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Infection Risk-Based Application of Plant Resistance Inducers for the Control of Downy and Powdery Mildews in Vineyards

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Abstract: Plant resistance inducers (PRIs) are potential alternatives for controlling grapevine downy (DM) and powdery (PM) mildews in vineyards. In a 3-year field study, we evaluated the field efficacy of six commercial PRIs of chemical and natural origin against DM and PM diseases when applied at designated vine growth stages in a mixture with low doses of copper and sulfur, and only when advised by weather-driven disease models. The disease severity and incidence were evaluated for each season at key growth stages (i.e., the end of flowering, berries pea-sized, veraison, and pre-harvest), and areas under the disease progress curves (AUDPC) were calculated and compared with those of nontreated vines. These risk-based applications resulted in a 41% and 61% reduction of interventions against DM and PM, respectively, compared to the official advice for integrated pest management in the growing area. These applications provided a disease control efficacy of 88% for DM and 93% for PM; the disease severity on bunches never exceeded 5%. Overall, when the disease severity was expressed as AUDPC, we observed higher efficacy of all the PRIs for PM, and of laminarin and cerevisane for DM. We also found that potassium phosphonate and fosetyl-Al (commonly used against DM) were effective against PM, and cos-oga (used against PM) was effective against DM. These results broaden the application and integration of PRIs in viticulture.

Keywords: cerevisane; cos-oga; fosetyl-Al; laminarin; potassium phosphonate; *Pythium oligandrum*

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1. Introduction

The Grapevine is affected by several diseases worldwide [1], including two biotrophic pathogens; the oomycete *Plasmopara viticola* which causes downy mildew (DM), and the ascomycete *Erysiphe necator* which causes powdery mildew (PM). Grapevine diseases can cause noteworthy yield and quality losses [2,3], which would require the intensive use of chemical fungicides to mitigate or prevent them [4]. Recently, pesticide application has become subject to many restrictions from regulatory authorities in an attempt to lower impacts on human health and the environment [4–6] and encourage the development of alternatives to achieve more sustainable plant protection [7].

Plant resistance inducers (PRIs) are eco-friendly alternatives for the control of DM and PM and are expected to play an important role in sustainable plant protection [8,9]. PRIs are molecules of various origins that prime a defense reaction in plants under attack by pathogens. This defense reaction takes two forms; pattern triggered immunity (PTI) and effector-triggered immunity (ETI).

PTI is based on molecular expression patterns activated upon the detection of microbial molecules or plant endogenous molecules including the pathogen itself, microbial and damage-associated molecular patterns, such as chitin or peptidoglycans from fungi or bacteria, oomycete β-glucans, or oligogalacturonides (OGs) and chitin monomers released from damaged plant cells [10,11]. On the other hand, ETI is a highly specific reaction resulting from direct and indirect interactions between effectors produced by the pathogen and the products of plant resistance genes during a strong and local defense response [12]. The recognition of pathogen patterns and the subsequent activation of the plant immunity leads to various reactions such as the accumulation of reactive oxygen species (ROS), ion fluxes, the occurrence of mitogen-activated protein kinase, protein phosphorylation cascades, callose deposition, and cell-wall reinforcement [13,14]. The resistance gene expression and the pathogenesis-related protein synthesis can also occur, as well as the synthesis of fungitoxic stilbenes, and a subsequent hypersensitivity reaction and programmed cell death (PCD) [14–16]. Phytohormones such as salicylic acid (SA), jasmonic acid, and ethylene (ET) regulate these pathways ensuring an effective basal plant resistance against bioagressors [15,17].

To date, some PRIs tested for the induction of resistance against *P. viticola* and *E. necator* are of natural origin; for example cerevisane, a compound from cell walls of *Saccharomyces cerevisiae* strain LAS 117 [18,19]; cos-oga, a chitosan oligosaccharide-oligogalacturonide complex found in fungal cell walls and crustacean exoskeletons, and a pectin-derived oga from plant cell walls [20,21]; laminarin (extracted from the brown algae *Laminaria digitata* [22,23]; or living propagula *Pythium oligandrum* (an oomycete originally isolated from soil [24]. Other PRIs are chemical compounds such as fosetyl-Aluminium and potassium phosphonates [25], as well as benzothiadiazole (BTH) and acibenzolar-S-methyl [26]. Recently, other alternatives have been investigated; volatile organic compounds such as essential oils [27], beneficial microorganisms such as the non-pathogenic fungi *Trichoderma harzianum* T39 and the bacterium *Pseudomonas fluorescens* PTA-CT2 [28], bioceramic silicon nitride (Si3N4) [29], grapevine plant extract [30], and RNA interference [31].

Most studies conducted on PRIs have been focused on the physiological, metabolic, transcriptomic, and proteomic reaction in the grapevine tissues following PRI application and the subsequent inoculation with *P. viticola* or *E. necator* [18,31–35]. However, few studies are available concerning the use of resistance inducers under vineyard conditions. Such studies focused on either DM or PM, have been based on the repeated use of PRIs on a regular or calendar basis, and usually included the application of chemical fungicides [20,36–40]. Overall, applied research on the integration of PRIs into practical disease management is still insufficient [8,9].

In previous studies, we evaluated the pre-infection efficiency of some commercial PRIs on *P. viticola* [41] and *E. necator* [42] under vineyard conditions. Plants were sprayed once with PRIs and the leaves were inoculated with the pathogen at 1 to 19 days after PRI treatment. These studies demonstrated that PRIs can be used for preventative applications against mildew, with the highest efficacy when applied for 1–6 days before infection, depending on the product. When used preventatively, PRIs not only reduced infection severity on the leaves but also affected the production of secondary inocula on DM lesions or PM colonies thus reducing the further spread of these polycyclic diseases. Correct positioning of PRIs should benefit from combining them with mathematical models able to predict the risk of infections [43,44].

