Fungicide activity of milk and whey powders towards *Erysiphe necator,* the causal agent of powdery mildew of grapevine

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1 Introduction

The fungus *Erisyphe necator* (syn. *Uncinula necator*) (Schw.) Burr. is the causal agent of powdery mildew, a major grapevine disease throughout the world. Control of powdery mildew is traditionally based on the management of secondary infections. According to a survey carried out by the European Commission, in 2007 growers in Europe used 70,000 tons of fungicides for grapevine protection, of which 53,000 tons were used against *E. necator* (European Commission, 2007). Despite this large use of fungicides, powdery mildew epidemics are frequently difficult to control due to the explosive nature of the infection cycles caused by its clonal reproduction.
The directive on the sustainable use of the plant protection products (EU Dir. 128/09) asks growers to implement disease control strategies to go beyond the use of synthetic chemicals. The means for bio-control are valuable technical tools that can effectively complement good practices such as the prevention and monitoring of plant diseases. Growers can use two categories of plant protection substances as alternatives to the 'conventional' plant protection products: the low-risk substances and the basic substances. The basic substances benefit from the following peculiarities: unlike a phytosanitary product, no approval by the manufacturing companies or licence for their purchase and use is required; they are easily available in any market or supermarket; they are not dangerous; some of these are allowed in organic farming. The basic substances include, among others, the dairy product whey. Whey has always been studied to be used as source of nutrients to be potentially use in a circular economy (Sharratt *et al.*, 1959; Ahmed Hashim, 2019). More recently, the activity of milk has been investigated on pumpkin and courgette with respect to *Sphaeroteca fuliginea*, demonstrating a certain activity in controlling the disease (Bettiol, 1999; Ferrandino *et al.*, 2006). Subsequently, the milk was tested for its effectiveness for the first time in Australia against the powdery mildew of the vine (Crisp & Bruer, 2001), tomato (Khairy *et al.*, 2018), and soybean (Perina *et al.*, 2013). Whey has also been found to be effective in controlling Tomato Yellow Leaf Curl Virus (TYLCV) in tomato (Abdelbacki *et al.*, 2010). Finally, a study with electron spin resonance and scanning electron microscopy speculated that free radical production and the action of lactoferrin are associated with the control of powdery mildew by milk (Crisp *et al*., 2006). In Italy, a preliminary assessment of the activity of the powdered milk against powdery mildew was carried out with promising results in 2017, a year of medium-high disease pressure (Cavazza *et al.*, 2018). The present preliminary investigation aimed to verify and confirm the potential efficacy of powdered milk and whey, its derivative, against *Uncinula necator*.

2 Materials and Methods

In 2018 and 2019, two efficacy field trials were carried out to evaluate the activity of milk and whey powders against *Uncinula necator* to control the powdery mildew disease on grapevine. Two vineyards, one of cultivar Pinot Gris and the other of cultivar Sangiovese, were selected in the hilly area of Tebano (Ravenna province, Emilia-Romagna region, Italy), a typical grapevine area usually affected by severe powdery mildew infections due to the favourable environmental conditions.
This study was realized in agreement with the most relevant EPPO guidelines (PP1/152(4): design and analysis of efficacy evaluation trials; PP1/181(5): conduct and reporting of efficacy evaluation trials, including good experimental practice; PP1/135(4): phytotoxicity assessment; and PP1/004(4): *Uncinula necator*) (EPPO, 2022). Both trials considered 4 repetitions per treatment and 5 plants per plot, with a complete randomized blocks experimental design. The treatments under study are reported in table 1 and included an untreated control unsprayed with fungicides for the whole season. The milk powder (Ferga Super Elevage, Ouest Élevage, Ploudaniel, France) and the whey powder (Quality Milk, Kalmi Italia) were both commercial calf feeds used for animal nutrition. The products under study were distributed with a backpack nebulizer sprayer (brand Stihl model SR420 with 1 nozzle of 2 mm in 2018 or model SR430 with 1 nozzle of 2.3 mm in 2019), simulating a water volume ranging between 500 L/ha and 1000 L/ha, according to the crop phenological stage. The spray interval between applications was of 6-8 days, according to the weather trend.

Formulated product (f.p.)	Active ingredient concentration	Dosage f.p. in g/L	Year	
			2018	2019
Untreated control	-	-	x	x
Tioflor	sulphur 80%	5		x
Tioflor	sulphur 80%	6	x	
Milk powder	n.a.	10	x	
Milk powder	n.a.	20	x	
Milk powder	n.a.	30	x	x
Whey powder	n.a.	30		x
Whey powder	n.a.	45		x

Table 1: Treatments under study during 2018-2019 to evaluate the milk powder and whey powder activity against *Uncinula necator* in grapevine crop.

