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SHORT COMMUNICATION

Sulfur – a potential additive to increase the efficacy of copper-based fungicides against grapevine downy mildew

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ABSTRACT

Control of downy mildew on grapevines in organic viticulture in Europe is mainly based on using copper-based fungicides. However, the effect of these agents is often insufficient to prevent losses when infestation pressure is high. The addition of sulfur, which is primarily used to control powdery mildew, improves the effect of copper on downy mildew. In three out of five trial years, sulfur had a clear positive effect when it was added during spray treatments against *Plasmopara viticola*, the causal agent of downy mildew on grapevines. This was evident mainly in the fruits; no further positive effect on the leaves was evident. In addition, the present study shows that copper-based fungicides are effective on leaves but have a rather poor effect on grapevine fruit. Furthermore, the study demonstrates that the effect of copper is significantly worse than that of the chemical synthetic contact fungicide folpet.

KEYWORDS: *Plasmopara viticola*, copper, grapevine, organic viticulture, sulfur, fungicide

INTRODUCTION

Grapevine downy mildew is one of the most threatening diseases of viticulture in humid climates. Since the pathogen *Plasmopara viticola* was only first introduced to Europe in the 19th century, *Vitis vinifera* cultivated there for many centuries has never been challenged to develop a defence against this pathogen and consequently is highly susceptible (Boso and Kassemeyer, 2008). Downy mildew is, therefore, controlled by several fungicide applications throughout the season to avoid significant yield losses (Bleyer *et al.*, 2022b). This is especially problematic in organic viticulture as the only extensively tested and reliable fungicides are based on the active agent copper (Schumacher *et al.,* 2022). As an antimicrobial compound, copper has been used in agriculture for more than a century to fight a broad range of plant diseases (La Torre *et al.*, 2018). These products have numerous advantages, such as low cost or their low risk of resistance development on the part of the pathogens. On the other hand, high application rates led to the accumulation of copper residuals in vineyards where such products have been used over decades (Flores-Vélez *et al.*, 1996). Elevated levels of copper in the ground have also been related to a reduction in the biodiversity of soil micro- and macroorganisms, such as gastropods and nematodes (Merrington *et al.*, 2002; Bünemann *et al.*, 2006). However, there are currently no approved alternatives to copper-containing fungicides to control downy mildew in organic viticulture. Quantitative limits for the use of copper formulations have been defined to reduce the negative effects caused by the excessive use of such fungicides. In the European Union, amounts of copper application have been restricted to a maximum of 28 kg within seven years (4 kg/ha/year) (EUR-Lex – 32018R1981). In Germany, organic farming and viticulture associations have made a further commitment to 3 kg/ha/year (Kuehne *et al.*, 2017). Seasons with particularly beneficial conditions for *P. viticola,* like frequent rain events during the susceptible phenological stages until BBCH 79 (berry touch), are, therefore, challenging for organic winegrowers. The decreased trafficability and wash-off effects of copper products have further increased pressure in these years (Weitbrecht *et al.*, 2021).

Referred to as the world's oldest fungicide, the effects of elemental sulfur on pests or the pathogens of plant diseases have been thoroughly described (Williams and Cooper, 2004). Due to its low toxicity to plants and animals, as well as to beneficial insects, sulfur is an attractive component for integrated plant protection until today (Williams and Cooper, 2004). While sulfur-based fungicides are available in a variety of chemical formulations, such as wettable powders of micronised sulfur or lime-sulfur, the direct toxicity is caused by the elemental form of sulfur (Hassall, 1982). In viticulture, the main use of sulfur is the prevention of grapevine powdery mildew. In organic viticulture, similar to copper and downy mildew, sulfur is more or less the only main substance to control this disease (Pertot *et al.*, 2017). In practice, sulfur- and copper-based fungicides are normally applied simultaneously as a tank mixture (Meissner *et al.*, 2019). Due to this fact, relatively little is known about the effect of sulfur against downy mildew. Therefore, this study's objective was to evaluate a possible beneficial effect of sulfur added to copper against downy mildew in the vineyard. This paper aims to initialise further studies about the role of sulfur in controlling this pathogen.

MATERIALS AND METHODS

The beneficial effect of sulfur, in addition to a sole copper treatment during the control of grapevine downy mildew, was evaluated in five independent field trials conducted in 2014, 2015, 2016, 2017 and 2021. Trials were performed in vineyards of the State Institute of Viticulture and Oenology in Freiburg (Germany), planted with *V. vinifera* cv. Pinot noir (row spacing 2 m, plant spacing 1.3 m) in the years 2014–2016 or *V. vinifera* cv. Mueller–Thurgau (row spacing 2 m, plant spacing 1 m) in 2017, as well as in a vineyard in Ihringen (Germany) planted with *V. vinifera* cv. Pinot noir (row spacing 2 m, plant spacing 1 m) in 2021. All vines are trained using a vertical shoot position (VSP) system. To ensure a high and uniform infection with downy mildew, vineyards were inoculated with *P. viticola* as published before (Bleyer *et al.*, 2020).

