Soil covering and biofumigant effect of Armoracia rusticana against spore dispersal and viability of *Downy mildew* inoculum in viticultural systems-BIOVINE

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1 Introduction

Downy mildew (*Plasmopara viticola*) is one of the major disease of grapevine, which causes severe yield losses and a great reduction in the final quality of grape/wine. Usually, symptoms appear on the upper side of the leaves as light-yellow oily spot, that later becomes yellow and necrotic and in case of severe attack infected leaves dry and drop (Winkler, 1965). The early infections are more dangerous, by causing defoliation of the canopy, which jeopardize fruit maturation (Thind et al., 2004). Infected branches take the classical S shape and infected parts turn brown due to cell necrosis (Belli et al., 2012). Downy mildew is a polycycle disease, with an optimal development at 25°C, but its insurgence, growth and spreading depend on temperature and rainfall. *P.viticola* overwinters as oospores in the fallen leaves and can survive at very low temperature, up to -20°C (Thind et al., 2004). Considering the cultural practices, plant density triggers vine susceptibility to disease insurgence. Therefore, it is better to avoid vigorous rootstocks, excessive nitrogen fertilization and vineyard establishment in too humid locations (because those conditions create the perfect microclimate for the pathogen development) (Belli et al., 2012).
Downy mildew control is traditionally based on the use of preventative fungicides, that prevent grapevine via contact in early stages of pathogen development, when is still on plant surface. The most used contact fungicides are copper based that create a protectant barrier on the plant killing the propagules of the pathogen, the zoospores, and avoiding the host penetration (Gisi, 2002). Usually, copper is used alone or combined with systemic fungicides, which affect pathogen in later stages of development by having a curative or eradicant effects (e.g. cymoxanil, fosetyl-Al, phenylamides etc.). *P.viticola* can produce several generations on leaves and for this reason many spray treatments are usually needed. Lately, organic viticulture started to have a positive trend on the market thanks to the consumers' care about healthier products, obtained in a more environmentally friendly way. According to the European regulation (Reg. EU 848/2018), the disease control in the organic viticulture must be achieved a) by choosing the best adapted variety or species for each area of production, by using rotation, mechanical tillage; b) by protecting the natural enemies of pests; c) by applying thermic treatment to control weeds etc. Moreover, the European Union adopted IPM (integrated pest management) strategies, that became mandatory in Europe in 2014 (Dir. EC 128/2009), describing the possible alternative strategies to control diseases in a more sustainable way, for both organic and conventional viticulture.
Because of an increasing concern about pesticides effects on human, environment and not target organisms, alternative solutions to chemicals started to be tested in field to check if they can be integrated in the disease management system, with the aim of reducing pesticide amount sprayed within a season to levels that are economically and environmentally acceptable (Villemaine et al., 2020). Recently, also the host plant resistance inducers started to be used to control downy mildew. These products are not directly effective against fungi, but they activate plant genes that regulate the plant disease reaction by interacting with salicylic and jasmonic acid pathways, that involved in the disease response (Gisi, 2002).
Nowadays a greater attention is focused on development of sustainable agricultural systems that preserve soil fertility, biodiversity and improves ecosystem functioning (Eckert et al., 2020). Application of cover crops in vineyards can be a promising strategy to improve sustainability in viticulture, soil aggregate stability, soil fertility, improving water infiltration and water reserve formation (Garcia et al., 2018, Hartwig et al., 2002). Cover crops presence in interrows have positive effects also on soil microbial community, increasing their presence and activity as well as on macro-organisms, for instance increasing beneficial nematodes presence, decreasing plant parasitic soil borne fungal population, increasing earthworm presence and activity as well as increasing mycorrhizal fungi association with plants (Garcia et al., 2018., Leon M. et al., 2021). Cover crops can prevent disease insurgence, by reducing the primary inoculum, by creating a physical barrier between soil and vines (Rossi et al., 2012), that should avoid or limit the splash effect that moves *P.viticola* oospores from ground to basal leaves, or by producing toxic compounds (biofumigation) against pathogens or by increasing the presence of natural micro-organisms able to fight with pathogens and to induce a higher plant resistance (Vukicevich et al., 2016). Many studies showed the effect of cover crops in reducing disease or pest presence, demonstrating that cover crops can be integrated in the disease management to avoid an excessive use of chemicals products (Ristaino et al., 1997; Rossi et al., 2012; Pertot et al., 2017; Wen et al., 2017; Hasanaliyeva et al., 2021). Due to the strictly limitations imposed by Europe in the last years, many pesticides are forbidden or limited as copper, for which a maximum dose applicable each year has been established (maximum 4kg/ha/year) (Reg. EU 1981/2018). Thus, disease control has become more complicated and difficult, especially in organic farming where copper is the most used fungicide (La Torre et al., 2008) and most of the other chemicals are not allowed. Thus, the research of alternative methods (as cover crops) is fundamental to reach an adequate production.
Therefore, objective of this study was to investigate physical barrier and biofimugation effect of Horseradish (*Armoracia Rusticana*) from Brassicaceae family. In general, plants from this family contain precursors of toxic compounds (e.g. glucosinolates) that are going to be hydrolyzed during the

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decomposition process and released into the soil. Their effectiveness depends on the variety/cultivar used, the biomass produced, the age of the plant at the incorporation time, the humidity, the fragment dimension, the depth and distribution in the soil after the incorporation (Van Bruggen et al., 2016).