In this study, we applied six commercial PRIs in the vineyard in combination with copper and sulfur for the preventative control of DM and PM over a 3-year term. Treatment timing was based on mathematical models so that products were applied prior to infection by *P. viticola* or *E. necator* as predicted from weather forecasts. Treatment efficacy was evaluated for both diseases on leaves and bunches.

2. Materials and Methods

2.1. Vineyard Characteristics, Experimental Design, and Treatments

Field experiments were carried out between 2020 and 2022 in an experimental vineyard located at the Res Uvae farm (Colli Piacentini area, Castell'Arquato, Italy; N 44°51′26.031″ E 9°51′21.779″), which was planted in 2006 with *Vitis vinifera* cv. Barbera; susceptible to both DM and PM [45]. Vines trained in a single guyot system were spaced with 2.4 m between rows and 1.3 m within the row, at a planting density of 3204 plants/ha. A standard weather station (iMetos, Pessl Instruments, Weiz, Austria) was installed in the vineyard to record hourly data on air temperature (T, °C), relative humidity (RH, %), rainfall (R, mm), leaf wetness (min), and wind speed (m/s). Weather forecasts were provided by a specialized provider (https://www.ilmeteo.it) and corrected for the geographic coordinates of the weather stations [46].

The trials were carried out using a randomized complete block design with 4 replicated plots, 5 plants per plot, and the plots separated by two border plants. The following treatments were compared: (i) control based on copper and sulfur only, both applied at the minimal dose indicated by the product label (designated as CHEM); (ii) CHEM plus each of the following resistance inducers: cerevisane (CER), cos-oga (COS), fosetyl-al (FOS), laminarin (LAM), potassium phosphonate (PHO), and *Pythium oligandrum* (PYT); and (iii) a nontreated control (NT, i.e., sprayed with water). The commercial products and the doses used are shown in Table 1).

Table 1. Characteristics of the tested plant protection products.

Treatment	Active Ingredient	Trade Name and Producer	Concentration	Dose
CHEM	Tribasic copper sulfate	Cupravit Bioadvanced, BAYER	30%	1.4–1.8 [1]
	Copper oxychloride	Verdram HI Bio, Belchim Crop Protection	30%	2–2.4 [1]
	Copper sulfate	Poltiglia Disperss, UPL	20%	2–6 [1]
		Microbagnabile, Green Ravenna s.r.l.	80%	100–200 [2]
	Sulfur	Tiogold disperss, UPL	80%	200–400 [2]
		Thiopron, UPL	57.3%	2–4 [3]
CER	Cerevisane	ROMEO, SUMITOMO Chemical	94.1%	0.25 [1]
COS	Cos-oga	IBISCO, GOWAN	12.5 g/L	2.5 [3]
FOS	Fosetyl-Al	ALIETTE, BAYER	80%	2.5 [1]
LAM	Laminarin	VACCIPLANT, UPL	45 g/L	1.5 [3]
PHO	Potassium phosphonate	CENTURY, BASF	755 g/L	2 [3]
PYT	*Pythium oligandrum*	POLYVERSUM, GOWAN	1×10^6 CFU/g	0.3 [1]

[1] in Kg/ha; [2] in g/hL; [3] in L/ha.

The CHEM treatment was applied preventatively based on infection predictions using the mathematical models of Caffi, et al. [47] for primary DM infections, Brischetto, et al. [43] for secondary DM infections, and Caffi, et al. [48] for primary PM infections. Treatments were carried out according to the fungicide model of Caffi et al. [49] and only when the efficacy of the previous fungicide treatment was <70%. All these models were implemented in the decision support system designated as “vite.net” [50]. CHEM treatments were applied with low doses of copper and sulfur included in the mixture; various copper- and sulfur-based products were used (Table 1), depending on the conditions at the time of spraying (e.g., weather conditions, plant growth stage) as indicated by good plant protection practices [51]. These treatments were mainly triggered by the prediction of primary DM or PM infections. Treatments for the control of secondary infections were avoided with the exception of a single application in 2020 and another in 2022, which were indicated by the model for secondary DM infections. No chemicals were applied after veraison because the bunches had acquired ontogenic resistance against both *P. viticola* and *E. necator* [52,53].

Resistance inducers were added to the CHEM mixture based on criteria derived from technical guidelines for the commercial products and advice from producers, as follows: CER, COS, and LAM can be used all season long for a maximum of 10, 8, and 20 applications, with intervals of 7–10 days between treatments; PYT can be applied from late flowering (80% of flower hoods fallen) to pre-harvesting, for a total of 3–4 applications; FOS can be applied from pre-flowering to pre-closure of the bunch with an interval of 7–12 days with a maximum of 4 applications per season; PHO can be applied from bud burst to pre-bunch closure at an interval of 12–14 days with a maximum of 5 applications per season. Therefore, based on the previous criteria, if a fungicide intervention was applied at a particular plant growth stage during which the product must be applied, then a 3-product mixture was sprayed (i.e., copper + sulfur + resistance inducer); otherwise, a 2-product mixture was used (i.e., copper + sulfur). As suggested by the producer, all treatments with PYT were delayed by 3 days with respect to copper and sulfur to avoid any possible negative interaction between the chemicals and the living propagules of *P. oligandrum*. In 2022, since the models did not provide any alarms after 22 June, additional applications of PYT (2 applications) and LAM (1 application) were done between GS 77 (i.e., a majority of berries touching) and post-veraison, as recommended by producers.

