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A Study on the Efficiency of Sustainable Wine Grape Vineyard Management Strategies

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Abstract: Crop protection strategies based on cupric products and mainly adopted in organic viticulture produce a consistent environmental impact due to the persistence of copper in soils and its negative effects on edaphic biodiversity. In this work, trials were carried out during the crop years 2018–2020 in a vineyard with an organic management by a low-copper strategy and in a conventional IPM management with an IPM strategy with reduced use of fungicides. Phytosanitary treatments have been strictly planned according to forecasting models, and fungicides have been partially substituted with substances improving the resistance mechanisms of plants. Different strategies of green manure management, in order to improve the health of vines, were also adopted. Results suggest the efficacy of the “GreenGrapes” plant protection strategy in conditions of low downy mildew pressure. Furthermore, no declines in the production quality have been recorded; conversely, the synergic effect of the green manure and the tested biostimulant substances (“GreenGrapes” protocols) and the green manure management improved yield and grape quality, compared with conventional conduction (IPM and Organic) with a grass covering.

Keywords: grapevine; Sangiovese; sustainability; plant protection

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1. Introduction

Viticulture represents one of the more widespread and economically strategic agricultural sectors in the Mediterranean area [1]; however, cultivation methods cause a high environmental impact, mainly due to the large use of phytosanitary products required to prevent cryptogamic infections [2] often responsible for production losses and consequent economic damages for producers [3].

According to 2016 ISTAT (Istituto Nazionale di Statistica) data, 26% of the total amount of fungicides distributed every year in the Italian agriculture is dispensed in the viticultural sector [4]. Therefore, in order to meet the increasing attention for the sustainability of agriculture, the implementation of innovative strategies to control vine fungal diseases is urgently necessary.

To date, cryptogamic diseases are mainly controlled with the use of copper and sulphur products [5], which are very effective to prevent downy mildew and powdery mildew, respectively, and represent up to the 70% of the total fungicide amount distributed in viticulture [4]. However, over the years, the large use of copper led to its over-accumulation in many vineyards soil, especially in soils with a high pH, due to its high persistence in the topsoil layer [6–9]. Besides its importance as a micronutrient, an excessive concentration of copper, especially in bioavailable forms, can even exert

phytotoxic effects on vines [10–13] and negatively influence the ecological balance of the vineyard [14]. Indeed, copper has been related to reduced vineyard fertility because of its impairing effects on nutrient cycles and biodiversity [15–20]. The use of copper in agriculture is strictly limited by current legislation (Regulation (EU) 2018/1981 of 13 December 2018). However, cupric fungicides represent the category of plant protection products most used in organic viticulture to combat downy mildew. [16,21–24].

On the other hand, Integrated Pest Management (IPM) viticulture, mandatory in the EU from 2014 after the adoption of the Sustainable Use of Pesticides Directive, is based on an innovative concept of agriculture, aimed to enhance the resilience capability of the vineyard, considered as an agroecosystem, and reduce the environmental impact of the crops. According to IPM principles, infections can be faced through multiple approaches [25] finalized to cut the use of synthetic fungicides (which use is allowed). Indeed, these products have been associated to negative effects on not-target organisms [26–29] and the development of fungicide resistance [5,30,31], as well as affecting the final products [32,33].

Therefore, in order to comply with the recent directive and improve viticulture sustainability, it is necessary to implement new approaches to protect vineyards from fungal diseases and examine their actual effectiveness. In this regard, some investigations reported evidence for the efficacy of natural substances, consisting of living microorganisms (biofungicides), bacterial derivatives, or botanicals, in contrasting pathogen attacks on grapevines [21,23,34–36]. However, their use is still limited, and commercial formulations have been scarcely developed. Biofungicides are known to exhibit different modes of action [37] such as pathogen parasitism, the production of toxic compounds, and competition for nutrients or space. Furthermore, some of these natural substances have been found to act as elicitors, reproducing the host–pathogen interactions and consequently stimulating the endogenous defence mechanisms of plants [25,36,38–43]. In particular, some evidence for their efficiency to control downy mildew infections has been obtained [44–47]. Other active molecules, known as biostimulants, can also contribute to the vine ability to resist to pathogens by enhancing their health status. Indeed, such substances can stimulate the physiological processes involved in the absorption and assimilation of nutrients, with an overall invigorating effect [48–51]. The mechanisms of action described above determined the choice of defense support products used in the protection strategies of the “GreenGrapes” protocols (here after GG) (Table 1). These protocols were set up in the trials carried out in the LIFE EU Life Green Grapes Project “New approaches for protection in a modern sustainable viticulture: from nursery to harvesting” on which the present paper was developed.

Table 1. Commercial products, doses and number of applications carried out during the three-year trial, on each treatment: integrated pest management (IPM), IPM with reduced distribution of agrochemicals (IPM-GG), classic organic (ORG), and classic organic with reduced employment of copper and sulphur (ORG-GG).

Active Ingredient	Commercial Product	Supplier	Years	Number of Applications for Treatment				Dose Kg-l ha ⁻¹
				IPM	IPM-GG	ORG	ORG-GG	
Dimetomorf, Metiram	Forum top	BASF Italia S.p.A.	2018	1	1			2.5
			2019	3	3			2.5
			2020	3	1			2.5
Fluopicolide, Fosetyl Al	R6 Erresei Albis	Bayer	2018	1	1			3
			2019	2	1			3
			2020	2	2			3
Cymoxanil, Fosetyl Al, Copper	Vitene triplo	Sipcam Italia	2018	1	1			4
			2019	1				4
			2020	1				4

