BIO Web of Conferences **50**, 03003 (2022) https://doi.org/10.1051/bioconf/20225003003
GDPM 2022

Activity of resistance inducers against *Plasmopara viticola* in vineyard

Taibi[1], O., Bardelloni[2], V., Bove[2], F., Scaglia[1], F., Caffi[1*], T., and V. Rossi[1]

[1]Università Cattolica del Sacro Cuore, DIPROVES, I-29122 Piacenza, Italy
[2] Horta Srl, via E. Gorra 55, 29122, Piacenza, Italy

1 Introduction

Plasmopara viticola (Berk. M. A. Curtis) Berl. & De Toni, the causal agent of grapevine downy mildew (DM), is an Oomycete responsible for serious yield and quality losses, and its management still largely depends on the use of fungicides (Gessler *et al.*, 2011). However, numerous alternative products are under investigation to comply with the European restrictions on pesticide use (Directive 2009/128/EC) for a more sustainable and safe agriculture. Among the alternatives to fungicides, the use of plant resistance inducers seems promising. Resistance inducers (RIs) are molecules able to trigger plant defense mechanisms through a cascade of processes and lead to the development of systemic acquired resistance (SAR) or induced systemic resistance (ISR) (Delaunois *et al.*, 2014). These processes are mediated by phytohormones such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and others that activate effective basal plant resistance mechanisms against pathogens (López *et al.*, 2008; Héloir *et al.*, 2019).

In grapevine, a number of compounds were reported to trigger plant resistance. Laminarin (a beta-glucan extracted from the brown alga *Laminaria digitata*) (Aziz *et al.*, 2003) and its sulfate derivatives, chitosan mixed with oligo-galacturonides (also known as COS-OGA) (van Aubel *et al.*, 2014), cerevisane (extracted from cell walls of *Saccharomyces cerevisiae* LAS 117) (Pujos *et al.*, 2014), and phosphonates (Lim *et al.*, 2013) have all been reported to induce a plant defense reaction against biotic and abiotic stresses. The metabolic pathways and defense mechanisms following infection by *P. viticola* in plants treated with RIs have been studied under controlled conditions and their efficacy have long been reported after inoculation experiments (Aziz *et al.*, 2003). In difference, studies devoted to field application tactics and strategies for an effective management in the field are less available (Aziz *et al.*, 2006; Bleyer *et al.*, 2020). Testing RIs under natural conditions is important because plants are already primed by various biotic and abiotic stresses in vineyards, and this may affect the commercial RIs efficacy compared to the laboratory experiments (Delaunois *et al.*, 2014).

In this study, we carried out experiments to evaluate the effect of resistance inducers (RIs) on downy mildew. In a first experiment, we focused on the use of the RIs in a protection strategy based on the combination with copper-based products applied following the indications of the decision support system (DSS) vite.net® provided by Horta (Rossi *et al.*, 2014). In a second experiment, we studied the efficacy of resistance inducers in controlling DM following artificial inoculation of *P.viticola* at different timings after treatment.

2 Efficacy of resistance inducers in combination with copper-based fungicides

In the years 2020 and 2021, we tested different protection strategies in a cv. Barbera vineyard located in the experimental farm *Res Uvae* in Castell'Arquato (Piacenza, Italy). The following RIs and copper-based products were applied: Fosetyl-Al (Aliette, Bayer Cropscience, 80%) at 2.5 kg/ha; Potassium phosphonates (Century, Basf, 51.7%) at 2 L/ha; COS-OGA (Ibisco, Gowan, 12.5 g/l) at 2 -3 L/ha; *Pythium oligandrum* (Polyversum, Gowan, $1x10^{6}$ CFU/g) at 0.3 kg/Ha; Cerevisane (Romeo, Sumitomo Chemical, 94.1%) at 0.25 kg/ha; Laminarine (Vacciplant, UPL, 5%) at 1.5 L/ha; Tribasic Copper Sulfate (Cupravit Bioadvanced, Bayer Crop science, 30%) at 1.4 kg/ha; Copper Oxychloride (Verdram Hi Bio, Belchim Crop protection, 30%) at 2 kg/ha; Copper Sulfate (Poltiglia Disperss, UPL, 20%) at 2 kg/ha.