Four treatments were carried out in every trial: untreated control (utc), folpet (Folpan® 80 WDG (800 g/kg folpet; Adama GmbH, Germany)), copper (Cuprozin progress® (383.8 g/l copper hydroxide; Certis Europe, Germany)) and copper + sulfur (2014, 2015, 2016, 2021: Cuprozin progress® + Kumulus® WG (800 g/kg sulfur; BASF SE, Germany), 2017: Cuprozin progress® + Netzschwefel Stulln 80 % WG (800 g/kg sulfur; agrostulln GmbH, Germany)). To avoid infections of the trial sites with powdery mildew, variants were treated with synthetic fungicides without side effects against downy mildew. Application of products was performed with a tunnel sprayer (Schachtner, Ludwigsburg, Germany) and TeeJet XR80015VS nozzles (TeeJet Technologies, Schorndorf, Germany). Plant protection applications were timed according to model-based strategies provided by the decision support system (DSS) VitiMeteo (https://www.vitimeteo.de; Bleyer *et al.*, 2022a). According to this strategy, a crop protection treatment is only carried out if the *P. viticola* model of VitiMeteo predicts an infection event and, at the same time, at least 400 cm^2 of unprotected leaf area per shoot has grown or if the product layer has been washed off due to at least 35 mm/m^2 of precipitation (Supplementary Table S1: Treatments according to model-based strategy). Deviations from this strategy may occur if thunderstorms were predicted. In consequence, the number of plant protection treatments between the trial years differed (2014: 9 treatments, 2015: 13 treatments, 2016: 13 treatments, 2017: 10 treatments, 2021: 12 treatments) (Exact dates and dose of application: Supplemental Table S2: Products and doses used for plant protection treatments).

Disease assessment was carried out according to the European and Mediterranean Plant Protection Organization (EPPO) standards in four randomised blocks for each treatment within the vineyards. Disease severity was rated for every treatment by visually determining the percentage of symptomatic surface area on 100 leaves and 100 clusters in every block at the end of the season (BBCH 85). Disease incidence was calculated by dividing symptomatic leaves or clusters by the total number of leaves or clusters examined for disease severity rating. Statistical analysis for significance was performed by one-way analysis of variance (ANOVA) with Newman–Keuls post-hoc test ($p \leq 0.05$) with the software piaf PSM (proPlant GmbH, Muenster, Germany).