Metalaxil, Copper	Planet C	Upl Europe Ltd.	2018					
			2019	1	1			4
			2020	1	1			4.5
Mandipropamid, Zoxamide	Ampexio	Syngenta Italia	2018	3				0.5
			2019	1	1			0.5
			2020	1				0.5
Copper (Bordeaux mixture)	Bordoflow new	Manica S.p.a.	2018	2				3.5
			2019	3	2	4	1	4
			2020	1				4
Tribasic copper sulphate	Cuprofix ultra dispress	Upl Europe Ltd.	2018		1 (2.1) *	10 (1.7)	10 (1.3)	
			2019			7	5	1
			2020	2 (1.5)	1 (1.5)	9 (1)	6 (1)	
Original blend of bioavailable nutrients (Mn. Zn). Combination of bacterial metabolites and enzymatic compounds	Pur'apres	Tailor'd Wine Design	2018		5			1.5
			2019		2			1.5
			2020		3		1	1.5
Original blend of bioavailable nutrients (Mn. Zn). Combination of bacterial metabolites and enzymatic compounds	Pur'avant	Tailor'd Wine Design	2018				1	1
			2019					
			2020				2	1
Solid extract of alfalfa, algae and molasses	K&A Oomisine 2.0	Kalos	2018				1	2.5
			2019				1	2.5
			2020				1	2.5
Solid extract of alfalfa, algae and molasses	K&A Evidence 2.0	Kalos	2018				2	2
			2019				1	2
			2020				2	2
Fluid yeast extract containing brown algae	K&A Frontiere 2.0	Kalos	2018		2		2	0.75
			2019				1	0.75
			2020		2		1	0.75
Plant extracts	Dinamico	Fertenia	2018		1		2	2.5
			2019					
			2020		2		2	2.5
Zeolite	Zeolite Fertenia	Fertenia	2018		5 (3)	1 (2.5)	1 (3)	
			2019	1 (2.5)	4 (5.25)	2 (4.5)	3 (4)	
			2020	2 (4.5)	4	1	6	6
Sweet orange oil	Prev'am	Nufarm Italy	2018		1		1	1.6
			2019				2	1.6
			2020		1		2	1.6

* Bracket-reported values indicate the dose of application when it was not the same for all treatments.

To date, in order to aid a rational recourse to phytosanitary products, forecasting models and Decision Support Systems represent an effective strategy in both IPM and organic viticulture; essentially, those tools process climatic data in accordance with mathematical models identifying the conditions predisposing pathogen infections [52–54]. Consequently, relying on DSS systems helps producers to conduct phytosanitary treatments only if strictly necessary, and to select the most suitable commercial products to be used.

Besides the adverse effects caused using cupric fungicides, soil tillage and the excessive use of chemical fertilizers also contribute to soil erosion and degradation, whereas the recourse to alternative management methods, such as grass cover and green manure, represents a strategic solution for maintaining soil quality and health. Grass

covers with periodical cuttings reduce soil erosion and nutrient leaching, especially in sloping vineyards, and alleviate soil compaction caused by mechanical operations, improve the habitat complexity, and thus increase the abundance and diversity of pathogen natural enemies that can exert a strong biological control [55,56]. Sowing different cover crops (green manure) that can finally be buried at the end of the season, on the other hand, allows for enrichment of the soil with organic matter [57–61].

In this work, we describe results obtained in three crop years (2018–2020) with different types of control management, following innovative strategies to reduce the use of synthetic/cupric phytosanitary products and to recover the resilience of the agroecosystem. The innovative defense strategies included using several commercial formulations, mainly classified as foliar fertilizers by the current legislation, based on natural substances known to elicit the natural systemic resistance mechanisms of plants against pathogens. In particular, the products employed belong to three main categories: seaweed extracts, plant extracts, and products containing enzymes and metabolites obtained from the biotechnological processing of the yeast *Saccharomyces cerevisiae* yeast; those substances will be classified as *supporting substances*.

Finally, the sustainability of each management was assessed by estimating their impact on both the human health and the environment.

2. Materials and Methods

2.1. Experimental Site

Trials were conducted over three years (2018–2020) in a vineyard, located in the Chianti Classico wine district (43°37'42" N, 11°15'38" E; San Casciano, Firenze, Italy), on a 0.7 m × 2.4 m spaced vineyard, characterized by a loamy-clay soil (39.9% clay, 36.2% silt and 24.2% sand) cultivated with Sangiovese variety grafted on 110R rootstock, East–West-oriented, trained on an upward-vertical-shoot-positioned trellis, with spur cordon pruning, planted in 2011. Vines were pruned with eight buds per vine (two nodes per spur and four spurs per vine). The vineyard was subdivided into plots to apply the discrete management strategies foreseen in the project proposal and described below.

A meteorological station was installed close to the vineyard to monitor the climatic parameters and consequently plan phytosanitary treatments based on real infection risks. Indeed, data collected by the weather sensors were processed by the Vite.net® system, a Decision Support System (DSS) developed by Horta s.r.l., providing daily updated information aiding to carefully scheduling of the antifungal treatments.

2.2. The “GreenGrapes” Strategies for the Vineyard Management

Four types of crop protection management (henceforth referred to as *protocols*) were implemented in the vineyard: IPM (integrated pest management); IPM-GG (the classic IPM management characterized by a substantial reduction in fungicides distribution and by the use of supporting substances); ORG (organic management); ORG-GG (the organic management with a reduction in copper distribution, implementing supporting substances) (Table 1).

The commercial formulations were applied with a low-volume articulated sprayer (VMA Power 50) calibrated to deliver 300 l ha^{−1}, with a forward speed of 5.3 Km/h and an operating pressure of 1, 5 bar and 2050 rpm. The applications of these treatments were performed based on the risk assessment given by the DSS.

The first phytosanitary treatments were applied between 23 and 27 April in all tested seasons, when the plants were at the phenological stage between the emission of the 5th and 6th leaf, and thus susceptible to the attack of the pathogen *Plasmopara viticola*, and continued until the infection risk was reported by the DSS.

