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ORIGINAL RESEARCH ARTICLE

Efficacy of using grape cane extracts against *Plasmopara viticola* under field conditions and their impact on the composition of berries and musts of *Vitis vinifera* L. cv. Riesling

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ABSTRACT

Grapevine downy mildew caused by *Plasmopara viticola* is a deleterious vine disease currently controlled using synthetic and copper-based fungicides. Environmental concerns surrounding such fungicides necessitate sustainable alternatives like grape cane extracts. However, studies into their efficacy and effects on the product remain limited. This study aimed to assess the open-field efficacy of a novel grape cane extract formulation, alone and combined with a copper agent, against downy mildew, and to examine its impact on berry and must composition. The results were compared to standard fungicides frequently used in integrated and organic viticulture. In 2022, the grape cane extract formulation reduced downy mildew severity by 78 % on berry clusters, comparable to the maximum acceptable dose of copper for organic farming in Germany (3 kg/ha/a). Combining the grape cane extract with the copper agent was more effective than the copper agent alone, but not more effective than the grape cane extract alone. In 2023, disease development was marginal, despite artificial inoculation. Compositional differences detectable by FTIR were insignificant between musts derived from all treatments, increased copper levels were observed whenever copper had been applied. The phenolic content in freeze-dried, de-seeded berries of 2022 was significantly lower after copper (14.6 mg/g dry weight DW) and grape cane extract treatments (6.6 mg/g DW) when compared to the control (36.1 mg/g DW), with discrepancies in stilbenoid and flavan-3-ol levels in particular, as analysed by HPLC-DAD. However, these differences were not confirmed in 2023, with results showing no correlations between different treatments and phenolic contents. These findings suggest that applications of the phenolic grape cane extract do not lead to artificially altered levels or relevant residues of the active constituents; *i.e.*, the phenolic compounds in the product. In brief, grape cane extracts might represent a promising natural alternative to controlling grapevine downy mildew, particularly in organic viticulture, which, to date, heavily relies on (eco-)toxic copper-pesticides. Further multi-year studies including wine analyses are warranted to follow the entire flow towards the final product.

KEYWORDS: *downy mildew*, organic viticulture, copper fungicides, biopesticides, polyphenols, stilbenoids, biostimulants

INTRODUCTION

Grapevine downy mildew is one of the most harmful fungal diseases in European viticulture, affecting both crop quality and quantity. It is caused by the oomycete *Plasmopara viticola* and impacts leaves and berries, leading to yield losses of up to 75 %, and thus to significant economic damages for winegrowers (Koledenkova *et al*., 2022). Infection on leaves often results in substantial losses of the effective total leaf surface. The resulting imbalance in leaf area and fruit weight of the vines has been shown to exert a negative impact on berry quality. For instance, ripening might be delayed, sugar accumulation decelerated, and malic acid respiration enhanced (Rienth *et al*., 2021). Infected berries, moreover, directly reduce grape yield and affect organoleptic properties in terms of the aroma and flavour of the derived wines (Pons *et al*., 2018).

Thus, viticulture relies on multiple fungicide applications per year to ensure sustainable yield and fruit quality, rendering it one of the most heavily treated cropping systems (Julius Kühn-Institut, 2022). While various synthetic fungicides can be effectively used to fight diseases like downy mildew in integrated viticulture, very few and often less effective fungicides are authorised for organic viticulture. In the latter, the application of salts of copper with antifungal effects is still tolerated due to their effectiveness for downy mildew control and their relatively low plant and human toxicity. In the EU, they are authorised for use in both integrated and organic viticulture to date (Tamm *et al*., 2022). However, intensive copper use over the last two centuries has led to its accumulation in soils, provoking negative ecological effects, such as reduced earthworm abundance and modifications to soil microbiota (Burandt *et al*., 2024; Droz *et al*., 2021; Dumestre *et al*., 1999; Paoletti *et al*., 1998; Robinson *et al*., 2006). Moreover, soil copper excess has been reported to affect the quality of berries, must and wine, provoking, for example, undesired oxidative spoilage, diminished levels of several amino acid, and haze formation (Provenzano *et al*., 2010). Although being quite unlikely when used at the currently authorised doses, excessive copper usage might involve the risk of it accumulating in annual organs of the grapevines (Miotto *et al*., 2014).

Consequently, European Union authorities have imposed restrictions on copper use in organic viticulture, limiting its application to a total application dose of 28 kg/ha within a time frame of seven years, which averages to 4 kg/ha/a. Frequently, organic farming associations request even stricter application limits. For instance, only 3 kg/ha/a should be used according to most German organic associations (Tamm *et al*., 2022). In response, research has concentrated on ecofriendly alternatives that can reduce or even supersede copper applications (Gutiérrez-Gamboa *et al*., 2019). In particular, the use of natural compounds or inorganic substances functioning as biostimulants or resistance inducers (*e.g.*, chitosans, carbonates) has been proposed to support inhibiting *P. viticola* growth on vines (Jindo *et al*., 2022; La Torre *et al*., 2019; Romanazzi *et al*., 2016).

Furthermore, plant extracts comprising bioactive constituents have gained a lot of attention due to their renewability, as well as their biocompatibility and biodegradability. In particular, extracts from grape cane became the topic of previous studies focusing on downy mildew inhibition. Grape cane annually accrue at up to 2 to 5 tonnes per hectare as a viticultural by-product, being rich in bioactive phytoalexins stilbenoids, like resveratrol (Aliaño-González *et al*., 2020). It is worth noting that stilbenoid levels in grape cane vary depending on different genetic and environmental parameters, as described elsewhere (Besrukow *et al*., 2022).

Along with several *in vitro* experiments (Chalal *et al*., 2014; Schnee *et al*., 2013), first greenhouse studies have shown a fungicidal efficacy of grape cane extracts *in situ* comparable to those of copper-based agents (Richard *et al*., 2016). These results have been recently confirmed by our working group, who additionally showed a synergistic effect of a specific grape cane extract formulation and a copper-based agent (Besrukow *et al*., 2023). Such combinations could contribute to complying with EU's stipulation of a 50 % pesticide-use reduction by the year 2030 (European Commission, 2023). However, a major drawback of greenhouse experiments is that they do not take into account the impact of often uncontrollable environmental factors (*e.g.*, precipitation, UV-radiation). To the best of our knowledge, Richard *et al*. (2016) were the first to report that a commercially available grape cane extract applied on a vineyard (cv. Merlot) reduced downy mildew incidence by up to 39 % and disease severity by up to 61 % on leaves. At the same time, incidence and severity were 75 % and 21 %, and 23 % and 2 % for the untreated control and the copper-based fungicide, respectively. A similar disease incidence reduction of 40 % on leaves and clusters has been reported by Billet *et al*. (2019), who applied a stilbenoid-rich grape cane extract (GCE) on vineyards planted with the cvs. Malbec, Cabernet franc and Folle-blanche. However, to our knowledge, experiments on the antifungal activity of grape cane extracts in combination with copper-based agents in field trials, as well as on the impact of grape cane extract treatments on berry and must quality are still lacking.

In the present study, we first sought to investigate the fungicidal effects of both a formulated grape cane extract used alone or in combination with a copper agent against *P. viticola* in field trials, comparing the results to those obtained with fungicides typically used in integrated (*i.e.*, Folpan®) and organic (*i.e.*, copper-based Cuprozin® progress) viticulture. In a second step, we aimed to carry out a compositional characterisation of the berries and musts obtained from the differently treated vines, focusing on oenological quality parameters (*e.g.*, levels of reducing sugars, organic acids and metal ions), and in particular on individual phenolic compounds.

