.
Mian, G., Musetti, R., Belfiore, N., Boscaro, D., Lovat, L., Tomasi, D., 2023. Chitosan application reduces downy mildew severity on grapevine leaves by positively affecting gene expression pattern. Physiol. Mol. Plant Pathol. 125, 102025.
Negm, N.A., El Sheikh, R., El-Farargy, A.F., Hefni, H.H., Bekhit, M., 2015. Treatment of industrial wastewater containing copper and cobalt ions using modified chitosan. J. Ind. Eng. Chem. 21, 526–534. https://doi.org/10.1016/j.jiec.2014.03.015.
Petrasch, S., Silva, C.J., Mesquida-Pesci, S.D., Gallegos, K., Van Den Abeele, C., Papin, V., Fernandez-Acero, F.J., Knapp, S.J., Blanco-Ulate, B., 2019. Infection strategies deployed by *Botrytis cinerea, Fusarium acuminatum*, and *Rhizopus stolonifer* as a function of tomato fruit ripening stage. Front. Plant Sci. 10, 223. https://doi.org/10.3389/fpls.2019.00223.
Provenzano, M.R., El Bilali, H., Simeone, V., Baser, N., Mondelli, D., Cesari, G., 2010. Copper contents in grapes and wines from a Mediterranean organic vineyard. Food Chem. 122, 1338–1343. https://doi.org/10.1016/j.foodchem.2010.03.103.
Prusky, D., Romanazzi, G., 2023. Induced resistance in fruit and vegetables: a host physiological response limiting postharvest disease development. Annu. Rev. Phytopathol. 61, 279–300. https://doi.org/10.1146/annurev-phyto-021722-035135.
Puopolo, G., Giovannini, O., Pertot, I., 2014. *Lysobacter capsici* AZ78 can be combined with copper to effectively control *Plasmopara viticola* on grapevine. Microbiol. Res. 169, 633–642. https://doi.org/10.1016/j.micres.2013.09.013.
Rajestary, R., Landi, L., Romanazzi, G., 2021. Chitosan and postharvest decay of fresh fruit: meta-analysis of disease control and antimicrobial and eliciting activities. Compr. Rev. Food Sci. Food Saf. 20, 563–582. https://doi.org/10.1111/1541-4337.12672.
Romanazzi, G., Mancini, V., Feliziani, E., Servili, A., Endeshaw, S., Neri, D., 2016. Impact of alternative fungicides on grape downy mildew control and vine growth and development. Plant Dis. 100, 739–748. https://doi.org/10.1094/PDIS-05-15-0564-RE.
Romanazzi, G., Feliziani, E., Sivakumar, D., 2018. Chitosan, a biopolymer with triple action on postharvest decay of fruit and vegetables: eliciting, antimicrobial and film-forming properties. Front. Microbiol. 9, 2745. https://doi.org/10.3389/fmicb.2018.02745.
Romanazzi, G., Mancini, V., Foglia, R., Marcolini, D., Kavari, M., Piancatelli, S., 2021. Use of chitosan and other natural compounds alone or in different strategies with copper hydroxide for control of grapevine downy mildew. Plant Dis. 105, 3261–3268. https://doi.org/10.1094/PDIS-06-20-1268-RE.
Romanazzi, G., Moumni, M., 2022. Chitosan and other edible coatings to extend shelf life, manage postharvest decay, and reduce loss and waste of fresh fruits and vegetables. Curr. Opin. Biotechnol. 78, 102834 https://doi.org/10.1016/j.copbio.2022.102834.
Romanazzi, G., Orçonneau, Y., Moumni, M., Davillerd, Y., Marchand, P.A., 2022. Basic substances, a sustainable tool to complement and eventually replace synthetic pesticides in the management of pre and postharvest diseases: reviewed instructions for users. Molecules 27, 3484. https://doi.org/10.3390/molecules27113484.
Ruano-Rosa, D., Sánchez-Hernández, E., Baquero-Foz, R., Martín-Ramos, P., Martín-Gil, J., Torres-Sánchez, S., Casanova-Gascón, J., 2022. Chitosan-based bioactive formulations for the control of powdery mildew in viticulture. Agronomy 12, 495. https://doi.org/10.3390/agronomy12020495.
Saint-Ges, V., Bélis-Bergouignan, M.C., 2009. Ways of reducing pesticides use in Bordeaux vineyards. J. Clean. Prod. 17, 1644–1653. https://doi.org/10.1016/j.jclepro.2009.05.006.
Schäufele, I., Hamm, U., 2017. Consumers' perceptions, preferences and willingness-to-pay for wine with sustainability characteristics: a review. J. Clean. Prod. 147, 379–394. https://doi.org/10.1016/j.jclepro.2017.01.118.
Vogelweith, F., Thiéry, D., 2018. An assessment of the non-target effects of copper on the leaf arthropod community in a vineyard. Biol. Control 127, 94–100. https://doi.org/10.1016/j.biocontrol.2018.08.011.
Wang, L., Cheng, W.C., Xue, Z.F., Xie, Y.X., Lv, X.J., 2023. Study on Cu-and Pb-contaminated loess remediation using electrokinetic technology coupled with biological permeable reactive barrier. J. Environ. Manag. 348, 119348 https://doi.org/10.1016/j.jenvman.2023.119348.
Xie, Y.X., Cheng, W.C., Wang, L., Xue, Z.F., Xu, Y.L., 2023. Biopolymer-assisted enzyme-induced carbonate precipitation for immobilizing Cu ions in aqueous solution and loess. Esprit 30, 116134–116146. https://doi.org/10.1007/s11356-023-30665-8.