The active substances adopted, the number of treatments applied, and the doses used are listed in Table 1; the last phytosanitary treatment was applied on 12 July, 13 July, and 10 July in the three years of the trial, respectively.

For each of the 4 treatment plots which covered an area of approximately 10,000 m², a grass-covered sub-plot (g) and a green-manured sub-plot (m) were identified. For the former, the agronomical practices basically consisted of 2/3 grass cutting operations.

The green manured rows consisted in a cover crop mixture composed by species belonging to the botanical families *Fabaceae*, *Graminaceae*, *Brassicaceae*, and *Hydrophyllaceae* (Table 2) sowed in late autumn (October–November). Subsequently, in late spring (April–May), the inter-rows were subjected to disc harrow processing to plough the vegetation, and, after few weeks, to a weeding towel operation to promote the embedding of the crops to the soil (the last operation was repeated even during the summer).

Table 2. Composition of the cover crop mixture employed in the “m” (green manure) sub-plot rows.

Botanical Family	Species
<i>Fabaceae</i> (55%)	<i>Vicia faba</i> L. (30%)
	<i>Vicia sativa</i> L. (10%)
	<i>Pisum sativum</i> L. (10%)
	<i>Trifolium incarnatum</i> L. (5%)
<i>Graminaceae</i> (38%)	<i>Avena sativa</i> L. (12–18%)
	<i>Hordeum vulgare</i> L. (16–20%)
<i>Brassicaceae</i> (5%)	<i>Sinapis alba</i> L. (3%)
	<i>Eruca sativa</i> M. (2%)
<i>Hydrophyllaceae</i> (2%)	<i>Phacelia</i> spp (2%)

2.3. Plant Disease Monitoring Activity and Evaluation of Infections

During the three years of experimentation, the main diseases of the vine (downy mildew, powdery mildew, and gray mold) were monitored during the most susceptible phenological phases.

The treatments were carried out on an area of 40,000 m², divided into 4 blocks of about 10,000 m² each, homogeneous in terms of soil characteristics and environmental conditions.

Each of the 4 treatments underwent a different protection strategy.

The incidence (percentage of leaves/clusters affected by symptoms) and severity (percentage of leaf surface/cluster affected by symptoms) of diseases (downy mildew, powdery mildew, and gray mold) on 25 leaves and 25 clusters for 4 random replicates were evaluated in each block, at each sampling time (Table 3).

Table 3. Monitoring activity scheme of *Plasmopara viticola* infections for each trial year: organs, number of organs and phenological stage.

Phenological State	Organ	Number of Organs Monitored	2018	2019	2020
BBCH 107-109	Leaves	100	06-may	07-may	05-may
BBCH 110-113	Leaves	100	18-may	15-may	13-may
BBCH 57-69	Leaves/Cluster	100/100	31-may	30-may	28-may
BBCH 65-73	Leaves/Clusters	100/100	06-june	07-june	03-june
BBCH 72-77	Leaves/Clusters	100/100	20-june	19-june	19-june
BBCH 79	Leaves/Clusters	100/100	09-july	05-july	03-july
BBCH 85-89	Leaves/Clusters	100/100	24-july	28-july	27-july

The severity of the disease was assessed, following the indications provided by the EPPO guidelines, based on the extension of the leaf or cluster area affected [62].

2.4. Quality of Final Production and Vine Balance

For each trial (defense protocol X soil management), three inter-rows portions have been randomly selected in different sectors of the same row, excluding the terminal portions to avoid edge effects. In each inter-row, three consecutive plants were subjected to the sampling: the clusters were counted, manually picked, and weighted with a portable electronic scale. Grapevines were sampled in order to assess yield (kg/plant), clusters per plant (n), cluster and berry weight (g). Musts obtained were analyzed for TSS, pH, Titratable acidity, total and extractable anthocyanins, and phenolic maturity. In the last two crop years, the Ravaz Index was also calculated, weighing the one-year-old pruning wood, sampled in December from the same vines previously subjected to harvest. The following instruments/methods were employed for must analysis: a digital refractometer (DBR 95, XS Instruments, Carpi, Italy); pH meter (microPH 2002, Crison Instruments, s.a., Alella, Spain). Three replicates, each one composed of 100 filtered randomly chosen berries were employed in order to assess TSS ($^{\circ}$ Brix), titratable acidity, organic acids, and phenolic maturity. Some drops of musts were poured on the digital refractometer in order to assess TSS; titratable acidity was calculated by acid-base titration of 10 mL of must with 0.1N NaOH and blue bromothymol used as an acid-base indicator at pH 7.0. Phenolic maturity (total and extractable anthocyanins) was assessed according to O.I.V. (International Organization of Vine and Wine) official methods (see Compendium of International Methods of Analyses—OIV—OENO 21/2004) by analyzing musts achieved from pressing and filtering 100 randomly chosen berries. Phenolic maturity was evaluated by following the partially modified method proposed by Glories [63].

2.5. Sustainability of the Production Processes Assessment

The sustainability of the production processes carried out in each crop year was evaluated individually for all the different protocols relying on Yousustain.net® (Available online: <https://www.horta-srl.it/yousustain-net/> (accessed on February 26, 2021), a tool implemented to quantify the sustainability, examining agronomic factors (such as soil erosion and water use efficiency), biodiversity-related aspects, risks for human health associated with the phytosanitary products used, and a LCA (Life Cycle Assessment) analysis. The software gave a response by different indicators (carbon footprint, human tox score, just to name a few), assembled on Compartments (health, air, water, soil, biodiversity and energy consumption). The less the Indicator or Compartment score, the more the sustainability.

2.6. Statistical Analysis

Statistical analyses were carried out by using SPSS Statistics software (IBM SPSS Statistics V20, Chicago, IL, USA). Data were analyzed by a one-way ANOVA in order to evaluate the statistical relevance of differences between treatments. Mean values of protocols (IPM, IPM-GG, ORG, ORG-GG), years, soil conduction (g/m) were then separated by the Duncan multiple range post-hoc test. Differences were assumed as statistically significant for $p < 0.05$.