MATERIALS AND METHODS

1. Chemicals

Cuprozin® progress was obtained from Certis (Hamburg, Germany), while Folpan® 500 was purchased from Adama (Cologne, Germany). Vineatrol®, a commercial grape cane extract, was acquired from Actichem (Montauban, France). Sodium ligninsulfonate was purchased from Otto Dille (Norderstedt, Germany). Lanthanum oxide and cesium chloride were obtained from Carl Roth (Karlsruhe, Germany), while the multielement standard solution was purchased from AnalytiChem (Oberhausen, Germany).

Analytical standards (all ≥ 97% purity) of quercetin-3-*O*-galactoside and -glucuronide, catechin, epicatechin and procyanidin B1, B2 and C1, as well as (*E*)-resveratrol, (*E*)-ε-viniferin and (*E*)-piceatannol, were purchased from Extrasynthese (Genay, France). Gallic, coutaric and ferulic acid, as well as kaempferol-3-*O*-rutinoside, acetonitrile and formic acid (all HPLC-grade) were acquired from Merck (Darmstadt, Germany). Ultrapure water (Elga Purelab, Celle, Germany) was used throughout the study.

Grape cane extracts were produced as described in detail by our research group in an earlier study (Besrukow *et al*., 2023). In brief, grape canes (cv. Pinot noir) from a controlled experimental organic vineyard of Geisenheim University were dried and milled to an average particle size of 1 mm. Extraction was performed by stirring the grape cane material in denatured aqueous ethanol (80 % vol., solid-to-solvent-ratio 1:5, w/v) for four hours at room temperature without light exposure. The crude extract was then evaporated to ca. 15 % total solids concentration, supplemented with sodium ligninsulfonate, and fed to a laboratory scale spray dryer (Büchi B-290, Flawil, Switzerland) to receive the powdery grape cane extract formulation, which was stored at 5 °C until application.

2. Investigations on biological efficacy of grape cane extract against *P. viticola* in the vineyard

In 2022 and 2023, field trials were performed according to EPPO Guidelines (EPPO, 1997a) on a randomised block-design experimental vineyard (49° 98′ 70″ N, 7° 95′ 25″ E) containing *V. vinifera* L. cv. Riesling (clone 198-10 Gm, rootstock SO 4) at Geisenheim, Germany (planted in 2014, planting density: 4,160 plants per ha). One shoot of every fourth vine was inoculated with *P. viticola* (total 2.3 L/ha with 10^5 sporangia/mL inoculation solution) at grapevine growth stages E-L 17 (Coombe, 1995). Background protection of the plants was conducted according to organic standards with wettable sulphur (Netzschwefel Stulln 80 % WG, AgroStulln, Stulln, Germany; 2.9 kg/ha in 2022, 4.0 kg/ha in 2023). All

TABLE 1. Treatment strategies, doses and active substances of testing media (including producers) applied on the grapevine canopy and cluster zone. For a given treatment, the respective first row in the table refers to application in 2022, the second to that in 2023.

Treatment*	Pesticidal formulation	Producer	Year	Concentration in use (g testing media/L spraying solution)#	Total active sub-stance applied (kg active substance per ha/a)
A	Water (control)	-	2022 2023	- -	- -
B	Folpan® (synthetic standard)	Adama (Cologne, DE)	2022 2023	0.4-1.6 g/L 0.6-1.6 g/L	3.84 kg folpet 4.56 kg folpet
C	Cuprozin® progress 3 kg (organic crop management standard)	Certis (Hamburg, DE)	2022 2023	0.4-2.3 g/L 0.5-1.8 g/L	2.88 kg copper 2.24 kg copper
D	Cuprozin® progress 2 kg (organic crop management standard, reduced)	Certis (Hamburg, DE)	2022 2023	0.3-1.5 g/L 0.3-1.2 g/L	1.92 kg copper 1.49 kg copper
E	Cuprozin® progress 1.5 kg	Certis (Hamburg, DE)	2022 2023	0.2-1.2 g/L 0.2-0.9 g/L	1.44 kg copper 1.12 kg copper
F	Cuprozin® progress 1.5 kg	Certis (Hamburg, DE)	2022 2023	0.2-1.2 g/L 0.2-0.9 g/L	1.44 kg copper 1.12 kg copper
	+ Grape Cane Extract formulation (GCE)	Self-made extract	2022 2023	20 g/L 20 g/L	13.88 kg stilbenoids 13.88 kg stilbenoids
G	Cuprozin® progress 1.5 kg	Certis (Hamburg, DE)	2022 2023	0.2-1.2 g/L 0.2-0.9 g/L	1.44 kg copper 1.12 kg copper
	+ Vineatrol®	Actichem (Montauban, F)	2022 2023	3.3 g/L 3.3 g/L	13.90 kg stilbenoids 13.90 kg stilbenoids
H	Grape Cane Extract Formulation (GCE)	Self-made-extract	2022 2023	20 g/L 20 g/L	13.88 kg stilbenoids 13.88 kg stilbenoids

*All treatments included background protection with wettable sulphur.

Used according to producer's instructions (varied throughout the season depending on phenological state).

treatments were carried out on four different, randomly located block locations within the vineyard with a total of 15 vines per replication; *i.e*., in total, 60 vines per treatment.

Experimental treatments, control plots and the corresponding details are listed in Table 1. Applications were performed every ten days from May to August in 2022 and 2023 during phenological stages E-L 18 to 34. To this end, a widely automatised pneumatic application gear [Schachtner, Ludwigsburg, Germany; *cf*. further information by Hochschule Geisenheim (2022)] was used, applying an approximate total spray solution volume of 500 L/ha on the first two dates and 650 L/ha on the other six dates.

Disease severity and incidence were assessed visually on 100 leaves and 100 berry clusters per replication according to the EPPO scheme (EPPO, 1997b) at different phenological stages on 11 July 2022 and 10 July 2023 (E-L 33), as well as 15 August 2022 and 14 August 2023 (E-L 37). Final disease quantification was calculated on data of four replicates. Efficacy (≙ disease reduction) levels were indicated as a percentage in relation to the control, which was set at 100 %. Phytotoxicity of the treatments was assessed according to EPPO (2014) by alterations in leaves and clusters throughout the season.

3. Analytical characterisation of must and berry samples and the grape cane extract

A total of approximately 1 kg of randomly picked berries was collected at full ripeness on 19 September 2022 and 25 September 2023, from the four vineyard blocks of all treatments, except for those treated with Folpan® and 2 kg Cuprozin® progress per ha and year. Berries collected from blocks of the same treatment were pooled and immediately pressed to obtain the must, which was centrifuged (Eppendorf 5430 R, Hamburg, Germany) at 2600 RCF and 20 °C for 5 min. Density, pH value, extract, sugar-free extract, total reducing sugars, total titratable acidity, as well as levels of malic acid, tartaric acid, gluconic acid, total volatile acids, glycerin and ethanol, were determined by Fourier-transform infrared spectroscopy (FTIR), as described by Patz *et al*. (2004). After diluting the musts in a mixture of a hydrochloric acid solution of lanthanum oxide with a cesium chloride solution (1/50, v/v), minerals were measured by atomic absorption spectroscopy (contrAA 300, Analytik Jena, Jena, Germany) using a multi-element standard solution. Content in total phenolic compounds was determined spectrophotometrically with the Folin-Ciocalteu reagent according to Singleton and Rossi (1965).