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The efficacy assessments were carried out on 50 bunches per plot, observing the powdery mildew disease symptoms and visually estimating the affected area. Each bunch was scored using the following damage classes: 0 = no symptoms; 1 = 0.1÷5% of symptomatic surface; 2 = 5.1÷15% of symptomatic surface, 3 = 15.1÷40% of symptomatic surface; 4 = 40.1÷70% of symptomatic surface; 5 = 70.1÷100% of symptomatic surface. Disease incidence (percentage of symptomatic grape bunches out of the total observed) and disease severity (percentage of symptomatic grape bunch surface according to the mean value of each damage class) were calculated.
In the same date of the efficacy assessments, also the phytotoxicity assessments were carried out. Any symptom due to lack of crop selectivity was recorded both on leaves and bunches, observing 100 and 50 organs per plot, respectively. The same approach used to score the disease symptoms was adopted for the phytotoxicity symptoms.
The collected data were analysed with the Analysis of Variance (Anova) and subsequent S.N.K. test ($p \leq 0.05$) for the separation of the means, transforming the percentages data with square root or arcsine of the root square percentage if required to satisfy the Bartlett's test. The degree of action (percentage of efficacy) of the treatments under study was calculated on the untreated control using the Abbott's formula. All the results are reported as mean values (± SEM).

3 Results and discussion

In both years, the disease pressure recorded in the vineyard under study can be considered of medium level. During spring, periods with temperatures above the norm have led to an advance of crop vegetation, more marked in the 2019 vintage, and a consequent advance of susceptibility to primary infections. In both years, the weather conditions continued in a similar way, with a very rainy and cool period during May (which in 2019 had exceptional characteristics of precipitations above the norm) and which caused an obstacle to the normal development of the disease. From June onward, the establishment of a favourable weather trend with low rainfall and high humidity parameters has allowed the epidemic development of the disease.
The first disease symptoms on the grapevine leaves were observed on May 23rd in 2018 and on May 27th in 2019. In both years, the uneven disease occurrence on the leaves has not allowed any evaluation of the tested products performance on the leaves. The first disease symptoms on the grape bunches were observed on May 30th in 2018, while only on June 17th in 2019. The evolution of the disease on the grape bunches, very rapid in 2018, was slower in 2019.
The results obtained in the 2018 trial are reported in figure 1 and figure 2 for the disease incidence and disease severity, respectively. Similarly, in figure 3 and 4 the results obtained in 2019 are presented.
In 2018, ten experimental applications were carried out between April and June (4/24, 4/30, 5/7, 5/15, 5/23, 5/30, 6/7, 6/13, 6/20, 6/27). Two efficacy assessments were carried out between late June (6/22) and early July (7/9). In both the assessments, the milk powder was significantly different from the untreated control, providing a disease control similar to that provided by the sulphur-based plant protection product. The efficacy in reducing the disease severity (figure 2) was close to 95-99% regardless the tested concentration of the milk powder, while concerning the disease incidence (figure 1) a numerical dose-effect was observed among the three tested dosages (milk powder at 10 g/L, 20 g/L and 30 g/L, the latter with the highest performance). In the second and last assessment (7/9), when the crop was at BBCH 81 (beginning of berries ripening), the milk powder applied at 30 g/L provided a protection of the grape bunches numerically better than the sulphur reference standard.

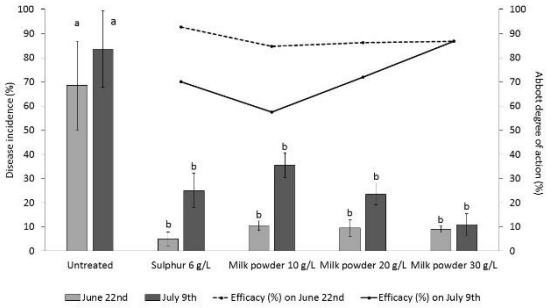

Figure 1: Disease incidence on grape bunches (histograms) and tested products efficacy (lines) recorded in 2018.