Products were applied following advices provided by the Decision Support System (DSS) vite.net® provided by Horta (https://www.horta-srl.it/en/) based on the models for primary and secondary infections (Caffi *et al.*, 2010; Brischetto *et al.*, 2021) and only when the previous application coverage fall below 70% efficacy (as determined by the model of Caffi and Rossi (2018). The treatment schedules are presented in table 1.

Date	Cerevisane	COS-OGA	Fosetyl-Al	Laminarin	*Pythium oligandrum*	Potassium phosphonate
17-April-20	*	*				*
27-Apr	*	*				*
08-May	*	*	*			*
18-May	*	*				*
28-May	*	*	*			
03-June	*	*	*		*	
10-June	*	*	*		*	
01-July	*	*		*	*	
16-July	*	*		*	*	
23-April-21	*	*			*	
07-May	*	*	*		*	
13-May	*	*	*		*	
01-June	*	*	*	*	*	
17-July		*	*	*	*	

Table 1: Timing of application of resistance inducers in combination with copper-based fungicides

May 2020 was dry and the average temperature was around 19°C; in June, there were 85.8 mm rain, and the average temperature raised to 21°C. In July, there were five isolate rainfall events, and a temperatures peak at 36°C. The flowering started in the second decade of May, while veraison at the end of July. The year 2021 was characterized by lower rainfall than 2020, with a total of 268 mm, mainly distributed in April, May and September. In June there was only one rain of 15.8 mm, and the average temperature was 25.7°C; in July, there were 11 rainfall events with a total of 49.3 mm rain, with

*Corresponding author: tito.caffi@unicatt.it maximum temperatures below 35°C. In August there were 3 rainfall events for a total of 11.2 mm rain. The beginning of flowering was observed in the second decade of June, while veraison started between the end of July and the beginning of August.

Disease severity was assessed at weekly intervals from the flowering at 65 BBCH (Growth stage) to berries ripe at 85 BBCH in the two years. The area under the disease progress curve (AUDPC) (Madden *et al.*, 2007) was calculated for the different treatments based on disease severity assessments. These data were used in an analysis of variance, and means were separated using the Student-Newman-Keul (SNK) test with α=0.05.

Overall, the untreated control (TNT) showed higher disease severity on leaves in 2021 than in 2020, while the disease severity on clusters was similar for the two years (Figure 1).

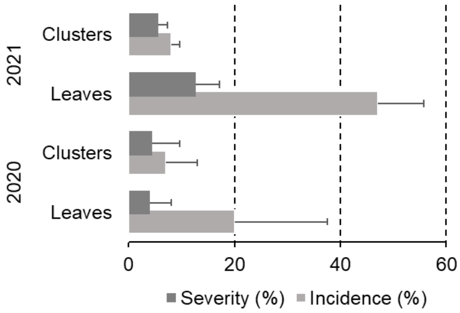

Figure 1: Mean disease severity and incidence of downy mildew on untreated controls (cv. Barbera) at the end of the growing seasons 2020 and 2021. Whiskers represent standard errors.

There was a significant difference between treatments and the TNT ($P \leq 0.05$), with the treatments based on Cerevisane and Laminarine combined with copper showing the lowest AUDPC (34.4 and 50.0, respectively) compared to copper with Fosethyl-Al or copper alone (AUDPC of 160.5 and 171.8, respectively) (figure 2).