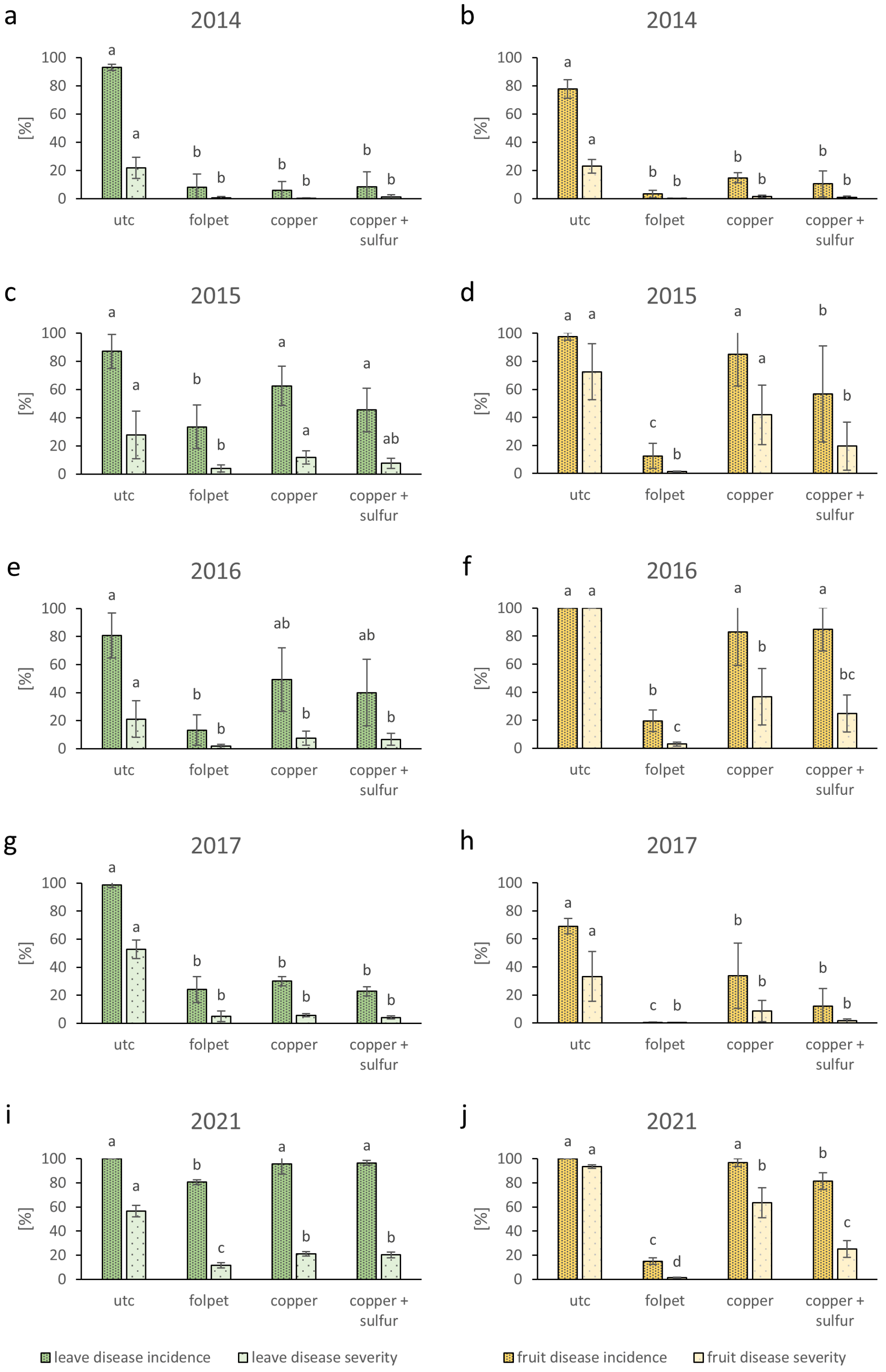

FIGURE 1. Sulfur significantly improves the effect of copper against downy mildew on the fruit of grapevine in years with particularly high infection pressure.

A,c,e,g,i: means of 4 × 100 leaves per treatment rated by the end of the season (BBCH 85); b,d,f,h,j: means of 4 × 100 clusters per treatment rated by the end of the season (BBCH 85). Utc = untreated control. Error bars show standard deviation; different letters indicate significant differences between the treatments (ANOVA with Newman–Keuls post-hoc test; $p \leq 0.05$).

RESULTS

Evaluation of a potentially beneficial effect of the addition of a sulfur-containing fungicide to a fungicide based on copper hydroxide during the control of grapevine downy mildew was carried out in vineyards in southern Germany in the four consecutive years 2014–2017 as well as in 2021 (Figure 1). All five years showed high levels of downy mildew infestation, with 2016 and 2021, in particular, leading to serious problems in the wine industry in southern Germany (Supplemental Figure S3: Epidemiology of *Plasmopara viticola* and precipitation during trial years). This is reflected in the high levels of fruit disease severity (> 90 %) in the untreated controls of 2016 and 2021.

Associated with periods characterised by high infection pressure, significant differences were observed between treatments, particularly in disease incidence and severity of clusters. In 2015, fruit disease severity was 73 % in the untreated control, 42 % in the sole copper treatment, and 20 % in plants with a combined copper and sulfur treatment. Losses on a similar level were rated in 2016 with a fruit disease severity in the untreated control of 100 %, while copper reduced the losses to 37 % and copper in combination with sulfur to 25 %. In 2021, losses in the untreated control were 93 %, while copper reduced disease severity to 64 % and the copper plus sulfur treatment to 25%. The synthetic fungicide folpet kept disease severity below 5 % in every year (2015: 1 %, 2016: 3 %, 2021: 1%). In the years with low infection pressure, 2014 and 2017, no significant differences between the treatments were observed. In 2015, 2016 and 2021, copper performed significantly worse on clusters than the positive control folpet, while the combination with sulfur significantly improved the effect against downy mildew.

Regarding leaf infestation, an improved efficacy against downy mildew by the addition of a sulfur fungicide to a product based on copper hydroxide was not detected in any of the trial years. Significant differences in leaf infestation between the copper variants and the synthetic fungicide folpet were visible in 2015 (untreated control: 28 %, copper: 12 %, folpet 4 %) and 2021 (untreated control: 56 %, copper: 21 %, folpet 12 %) but not as relevant as on grape clusters.

Taken together, results of three out of five trials with especially high downy mildew infection pressure demonstrated that the addition of a sulfur-based fungicide to a copper hydroxide-based fungicide could significantly improve the rather weak performance of copper fungicides on *P. viticola* fruit infestation of grapevine.