3. Results

3.1. Plant Diseases and Evaluation of Infection

3.1.1. Climate Data: 2018

The crop year 2018 was characterized by numerous rainy events which were most concentrated in March (18 rainy events), April (8 rainy events), and May (14 rainy events), with a total of 374 mm of rain fallen between 1 March and 30 May (Figure 1B). According to the indications of the DSS and the data recorded with field monitoring, the plant, based on the BBCH, was susceptible to the disease, starting from the last week of April. In June, there were three rain events for a total of 20 mm of rain; in July, there were four rain events and 26 mm of rain. In August, there were nine rainy days for a total of 88 mm. In the same

period, the average temperatures were above 24 °C for most days (Supplementary Table S1). The meteorological data clearly suggest that 2018 was a year of high disease pressure for downy mildew (Supplementary Table S1).

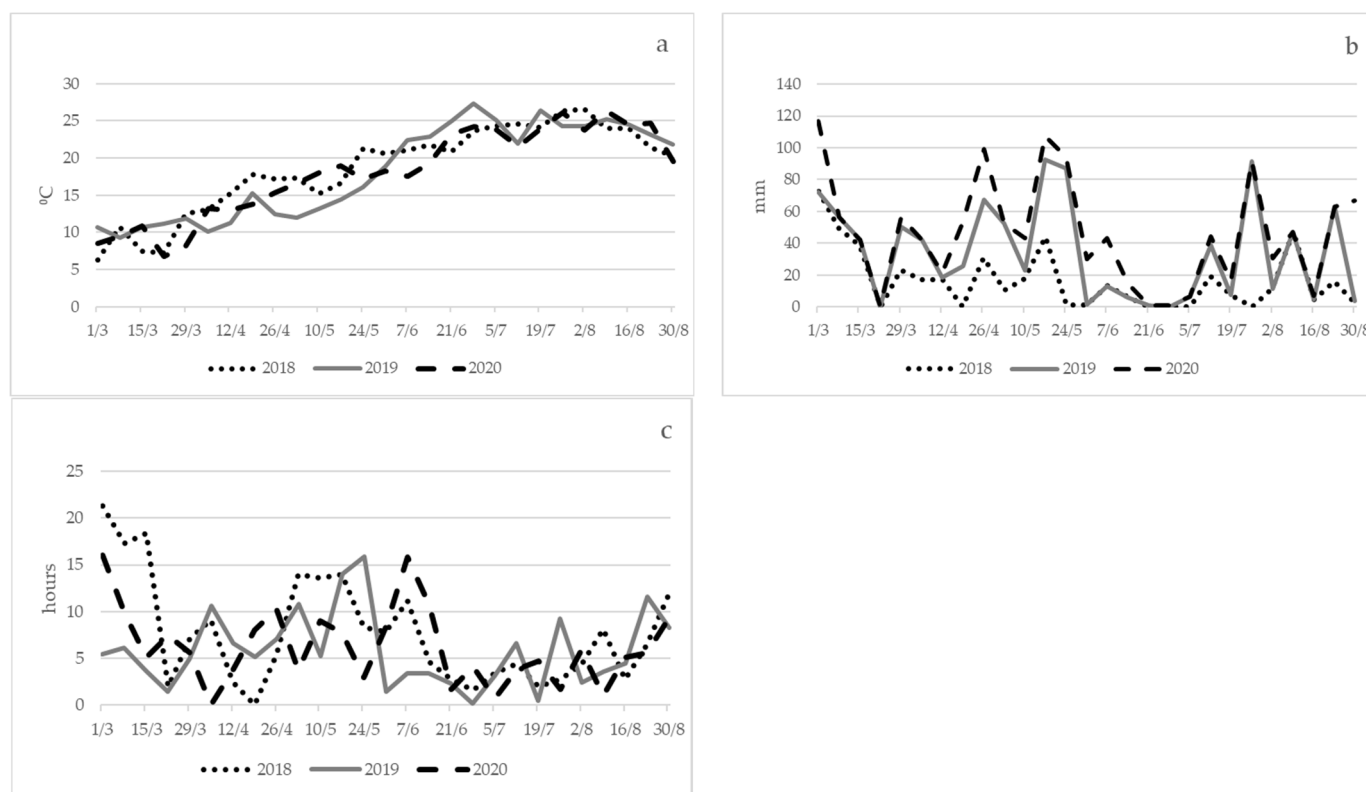


Figure 1. Comparison of the meteorological parameters for each year: average temperatures (a), mm of rain fallen (b), hours of leaf wetness (c), weekly gathered.

The analysis of the data deriving from the monitoring carried out both on the leaves and on the clusters (Table 3), highlighted statistically significant differences between the treatments (Table 4).

Table 4. Average percentage of incidence and severity of *Plasmopara viticola* calculated both on leaves and on bunches of the vineyards treated with the four control strategies: IPM; integrated pest management with pesticide reduction and supporting substances addition (IPM-GG); defense management in organic viticulture (ORG); disease control management adding supporting substances and copper reduction (ORG-GG). The data were subjected to univariate analysis (ANOVA): $p < 0.05$. In each column, values followed by the same letter indicate homogeneous subgroups (Duncan test, $p < 0.05$).