All applications were accomplished with a battery-powered backpack sprayer (Volpi S.p.a., Casalromano, Italy) using a pressure of 4.5 bar, and the spray volumes varied between 400–600 L/ha depending on the growth stage of the vineyard.

2.2. Disease Assessments

Disease incidence (% affected leaves or bunches) and severity (% affected area) were assessed during each season on leaves and bunches at the end of flowering (growth stage GS 69 of Lorenz, et al. [54]), when the berries were pea-sized (GS 75), at veraison (GS 83, berries developing color), and at pre-harvest [54], on a random sample of 50 leaves and bunches per plot. Different leaves and bunches were used for each assessment date. Disease severity was assessed by using EPPO [55–57] scales. The central value of the EPPO scale was expressed as a proportion (i.e., 0–1, in which 1 is 100% severity) for the calculation of the Area Under the Disease Progress Curve (AUDPC) (i.e., 0.025, 0.075, 0.175, 0.325, and 0.875 proportion of affected leaf or bunch area).

2.3. Data Analysis

AUDPC data were subjected to an analysis of variance (ANOVA) for a randomized complete block design repeated over years, with the 3 years considered as a random factor, by using IBM SPSS Statistic, version 27.0 (IBM Corp, Armonk, NY, USA). Treatments were compared by using the Fisher-protected Least Square Difference Test (LSD) at $p = 0.05$.

The AOV was repeated for disease incidence and severity data, and overall data. Overall data means that AUDPC for leaves and bunches was summed to estimate the overall effect of the treatments on the plants.

3. Results

3.1. Weather Conditions, Infection Risk, Treatments, and Disease Development

In 2020 (Figure 1A), the period between bud break (GS 09) and when the berries began to touch (GS 79) was rainy, with 65 rainy days and a total of 137 mm of rain; the average temperature was 16.8 °C (min 9.4, max 21.5). Based on weather forecasts, models for primary mildew infection predicted 16 possible infection periods (between 10 May and early July) for DM, and 15 possible infection periods (between 20 April and mid-June) for PM; one of these predictions (on 28 April) was not confirmed by the actual weather data (Supplementary Material Figure S1). According to these predictions, 7 applications of CHEM were done between 17 April and 16 July (Figure 1A). An additional application on 1 July was done to prevent a possible infection caused by a 3-day long rainy period comprising 15.3 mm of rain, 24 h wetness duration (WD) at a temperature of 22.4 °C. An application at veraison was done to prevent an infection that was not confirmed based on the actual weather data. In the nontreated group (NT) (Figure 2), symptoms of both mildew diseases were mild (<2%) at GS 69 and GS 75, except for PM on bunches that showed a 28.0% incidence and 9.6% severity (Figure 2D). At GS 81, mildew incidence and severity increased on both leaves and bunches; e.g., 5% and 35% of bunches were affected by DM and PM with 1.2% and 10.7% severity, respectively (Figure 2B,D), with no further increase at GS 89.

In 2021 (Figure 1B), grapevine growth was delayed compared to 2020. Only 22 rainy days occurred between bud break (GS 09) and veraison (GS 81) and comprised 132.8 mm of rain, most of which fell during 5 rainy periods; the temperature was lower than in 2020, averaging 20.2 °C, min 6.2 °C, max 28.4 °C. Model predictions indicated 6 possible infection periods for both DM and PM between late April and the second decade of July

(Figure S2), which resulted in 5 CHEM treatments between 23 April and 17 July (Figure 1B). There was another rainy period in late July for which the model indicated alarms for secondary DM infections (Figure S2, but because the plants were at veraison, no additional fungicides were applied). Mildew was observed sporadically at GS 69 on NT, but the disease progressively increased over the season, e.g., at GS 89, 47.0% of leaves (with 12.6% severity) and 8.0% of bunches (with 5.6% severity) were affected by DM, and 18.1% of leaves (with 10.0% severity) and 15.2% of bunches (with 6.3% severity) were affected by PM (Figure 2).