For the analyses of the (poly)phenolic compounds, a second batch of berries was collected as described above. It was immediately freeze dried (Beta 2-8 LD plus, Martin Christ, Osterode, Germany), according to Otto *et al*. (2022), to obtain a powder with a residual moisture of 4-5 %. Extraction was performed as reported by Serni *et al*. (2022) with modifications: 200 mg of deseeded, freeze-dried berry samples were utilised (instead of 20 mg of grape skins as used by Serni *et al*.) with 10 mL of a mixture of methanol and deionised water (1:1, v/v) acidified with 1% (v/v) formic acid in a 15 mL plastic tube. After extraction, stilbenoid analyses were performed by HPLC-DAD as described previously (Besrukow *et al*., 2023), using 0.1 % (v/v) aqueous formic acid and acetonitrile for gradient elution on a Hypersil GOLD RP-C18 column (Thermo Fisher Scientific, Dreieich, Germany) operated at 40 °C. In parallel, further (poly)phenols were analysed following Friedel *et al*. (2015), utilising a gradient elution with 2 % (v/v) aqueous formic acid and acetonitrile/formic acid (98/2, v/v) on a Luna Omega RP-C18 column (Phenomenex, Aschaffenburg, Germany) at 40 °C.

For the (poly)phenol analyses of the grape cane extract, the extract was thoroughly dispersed in a mixture of water and acetonitrile (85/15, v/v) and filtered through a glass fiber membrane (0.2 µm pore size, Chromafil Xtra, Macherey-Nagel, Düren, Germany) into amber glass vials prior to HPLC analyses as described by Besrukow *et al*. (2023).

4. Meteorological data

Meteorological data were recorded by a weather station of the German Meteorological Service located 20 m from the experimental vineyard (49° 98′ 70″ N, 7° 95′ 25″ E). Hourly, air temperatures (measured at 0.7 m and 2.0 m above ground), precipitation (measured at 1.0 m above ground), relative humidity, wind velocity and global radiation were recorded (Hochschule Geisenheim, 2023).

5. Statistical analysis

Means, standard deviations and statistically significant differences of means ($p < 0.05$) were calculated by ANOVA, applying the *post hoc* Tukey test. Statistical processing of results from vineyard disease assessments were performed in Statistica 13 (StatSoft, Germany), whilst data regarding musts and berries composition were analysed with Excel 2019 (Microsoft, USA). Principle Component Analyses (PCAs) were performed with RStudio software (version 4.1.2).

Efficacies of treatments on leaves and berries were calculated with Abbott's formula (Abbott, 1925):

$$\text{Efficacy (\%)} = \left(1 - \frac{S_{\text{treatment}}}{S_{\text{control}}}\right) \text{x } 100$$

where $S_{treatment}$ is the disease severity after a given treatment as described and assessed above, and $S_{control}$ stands for the disease severity of the untreated control.

RESULTS AND DISCUSSION

1. Protection against downy mildew

1.1. Disease assessment on berry clusters

The potential of grape cane extracts and combinations with copper-based agents was tested in a field trial on vines previously inoculated with *P. viticola* to force the emergence of downy mildew. On monthly average, the vegetation periods (May–August) in these years were significantly warmer (2022: +2.4 °C, 2023: +4.6 °C), drier (2022: -25.7 mm, 2023: -13.4 mm precipitation) and had more sunshine hours (2022: +78.6 h, 2023: +121.4 h) compared to the long-term average of 1991–2020 in that area (Hochschule Geisenheim, 2023).

The following results are discussed individually for 2022 due to the virtually absent infections in the year 2023. In 2022, downy mildew assessments of berry clusters showed a rather pronounced infection pressure when compared to those of leaves (see below). In the first assessment (hereafter referred to as '1st') in July 2022, disease severity and incidence in the untreated control berry clusters were found to be 19 % and 80 %, respectively (Figure 1A). While disease incidence remained at this level until the second assessment (hereafter referred to as '2nd'), severity increased to 39 %, providing an adequate basis for efficacy trials (Figure 1B). The fungicide Folpan® led to a virtually complete suppression of downy mildew, as indicated by the very low disease severity (both assessments: 0 %) and incidence levels (1st: 4 %; 2nd: 3 %). As expected, applications of copper at 3.0, 2.0 and 1.5 kg/ha/a were all less effective than those of Folpan®, showing disease severity levels of 10 %, 14 % and 21 % (1st: 2 %, 5 % and 6 %) and incidence levels of 49 %, 62 % and 81 % (1st: 22 %, 36 % and 45 %), respectively. These data evinced a dose-response relationship between the application levels of the fungicide Cuprozin® progress and the incidence and severity of grapevine downy mildew disease.

Applications of both Vineatrol® (1st: 20 %; 2nd: 51 %) and our grape cane extract formulation (1st: 29 %; 2nd: 65 %) together with the copper agent at 1.5 kg/ha/a led to significantly lower disease incidence levels compared to applying copper alone. In parallel, disease severity levels were reduced to 9 % (1st: 3 %) when supplementing Vineatrol® and to 12 % (1st: 3 %) when supplementing our grape cane extract formulation (Figure 1A and Figure 1B). In all treatments, no visible phytotoxic effects on berries were observed.

Plant treatment with our grape cane extract formulation alone was also highly effective, diminishing disease severity to 10 % (1st: 3 %) and incidence to 70 % (1st: 33 %). This efficacy when protecting against downy mildew (78 % efficacy) was comparable to that of copper at 3 kg/ha/a (77 % efficacy), which is the highest dose approved by organic farming associations in Germany. Billet *et al*. (2019) have earlier studied the downy mildew-inhibiting effect of grape cane extracts on both leaves and berry clusters, reporting an efficacy of 43 % on berry clusters after treatment with a grape cane extract, corresponding to the efficacy of a 1 kg/ha/a copper application. Given the fact that Billet *et al*. (2019) applied their extract at 8 g/L (total stilbenoid amount: 1.1 g/L) and grape cane extract formulations in our trials were used at 20 g/L (total stilbenoid amount: 2.5 g/L), the aforementioned discrepancies in protection levels between the studies appear to be plausible. It is worth noting that observable protection levels might depend on a series of factors, like weather conditions, location, virulence of the inoculated pathogen, vintages and the studied cultivars, as stated before (Billet *et al*., 2019), making direct comparison difficult and further systematic studies necessary.