Year	Treatment	Leaf Incidence %	Leaf Severity %	Cluster Incidence %	Cluster Severity %
2018	IPM	19.5 ± 4.55 b	1.79 ± 0.98 b	4.0 ± 3.16 c	0.22 ± 0.23 b
	ORG	20.56 ± 5.50 b	2.32 ± 0.47 b	12.5 ± 6.69 c	0.91 ± 0.29 b
	IPM-GG	43.47 ± 8.5 a	3.74 ± 0.99 ab	32.0 ± 6.32 b	4.05 ± 1.97 a
	ORG-GG	45.50 ± 10.23 a	4.94 ± 1.83 a	53.5 ± 20.15 a	5.20 ± 2.26 a
2019	IPM	3.0 ± 1.0 a	0.075 ± 0.02 b	0.0	0.0
	ORG	2.0 ± 2.0 a	0.325 ± 0.33 b	0.0	0.0
	IPM-GG	2.5 ± 0.87 a	0.150 ± 0.59 b	0.0	0.0
	ORG-GG	3.5 ± 0.93 b	1.0 ± 0.37 a	0.0	0.0
2020	IPM	1.88 ± 0.65 c	0.10 ± 0.02 c	0.38 ± 0.09 c	0.02 ± 0.004 c
	ORG	24.75 ± 1.80 b	3.38 ± 0.45 b	6.88 ± 1.24 b	1.49 ± 0.10 ab
	IPM-GG	22.75 ± 4.87 b	3.03 ± 0.9 b	5.75 ± 1.30 b	1.30 ± 0.18 b
	ORG-GG	61.75 ± 2.50 a	9.13 ± 1.65 a	41.40 ± 5.63 a	17.98 ± 1.44 a

In 2018, the incidence of downy mildew on leaves was higher in the “GreenGrapes” treatments (IPM-GG and ORG-GG), which statistically belong to the same subset and differ from the company treatments (IPM and ORG). Statistical differences were also recorded on the clusters. In particular, ORG-GG showed the highest value of incidence and severity both on leaves and on bunches.

Figure 2 shows the trend in the incidence of the disease on leaves based on the monitoring carried out during the growth season.

3.1.2. Climate Data: 2019

The crop year 2019 was characterized by a rainy period between the beginning of April and the end of May. During these months, there were 27 rain events for a total of 271 mm. The average weekly temperature in this period was around 13 °C; after only a few days, it exceeded 15 °C (Supplementary Table S2).

The meteorological data of the three years of trials (Figure 1B) showed the differences that allowed us to better understand the evolution of the disease.

In 2019, compared with the other two years, a lower temperature was noted from mid-April to the end of May (Figure 1A). In the following period, from the end of May to the first week of July, there was a higher increase in temperatures. Between the middle and the end of May, a period of greater rain intensity (millimeters of rain fallen, Figure 2B) than in the other two years was recorded, and from the end of May–beginning of June to the end of June, the leaf wetness hours were lower than in the same period in 2018 and 2020 (Figure 2C).

During the 2019 season, there were no significant increases in the incidence of the disease, which remained very low until harvest (Figure 2B).

During the most intense rainy period, plants were in a phenological phase between the emission of the first leaf, recorded on May 18, and the emission of the 12th leaf recorded on 31/05.

No statistically significant differences were recorded on the incidence and severity of downy mildew on the leaves (Table 4), both in treatments with or without copper and/or synthetic fungicides reduction.

No infections were recorded on the bunch in the 2019 season in any of the four treatments compared.

