VITIFIT: Aiming for copper reduction in organic viticulture - Improvement of established strategies and new techniques for plant protection against *Plasmopara viticola*

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1 Introduction

Downy mildew of grapevine is one of the most devastating diseases in viticulture. The disease-causing pathogen *Plasmopara viticola* finds especially good conditions in humid years with frequent rainfalls and can then lead to severe damage in the vineyard. This is particularly problematic because all traditional *Vitis vinifera* varieties cultivated in European countries have a high susceptibility to this pathogen. Therefore, winegrowers treat these grape varieties with fungicides at an average of 12-15 times per year to keep leaves and grapes healthy until harvest (Pertot et al., 2016). Whereas conventional estates have access to a wide range of synthetic fungicides, organic winegrowers rely mainly on copper fungicides (Gessler et al., 2011). However, the amounts for copper application are restricted to a maximum of 28 kg within seven years (4 kg/ha/year) in the European Union (Commission Implementing Regulation (EU) 2018/1981). German eco-associations have furthermore committed themselves to 17.5 kg within five years (3 kg/ha/year with option to increase up to 4 kg/ha/year at high infection pressure). Years with particularly high *P. viticola* infection pressure like 2016 and 2021 in Germany therefore lead to existential problems in the organic wine-growing industry, especially since potassium phosphonate is banned in organic winegrowing since 2013 (Bleyer et al., 2020). Moreover, negative effects of copper on the environment like a reduction in biodiversity of soil micro- and macroorganisms (Merrington et al., 2002; Buenemann et al., 2006) were reported. Authorities and environmental organizations have therefore been calling for a reduction in copper fungicides for many years.

Since 2019, major research institutes in the winegrowing sector in Germany have joined in the VITIFIT project (www.vitifit.de). The aim of this project is to develop new strategies, particularly suitable for organic viticulture, to reduce the amount of copper fungicides in *P. viticola* control. In cooperation with industrial partners, associations of organic growers, and various model wineries, existing strategies are to be further developed, supplemented by new approaches and finally made accessible to practice within five years from project start. The German Federal Ministry of Food and Agriculture (BMEL) funds the project with 6.3 million Euro based on a resolution passed by the German Bundestag as part of the federal program Organic Farming and Other Forms of Sustainable Agriculture (BOELN). Hochschule Geisenheim University (HGU) heads the consortium. Also involved are the Bavarian Office for Winegrowing and Horticulture (LWG) in Veitshoechheim (D), the Rural Service Centers (DLR) Rheinpfalz and Rheinhessen-Nahe-Hunsrueck, the Friedrich-Alexander-University (FAU) in Erlangen-Nuremberg (D), the Julius-Kuehn-Institute (JKI) in Siebeldingen (D) and the State Institute of Viticulture and Enology (WBI) in Freiburg (D). The associations for organic agriculture Bioland, Demeter and Naturland as well as ECOVIN for organic viticulture are important partners within the consortium due to their extensive networking. The consortium is completed by the industrial partners GEOsens GmbH from Schallstadt (D), Trifolio-M GmbH from Lahnau (D) and uv-technik meyer gmbh from Ortenberg (D).

The research topics are divided into four parts:
Topic A: Strategies for maintaining good vine health will be developed as part of this topic. New substances suitable for organic viticulture are tested for their biological efficacy, and strategies for plant protection in robust, fungus-resistant grape varieties (so called PIWIs) are developed. The potential to reduce the application of plant protection products by means of cultivation techniques or physical methods is analysed. As part of this topic, strategy trials are being carried out at five different locations in Germany to show which measure is most effective in saving copper applications. In addition, the efficacy of microencapsulated copper salts (CuCaps) is being optimized to develop a product that significantly reduces the amount of copper applied while maintaining the same effect against *P. viticola*.
Topic B: This topic deals with the further development of new, fungus-resitant grape varieties (PIWIs). In addition to the identification of new genetic resources and breeding/selection of new clones, research activities are also focussed on the development of PIWI specific oenological wine styles as well as on market introduction, marketing and consumer acceptance of PIWIs.

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Topic C: Deals with the extension and adaptation of the forecast model "VitiMeteo Plasmopara" to PIWIs and special requirements of organic viticulture. Differences in resistance of several PIWIs against *P. viticola* are therefore identified and characterized. The corresponding algorithms can then be adapted and integrated into the forecast model. Based on the extended information provided by the decision support system, plant protection treatments during resistant developmental stages can be saved while sensitive stages can be protected. Thereby, this protects the underlying resistance from being overcome by *P. viticola*.
Topic D: The fourth topic deals with the development of measures to optimize knowledge transfer, networking and communication between science and practice. The channels of the various associations and partners are used in order to transfer and implement acquired research results into practice. The eco-associations and professional winegrowers not only serve as multipliers, but also provide important suggestions for research based on their own experience. A major goal of the project is that a comprehensive practice-research network will finally be established within the framework of VITIFIT.

