Characterization of the Folpet fungicidal activity against *Plasmopara viticola*

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1 Introduction

Grapevine downy mildew is an important disease in vineyards. It requires continuous work on plant protection products to supply alternative solutions for pest management, avoid fungicides extensive use, and comply with the EU regulations (128/2009/EC) (Gessler *et al*., 2011; Pertot *et al*., 2017). Fungicides used to control *Plasmopara viticula*, the causal agent of downy mildew in grapevine (Gessler *et al*., 2011) are well known in their mode of action (Gisi and Sierotzki, 2008). However, a further understanding of the fungicide characteristics, namely, the physical mode of action (PMoA) is important to consider also. The PMoA refers to aspects other than the biochemical mode of action (Moa), that impact the molecule activity within the plant, pathogen, and environment relationships, namely the protection temporal efficacy, the application time, and the product retention and movement (Szkolnik, 1978). The integration of this information in a plant protection strategy allows for timely and precise management of grapevine pathogens (Rossi *et al*., 2019). Moreover, alternative solutions' availability is a determinant factor in the setting of a vineyard protection strategy (Dagostin *et al*., 2011; Romanazzi *et al*., 2016; Massi *et al*., 2021). It is important to assess these products and their new formulations to provide a comprehensive analysis of their field efficacy (Ahmed and El-Hassawy, 2021). Subsequently and therefore, this work had the purpose to study the Folpet, a well-known contact fungicide (NCBI, 2021), and describing its protection temporal dynamic on grapevine plant material.

Through the seasons 2020 and 2021, we carried out a series of experiments on *Vitis vinifera* cv. Merlot, grapevine plants grown in outdoor pots and weather conditions similar to those of open fields, at the Sustainable Plant Production Department of the Catholic University, located in Piacenza, Italy. A complete randomized design was adopted for the trials, including 2 plots and 7 plants per plot. To evaluate the preventive efficacy, grapevine plants in a 65 BBCH-growth stage (Lorenz *et al*., 1995) were separated into plots and treated with (Vinifol 80 WDG, Folpet 80%, BASF ITALIA) at the indicated dose (1,875 kg/ha) using a manual shoulder pump (VOLPI) with a full volume to cover the plants thoroughly. Moreover, the untreated control plots (TNT) were treated with neutral water. Thereafter, 1-, 3-, 5-, 7-, 9-, and 11-days post-treatment (DPT), the 3rd to 4^{rthe} leaves from the apex of a developing shoot and developing bunches were sampled from randomly selected plants in each plot and prepared for the inoculation as follow: Leaves were repeatedly washed under tap water, and dried until the surface gets completely dry. Then, 15 Leaf disks (25 mm ø) were excised using a cork borer and placed, the abaxial side up along with 15 parts of bunches in sterile Petri dishes at 5 leaf discs per Petri dish prepared with two layers of filter paper moistened with 3 mL of sterile demineralized water for each treatment. The leaf disks were then inoculated with 4 x 10 μL sporangial solution drops distributed at equal distances and the bunch parts were sprayed with a 1 ml of sporangia suspension (10^4/mL) of *P.viticula* per petri dish. Afterwards, Petri dishes were firmly closed with parafilm, and incubated in a growth chamber at 20°C, with a 12h L/D photoperiod. The plant materials were dried and surface inoculum moisture was removed without touching the surface 24 hours post-inoculation, monitored for 7 days until infection appearance, and assessed to calculate the ratio of infected per inoculated sites. Differently, and to study the curative and anti-sporulation efficacy this time, leaf disks and bunche portions from healthy grapevine plants were inoculated according to the above-cited protocol. Yet, the treatment application in this experiment followed the inoculation, drying of the surface, and then treatment by 1-, 3-, 6-, -12, 24-, and 96-hour post-inoculation (HPI). Subsequently, the plant materials were incubated till infection occurrence and assessed as previously described. Finally, in our characterization study, the grapevine potted plants were subject to a rain-washing simulation (from 10 mm, 20, 40, 60, to 80 mm of artificial rain) post-treatment to define the product retention capacity. The artificial rain simulation was obtained using an irrigation pipe mounted at 2m height with 13 watering spray nozzles (1.9 l/h) (Gardena, Husqvarna Italia S.p.A.), which provided a 9 m^2 coverage. To assess the product retention, we challenged the grapevine leaves with *P.viticola* following the same protocol. Analysis of variance was carried out with the statistical program SPSS 27.0. After one-way ANOVA, SNK "Student-Newman-Keuls" Post-hoc test was applied, at a significant difference defined at the $p \leq 0.05$ level. Abbott's formula was used to calculate the corrected efficacy % (Abbott, 1925).