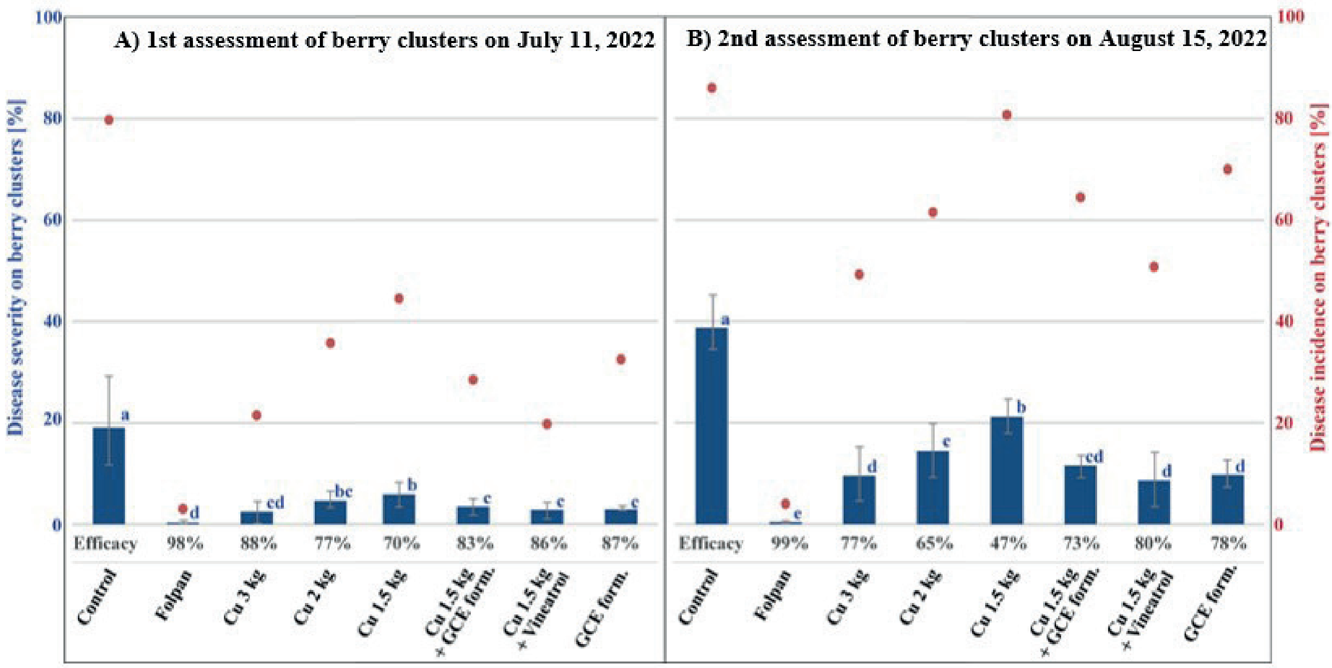

FIGURE 1. Downy mildew disease severity (blue columns) and incidence (red dots) assessed on cv. Riesling berry clusters on (A) 11 July (E-L 34), and (B) 15 August (E-L 37) in 2022 after treatments with Folpan® (standard for integrated viticulture), copper alone at 3, 2 and 1.5 kg/ha/a (Cu, Cuprozin® progress, standard for organic viticulture), and copper at 1.5 kg/ha/a in combination with our novel Grape Cane Extract formulation (GCE) or Vineatrol®, as well as the GCE alone. The GCE showed an inhibition in both disease incidence and severity comparable to those of the copper agent applied at maximum level (3 kg/ha/a), but inferior to that achieved by the synthetic fungicide Folpan®. Different letters indicate significant differences of means (Tukey test; $p < 0.05$).

In addition to the grape cane extract treatments, the supplementation of both Vineatrol® (80 % efficacy) and our grape cane extract formulation (73 % efficacy) to copper at 1.5 kg/ha/a led to an efficacy only insignificantly lower than that of the maximum copper application at 3 kg/ha/a (77 % efficacy). However, the efficacy of grape cane extract-copper combinations (73-80 %) was significantly higher when compared to that of copper applied alone at 1.5 kg/ha/a (47 % efficacy), but not higher than that of grape cane extract applications alone (78 % efficacy). We believe that the dosages of both grape cane extracts might have been relatively high for the actual disease pressure in 2022, thus exerting a very powerful impact on grapevine protection whether applied alone or in combination with copper. This highlights the need for research on threshold concentrations of such extracts related to varying weather conditions, at which plant protection is still ensured. Nevertheless, in view of the EU's aim to halve pesticide usage by the year 2030 (European Commission, 2023), as well as the hitherto challenging logistical availability of grape cane, a combination of these agents might be a promising first step in fulfilling environmental and regulatory purposes.

In 2023, downy mildew assessments evinced no disease development, with both incidence and severity levels of 0 % on berry clusters of the differently treated plants including the control (data not shown).

1.2. Disease assessment on leaves

On leaves, both disease severity (2 %) and incidence (24 %) were low in the control plots in the first assessment in July 2022, increasing to 8 % and 49 % in the second assessment in August 2022, respectively (Figure 2). Generally, the low disease pressure meant that it was difficult to carry out a meaningful evaluation of the results regarding the leaves. Depending on the concentration used, levels of infestation for post-Folpan® or -copper application ranged from 1-4 % with infestation frequency rates from 18-29 %.

The application of our grape cane extract alone reduced disease severity to 1 % (1st: 1 %) and disease incidence to 17 % (1st: 12 %), corresponding to a reduction efficacy related to disease severity of 87 % and that of disease incidence of 50 %. However, the results of our study need to be interpreted carefully due to the comparably low disease severity and incidence as seen in those of the control treatments. For comparison, Billet *et al*. (2019) reported a disease incidence reaching 80 % and a disease severity of up to 40 % on water treated leaves (control), while both indicators evinced an approximate 35 % efficacy for grape cane extract applications. Nevertheless, treatments with the self-made grape cane extract in our study showed highest disease incidence and severity reduction levels across all tested treatments on leaves.

The addition of the grape cane extract formulation to the copper agent at 1.5 kg/ha/a led to 3 % (1st assessment: 2 %) disease severity and 24 % (1st: 20 %) disease incidence. In relation to the control (100 %), this indicates a significantly higher efficacy of both incidence (65 % efficacy) and severity (62 % efficacy) when compared to those of