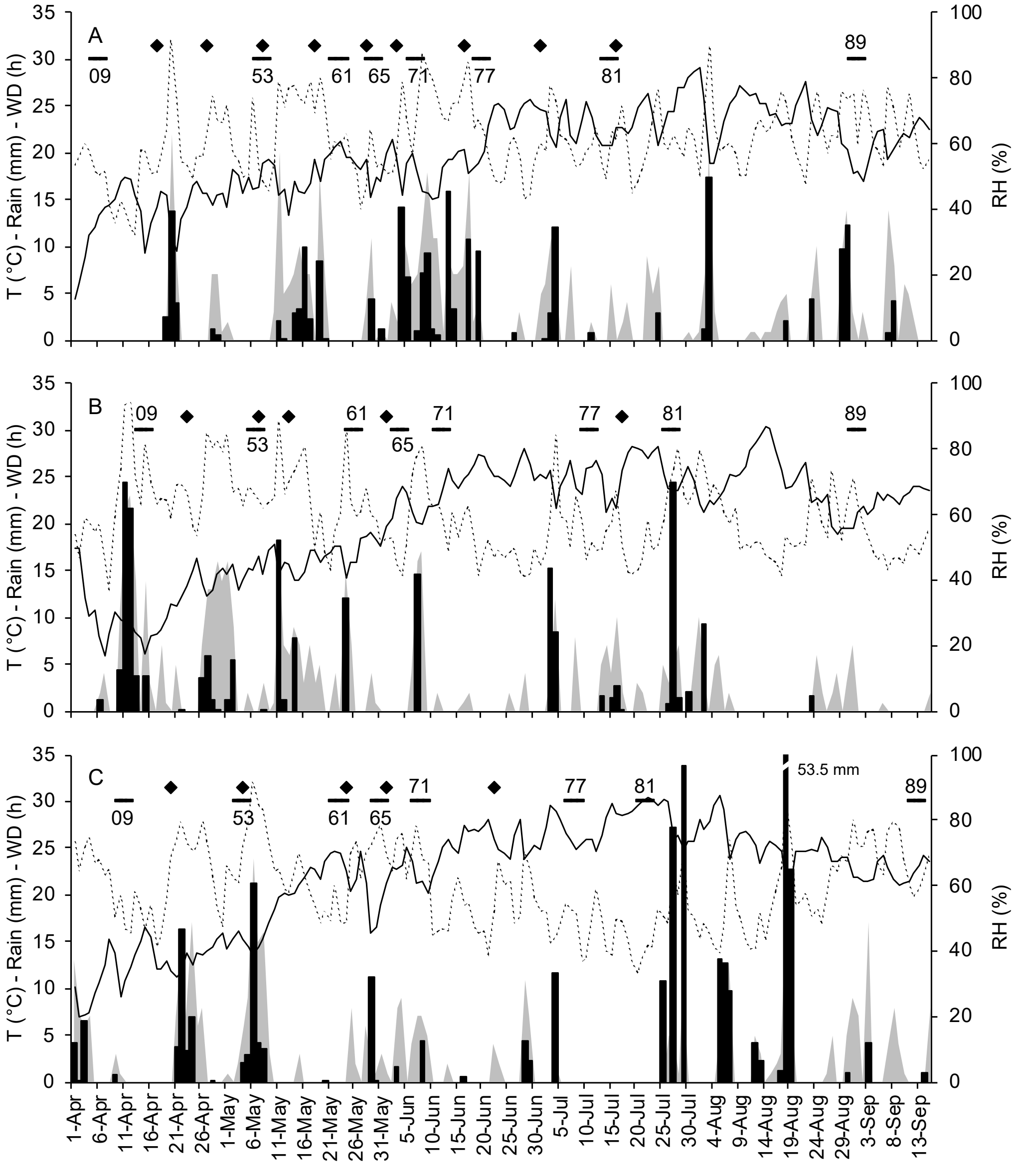

Figure 1. Daily data for temperature (T, °C, full line), relative humidity (RH, %, dotted line), rain (mm, bars), and wetness duration (WD, h, gray area) during the grapevine-growing season in 2020 (**A**), 2021 (**B**), and 2022 (**C**). Segments show the main grape growth stages: bud break (09), inflorescences clearly visible (53), the beginning of flowering (61), full flowering (65), fruit set (71), berries beginning to touch (77), veraison (81), and berries ripe for harvest (89) [54]. Black diamonds show the fungicide intervention with a mixture of copper and sulfur.

The weather was drier in 2022 (Figure 1C) than in 2021, with 15 rainy days between bud break (GS 09) and fruit set (GS 71), comprising 82.5 mm of rain with an average temperature of 17.7 °C (min 9.1 °C, max 25.1 °C). Eight primary infection periods for DM and 10 for PM were predicted between April 20 and the first decade of June and one additional possible infection period was predicted for secondary DM infections on 22 June (Figure S3). Consequently, 5 applications of CHEM were done between 20 April and 22 June. Fungicide interventions were not done for a primary DM infection predicted on 28 July and for several secondary DM infections over the following days caused by repeated and abundant rain between 25 July and 19 August (191.5 mm of rain in total) in the presence of a null risk of PM (Figure S3), because the grapevines were at a stage in which bunches are ontogenetically resistant to *P. viticola* (Figure 1C). PM caused very mild infections, with <1% incidence on both leaves and bunches. DM was also mild until GS 81 (<4% incidence) but increased to a 67.4% incidence on the leaves (with 18.9% severity) due to secondary infections on lateral shoots that were predicted by the models between late July and August, and 18.9% of bunches (with 4.2% severity) due to the onset of "larvata" symptoms caused by the infections that occurred during the first decade of June (Figure 2).