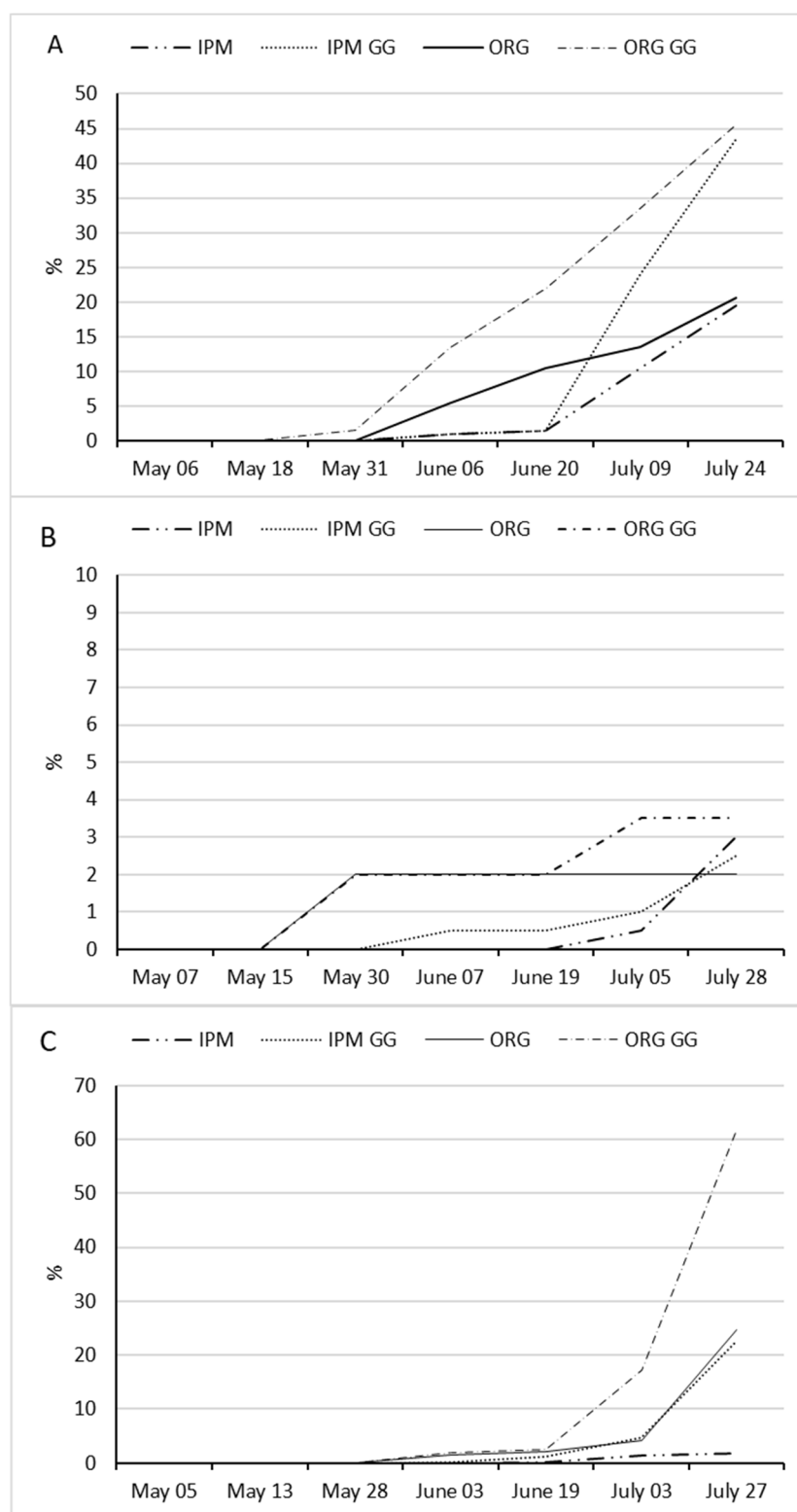


Figure 2. Incidence trend of downy mildew on leaves for the years 2018 (A), 2019 (B) and 2020 (C), for each trial: IPM; integrated pest management with pesticide reduction and supporting substances addition (IPM-GG); disease control management in organic viticulture (ORG); disease control management adding supporting substances and copper reduction (ORG-GG).

3.1.3. Climate Data: 2020

In 2020, the most intense rainy period was recorded between 10/05 and 14/06 (Figure 2B), during which 14 rain events occurred for a total of 108.6 mm, with an average temperature for most days above 18 °C and leaf wetness (weekly average) mostly for 7 to 10 h a day (Figure 1A–C).

From the second half of June to the end of August, only eight rainy days were recorded (99.9 mm rain). During the period of greatest rain intensity, the plant was in a phenological phase between the emission of the 9th leaf on 10/05 and the pea-sized grape on 20/06.

In 2020, the incidence of downy mildew was reduced in the first part of the season in all the treatments considered, but it increased since the end of June in all the treatments except the ones with integrated management (Figure 2C), where the incidence remained low until harvest.

In 2020, the incidence of downy mildew both on leaves and on clusters showed the same differences between the company treatments (IPM and ORG) and the GreenGrapes treatments (IPM-GG and ORG-GG) (Table 4). ORG-GG protocol was the one with the highest and statistically different incidence. The IPM-GG and the ORG managements did not show statistically significant differences. The IPM protocol is the one where the lowest incidence of the disease was recorded both on bunches and leaves.

ORG-GG management showed the highest severity both on bunches and leaves and IPM showed the lowest, which was in line with the results obtained on the incidence. No statistically significant differences were recorded between the IPM-GG and the ORG-GG protocols.

3.2. Quality of Final Production and Vine Balance

3.2.1. Soil Management

Significant differences have been observed for mean yield and bunch weight (Table 5) where the trials conducted with green manure management gave a better performance. Regarding vine pruning, GG treatments positively affected wood production only in IPM managed with green manure (419.6 g/vine), whereas other trials showed no significant differences (from 275 to 332.5 g/plant) (Table 5); the difference appeared in the third year of management.

Table 5. Three years means production components: yield (Kg/vine), bunch weight (g), bunch number; pruning wood production (g/plant) and Ravaz index calculated for each Protection protocol X soil (grass covering–g; green manure–m) management trial. Different letters show significant difference for $p < 0.05$.

Protection Protocol	Soil Management	Yield (Kg/vine)	Bunch Weight (g)	Bunch (n/Plant)	Wood Production (g/Plant)	Ravaz Index
IPM	g	1.3 ± 0.5 a,b	111.1 ± 36.9 a	12 ± 5.2 e	276.7 ± 57.9 a	5.1 ± 1.6 a
	m	1.6 ± 0.7 c	154 ± 37.3 b,c	10.9 ± 4.7 d	295.5 ± 100.9 a	5.8 ± 1.6 a
IPM-GG	g	1.1 ± 0.5 a	130.7 ± 36.8 a,b	8.7 ± 3.3 a,b	282.5 ± 112.6 a	5.8 ± 1.9 a
	m	1.7 ± 1 c	165.3 ± 55.2 c,d	10.1 ± 4 c,d	419.6 ± 47.3 b	4.1 ± 2.3 a
ORG	g	1.5 ± 0.6 b,c	183.1 ± 57 d,e	8.5 ± 2.8 a,b,c	277.3 ± 33.6 a	4.4 ± 1.7 a
	m	1.5 ± 0.8 b,c	225.7 ± 60.8 f	6.9 ± 2.9 a	332.5 ± 69.9 a	5.5 ± 2.1 a
ORG-GG	g	1.2 ± 0.5 a	155.4 ± 37.1 b,c	7.2 ± 2 a,b	275 ± 51.7 a	4.1 ± 1.2 a
	m	1.7 ± 0.6 c	195.9 ± 61.7 e	8.9 ± 2.8 b,c	317.3 ± 33.6 a	5.6 ± 1.7 a

The performance of green manure or grass coverings did not reveal any significant difference within the same protocol over three years of TSS, titratable acidity, grape weight, bunch number per plant, organic acids (except for shikimic and citric, in the ORG

management), total anthocyanins (except in the ORG management), or phenolic richness (except in the ORG management) (Table 6).

Table 6. Mean over three years means TSS, total acidity, total and extractable anthocyanins for each protection protocol X soil (grass covering–g; green manure–m) management trial. Different letters show significant difference for $p < 0.05$.