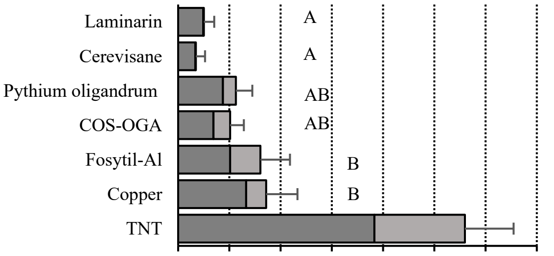

Figure 2: Mean AUDPC values for downy mildew severity on leaves and clusters (cv. Barbera). Whiskers represent the standard error. Letters indicate significant differences between treatments based on the SNK test with α=0.05.

3 Efficacy of resistance inducers following artificial inoculation of *Plasmopara viticola*

In 2020 and 2021, three experiments were performed *in planta* in the growth stages BBCH 71, 79 (2020) and 73 (2021) (Lorenz *et al.*, 1995). At each of these stages, the RIs were applied as indicated before. After 1, 3, 6, 9, 12 and 19 days post-treatment (DPT), the abaxial side of 15 leaves per treatment were inoculated using a *P.viticola* sporangial suspension (5×10^4). At disease onset, downy mildew severity (%) was assessed on individual leaves.

The mean severity of downy mildew on the TNT leaves in 2020 approximated 35% for the trial at BBCH 71, and 5% at BBCH of 79; in 2021, it was 15% at BBCH of 73. Our evaluations then covered a wide range of disease severities.

The analysis of variance (AOV) showed a highly significant ($P \leq 0.01$) effects of the treatments, timings (DPT), and their interaction. The interaction accounted for 28% of the total variance, demonstrating that the different RIs had varying effects on the disease when applied at different timings with respect to *P. viticola* infection. The area of inoculated leaves was used as a covariate in the AOV, and had a highly significant ($P \leq 0.01$) effect, indicating that although the leaves selected for inoculation had a similar position on the shoot (the 4th position from the apex), the leaf area influence the response to the infection; in particular, smaller leaves were more affected than larger leaves. Overall, leaves treated with Potassium phosphonate and Laminarin showed the highest efficacy and some efficacy even when *P. viticola* was inoculated at 1 DPT (figure 3). Potassium phosphonate had about 40% efficacy as early as 1 DPT, and 80% efficacy from 3 to 12 DPT; efficacy then decreased to 60% at 19 DPT. The laminarin-based product showed 73% efficacy at 1 DPT and > 80% efficacy at 3 and 6 DPT; efficacy then decreased to 57% at 12 DPT and 30% at 19 DPT. Fosetyl Al and Cerevisane had no efficacy at 1 DP1, and 50% to 70% efficacy for inoculations at 3 to 19 DPT (figure 3). Finally, COS-OGA and *P. oligandrum* overall showed a lower efficacy compared to other RIs (figure 3).

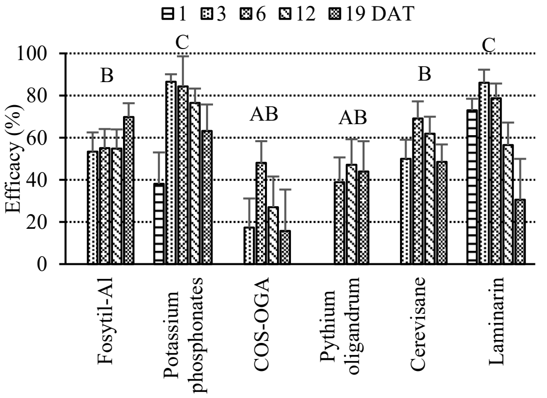

Figure 3: Efficacy (%, Abbott, 1925) of RIs on downy mildew severity on grapevine leaves (cv. Barbera) following artificial inoculations of *Plasmopara viticola* at 1, 3, 6, 12 and 19 days post-treatment (DPT). Whiskers indicate standard errors. Letters indicate significant differences between treatments based on the SNK test with α=0.05.