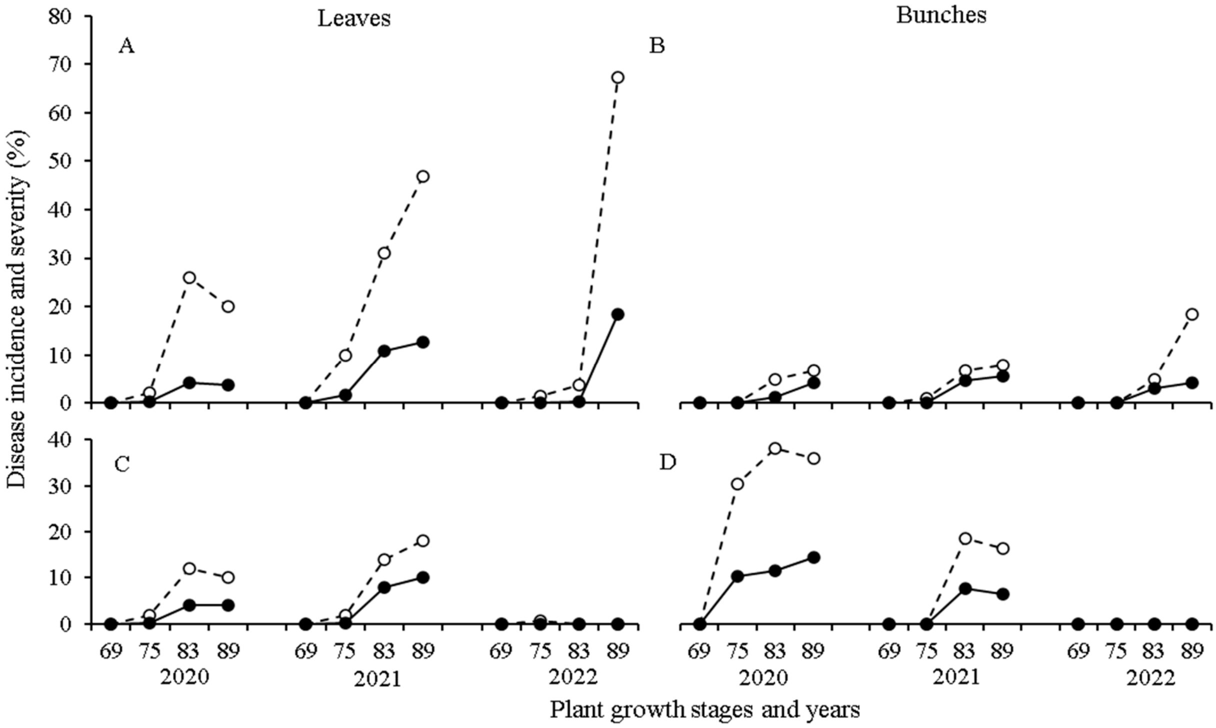

Figure 2. Incidence (as % of affected leaves or bunches, white dots) and severity (as % of affected leaf or bunch area, black dots) of downy (**A**,**B**) and powdery (**C**,**D**) mildews on leaves (**A**,**C**) and bunches (**B**,**D**) of nontreated plots, at different growth stages of vines in 2020, 2021, and 2022: end of flowering (69), berries pea-sized (75), berries developing colors (83) and berries ripe for harvest (89) [54]. Data are averages of 4 replicate plots per year.

3.2. Treatments with PRIs

Based on the previously described application guidelines, 2 applications per season of LAM in a mixture with copper and sulfur, 3–4 of PYT and FOS depending on the season, 4 of PHO, and 4–9 of CER and COS were done during the various years, as shown in Table 2.

Disease incidence and severity data resulting from these applications are shown at pre-harvest for brevity; disease progress during the season was summarized by the AUDPC and is discussed below. All treatments reduced disease in comparison to the nontreated group NT (Figure 3). It is noteworthy that the cumulative mildew severity on bunches treated with CHEM or CHEM + PRIs was less than 5% of the affected bunch area, while the cumulative severity was >10% for NT (Figure 3D).

Table 2. Dates of the grapevine foliar application in the 3 years.

Treatment	Date of Application		
	Year 2020	**Year 2021**	**Year 2022**
Copper + sulfur (CHEM)	17 and 27 April; 8, 18 and 28 May; 3 and 9 June; 1 and 16 July	23 April; 7 and 13 May; 1 June; 17 July	20 April; 4 and 24 May; 1 and 22 June; 12, 20 and 26 July
Cerevisane (CER)	17 and 27 April; 8, 18 and 28 May; 3 and 9 June; 1 and 16 July	23 April; 7 and 13 May; 1 June	4 and 24 May; 1 and 22 June
Cos-oga (COS)	17 and 27 April; 8, 18 and 28 May; 3 and 9 June; 1 and 16 July	23 April; 7 and 13 May; 1 June; 17 July	20 April; 4 and 24 May; 1 June
Fosetyl-Al (FOS)	8 and 28 May; 3 and 9 June	13 May; 1 June; 17 July	4 May; 1 and 22 June
Laminarin (LAM)	1 and 16 July	1 June; 17 July	22 June; 20 July
Potassium phosphonate (PHO)	17 and 27 April; 8 and 18 May	23 May; 7 and 13 May; 1 June	4 and 24 May; 1 and 22 June
Pythium oligandrum (PYT)	3 and 9 June; 16 July	23 April; 7 and 13 May; 1 June; 17 July	22 June; 12 and 26 July

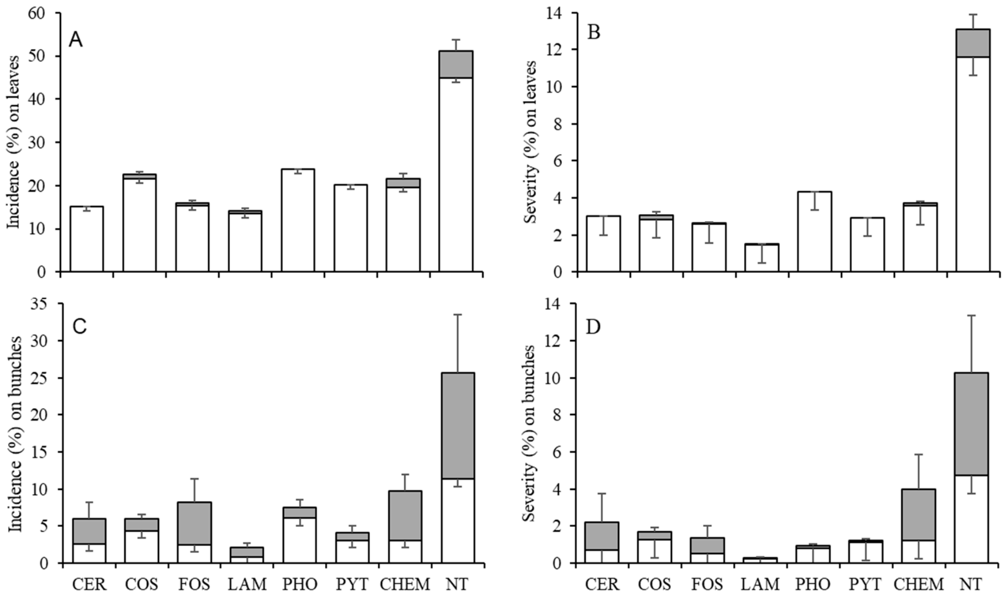

Figure 3. Incidence (expressed as % of affected leaves, panel (**A**), or bunches, (**C**) and severity (expressed as % of affected leaf, (**B**), or bunch area, (**D**) for downy (white bars) and powdery (gray bars) mildew in nontreated plots (NT), plots treated with copper and sulfur alone (CHEM) or in combination with plant resistance inducers (CER = cerevisane; COS = cos-oga; FOS = fosetyl-Al; LAM = laminarin; PHO = potassium phosphonate; PYT = *Pythium oligandrum*) at pre-harvest. The values are expressed as 3-year averages (i.e., 2020–2022) and 4 replicate plots per year. The whiskers show the standard error.