Furthermore, the obtained information was integrated into a fungicides persistence model published by Caffi and Rossi (2018) and thereby, merged into the decision support system (DSS) vite.net® (Rossi *et al*., 2014). The fungicides persistence model is based on estimates of the treatment residual efficacy according to the product characteristics, the weather conditions, the disease development, and the grapevine phenological growth.

2 The preventive efficacy

The experiments on the preventive activity showed a highly significant effect of the product on the severity with a ($p \leq 0.01$) on leaf disks and significant ($p \leq 0.05$) on the bunches. The product showed a considerable reduction of infections (higher than 70%) from the first day to the day 9 post-treatment in both leaves and bunches while the

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control showed a mean severity of 39% on leaf disks and 23% on bunch portions (Figure 1).

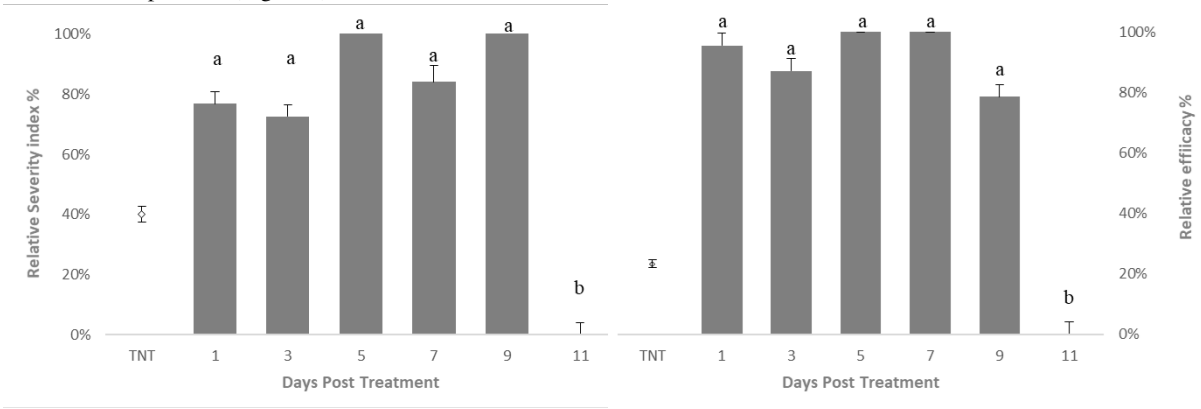

Figure 1: Relative severity index of *P.viticola* in the control on leaf disks (Left) and bunch portions (Right) and the relative protection efficacy of the product in preventive applications, at 1-, 3-, 5-, 7-, 9-, and 11-days post-treatment. The bars indicate the standard error (SE). Letters represent the significant difference between the control (TNT) and the treatment at (one-way ANOVA; $p \leq 0.05$) when tested for an SNK post hoc test.

3 The curative efficacy

Regarding the curative activity experiment, the product showed also a highly significant effect ($p \leq 0.01$) on the relative severity at different hours post-infection. Despite the fluctuating efficacy starting from a 100% reduction in the first-hour post-inoculation to a 30% in the 3rd and 12 hours. However, a treatment intervention after 96 hours post-inoculation showed weak protection. Moreover, the analysis resulted in a significant interaction ($p \leq 0.01$). between the periods, which demonstrates the product's time-dependent efficacy (Figure 2).