4 Conclusions

Our experiments based on vineyard applications of RIs in combination with copper-based fungicides applied at low dosages confirmed that alternatives to the chemical fungicides may contribute to downy mildew control (Bleyer *et al.*, 2020) when used as preventative, i.e., prior *P. viticola* infection periods predicted by mathematical models. This is a new finding, because in previous research and in technical advices, these products have been and still are recommended as calendar-based, repeated applications (Romanazzi *et al.*, 2016; Gutiérrez-Gamboa *et al.*, 2019).

The artificial inoculation experiments confirmed the preventive efficacy of RIs when applied 1 to 19 before infection. In general, the efficacy increased towards the infections occurring from 3 to 6 DPT and then decreased in subsequent days even if, in some cases, high efficacy was registered at 19 DPT too. Laminarin and Potassium phosphonate were characterized by a high, fast, and long-lasting efficacy. Unlike all the other products, they provided effective downy mildew control as early as 24 hours post-treatment. Studies on Laminarin had already documented a rapid reaction (several hours) in the plant after treatment (Aziz *et al.*, 2003). Potassium phosphonate is known to have also a direct action on the pathogen, and this may explain a prompt efficacy. Fosetyl-Al and Cerevisane also showed good efficacy, less prompt but long-lasting, which is still > 50% at 19 DPT (similar to the Potassium phosphonate). These observations are, overall, consistent with what has been previously documented about the ability of these active ingredients to induce resistance against downy mildew (Lim *et al.*, 2013; Pujos *et al.*, 2014). The COS-OGA and *P. oligandrum* showed a lower and later efficacy. It may be considered that these products are usually recommended for the control of *Erysiphe necator* and *Botrytis cinerea* (Mohamed *et al*., 2007; van Aubel *et al*., 2014); therefore, this activity against *P. viticola* is noteworthy. Cerevisane (commercial product Romeo) is registered in Italy for downy mildew, powdery mildew, and grey mold, and Laminarin (commercial product Vacciplant) for downy mildew and powdery mildew; their broad spectrum makes them interesting from an integrated pest management perspective.

The integration of RIs in vineyard protection strategies along with the use of DSSs for precise and preventive interventions against *P.viticola* may help overcome current strategies based on conventional (calendar-based) plant protection rather than on actual disease risk. Finally, moving to a pre-infectional use of these substances allows also compensate the metabolic cost related to the plant immunity activation, energy allocation and metabolites associated with priming (Nogueira Júnior et al., 2020), which is otherwise unjustified in the absence of infection risk.

Acknowledgement

This work was supported by the European project NOVATERRA ID: 101000554 funded by the H2020-EU.3.2.1.1 through the Università Cattolica del Sacro Cuore di Piacenza and its spin-off company HORTA srl.

Othmane Taibi was supported by the Doctoral School on the Agro-Food System (Agrisystem) of Università Cattolica del Sacro Cuore (Piacenza, Italy).