All treatments significantly affected the AUDPC of DM on the leaves and bunches (Table 3). The effect of year was also significant in most cases, while the interaction "treatments × years" was not significant, indicating that the effect of treatments was consistent over the 3 years characterized by different disease epidemics.

For DM, all treatments with CHEM or CHEM + PRIs reduced the AUDPC in comparison to NT, by 53.5–70.4% for disease incidence and by 60.5–88.3% for disease severity, depending on the treatment (Figure 4A,B). The use of PRIs did not significantly affect the AUDPC for DM incidence on both leaves and bunches because no differences were found between CHEM and CHEM plus any of the PRIs (Figure 4A). Differently from the DM incidence, the AUDPC for disease severity was lower for LAM and CER, showing an 88.3% and 83.6% reduction with respect to NT, respectively, than for CHEM with a 60.5% reduction.

Table 3. ANOVA results for the AUDPC calculated for incidence and severity data and for downy and powdery mildews for nontreated plots, plots treated with copper and sulfur alone, or in combination with plant resistance inducers (as indicated in Table 1). Treatments were applied for a 3-year period (2020 to 2022), which was considered as a random variable.

Source of Variation	df	Downy Mildew				Powdery Mildew			
		Incidence		Severity		Incidence		Severity	
		p [1]	% [2]	*p*	%	*p*	%	*p*	%
		Leaves							
Treatment (1)	7	<0.001	54.6	<0.001	65.4	0.019	44.5	0.033	52.5
Year (2)	2	<0.001	35.1	0.004	18.9	0.003	31.0	0.09	13.8
Interaction (1 × 2)	14	0.907	10.3	0.790	15.7	0.241	24.5	0.613	33.7
		Bunches							
Treatment (1)	7	0.010	62.1	0.021	53.5	0.025	31.4	0.019	43.7
Year (2)	2	0.145	9.1	0.049	16.3	0.001	51.1	0.031	26.0
Interaction (1 × 2)	14	0.814	28.8	0.902	30.2	0.266	17.5	0.247	30.3
		Overall [3]							
Treatment (1)	7	<0.001	59.8	0.001	63.7	0.042	36.3	0.031	49.1
Year (2)	2	<0.001	30.8	0.010	17.6	0.001	43.3	0.029	20.2
Interaction (1 × 2)	14	0.865	9.4	0.723	18.7	0.262	20.4	0.431	30.7

[1] *p*-value; [2] % of variance; [3] AUDPC data for leaves and bunches were summed up.

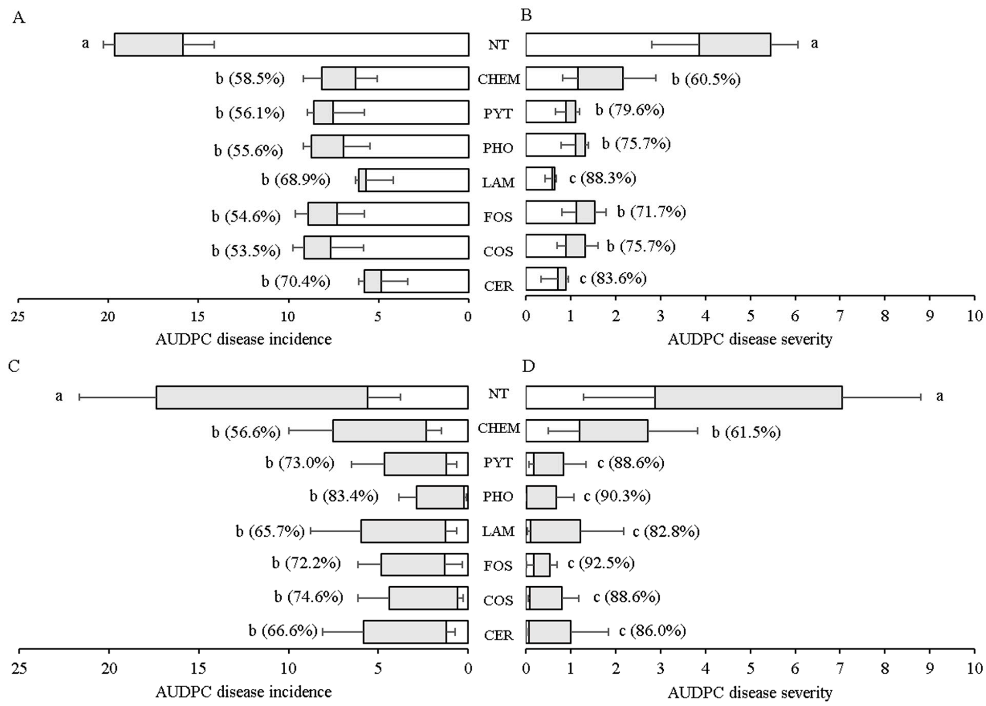

Figure 4. AUDPC data calculated for disease incidence (as % of affected leaves, white bars, or bunches, gray bars) and severity (as % of affected leaf, white bars, or bunch area, gray bars) for downy (**A**,**B**) and powdery (**C**,**D**) mildew in nontreated plots (NT), plots treated with copper and sulfur alone (CHEM) or in combination with plant resistance inducers (CER = cerevisane; COS = cos-oga; FOS = fosetyl-Al; LAM = laminarin; PHO = potassium phosphonate; PYT = *Pythium oligandrum*) at pre-harvest. Data are averages of 3 years (2020–2022) and 4 replicate plots per year; whiskers are standard errors; AUDPC data were calculated by using the disease assessments at different plant growth stages: end of flowering, berries pea-sized, veraison, and pre-harvest. Letters show significant differences at the LSD test for overall data (AUDPC for leaves and bunches were summed up), with $p = 0.05$; % between brackets show reduction with respect to NT.