In this context, the product showed high efficacy in the first-hour post-inoculation, then declined to 40% at 3 HPI and 33% at 12 HPI with unreliable protection at 6, 24, and 96 HPI. The treatment at 96 HPI did not show significant protection against the infection (Figure 2).

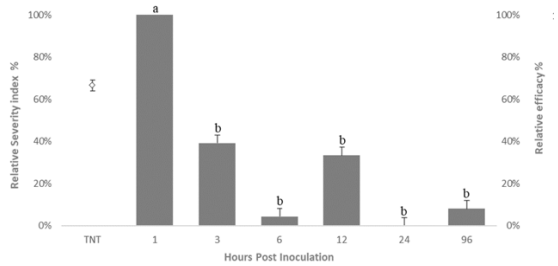

Figure 2: Relative severity index of *P.viticola* in the control and the relative protection efficacy of the product in the different periods (1, 3-, 6-, 12-, 24-, and 96-hours post-inoculation). The bars indicate the standard error (SE). Letters represent the significant difference between the control (TNT) and the treatment at (one-way ANOVA; $p \leq 0.01$) when tested for an SNK post hoc test.

4 The Rain fastness experiment

With no less interest, the wash-away experiment showed a highly significant ($p \leq 0.01$) reduction of the disease severity on leaves at different artificial rain intensity simulations. The product showed a high rain fastness to the washing effect simulated by a 60 mm rainfall. The fungicide showed a high persistent to the rain wash away effect until 80 mm rainfall where it drops sharply to a non-significant protection level (Figure 3).

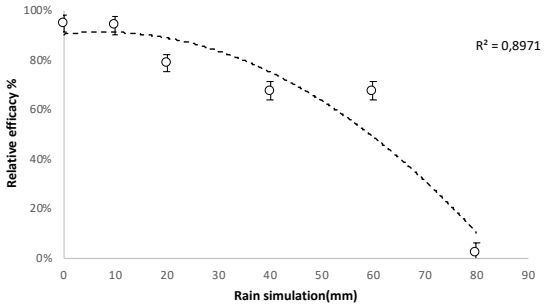

Figure 3: Relative protection efficacy of the product at different rainfall scenarios (0, 10,20, 40, 60, 80 mm). The bars indicate the standard error (SE). Dotted line represents the regression (R^2=0,8971).

5 Conclusions

The experiments carried out to characterize the PMoA of the commercialized active ingredient Folpet allowed for a better understanding of the product's effectiveness and its time-dependent fungicidal activity. The importance of the physical mode action studies on disease suppression was already confirmed by other studies confirmed (Caffi and Rossi, 2018; Claassen *et al*., 2021). The obtained results confirmed the long-lasting efficacy of the Folpet in the control of the downy mildew on grapevine leaves and bunches up to 9 days period post-treatment. Similarly, the Folpet efficacy on leaves and berries infection was demonstrated in a field study (Bleyer *et al*., 2020). The Folpet may, therefore, represent a solution to compensate for the restrictions on copper-based fungicides without compromising grapevine protection. Furthermore, the contact fungicide provided very good protection in our study when grapevine leaves were challenged following rainfall simulations up to 60 mm. Despite the proven reduction in the infections 1- and 3-hours post-inoculation, the Folpet did not succeed to reduce the downy mildew infections in the later periods. The first hours post-inoculation are important for the pathogen to penetrate the leave tissue (Rossi *et al*., 2008), however, the high variability in the Folpet effectiveness demonstrates the unadopted use of the molecule for a pre-existing infection. Most probably, combined use of the Folpet with other molecules would be more effective to protect the

grapevine against infections (Bleyer *et al*., 2020). Nevertheless, the study of the PMoA confirmed the strong standing of the Folpet on the grapevine tissues when tested for its preventive efficacy.

The washing-effect scenarios, in turn, were important to dress the curve of the efficacy reduction with rain-falls, which feed the DSS vite.net®(Rossi *et al*., 2014; Caffi and Rossi, 2018). This knowledge is capital for the improvement of the grapevine protection strategies, reducing the pressure on the environment, and supporting the growers for sustainable viticulture.

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