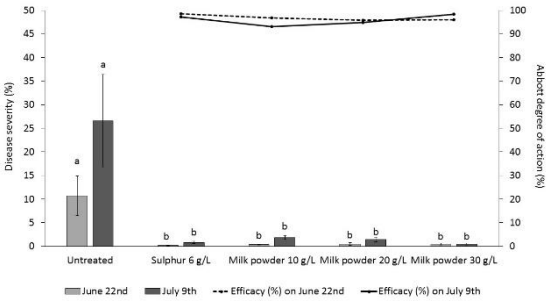

Figure 2: Disease severity on grape bunches (histograms) and tested products efficacy (lines) recorded in 2018.

In 2019, eleven experimental applications were carried out between April and July (4/19, 4/26, 5/6, 5/16, 5/22, 5/30, 6/6, 6/13, 6/21, 6/29, 7/8). Two efficacy assessments were carried out between late June (6/28) and mid-July (7/19). In this trial, in terms of percentage of affected grape bunches, the sulphur-based product provided a significantly better performance compared to the two dairy products, which however were significantly different from the untreated control. Regarding the percentage of symptomatic grape bunch surface, both the milk and whey powders provided a similar activity to the sulphur-based reference standard. The two dosages of whey powder (30 g/L and 45 g/L) provided a performance comparable to that of the milk powder applied at 30 g/L, with a numerical better control of the highest tested dosage.
Both 2018 and 2019 results confirm the good performance of these dairy powders observed in mid-June 2017, where eight applications of milk powder at 30 g/L provided a protection of the grape bunches comparable to that of a sulphur-based plant protection product. In that screening the milk powder showed an efficacy higher than 90% in reducing the disease incidence and an Abbott degree of action close to 99% in reducing the disease severity (Cavazza *et al.*, 2018). Nevertheless, it has to be noted that in the 2017 screening a

milk powder for human use (as food for new-borns) was considered, while in the present study the milk powder tested was chosen among the ones available for zootechnical uses.

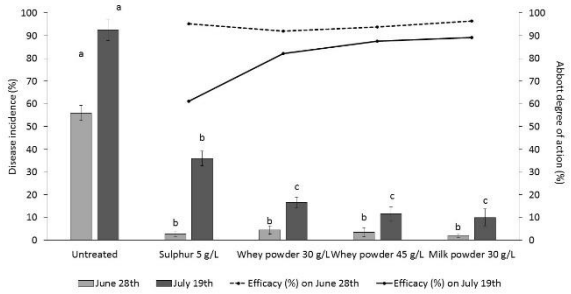

Figure 3: Disease incidence on grape bunches (histograms) and tested products efficacy (lines) recorded in 2019.

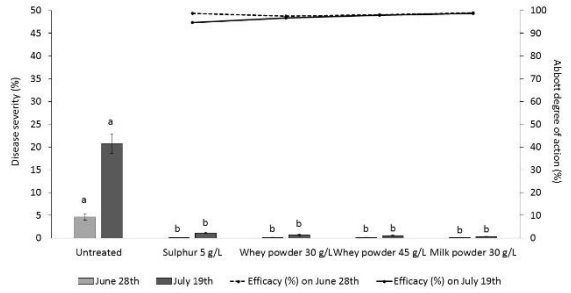

Figure 4: Disease severity on grape bunches (histograms) and tested products efficacy (lines) recorded in 2019.

Regarding the phytotoxicity symptoms, no lack of crop selectivity was observed in the grapevine plants repeatedly treated with neither the milk powder not with the whey powder.

4 Conclusions

During 2018-2019 seasons, in two vineyards characterized by a medium pathogen pressure, the sulphur-based plant protection product provided a good control of powdery mildew disease when applied straight with weekly sprays. The zootechnical milk and whey powders (dairy products used as calf feeds) tested in this study on grapevine crop provided significantly effective disease reduction (compared to the untreated control), with a good performance when tested at concentrations of 30 g/L and 45 g/L, respectively. The present study allowed to define a preliminary minimum effective dose of such basic substances that could be potentially be exploited in crop protection. Further studies are needed to better investigate and understand the role of milk and whey powders in the control of powdery mildew on grapevine. Similarly, new screenings are required to widen the target plant pathogens (*i.e.,* downy mildew disease caused by *Plasmopara viticola*) that could be affected by these substances and the crops potentially suitable for their usage without causing adverse side-effects. In fact, the European commission following the EFSA analysis, only recently authorized whey and cow milk as basic substances for the control of powdery mildew disease in grapevine. This authorization indicates as period as suitable for application the spring from 1st shoots occurrence to cluster tightening (BBCH 10-57), at 7 to 10 days interval (EFSA, 2020; European Commission, 2021), a phenological period more at risk for downy mildew and powdery mildew primary infections rather than for powdery mildew secondary infections.

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