For PM, treatments significantly reduced the AUDPC of disease incidence for leaves and bunches (Table 3) by 56.6% (CHEM) to 83.8% (PHO), and by 61.5% (CHEM) to 90.3% (PHO) and 92.5% (FOS) for disease severity with respect to NT (Figure 4C,D). None of the PRIs significantly lowered the AUDPC for PM incidence in comparison to CHEM (Figure 4C). The AUDPC for disease severity was significantly reduced by all the PRIs in comparison to CHEM (with a 61.5% reduction with respect to NT), by 82.8% (LAM) to 92.5% (FOS) (Figure 4D).

4. Discussion

In this study, we examined six commercial PRIs authorized for use in vineyards against DM (i.e., PHO and FOS), PM (i.e., COS and PYT), or both (i.e., CER and LAM). These products were used in combination with copper and sulfur at low doses. Most studies on PRIs have been conducted under controlled environmental conditions using artificial inoculation in monocycle experiments [22,23,26,32,34,58], while limited information is available about the effectiveness of PRIs in the field with a natural inoculum [20,36,39,40,59–62], so concern still exists on how PRIs can be integrated in practical disease control in vineyards [8,17,63].

Previous field studies were conducted by using PRIs on a calendar basis, with repeated applications throughout the grape-growing season. For instance, to control PM, LAM was used 7–14 times per season at 7–9-day intervals, from inflorescences clearly visible to bunch closure or veraison stages, alone or in combination with sulfur [23,60]. To control DM, LAM was used 10 or 11 times per season from mid-May to the end of July alone or in a mixture with copper or other biological products [38]. In these experiments, the control of DM (when studying PM) or PM (when studying DM) was achieved with specific fungicides, so it was impossible to study the effect of LAM on both pathogens simultaneously. However, farmers prefer to control both diseases with a single intervention.

Conversely, in our experiments, we targeted both mildew diseases by using a mixture of copper and sulfur at low doses, and by adding PRIs to this mixture when indicated by the guidelines provided by the producers. We decided to mix PRIs with fungicides because that is the method recommended on the product label. We used copper and sulfur at low doses to obtain partial disease control so we could better estimate the contribution of the addition of the PRIs; indeed, full disease control in CHEM would have masked the contribution of the PRIs. Besides, both consumer concerns and legislation require farmers to restrict the use of both copper and sulfur in agriculture to reduce negative environmental impacts [5,64,65].

We also shifted from a calendar to a risk-based application by using mathematical models to predict potential infection by *P. viticola* and *E. necator*, as well as the residual efficacy of a previous fungicide treatment. By using weather forecasts, model predictions anticipated the possible risk so that treatments were applied preventatively, 1–3 days before an infection prognosis, as we suggested in previous studies [41,42]. In those studies, we evaluated disease and sporulation severity following the artificial inoculation of leaves collected from PRI-treated vineyard plants with *P. viticola* sporangia [41] or *E. necator* conidia [42] at 1–19 or 1–12 days after treatment (DAT), respectively. The results clearly showed that PRIs can be used preventatively, with the highest efficacy at 3–6 DAT, depending on the product. The effect of PRIs on the sporulation of both pathogens also suggested a broader and longer effect on mildew epidemics, which we investigated in the present study.

The risk-based application of fungicides resulted in 9 applications in 2020, the rainiest year, and 5 in 2021 and 2022, which was a considerable reduction in comparison to common practice. In 2020, the official advice for IPM recommended 15 applications against DM (from the second decade of April to the first decade of August), and 18 against PM (from mid-April to early August), with two additional applications in vineyards severely affected by the disease [66]. In 2021, 8 applications were recommended against DM (between early May and the last decade of July), with three additional precautionary interventions; 17 applications were recommended for PM from the last decade of April to mid-August, the last two only in infected vineyards [67]. In 2022, 9 interventions were recommended for

DM, and 13 for PM (between early May and the end of July) [68]. Therefore, we estimate that our risk-based fungicide schedule halved the number of interventions with respect to common practice. Even if the number of interventions was lower, the efficacy of control reached a maximum of 88% for DM, and 93% for PM, and disease severity on bunches never exceeded 5%, which is considered the threshold for insignificant damage for winemaking [69]. These results confirm that the use of model-based decision support systems can reduce fungicide treatments by at least 50% without compromising disease control [70].

Overall, we observed a significant contribution of adding any of the PRIs for PM control and of adding LAM and CER for DM control. These results were obtained when the epidemics were expressed as the AUDPC calculated for disease severity, but not for disease incidence. This can be explained by considering that both mildews are dual (i.e., they develop on two plant organs during a cropping season), polycyclic diseases (i.e., that complete more infection cycles in a season), and that incidence is not an appropriate estimator of disease for such diseases [71]. The relationship between incidence and severity is not linear; disease severity increases exponentially when the incidence is approximately >50% [72], and nonlinearity exists for foliage–bunch disease relationships [73]. In consequence, results for disease severity may be more robust than those for disease incidence.