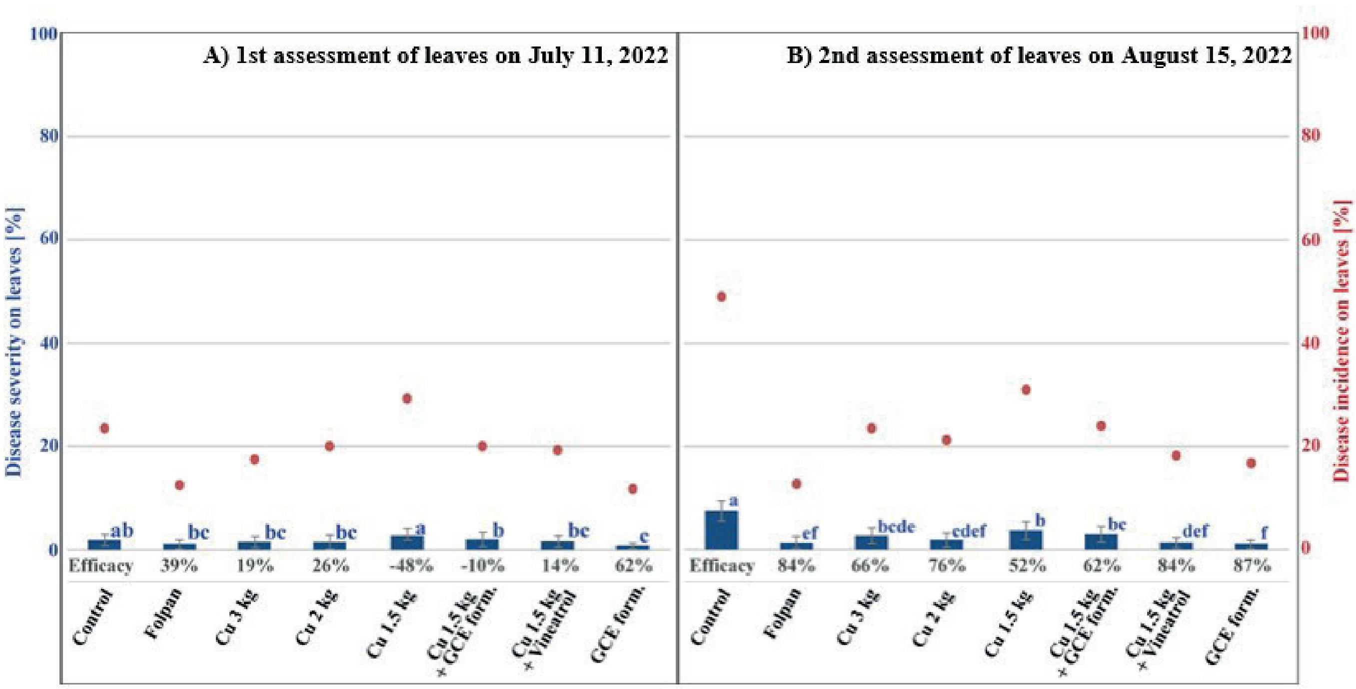

FIGURE 2. Downy mildew disease severity (blue columns) and incidence (red dots) assessed on cv. Riesling leaves on (A) 11 July (E-L 34), and (B) 15 August (E-L 37) 2022 after treatments with Folpan® (standard for integrated viticulture), copper alone at 3, 2 and 1.5 kg/ha/a (Cu, Cuprozin® progress, standard for organic viticulture), and copper at 1.5 kg/ha/a in combination with our novel grape cane extract (GCE) formulation or Vineatrol®, as well as the GCE alone. Different letters indicate significant differences of means (Tukey test; $p < 0.05$).

the sole copper treatment at 1.5 kg/ha/a (25 % disease incidence increase, 52 % disease severity reduction). Unexpectedly, the grape cane extract applied alone appeared to be even more effective than when combined with the copper-based agent. A significant synergistic effect of this grape cane extract/copper-agent mixture with an efficacy of 92 %, which has been previously observed by our group in greenhouse trials (Besrukow *et al*., 2023), was not seen when observing the leaves of this field study (62 % disease reduction in relation to control [100 %]). One explanation for these apparent discrepancies between greenhouse and field trials might be found in the low basal disease levels (8 %) of the field trial. Due to warm and dry weather conditions and, thus, practically non-existent primary and secondary *P. viticola* infections (Hochschule Geisenheim, 2023), disease pressure in vineyards was significantly lower (8 %) than in greenhouse trials (80 %). As a result, disease protection by individual copper and grape cane extract treatments might already have been sufficiently comprehensive, not being further amplifiable by combining these agents.

When adding the commercially available Vineatrol® to copper instead of our self-made extract formulation, severity was found at 1 % (1st: 2 %) and incidence at 18 % (1st: 19 %), achieving a more pronounced efficacy (84 %) than that from combining copper with our (self-made) grape cane extract formulation (62 %). The addition of Vineatrol® to the copper-based fungicide (at 1.5 kg/ha/a) significantly improved its efficacy against grapevine downy mildew (Figure 2). In all treatments, no visible phytotoxic effects on leaves were observed.

Similar to the assessments on berry clusters, no relevant disease development was observed on leaves in 2023 (data not shown).

2. Quality parameters of musts and berries

2.1. Must characterisation

In order to determine the potential effects of our studied fungicidal treatments on the quality of the product to be used for human consumption, we analysed must samples from berries of both years (2022 and 2023) for several oenologically relevant physico-chemical parameters and metal contents, as shown in Table 2.

In 2022, differences in density (1.07-1.08 g/L), sugar-free extract (20-21 g/L), malic acid (2.3-2.8 g/L) and pH levels (2.5-2.7) were insignificant between treatments, similar to the results of previous investigations on the impact of biopesticides like chitosans or laminarin on must composition (Rantsiou *et al*., 2020). This also applies to the levels of microbial metabolites, like ethanol, gluconic acid, glycerin and volatile acids, all being below the limit of quantification (0.1 g/L) in this study.

However, discrepancies were found in the must levels of the total extract (177-220 g/L) and reducing sugars (156-200 g/L), which uniformly showed significantly lower levels after copper treatments, and lowest contents after grape cane extract applications when compared to the control. Inversely, amounts of total titratable acidity (7.6-8.4 g/L) and specifically tartaric acid (5.6-6.5 g/L) were generally higher in musts obtained from the vines treated with grape cane extract-containing agents than that from vines treated with copper or water (Table 2). In 2023, however, the differences between the levels of total extract (237-252 g/L) and reducing sugars (216-231 g/L, Table 2) after copper treatments and those after grape cane extract applications were negligible. Likewise, differences in the levels of total titratable acidity and tartaric acid were marginal. The absolute levels of some parameters like volatile acids (0.1-0.2 g/L), gluconic acid (1.3-1.3 g/L) and glycerin (1.7-2.4 g/L) were significantly higher in 2023 compared to the results of 2022. This also applies to further parameters including reducing sugars (216-231 g/L), pH (3.1-3.2) and total acidity (9.4-10.4 g/L). Such variations between vintages have been described before (Seeber *et al*., 1991). In view of the importance of these parameters for the fermentation process and organoleptic properties of the future wine (Fowles, 1992; Herrera *et al*., 2003), however, the potentially varying sugar and acidity levels resulting from grape cane extract treatments, as observed in this study, warrant further study.

Contents of metals in musts evinced partly significant variations between samples of differently treated vines (Table 2). No clear correlations between different treatments and levels of potassium (1635-1860 mg/L), calcium (72-84 mg/L) and magnesium (50-58 mg/L) were observed. However, the determined levels of copper (2022: 0.2-1.1 mg/L, 2023: 0.1-0.6 mg/L) indicated a dose-response relationship to the actually plant-applied copper dose, with higher doses leading to higher levels of copper in the must (Table 2). For instance, in 2022, must copper levels after copper treatment with 3 kg/ha/a (1.1 mg/L) were approximately twice as high as the treatments with 1.5 kg/ha/a (0.6 mg/L), with or without added grape cane extracts or Vineatrol®. Analogously, in 2023, copper levels after 3 kg/ha/a (0.6 mg/L) copper treatment were twice (0.3 mg/L) and six times (0.1 mg/L) higher than those of the treatments with 1.5 kg/ha/a or without copper, respectively; the results were thus uniformly lower than those of 2022. These findings are in agreement with those of Miotto *et al*. (2014), who had investigated copper concentrations and contents in perennial and annual organs of grapevines, reporting a seven- to twelve-fold increase in copper concentrations in grape bunches upon copper-fungicide application. In our study, treatments omitting copper application (i.e., both the control treatment and the application of the individual grape cane extract formulation) resulted in a must copper level of 0.1-0.2 mg/L. These results comply with the maximum residue levels of 2 mg/L for grape musts and 1 mg/L for wines (International Organisation of Vine and Wine, 2022) and confirm amounts previously reported in literature (Sun *et al*., 2018). Generally, sources of copper in wine have been reported to be attributable to both vineyard (*e.g*., soil, copper-fungicide application) and vinification treatments (*e.g*., removing sulfur off-flavours). It is worth noting that levels of this heavy metal are known to decrease during clarification processes (Čuš *et al*., 2022).

TABLE 2. Physico-chemical parameters and metal levels of musts derived from cv. Riesling vines treated in 2022 and 2023 with water (control), Cuprozin® progress (copper) at 3 kg/ha/a, 1.5 kg/ha/a incl. added grape cane extract formulation (GCE) or Vineatrol®, as well as the grape cane extract (GCE) formulation (20 g/L) alone. For a given parameter, the respective first line in each row of the table refers to data from 2022 and the second to data from 2023.