In the following part of this paper, only the work of topic A is described in more detail. One fundamental innovation involved in this work package are microencapsulated copper salts (CuCaps) developed and produced by iPAT (FAU). Ten years ago, CuCaps were first developed by iPAT and since then continously optimised for downy mildew control in cooperation with WBI (Weitbrecht et al., 2020). Although the original formulation showed good efficacy against *P. viticola,* the final product was not economically attractive due to its composition and manufacturing process, and thus not suitable for practical use. In the course of VITIFIT, iPAT therefore developed numerous new formulations and optimized them both in terms of Cu^{2+}-release and suspendability in the laboratory. Candidates with suitable technical properties were afterwards analysed for their efficacy against *P. viticola* in leaf disc assays and on potted vines by DLR Rheinpfalz, HGU and WBI. The goal of this work was to develop an applicable and effective formulation for further evaluation in field trials. In addition, this paper presents the results of four experimental variants from field trials conducted at HGU and WBI. The aim during this part of the project was the validation of CuCaps under practical field conditions as well as the evaluation of a potential copper reduction by the use of potassium phosphonate during downy mildew control.

2 Materials & Methods

Efficacy tests of CuCaps

Out of more than 200 different formulations tested for sufficient Cu^{2+}-release and suspendability at iPAT, 25 candidates were analysed for their efficacy against *P. viticola* in leaf disc assays. Leaf disc assays were performed at WBI as mentioned before (Weitbrecht et al., 2020). HGU and DLR Rheinpfalz further investigated particularly promising candidates on potted vines. Plants were therefore treated manually (DLR) or with the same automated application system as used for leaf disc assays (HGU). CuCaps were provided by iPAT, the commercial copper fungicide Cuprozin progress® (copper hydroxide; Certis Europe, Germany) was used for comparison. 24 hours after treatment with plant protection agents and new microencapsulated products, respectively, all leaves of the potted vines were inoculated with a solution of 10^5 sporangia of *P. viticola* per millilitre by means of a commercially available pump sprayer. The youngest unfolded leaf was tagged at the time point of inoculation in order to exclude it during disease severity assessment. Seven days after inoculation, potted vines were exposed to high humidity overnight. In the next morning, disease severity was assessed on five leaves per vine below the tagged leaf.

Vineyard experiments

In 2020 and 2021, a total of eleven plant protection variants against downy mildew were assessed and compared to an untreated control. As mentioned above, only selected variants carried out at HGU and WBI are highlighted here (Table 1).

v	treatment
utc	untreated control
1	max. 3 kg copper (Cu) / hectare (ha) / year (a)
2	2 kg Cu/ha/a
3	2 kg Cu/ha/a + potassium phosphonate
4	2 kg Cu/ha/a (microencapsulated copper (CuCaps))

Table 1: Plant protection treatments applied against *P. viticola*. v = variant

Trials of HGU were performed in an organic vineyard at Geisenheim (Germany) planted in 2008 with *Vitis vinifera* cv. Riesling. Trials of WBI were carried out in two different experimental sites. In 2020 experiments were conducted in a vineyard in Freiburg (Germany), planted in 2011 with *V. vinifera* cv. Mueller-Thurgau. Due to frost injuries in Freiburg in spring, trials of 2021 were performed in a vineyard in Ihringen (Germany), planted in 2014 with *V. vinifera* cv. Pinot noir. All vines are trained using a vertical shoot position (VSP) system. Management of the vineyards was carried out in accordance with the European Union regulations (EEC) No. 834/2007 and No. 889/2008 on organic production of agricultural products. In addition, the stricter guidelines of the ECOVIN association were applied (ECOVIN, 2020).
To ensure a high and uniform infection with downy mildew, vineyards were inoculated with *P. viticola*. For this purpose, one leaf from a single shoot of every third (HGU) or fourth (WBI) vine was inoculated by spraying with a sporangial solution, collected from infected greenhouse plants. To ensure the viability of the used isolate, it was refreshed with newly collected sporangia every year. The sporangial solution was produced by rinsing infected leaves with desalted water. The solution was adjusted to a concentration of 25,000 sporangia/mL. Inoculation was achieved with a commercially available pump-sprayer on a spot that was 3 cm in diameter on the lower side of the leaf.
Plant protection applications were timed according to weather conditions and calculated/predicted infections provided by the decision support systems (DSS) from HGU (https://rebschutz.hs-geisenheim.de/pero/pero-radolan.php; Berkelmann-Loehnertz et al., 2011) for Geisenheim and VitiMeteo (https://www.vitimeteo.de; Dubuis et al., 2019) for Freiburg and Ihringen, respectively. Applications of commercially available products were performed with tunnel