DISCUSSION

Fungicides based on copper and sulfur are the most frequently applied products in organic viticulture, and their effect has been known for more than a century. While copper is the most effective agent against downy mildew (*P. viticola*), sulfur is mainly used to treat powdery mildew (*E. necator*) (Dagostin *et al.*, 2011; Gadoury *et al.*, 2011). Whereas copper is also used to control powdery mildew in some crops, the effect of sulfur on downy mildew control has been studied little (Lamichhane *et al.*, 2018). The results of the present study show a beneficial effect for the control of grapevine downy mildew, especially in years with high infection pressure. When a sulfur-containing fungicide was applied together with a product based on copper hydroxide, the disease was significantly better controlled on clusters in three out of five years; an improvement in efficacy on leaves was not visible.

As sulfur was never applied solely during this study, it is not possible to distinguish between a direct effect of the sulfur fungicide or an improvement in the efficacy of the copper fungicide. While sulfur has been used for centuries for the control of fungal plant pathogens, studies on the effect of sulfur on oomycetes are rarely available. One study that examines and summarises the effect of sulfur in detail demonstrates high toxicity of elemental sulfur to several fungi from the Ascomycota, Basidiomycota, and Deuteromycota but not to the oomycete *Phytophthora palmivora* (Williams and Cooper, 2004). Williams and Cooper (2004) assume that “the absence of reports in the literature may merely reflect its lack of effect” on other oomycetes or that the effect at least was not absolutely convincing or demonstrable beyond reasonable doubt. The efficacy of sulfur on the germination of all tested fungal spores in the aforementioned study was high, while activity on the encysted zoospores on *P. palmivora* regarding germination and germ tube growth was not recognised (Williams and Cooper, 2004). This was also further demonstrated with a thin-layer chromatography plate bioassay where all fungi were affected in different degrees while *P. palmivora* grew unhindered (Williams and Cooper, 2004). In *Blumeria graminis*, which was chosen to represent powdery mildews, spore germination was almost completely suppressed with the lowest used concentration (Williams and Cooper, 2004). The mode of action of elemental sulfur is still not completely understood. As extensively reviewed by Williams and Cooper (2004), the currently accepted theory relies on the uptake of sulfur into the fungal cytoplasm, where it affects several cellular processes in the mitochondrial respiratory chain and may react with hydrogen to form toxic hydrogen sulphide. Differences in the cell wall between the different microorganisms, which provides protection against the penetration of sulfur into the cytoplasm, are suggested as a possible explanation for the poor efficacy against oomycetes (Williams and Cooper, 2004). However, *P. palmivora* is a necrotroph and other oomycetes like the biotrophic *P. viticola* were not analysed.

Both a direct or indirect effect on *P. viticola* could be plausible. Sulfur may serve as a precursor for the metabolisation of other complex sulfur compounds, like the recently published volatile S-methyl methane thiosulfonate found in potato-associated bacteria, which has direct anti-oomycete activity against *Phytophthora infestans* (Chinchilla *et al.*, 2019). Furthermore, the influence of sulfur on various processes in the plant, for example, the transcription of various defence-related genes in grapes, has been demonstrated (Giraud *et al.*, 2012). Finally, yet importantly, since the sulfur in this study was applied in the form of commercially available fungicides, it is possible that the formulation of these fungicides also increase, for example, the adhesion of the copper to the clusters, resulting in an improvement of the copper fungicide.

Even though the design of this study does not allow an estimation of the mode of action of sulfur against grapevine downy mildew and more in-depth studies in the lab are needed, the effect on grape clusters was noteworthy. The weak efficacy of copper on grape clusters, in particular, has already been shown in previous studies (Bleyer *et al.*, 2020). Especially in years with high pressure, organic wine growers must fear existence-threatening consequences due to the few possibilities for safe control of grapevine downy mildew. The results of this study once again show that the use of a sole copper treatment is not sufficient to protect the fruits in years with high infection pressure. Furthermore, the data provided by the trials of this work suggest that the addition of sulfur to a copper fungicide might increase the protection of clusters from *P. viticola* damage. The reason for this phenomenon is unknown. Since copper fungicides provide relatively good protection of leaves, a further beneficial effect might not be detectable. On clusters, where the effect of copper is rather poor, the effect of sulfur becomes visible. Nevertheless, in long-term field trials of the State Institute of Viticulture and Oenology in Freiburg (Germany), sulfur was the most effective additive to copper products compared to other substances approved for organic viticulture, for example, plant strengthening agents (unpublished data).

Copper-based plant protection products are still the only effective and approved option for controlling grapevine downy mildew in organic viticulture. However, copper-containing plant protection products are to be substituted in the European Union in the medium term (EUR-Lex - 32018R1981). Therefore, many countries in Europe are currently researching effective crop protection strategies for copper minimisation and alternatives to copper-based crop protection products. Especially under the circumstances of a desired minimisation of copper-containing pesticides, further studies should be conducted to evaluate the effect of sulfur in the field against downy mildew without the addition of copper as well as in comparison and combination with the new alternative crop protection products. Moreover, these studies should elucidate the role of sulfur as a direct or indirect actor in the downy mildew pathosystem.

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