		Musts obtained from grapevine plants treated with					
		Water (control)	Copper at 3 kg/ha/a	Copper at 1.5 kg/ha/a	Copper at 1.5 kg/ha/a + GCE formulation	Copper at 1.5 kg/ha/a + Vineatrol®	GCE formulation
Density (g/L)	2022 2023	1.084 ± 0.0^{a*} 1.095 ± 0.0^{a}	1.080 ± 0.0^{a} 1.092 ± 0.0^{a}	1.077 ± 0.0^{a} 1.096 ± 0.0^{a}	1.071 ± 0.0^{a} 1.091 ± 0.0^{a}	1.068 ± 0.0^{a} 1.096 ± 0.0^{a}	1.073 ± 0.0^{a} 1.097 ± 0.0^{a}
Extract (g/L)	2022 2023	219.9 ± 0.0^{a} 247.1 ± 0.0^{c}	208.8 ± 0.0^{b} 239.1 ± 1.4^{a}	200.5 ± 0.0^{c} 249.7 ± 0.0^{d}	185.5 ± 3.1^{d} 237.1 ± 0.0^{e}	176.6 ± 0.2^{e} 250.9 ± 0.0^{b}	190.7 ± 2.0^{d} 252.3 ± 0.0^{a}
Sugar-free extract (g/L)	2022 2023	21.1 21.5	20.3 21.6	21.3 21.3	20.9 21.2	21.0 20.1	21.1 21.5
Reducing sugars (g/L)	2022 2023	198.8 ± 0.1^{a} 225.6 ± 0.0^{c}	188.5 ± 0.0^{a} 217.5 ± 0.0^{d}	179.2 ± 1.0^{b} 228.4 ± 0.0^{b}	164.6 ± 0.4^{c} 215.9 ± 0.0^{a}	155.6 ± 0.3^{d} 230.8 ± 0.0^{a}	169.6 ± 0.4^{c} 230.8 ± 0.0^{a}
pH	2022 2023	2.7 ± 0.0^{a} 3.1 ± 0.0^{a}	2.6 ± 0.0^{a} 3.1 ± 0.0^{a}	2.6 ± 0.0^{a} 3.1 ± 0.0^{a}	2.6 ± 0.0^{a} 3.2 ± 0.0^{a}	2.6 ± 0.0^{a} 3.2 ± 0.0^{a}	2.5 ± 0.0^{a} 3.2 ± 0.0^{a}
Total titratable acidity (g/L)	2022 2023	7.6 ± 0.0^{c} 9.5 ± 0.0^{c}	7.9 ± 0.1^{b} 10.4 ± 0.0^{b}	7.8 ± 0.0^{b} 9.4 ± 0.0^{c}	8.3 ± 0.0^{a} 10.7 ± 0.0^{a}	8.1 ± 0.0^{b} 9.5 ± 0.0^{c}	8.4 ± 0.0^{a} 10.3 ± 0.0^{b}
Red. sugars *vs.* total titratable acidity	2022 2023	26.2 23.7	23.9 20.9	23.0 24.3	19.8 20.2	19.2 24.3	20.2 22.4
Tartaric acid (g/L)	2022 2023	5.6 ± 0.0^{c} 6.9 ± 0.0^{d}	6.1 ± 0.0^{b} 7.8 ± 0.0^{a}	6.1 ± 0.0^{b} 7.5 ± 0.0^{b}	6.1 ± 0.0^{b} 7.6 ± 0.0^{b}	6.5 ± 0.0^{a} 7.3 ± 0.0^{c}	6.5 ± 0.0^{a} 7.4 ± 0.0^{b}
Malic acid (g/L)	2022 2023	2.6 ± 0.0^{b} 4.0 ± 0.0^{c}	2.4 ± 0.1^{c} 4.1 ± 0.0^{c}	2.3 ± 0.0^{c} 3.5 ± 0.0^{d}	2.8 ± 0.0^{a} 4.6 ± 0.0^{a}	2.3 ± 0.0^{c} 3.9 ± 0.0^{c}	2.3 ± 0.0^{c} 4.4 ± 0.0^{b}
Tartaric *vs.* malic acid	2022 2023	2.2 1.8	2.5 1.9	2.7 2.1	2.2 1.7	2.8 1.9	2.8 1.7
Volatile acids (g/L)	2022 2023	< 0.1 0.2 ± 0.0^{a}	< 0.1 0.2 ± 0.0^{a}	< 0.1 0.1 ± 0.0^{a}	< 0.1 0.2 ± 0.0^{a}	< 0.1 0.2 ± 0.0^{a}	< 0.1 0.2 ± 0.0^{a}
Ethanol (g/L)	2022 2023	< 0.1 < 0.2	< 0.1 < 0.5	< 0.1 < 0.2	< 0.1 < 1.0	< 0.1 < 0.2	< 0.1 < 1.0
Gluconic acid (g/L)	2022 2023	< 1.0 1.3 ± 0.0^{b}	< 1.0 1.5 ± 0.0^{b}	< 1.0 < 1.0	< 1.0 1.9 ± 0.0^{a}	< 1.0 1.4 ± 0.0^{b}	< 1.0 1.5 ± 0.0^{b}
Glycerin (g/L)	2022 2023	< 0.1 2.1 ± 0.0^{b}	< 0.1 1.8 ± 0.0^{c}	< 0.1 1.7 ± 0.0^{d}	< 0.1 2.0 ± 0.0^{bc}	< 0.1 1.9 ± 0.0^{c}	< 0.1 2.4 ± 0.0^{a}
		Metals					
Copper (Cu, mg/L)	2022 2023	0.2 ± 0.0^{c} 0.1 ± 0.0^{c}	1.1 ± 0.0^{a} 0.6 ± 0.0^{a}	0.6 ± 0.0^{b} 0.3 ± 0.0^{b}	0.6 ± 0.0^{b} 0.3 ± 0.0^{b}	0.6 ± 0.0^{b} 0.3 ± 0.0^{b}	0.2 ± 0.0^{c} 0.1 ± 0.0^{c}
Potassium (K, mg/L)	2022 2023	1860 ± 9^{b} 1397 ± 0^{f}	1836 ± 5^{b} 1664 ± 0^{d}	1636 ± 20^{c} 1632 ± 12^{e}	1829 ± 6^{b} 1779 ± 3^{a}	1961 ± 8^{a} 1683 ± 10^{c}	1678 ± 6^{c} 1746 ± 2^{b}
Calcium (Ca, mg/L)	2022 2023	72.3 ± 0.2^{c} 76.9 ± 0.2^{d}	80.9 ± 0.6^{b} 72.4 ± 0.2^{f}	82.8 ± 0.4^{ab} 79.3 ± 0.2^{c}	72.2 ± 0.4^{c} 73.6 ± 0.1^{d}	81.1 ± 0.7^{b} 79.9 ± 0.0^{b}	84.4 ± 0.4^{a} 84.1 ± 0.3^{a}
Magnesium (Mg, mg/L)	2022 2023	57.7 ± 0.9^{a} 65.6 ± 0.4^{b}	56.2 ± 0.6^{a} 59.7 ± 0.4^{d}	50.2 ± 0.1^{b} 66.5 ± 0.1^{b}	50.6 ± 0.1^{b} 63.7 ± 0.2^{c}	49.7 ± 0.2^{b} 66.3 ± 0.4^{b}	50.8 ± 0.9^{b} 67.8 ± 0.2^{a}
Sodium (Na, mg/L)	2022 2023	7.1 ± 0.1^{b} 3.1 ± 0.1^{a}	7.4 ± 0.2^{ab} 2.9 ± 0.1^{a}	7.5 ± 0.2^{ab} 2.9 ± 0.1^{a}	8.1 ± 0.2^{a} 3.1 ± 0.2^{a}	7.2 ± 0.2^{ab} 3.2 ± 0.2^{a}	8.0 ± 0.2^{a} 2.9 ± 0.1^{a}
Zinc (Zn, mg/L)	2022 2023	0.5 ± 0.0^{c} 2.1 ± 0.0^{a}	0.5 ± 0.0^{c} 1.4 ± 0.0^{b}	0.6 ± 0.0^{b} 0.9 ± 0.0^{c}	0.7 ± 0.0^{a} 0.8 ± 0.1^{c}	0.5 ± 0.0^{c} 0.6 ± 0.1^{c}	0.7 ± 0.0^{a} 0.0 ± 0.0^{d}
Iron (Fe, mg/L)	2022 2023	< 0.1 0.1 ± 0.0^{a}	< 0.1 0.2 ± 0.1^{a}	< 0.1 0.3 ± 0.0^{a}	< 0.1 0.2 ± 0.0^{a}	< 0.1 0.2 ± 0.0^{a}	< 0.1 0.3 ± 0.0^{a}