References

1. Abbott W. S. (1925). A method of computing the effectiveness of an insecticide. *J. econ. Entomol,* 18(2): 265-267.
2. Aziz A., Poinssot B., Daire X., Adrian M., Bézier A., Lambert B., Joubert J.-M. and Pugin A. (2003). Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and Plasmopara viticola. *Molecular plant-microbe interactions,* 16(12): 1118-1128.
3. Aziz A., Trotel-Aziz P., Dhuicq L., Jeandet P., Couderchet M. and Vernet G. (2006). Chitosan oligomers and copper sulfate induce grapevine defense reactions and resistance to gray mold and downy mildew. *Phytopathology,* 96(11): 1188-1194.
4. Bleyer G., Lösch F., Schumacher S. and Fuchs R. (2020). Together for the Better: Improvement of a Model Based Strategy for Grapevine Downy Mildew Control by Addition of Potassium Phosphonates. *Plants,* 9(6): 710.
5. Brischetto C., Bove F., Fedele G. and Rossi V. (2021). A Weather-Driven Model for Predicting Infections of Grapevines by Sporangia of *Plasmopara viticola*. *Frontiers in plant science,* 12: 317.
6. Caffi T. and Rossi V. (2018). Fungicide models are key components of multiple modelling approaches for decision-making in crop protection. *Phytopathologia Mediterranea,* 57(1): 153-169.
7. Caffi T., Rossi V. and Bugiani R. (2010). Evaluation of a warning system for controlling primary infections of grapevine downy mildew. *Plant disease*, 94(6): 709-716.
8. Delaunois B., Farace G., Jeandet P., Clément C., Baillieul F., Dorey S. and Cordelier S. (2014). Elicitors as alternative strategy to pesticides in grapevine? Current knowledge on their mode of action from controlled conditions to vineyard. *Environmental Science and Pollution Research,* 21(7): 4837-4846.
9. Gessler C., Pertot I. and Perazzolli M. (2011). Plasmopara viticola: a review of knowledge on downy mildew of grapevine and effective disease management. *Phytopathologia Mediterranea,* 50(1): 3-44.
10. Gutiérrez-Gamboa G., Romanazzi G., Garde-Cerdán T. and Pérez-Álvarez E. P. (2019). A review of the use of biostimulants in the vineyard for improved grape and wine quality: effects on prevention of grapevine diseases. *Journal of the Science of Food and Agriculture*, 99(3): 1001-1009.
11. Héloir M.-C., Adrian M., Brulé D., Claverie J., Cordelier S., Daire X., Dorey S., Gauthier A., Lemaître-Guillier C. and Negrel J. (2019). Recognition of elicitors in grapevine: from MAMP and DAMP perception to induced resistance. *Frontiers in plant science,* 10: 1117.
12. Lim S., Borza T., Peters R. D., Coffin R. H., Al-Mughrabi K. I., Pinto D. M. and Wang-Pruski G. (2013). Proteomics analysis suggests broad functional changes in potato leaves triggered by phosphites and a complex indirect mode of action against *Phytophthora infestans*. *Journal of proteomics,* 93: 207-223.
13. López M. A., Bannenberg G. and Castresana C. (2008). Controlling hormone signaling is a plant and

pathogen challenge for growth and survival. *Current opinion in plant biology*, 11(4): 420-427.

14. Lorenz D., Eichhorn K., Bleiholder H., Klose R., Meier U. and Weber E. (1995). Growth Stages of the Grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. vinifera)—Codes and descriptions according to the extended BBCH scale. *Australian Journal of Grape and Wine Research*, 1(2): 100-103.
15. Madden L., Hughes G. and Bosch F. (2007). Van den, B. The Study of Plant Disease Epidemics: APS Press: Minnesota, MN, USA.
16. Mohamed N., Lherminier J., Farmer M.-J., Fromentin J., Béno N., Houot V., Milat M.-L. and Blein J.-P. (2007). Defense responses in grapevine leaves against Botrytis cinerea induced by application of a *Pythium oligandrum* strain or its elicitin, oligandrin, to roots. *Phytopathology*, 97(5): 611-620.
17. Pujos P., Martin A., Farabullini F. and Pizzi M. (2014). RomeoTM, cerevisane-based biofungicide against the main diseases of grape and of other crops: general description. *Atti, Giornate Fitopatologiche, Chianciano Terme (Siena), 18-21 marzo 2014, Volume secondo*: 51-56.
18. Romanazzi G., Mancini V., Feliziani E., Servili A., Endeshaw S. and Neri D. (2016). Impact of alternative fungicides on grape downy mildew control and vine growth and development. *Plant disease*, 100(4): 739-748.
19. Rossi V., Salinari F., Poni S., Caffi T. and Bettati T. (2014). Addressing the implementation problem in agricultural decision support systems: the example of vite. net®. *Computers and Electronics in Agriculture*, 100: 88-99.
20. van Aubel G., Buonatesta R. and Van Cutsem P. (2014). COS-OGA: a novel oligosaccharidic elicitor that protects grapes and cucumbers against powdery mildew. *Crop Protection*, 65: 129-137.