Defense Protocol	Soil Conduction	TSS (°Brix)	TA (g/l)	Total Anthocyanins (mg/Kg (+)-Malvidin)	Extractable Anthocyanins (mg/Kg Catechin 3 Glucoside)
ORG-GG	g	24.2 ± 1.5 b	5.6 ± 0.5 a,b	1542 ± 271 a,b,c	754 ± 158 a,b,c
	m	23.7 ± 1.6 a,b	5.5 ± 0.4 a,b	1430 ± 262 a,b	727 ± 128 a,b
ORG	g	23.9 ± 0.9 a,b	5.6 ± 0.5 a,b	1681 ± 242 c	792 ± 103 a
	m	23.7 ± 0.8 a,b	5.6 ± 0.5 a	1386 ± 225 a	707 ± 141 a,b,c
IPM-GG	g	23.9 ± 1.6 a,b	5.9 ± 0.7 a,b	1655 ± 243 b,c	847 ± 167 c
	m	23.6 ± 1.4 a,b	5.8 ± 0.5 a,b	1576 ± 285 a,b,c	761 ± 172 a,b,c
IPM	g	23.9 ± 1.3 a,b	5.5 ± 0.5 b	1641 ± 291 b,c	827 ± 132 b,c
	m	23.0 ± 2.2 a	5.7 ± 0.6 a,b	1532 ± 247 a,b,c	793 ± 168 a,b,c

3.2.2. Disease Control Management

Application of GG treatments increased pruning wood production significantly only on IPM management (351.1 g/vine of IPM-GG against 286.1 of IPM), whereas no effect appeared on ORG management (respective means of m/g in Table 5). The Ravaz index showed no significant differences inside all the trials.

The implementation of the GG management did not change the yield significantly (respective means of m/g in Table 5) while negatively influencing the number of bunches per plant, whereas IPM obtained the best performance (11.5) and ORG the lowest (7.7). For the mean bunch weight, ORG management showed the heaviest (204.4 g) and IPM the lightest (132.6 g). ORG-GG showed grapevines with higher sizes (2.24 g) and IMP the lowest (1.53 g) (respective means of m/g in Table 5). No significant differences were found for TSS (respective means of m/g in Table 6), whereas for titratable acidity only IPM-GG (5.8 g/l) showed a difference with respect to the other trials. The highest anthocyanins content derived from IPM-GG (1615.9), , ORG-GG contained lower anthocyanins (1486.38) ($p < 0.05$); extractable anthocyanins were higher in IMP (810.61) and lower in ORG-GG ($p < 0.05$) (respective means of m/g in Table 6).

3.3. Sustainability of the Production Processes

Employment of GG protocols applied to IPM or GG allowed an important reduction in agrochemicals or copper distribution, fully answering to the need of a more sustainable production (Table 7).

Table 7. Mean reduction (%) per year in antifungal active molecules in the IPM vineyard, and in copper and sulphur in the organic vineyard relating the “Green Grapes” (GG) defense protocol.

Disease control management	Active Molecule	Farm (kg/ha)	GG (kg/ha)	Reduction (%)
IPM	Ametoctradin	0.09	0.00	100
	Boscalid	0.20	0.00	100
	Cymoxanil	0.14	0.07	47
	Dimethomorph	0.45	0.23	48
	Fluopicolide	0.22	0.13	39
	Fosetyl-Al	4.54	3.07	32
	Mancozeb	0.33	0.00	100
	Mandipropamide	0.12	0.04	64

	Metiram	2.55	1.83	28
	Metrafenone	0.20	0.11	46
	Myclobutanil	0.08	0.00	100
	Copper oxychloride	0.79	0.39	50
	Quinoxifen	0.09	0.03	61
	Copper sulphate	2.22	0.88	60
	Zoxamide	0.12	0.04	66
ORGANIC	Copper	5.2	3.9	25.1
	Sulphur	21.89	17.55	20

Gathered data, once elaborated by Horta@, estimated the impact of the different production processes. The IPM management including green manure and grass covering) reached the highest Human tox index score, treatment frequency index, and Eco tox score, whereas for the same indicators, lower scores were reached by the application of GG protocols to IPM and ORG management (with green manure and grass coverings) (Table 8). ORG-GG with grass coverings and ORG with green manure reached the highest score for the carbon footprint indicator, whereas IPM with grass coverings and green manure reached the lowest. ORG and IPM-GG with grass covering and green manure, respectively, showed the highest score for carbon sequestration and grass-covered ORG/ grass-covered ORG-GG (equally), and the lowest score for IPM (Table 8). For the water footprint, a major score was reached by grass-covered and green-manured ORG-GG, whereas IPM-GG showed the lowest point. Finally, green-covered and green-manured IPM-GG and ORG reached major scores; ORG and ORG-GG grass coverings and green manure reached the lowest point (Table 8).

Table 8. Sustainability of each defense protocol x soil conduction (grass covering–g or green manure–m) relating the human tox score, treatment frequency index, eco tox score, carbon footprint, carbon sequestration and water footprint indicators; the more the value, the less the sustainability.

Disease control management	Soil Management	Human Tox Score	Treatment Frequency Index	Eco Tox Score	Carbon Footprint (t CO ₂ eq/t of Production)	Carbon Sequestration (t of C/ha)	Water footprint (m ³ of Water/t Production)
IPM	g	141.5	22.46	169.4	0.3	1.36	1419.67
	m	141.57	22.46	169.43	0.28	1.54	1348.00
IPM-GG	g	107.83	16	114.73	0.34	1.25	1181.33
	m	107.83	16	114.73	0.27	1.76	1021.00
ORG	g	63.83	21.8	39.83	0.31	1.45	1844.00
	m	63.83	21.8	39.93	0.33	1.59	1839.33
ORG-GG	g	60.77	21.26	38.83	0.37	1.26	1769.33
	m	61	21	38.83	0.28	1.61	1570.99

4. Discussion

In order to reduce the amount of copper and fungicides used for downy mildew control, various alternative products and different control strategies were tested [18]. The trials allowed us to better understand which are the most suitable moments for the application of the products used to control the disease, and a mean substantial reduction in anti-fungal molecule distribution (Table 8).