*different letters indicate significant ($p < 0.05$) differences of means obtained from duplicate analyses of a single pooled sample within a row.

TABLE 3. Phenolic compounds in freeze-dried grape berries derived from vines treated in 2022 or 2023 with water (control), Cuprozin® progress (copper) at 3 kg/ha/a, 1.5 kg/ha/a incl. added grape cane extract (GCE) formulation or Vineatrol®, as well as the grape cane extract (20 g/L) alone. The highest levels of (poly)phenols were found in dried berries of the control plot. For a given parameter, the respective first line of each row in the table refers to data from 2022, the second to data from 2023.

		Berries obtained from grapevine plants treated with:					
		Control (water)	Copper at 3 kg/ha/a	Copper at 1.5 kg/ha/a	Copper at 1.5 kg/ha/a + GCE formulation	Copper at 1.5 kg/ha/a + Vineatrol®	GCE formulation
Summed stilbenoids [µg/g dry weight DW]	2022	63.9 ± 0.8^{a}	25.2 ± 0.6^{b}	30.8 ± 8.6^{b}	15.4 ± 2.2^{c}	28.2 ± 2.3^{b}	12.0 ± 0.2^{c}
	2023	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Resveratrol	2022	15.1 ± 0.6^{a*}	n.d.[§]	n.d.	n.d.	n.d.	n.d.
	2023	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Piceid	2022	48.8 ± 0.2^{a}	25.2 ± 0.6^{b}	30.8 ± 8.6^{b}	15.4 ± 2.2^{c}	28.2 ± 2.3^{b}	12.0 ± 0.2^{c}
	2023	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Summed flavonols [mg/g DW]	2022	1.4 ± 0.1^{b}	1.5 ± 0.1^{b}	3.5 ± 1.4^{a}	1.5 ± 0.2^{b}	1.7 ± 0.3^{b}	1.2 ± 0.1^{c}
	2023	1.6 ± 0.1^{b}	1.7 ± 0.2^{b}	1.6 ± 0.2^{b}	1.7 ± 0.1^{b}	1.7 ± 0.0^{b}	1.5 ± 0.2^{b}
Quercetin (incl. glucosides)	2022	1.4 ± 0.1^{b}	1.5 ± 0.1^{b}	3.1 ± 1.4^{a}	1.5 ± 0.2^{b}	1.7 ± 0.3^{b}	1.2 ± 0.1^{c}
	2023	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}	1.6 ± 0.0^{a}	1.4 ± 0.2^{a}
Kaempferol (incl. glucosides)	2022	n.d.	n.d.	0.4 ± 0.0^{a}	n.d.	n.d.	n.d.
	2023	0.1 ± 0.0^{a}	0.1 ± 0.1^{a}	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
Total flavan-3-ols [mg/g DW]	2022	12.8 ± 2.8^{a}	5.7 ± 1.9^{b}	5.9 ± 0.7^{b}	3.6 ± 1.6^{bc}	4.5 ± 0.8^{b}	2.0 ± 0.1^{c}
	2023	4.7 ± 0.5^{a}	2.4 ± 0.3^{a}	2.4 ± 0.2^{a}	2.4 ± 0.1^{a}	2.4 ± 0.1^{a}	2.4 ± 0.1^{a}
Catechin	2022	2.1 ± 0.4^{a}	1.5 ± 0.3^{b}	1.1 ± 0.1^{bc}	1.1 ± 0.3^{bc}	1.0 ± 0.0^{c}	0.6 ± 0.0^{d}
	2023	0.9 ± 0.1^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}
Epicatechin	2022	1.5 ± 0.4^{a}	0.8 ± 0.4^{b}	1.0 ± 0.1^{b}	0.6 ± 0.3^{b}	0.6 ± 0.1^{b}	0.2 ± 0.0^{c}
	2023	0.9 ± 0.1^{a}	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}
(Epi)catechin gallate	2022	2.5 ± 0.5^{a}	1.5 ± 0.5^{b}	0.7 ± 0.1^{c}	1.2 ± 0.4^{b}	1.4 ± 0.5^{b}	0.5 ± 0.2^{c}
	2023	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Procyanidin B1	2022	1.2 ± 0.2^{a}	0.6 ± 0.2^{b}	1.4 ± 0.1^{a}	0.4 ± 0.2^{bc}	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}
	2023	0.8 ± 0.1^{a}	0.8 ± 0.2^{a}	0.8 ± 0.1^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}
Procyanidin B2	2022	1.0 ± 0.3^{a}	0.4 ± 0.2^{b}	0.4 ± 0.2^{b}	0.3 ± 0.2^{b}	0.4 ± 0.1^{b}	n.d.
	2023	1.0 ± 0.1^{a}	n.d.	n.d.	n.d.	n.d.	n.d.
Procyanidin C1	2022	4.6 ± 1.0^{a}	0.9 ± 0.3^{bc}	1.3 ± 0.1^{b}	0.8 ± 0.2^{c}	0.8 ± 0.1^{c}	0.4 ± 0.1^{d}
	2023	0.9 ± 0.1^{a}	n.d.	n.d.	n.d.	n.d.	n.d.
Summed phenolic acids [mg/g DW]	2022	6.5 ± 1.2^{a}	4.4 ± 0.1^{b}	4.0 ± 0.2^{b}	5.1 ± 0.4^{ab}	6.8 ± 0.3^{a}	3.4 ± 0.2^{c}
	2023	1.0 ± 0.2^{a}	0.8 ± 0.1^{a}	1.0 ± 0.2^{a}	1.0 ± 0.2^{a}	1.0 ± 0.1^{a}	1.1 ± 0.2^{a}
Caftaric acid	2022	3.8 ± 0.4^{b}	2.8 ± 0.0^{c}	2.5 ± 0.1^{d}	3.4 ± 0.2^{b}	4.4 ± 0.2^{a}	2.0 ± 0.1^{e}
	2023	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Coutaric acid	2022	1.3 ± 0.5^{a}	0.7 ± 0.1^{b}	0.5 ± 0.1^{b}	0.6 ± 0.1^{b}	1.2 ± 0.0^{a}	0.6 ± 0.0^{b}
	2023	0.8 ± 0.1^{a}	0.6 ± 0.1^{a}	0.8 ± 0.2^{a}	0.7 ± 0.1^{a}	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}
Gallic acid	2022	0.5 ± 0.2^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.1^{b}	0.1 ± 0.0^{b}
	2023	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fertaric acid	2022	0.9 ± 0.1^{a}	0.8 ± 0.0^{ab}	0.9 ± 0.0^{a}	1.0 ± 0.1^{a}	1.1 ± 0.0^{a}	0.7 ± 0.1^{b}
	2023	0.2 ± 0.1^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}
Total phenolic content[#] [mg/g DW]	2022	36.1 ± 6.2^{a}	14.6 ± 1.0^{c}	18.6 ± 1.2^{b}	10.3 ± 1.3^{cd}	13.3 ± 0.5^{c}	6.6 ± 0.3^{d}
	2023	11.5 ± 2.1^{a}	12.7 ± 1.9^{a}	15.6 ± 4.2^{a}	12.0 ± 1.1^{a}	12.9 ± 0.5^{a}	12.6 ± 1.1^{a}