sprayers (HGU: Lipco GmbH, Sasbach, Germany; WBI: Schachtner, Ludwigsburg, Germany). CuCaps were applied with a Solo Port 423 backpack sprayer (Solo Kleinmotoren GmbH, Sindelfingen, Germany) only in 2021 in the vineyard of HGU. Copper treatments were carried out with the commercially available products Funguran progress® (copper hydroxide; Certis Europe, Germany) at HGU and Cuprozin progress® at WBI, respectively. Potassium phosphonate treatments were performed with the product Veriphos® (Adama Deutschland GmbH, Germany) until end of bloom. CuCaps were produced and provided by iPAT (FAU, Germany). Furthermore, all plants, including utc (Table 1), were sprayed against powdery mildew with the commercially available sulphur fungicides Stulln® (Belchim Crop Protection Deutschland GmbH, Germany) at HGU and Kumulus® (BASF SE, Germany) at WBI. Due to a preharvest interval of 56 days of sulphur products in grapes in Germany, last treatments were performed with VitiSan® (potassium hydrogen carbonate; Biofa GmbH, Germany) in combination with Wetcit™ (narrow-range ethoxylate; Biofa GmbH, Germany).

Disease assessment was carried out according to the European and Mediterranean Plant Protection Organization (EPPO) standards in four replications per treatment within the randomized vineyards. Disease severity was rated for every treatment by visually determining the percentage of symptomatic leaf/grape surface area on 4 x 100 leaves or grapes. Disease incidence was calculated by dividing symptomatic leaves or grapes by the total number of leaves or grapes examined.

3 Results & Discussion

Microencapsulated copper (CuCaps) as a newly developed copper containing product was first tested in the field in 2013 (Weitbrecht et al., 2020). Although CuCaps showed good efficacy against *P. viticola*, the final product as published in Weitbrecht et al. (2020) was not economically feasible in practice and was therefore further developed during VITIFIT. The subsequently available new formulations of CuCaps were tested for their efficacy against *P. viticola* in leaf disc assays as well as on potted vines.

The first promising candidate (W143) was identified right before the growing period 2021. W143 showed equally good efficacy against *P. viticola* compared to the commercially available copper fungicide Cuprozin progress® under laboratory conditions (data not shown).

Due to sediments and flocculation, it became apparent that W143 was neither applicable by tunnel sprayers used for precise and standardised application at the experimental sites of VITIFIT, nor in field sprayers usually used in practice by wine estates. Therefore, CuCaps were not included in the vineyard experiments as planned before. However, at HGU field trials with W143 were performed on the experimental vineyard using a backpack sprayer. Even though this experiment is only limited comparable to the results obtained from the treatments sprayed with the tunnel sprayer, this single experiment showed a similar outcome as published before (Weitbrecht et al., 2020). While an amount of 2 kg copper from CuCaps performed equally as 2 kg copper from the commercial fungicide Funguran progress® on leaves, CuCaps showed a significantly better biological efficacy against *P. viticola* on grapes (Figure 1).

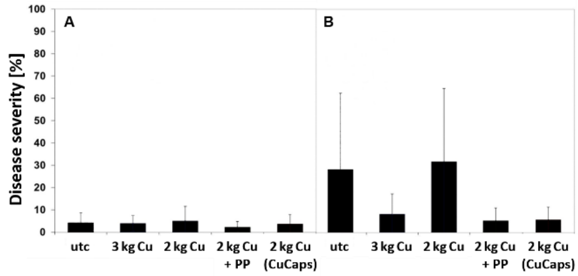

Figure 1: CuCaps showed a stronger biological efficacy on grapes. Graph shows disease severity of *P. viticola* in a field trial performed in a vineyard at HGU in 2021. Disease severity was assessed at BBCH 65 (22.06.2021). A: leaves, B: grapes, utc = untreated control, Cu = copper, PP = potassium phosphonate. Means ± SD.

While W143 was applied in the vineyard with a backpack sprayer, it was further developed in the laboratory of iPAT, resulting in the three CuCaps candidate batches W189, W191 and W195. These batches showed a significantly better applicability when tested in a newly developed test rig at iPAT (data not shown), suggesting an unproblematic usability in field sprayers used in practice. Furthermore, all of them performed better or at least equally than Cuprozin progress® on leaf discs (Figure 2) and on potted vines (data not shown), respectively.