2 Materials and methods

In the University campus of Piacenza, two small scale experiments plots were carried out: the former to monitor the effect of cover crop as a barrier for spread of spores of *P.viticola*, the latter to check biofumigation effects on *P. viticola* zoospores.

In the first experiment bare soil (control) was compared to three different plots sowed with Horseradish (*Armoricia Rusticana*) on different dates (15days, 30days, 50days) (Fig.1). Later, small strips of blotting paper were randomly placed on the ground in the different plots, then artificial rainfall was provided using a blue coloured water (Fig. 2)

Figure 1. Cover crops (horseradish) development at the time of experiments: different plots were created with different sowing time

Figure 2. Rain simulation-splash experiment: blotted-paper strips posed on the ground of each plot allowed to quantify the interference of the cover crop with simulated rainfall

In the secnd experiment secondary metabolites extraction was conducted in laboratory condition. Total glucosinolates were extracted from leaves and roots of horseradish using boiled water (Herzallah and Holley, 2012). This method was selected to be more close to what could happen in the soil when Horseradish is cut and incorporated into the soil, which is more likely to produce glucosinolates using heat and humidity (Manici, 1997; Tiznado et al., 2008). Healthy, young, unfolded leaves (3rd or 4th from the shoot apex) were collected from vine plants grown in greenhouse. Later leaves were washed, dried and 4 leaf discs was cut from each to be transferred to the Petri dishes. The inoculum suspension was obtained according to Caffi T. et al., (2016) from the leaves of the cv. Barbera plants used for inoculum maintenance. Leaf discs were inoculated with standard suspension (control) and worked suspension (mixed with total glucosinolates extract).

3 Results

The rainfall drops were able to reach the soil, in all the experiment repetitions performed, even if the average value was significantly lower in the plot where the cover crop was growing. Interestingly, the most relevant difference was observed in the plot where the horseradish was at the beginning of its development (about 10 cm in height, Figure 3). Soil coverage between 40-50% was monitored in cover cropped plots comparing to bare soil plot, which ended up with almost 80% of coverage. This was because of the almost perfect soil coverage performed by the cover crop that in this stage is expanding its leaves increasing the “shield” effect on the soil.

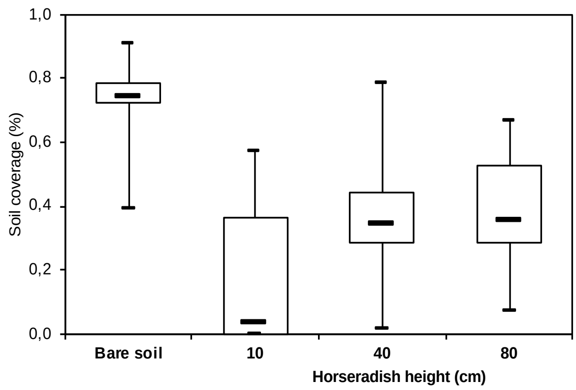

Figure 3. Soil coverage percentage: bare soil and horseradish at different growth plant heights (10 cm, 40cm and 80cm), depending on sowing day.

In the second experiment it was visually and statistically possible to see that leaf discs inoculated with mixture of downy mildew suspension and total glucosinolate extract resulted in 100% inhibition of sporulation process comparing to control (Fig. 4).

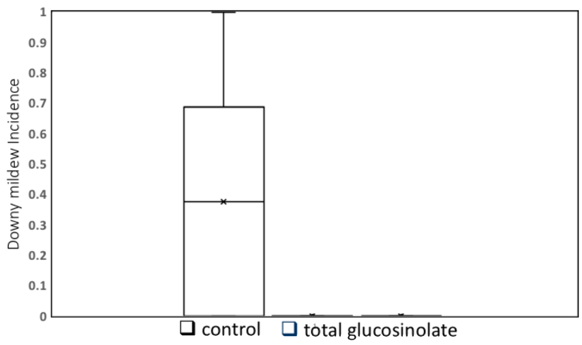

Figure 4. Downy mildew sporulation on control leaf discs (untreated) and on leaf discs treated with glucosinolates.

4 Conclusions

This experiment shows promising evidence that the canopy structure and plant coverage of the soil would allow a significant reduction of the dispersal of pathogens spores trough rain splashes, reducing the number of rain drops that can reach grapevine leaves.

The results obtained from glucosinolates experiment showed that these compounds potentially can be effective in suppressing downy mildew sporulation, and being natural may be used in organic viticulture as a bio-fungicide, to prevent *P.viticola* sporulation. This practice of cover cropping will eventually help farmers reduce pesticide/fungicide use and benefit them economically by preventing yield loss.

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