Different letters indicate significant ($p < 0.05$) mean differences obtained in duplicate within a row. [§]n.d. = not detected. [#]Determined with the Folin-Ciocalteu reagent according to Singleton and Rossi (1965).

However, copper fungicide use in European organic viticulture may exceed 4 kg/ha/a in seasons of high disease pressure, since yearly copper doses can be split over a period of seven years. Considering that it is continuously accumulated in soil and subsequently transported into the plant (Ballabio *et al*., 2018), a long-term copper reduction is crucial for making viticulture and vinification more sustainable than today.

While not particularly important from a (phyto)pathological or oenological point of view, sodium levels in musts (2022: 7.1-8.1 mg/L; 2023: 2.3-3.2 mg/L) were up to 15 % (0.6-1.0 g/L) higher after treatments with our grape cane extract formulation compared to the control in 2022. This increase might be due to the presence of the co-formulant sodium ligninsulfonate [7 % pure sodium, (Otto Dille, 2023)] in the respective grape cane extract formulation.

Considering the aforementioned dose-response relationship of applied copper to the respective must copper content, a “treatment-to-must content” carryover factor for copper might be estimated at $1/2.6x10^6$ [L/ha/a] in our experiment (2.88 kg applied copper/ha/a ÷ 1.1 mg copper /L must). When applying this factor to the 2.2 kg/ha of actually applied sodium that derived from our grape cane extract formulation, discrepancies between grape cane extract-including and -excluding treatments become plausible and might indicate that the co-formulant sodium ligninsulfonate had led to the increased must sodium levels observed in this study. Nevertheless, these differences between treatments were not observed in 2023, contradicting this hypothesis and thus requiring further study.

2.2. Phenolic compounds in berries

In 2022, the total levels of phenolic compounds (7-36 mg/g dry weight [DW]), stilbenoids like resveratrol and piceid (12-64 µg/g DW) and flavan-3-ols like catechin, epicatechin and procyanidin B1 (2-13 mg/g DW), were found to be up to six times higher in berries derived from the untreated control vines than in those obtained from vines treated with copper and grape cane extract (Table 3). Amounts of flavonols, such as quercetin (1-4 mg/g DW), were found to be insignificantly different across the different (bio-)fungicidal treatments, except for samples from low-dose (1.5 kg/ha/a) copper applications, with total flavonol levels more than twice those of the other treatments (3.5 *vs*. 1.5 mg/g [DW]. Likewise, differences in total phenolic acid levels (3-7 mg/g DW) were minimal, with slightly higher amounts found in samples obtained from vines treated with water (control) or the copper-Vineatrol® combination.

When analysing these results together with the disease assessment data from our study, a positive correlation between increased downy mildew severity levels and increased levels of phenolic compounds, particularly stilbenoids and flavan-3-ols, becomes apparent. This observation supports those of previous studies about increased levels of these polyphenols upon fungal infection (Atak *et al*., 2017; Dercks and Creasy, 989). In addition, our results confirm reports on their decrease after fungicide application (Mulero *et al*., 2015). Regarding individual stilbenoids, the predominance of piceid over its more prominent aglycone resveratrol observed in our study (Table 3) is also in accordance with the literature (Romero-Pérez *et al*., 2001). Alongside flavan-3-ols and certain phenolic acids, stilbenoids are known to play a vital role in grapevine defense mechanisms against biotic and abiotic stress, being biosynthesised as phytoalexins upon fungal attack and other stressors (Atak *et al*., 2021; Jeandet *et al*., 2010). Hence, low levels of these phenolics after treatments with copper or/ and grape cane extracts compared to the control in our study suggests that treated berries had required a less active intrinsic defense mechanism for self-protection against fungal attack, at least with regard to the reproductive plant organ, the berry.

In 2023, total levels of phenolic compounds (12-16 mg/g DW) did not differ significantly between treatments. However, these levels are comparable to those found in samples from 2022, which were derived from copper and grape cane extract formulation treatments, and likewise did not show a severe disease infestation. This confirms that in addition to induced production, there is also a certain constitutively present fraction of phenolic compounds in berries (Atak *et al*., 2017). In 2023, the levels of flavonols (1.5-1.7 mg/g DW) and flavan-3-ols (2-5 mg/g DW) were similar to those observed in 2022. However, phenolic acids (0.8-1.1 mg/g DW) were consistently lower across all treatments compared to 2022, with caftaric and gallic acids completely absent in 2023. In addition, stilbenoids remained undetectable in the musts of 2023 across all treatments, possibly due to the special role of this phenolic class as a phytoalexin; *i.e*., it being biosynthesised as result of biotic or abiotic stress. Conceivably, the general absence of biotic stress factors in 2023 made the production of the plant defense compounds stilbenoids obsolete. Nevertheless, stilbenoids were undetectable, even in musts from grapevines that were intensely sprayed with stilbenoid-rich extracts. This observation and the fact that stilbenoids might occur naturally in musts and wines point to the attractive potential of using biopesticides based on grape cane extracts. Residues of the bioactive stilbenoids seem to be either completely absent (data of 2023) or, at least, of no concern due to their natural occurrence in the product (data of 2022).

CONCLUSION

This study confirms that grape cane extract formulations should be further explored for their potential as alternatives to the currently used fungicides in grapevine downy mildew control. Such formulations might have the potential to partially or fully substitute downy mildew fungicides, especially eco-toxic copper fungicides, being particularly important for organic viticulture. Despite their efficacy being similar to that of the copper-based agent, grape-cane extract application might be intricate due to availability, cost or standardisation limitations and hurdles, which would need to be overcome prior to any commercialisation. Applications together with reduced dosages of copper-based agents might be a first option that is easier to achieve and that yields levels of efficacy similar to those of treatments with copper at maximum dosage, as observed in berries here, with inhibited efficacy usually being observed. While we have clearly stated that further multi-year studies should be encouraged to corroborate or disprove these findings, copper reduction efforts should be increased in our view. In this regard, we highlight that the grape cane extract had also led to significantly reduced copper amounts in the must. In addition, spraying grape cane extract formulations (*i.e*., an aqueous solution or dispersion rich in grapevine [poly-]phenols) onto the vines did not result in increased levels of these natural plant compounds, but rather in common phenolic levels, as observed in treatments with other fungicides, like copper-based agents. Alongside the importance of these compounds for wine quality, their fate in, for example, the soil might be of relevance in a discussion on environmental residues.

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