This was possible thanks to the combined effect of the DSS [64] application and field monitoring that allowed us to record and process a large number of data, relating weather conditions, plant protection and plant support products sprays, disease pressure, and impact in the field of the strategies applied.

The correct use of DSS systems favors the reduction in pesticides and working hours, providing the winegrower with an economic profit [64]. The adoption of management strategies of the “GreenGrapes” vineyard guarantees greater environmental sustainability of viticulture and allows the producer to adequately address the growing limits to the use of pesticides imposed by the legislator. Copper, for example, a candidate molecule for replacement but currently essential for disease control especially in organic farming, has been reduced in the “GreenGrapes” protocols compared with the maximum quantity allowed by the Implementing Regulation (EU) No. 1981 of European Commission (4 kg/ha).

Results obtained during the presented trials underline the importance of well-planned strategies also with a deep knowledge of the mode of action of the products that are applied in crop protection according to what was also reported by other authors [58] “GreenGrapes” strategies that involved the integration and/or alternation of products based on plant extracts, yeast extracts, and seaweed extracts, to plant protection products gave a lower control efficacy [23,36,41,47,65]. This is in accordance with research studies [66], showing that the elicitors have a lower protection than conventional pesticides.

Despite this aspect, it should be emphasized that the severity of the disease both on the leaves and on the clusters remained very low in all three years, guaranteeing the quality of production without affecting production amount. Only in 2020 in the organic production with copper reduction did the severity reach significantly higher values.

The greater effectiveness of synthetic fungicides is clearly shown examining, as an example, the disease development in 2018, showing a disease incidence increase occurring after periods of high infection risk: in the treatments managed with integrated control and fungicide reduction (IPM-GG), there were three periods of secondary infections risk, during which the official interval of efficacious protection by the treatments was no longer active: between 20/05 and 27/05; between 09/06 and 12/06 and between 04/07 and 23/07 (the last one with 9 days at infection risk). In the first two periods, the residual effect of the interaction among the previous sprays (contact, systemic, and translaminar penetration) kept the incidence of the disease low, probably due to their interaction as reported in [67,68], despite their official efficacy interval had expired.

Employment of supporting substances (seaweed extracts, hydrolyzed protein on yeast extracts) usually does not improve vine production, TSS, or total acidity, as reported by on cv Carmene [69, 70], on cv Sangiovese [71,72], on cv Solaris and Regent [73], on cv Narince, and on cv Merlot [74]. On the other hand, beneficial effects are principally reported for antioxidant components on grapevines or other species [71,75–78].

We could conclude that the treatments carried out in 2018 on 27/06, 09/07, and 19/07 including only elicitors, i.e., natural products with eliciting activity [79], failed to contain the attack of the pathogen. The monitoring carried out on 07/24 showed a significant increase in the incidence of the disease, which stood at 43.5%.

The pressure of the pathogen also plays a key role in the effectiveness of these type of treatments. During the three years of trials, it was possible to highlight how there is a threshold beyond which it is the natural plant defence action, even if supported by elicitors, which is able to control the epidemic. To confirm this, we can consider the disease development in 2019, when at the end of the growth season the incidence of the disease was very low in all the treatments, thanks to the low pressure of the pathogen [41].

As we know, the products with defense induction activity cannot simply replace the products with a direct antifungal activity even if they can often show a partial activity of this type [41,64,80]. In any case, as shown in this work, they can support the defense reaction of plants at a low disease pressure or improve the efficacy of plant protection products at a higher disease pressure.

Finally, it must be underlined that employment of supporting substances on IPM and ORG management allowed to maintain the same productivity (no differences for $p < 0.05$) and grapes with the same (TSS, total anthocyanins) or better quality (total acidity).

5. Conclusions

This research work was focused on reduction in chemicals in the downy mildew control. Nevertheless, in the three years of trials infections by botrytis and powdery mildew agents were also surveyed and, as stated above, no infection by the two pathogens was ever recorded, even in the strong reduction treatments. This may be due to the characteristics of the two pathogens that are particularly sensitive to the resistance reaction induced in the plant (more actively sensitive than usually reported in the plant reaction to downy mildew).

The overall results of the three-year study indicate that disease management protocols based solely on the use of resistance-inducing substances do not appear to ensure effective protection against downy mildew infections. In actual fact, when the environmental conditions were favorable to the disease for several days (high pressure of the disease according to DSS), the treatments based on defense support products did not guarantee the protection of the crop compared with the IPM treatments.

A careful retrospective analysis of the infection risk graphs originated by the model over the three years of experimentation made it possible to identify the critical periods for the use of defense support products. In particular, in 2018 and 2020, years particularly favorable to the development of downy mildew, in periods of high disease pressure, there was the greatest increase in the incidence of downy mildew in GG treatment, coinciding with treatments with defense support products.

This highlights the need to act on the pathogen under certain environmental conditions, integrate resistance-inducing products to a copper-based strategy or a classic IPM strategy. Proper interpretation of the DSS can ensure sustainable defence and savings in the amount of pesticide use reduction.

In seasons that are climatically unfavorable to the development and spread of downy mildew such as in 2019, the amount of fungicides necessary for the defense of the vineyard can be significantly reduced in the context of both integrated and organic pest management. The correct interpretation of the risk provided by the DSS and the knowledge of the characteristics of the products used are essential for the reduction in fungicides. It must always be kept in mind that defense support products have an action on the plant, but not on the pathogen.

The use of fungicides is now a proven practice in viticulture thanks to low costs and a greater guarantee of effectiveness compared with new substances such as elicitors. Today, however, the greater sensitivity of consumers to organic farming and the rules that regulate it, push towards testing and challenging new products with a low environmental impact.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/agronomy12020392/s1, Tables S1–S3: Climate trend in 2018–2019–2020. Weeekly data from Vite.net Horta S.r.l.

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