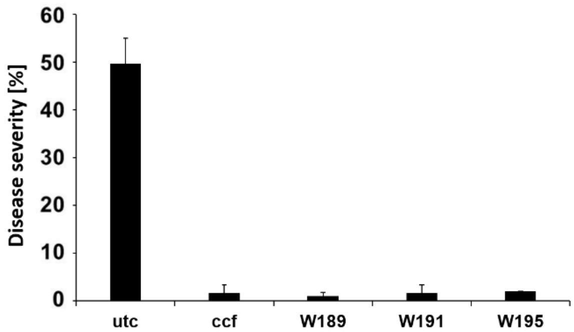

Figure 2: CuCaps batches W189, W191 and W195 show equal efficacy against *P. viticola* as the commercial copper fungicide. Graph shows means (± SD) of three independent leaf disc assays of WBI. utc = untreated control, ccf = commercial copper fungicide (Cuprozin progress®).

Another interesting result regarding the Cu^{2+}-release kinetics of CuCaps was observed in experiments on potted vines performed at HGU. While 2 kg copper from CuCaps batch W189 performed equally to 2 kg copper from Cuprozin progress® if *P. viticola* inoculation was performed one day after CuCaps application, efficacy remarkably improved if inoculation was performed 3 and 5 days after application, respectively (data not shown). These results suggest a potential depot effect of CuCaps and a continuous Cu^{2+}-release on the plant surface.

In summary, the current performance of CuCaps within the framework of VITIFIT indicate that by the start of the 2022 season an applicable formulation will be available for broad-scale field trials. Assuming favourable conditions for *P. viticola* in 2022, comparable data under standardized conditions could be available by the end of the year, opening the way for a promising new copper fungicide. Since currently available copper fungicides perform particularly weak on grapes this may be an important perspective for organic winegrowers (Bleyer et al., 2020).

The 2021 season in particular has shown once again that organic vintners face problems threatening their economical existence by the small portfolio of plant protection products currently available. VITIFIT's vineyard trials also showed that this situation has been significantly exacerbated by the ban on potassium phosphonate in organic viticulture. While in years with low infection pressure of downy mildew, such as 2020, a reduction in the amount of copper from 3 to 2 kg is easily possible, satisfactory control in difficult years like 2021 is challenging to achieve – even with the complete application dose (3 kg).
Even a slight deviation from the optimal time point of treatment, caused for example by poor passability of the vineyards after heavy rainfall, can lead to serious losses. Results from 2021 indicated that the addition of potassium phosphonate until the end of bloom significantly reduced losses (Figure 3). This was also reported by Bleyer et al. (2020) who demonstrated that the addition of potassium phosphonate can significantly extend the interval between two spray events. This could be confirmed within the 2021 field trials: 2 kg copper in combination with potassium phosphonate performed in the same way like a copper application of 3 kg against *P. viticola*. Even though the full application rate of 3 kg in combination with potassium phosphonate was not analysed in the scope of VITIFIT, it could be assumed that this combination may significantly improve the competitiveness of organic growers – especially in difficult years with high downy mildew pressure.

4 Conclusions

Copper-based fungicides are the most effective plant protection products against grapevine downy mildew that are approved in organic farming at the moment. Given the low amount of formulation choice and the need to nonetheless reduce the use of copper, CuCaps introduce a promising and innovative copper fungicide to control downy mildew. Furthermore, the addition of potassium phosphonates is an effective solution for increasing the effectiveness of a copper treatment, which, however, is currently not permitted in organic viticulture.

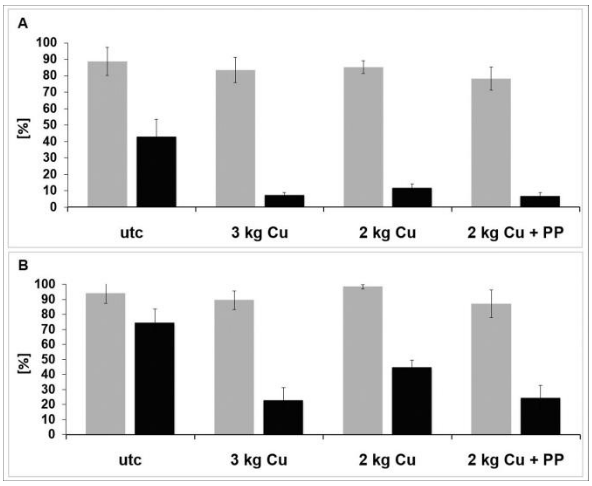

Figure 3: Combination of potassium phosphonate with copper increases efficacy against *P. viticola*. Graphs show results of a field trial of 2021 performed in a vineyard of WBI. Disease incidence and severity were assessed at BBCH79 (31.08.2021). A: leaves, B: grapes, grey bars: disease incidence, black bars: disease severity, Cu = copper, PP = potassium phosphonate, utc = untreated control. Means ± SD.

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