LAM is a water-soluble polysaccharide that consists of β-(1-3)-glucan derived from the brown alga *Laminaria digitata* [74], which has been previously shown to control both DM and PM [22,23,37], and to efficiently reduce infection by *P. viticola* and the production of sporangia on DM lesions, with prompt and long-lasting activity [41]. CER is a yeast-derived PRI made of purified cell walls of *Saccharomyces cerevisiae* strain LAS117 that has been found to effectively reduce DM [18,19,75] by mobilizing plant defense reactions that are more effective against *P. viticola* than *E. necator* [76]. Even though LAM and CER showed similar efficacy in controlling DM severity, it is noteworthy that LAM was used two times per season from flowering onwards, while CER was used in almost all applications (i.e., 9 in 2020, and 4 in 2021 and 2022).

Our results provide new insights into the spectrum of activity of some of the tested products. PHO (potassium phosphonate) and FOS (fosetyl-Al) have been studied for their capability to trigger a plant resistance response against *P. viticola* [26,36] and for PHO, to inhibit the pathogen directly [25,77,78]. In fact, the labels of the commercial products we used include DM only as the target disease. In this study, PHO and FOS effectively reduced PM incidence on leaves and bunches and showed similar disease severity control as the other PRIs known to protect plants against PM [20,40,42,58,61]. Rantsiou, et al. [60] and Moine, et al. [62] also documented the efficacy of PHO against *E. necator* in a vineyard study. Similar results were obtained with FOS but were limited to bunches. PHO is a salt of phosphonic acid and FOS is a salt of ethyl phosphonic acid, which is transformed into phosphonates in the plant; PHO and FOS then share the same active ingredient [25]. Phosphonates are distributed within the plant via the phloem and xylem, have a complex action (Guest and Grant, 1991), and induce multiple defense reactions in plants including cell-wall reinforcement, ethylene biosynthesis, and phytoalexin accumulation [78], which may provide a similar response following infection with both biotrophic pathogens *P. viticola* and *E. necator* [26,77].

Interestingly, PHO was used only four times per season, mostly between initial shoot growth and the end of flowering, the period when shoots grow very rapidly, and the distinct systemic effects of the product protect the new vegetation [41]. FOS was used 3–4 times depending on the season, between inflorescences clearly visible and when berries began to touch. The period between bud break and fruit set is also that in which primary (ascosporic) infections by *E. necator* occur [48]; controlling them enables effective control of PM epidemics during the season [44]. We can conclude that the use of PHO or FOS in the early season to control DM also provides optimal control of PM (particularly on bunches) when used in a mixture with copper and sulfur at low doses. However, this finding warrants further investigation.

In a similar manner to PHO and FOS (whose activity against PM has not been previously widely documented), COS control of PM was also similar to other PM-specific PRIs. COS is composed of chitosan oligosaccharides–oligogalacturonides complex and was found to induce resistance against biotrophic pathogens in different crop plants [79] through the activation of the SA pathway [17,34]. Its effectiveness in the control of PM has been previously studied [20,21]. Our results confirm the findings of Calderone, et al. [20] concerning the effectiveness of COS against PM in the vineyard, and those of van Aubel, et al. [21] and Taibi, et al. [42] who observed effects on PM. However, as suggested by the producer, we used COS intensively in our experiments. While knowledge of the ability of PYT to control both mildew diseases is still scarce [24,80–82], its potential against both DM and PM have been demonstrated in previous field studies with artificial inoculations [41,42] and the current work.

5. Conclusions

Our experiment was designed to meet the following farmers' needs: (i) prevent rather than stop infections; (ii) avoid unjustified applications and possibly reduce the usage of copper- and sulfur-based fungicides; and (iii) treat for both mildews simultaneously. Our results may thus contribute to a better integration of PRIs into practical plant disease management [9]. With respect to these aims, we used PRIs for preventative DM and PM control by anticipating the risk of infection predicted by mathematical models, shifting from the current calendar-based, repeated applications to risk-based scheduling. In consequence, we avoided unjustified treatments in most cases, with only two unjustified alarms resulting in treatments that likely might have been avoided. We used PRIs in a mixture of copper and sulfur at low doses, thus reducing their usage while maintaining high levels of disease control. It is worth noting that all the commercial PRIs we used increased the level of PM control, even though *E. necator* is not indicated as the target pathogen on all the product labels. We applied the PRIs precisely following manufacturers' recommendations; specifically, we added any PRI to copper and sulfur for all disease alarms occurring at those plant growth stages in which the PRI was recommended. Therefore, some PRIs were used more frequently than others. Further studies are necessary to better indicate the exact plant growth stages during which the PRIs would be more effective and the trade-off between the metabolic efforts for growth and/or immunity leverage [83]. This should improve their use and likely reduce the cost for farmers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13122959/s1, Figures S1a–S3a: The output of the DSS vite.net® in 2020, 2021, and 2022, respectively, at Res Uvae. The main events characterizing primary (A) and secondary (B) infection cycles of *Plasmopara viticola* are represented by dots; different colors (yellow, orange, and red) represent increasing risk. The blue bands represent the periods in which the plants are protected by fungicide treatments applied to the vineyard; Figures S1b–S3b: The output of the DSS vite.net® in 2020, 2021, and 2022, respectively, at Res Uvae. The main events characterizing ascosporic infections (A) and dynamics of disease pressure on clusters (B) and leaves (C) by *Erysiphe necator*; different colors (green, yellow, orange, and red) represent increasing risk. The blue bands represent the periods in which the plants are protected by fungicide treatments applied to the vineyard. The dark green dots on the top of panels B and C show the periods of likely onset of powdery mildew symptoms.

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