Soil management and plant protection strategies with reduced use of copper: productive and environmental aspects in a Sangiovese vineyard

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Abstract. Plant protection strategies in organic viticulture are based on the application of copper products, which is well known to generate a consistent environmental impact due to the accumulation of copper in soils and its negative effects on edaphic biodiversity. Life Green Grapes is a demonstrative project aiming to improve the sustainability of viticulture throughout the supply chain: from vine nursery to the table. In this paper, we report the main results obtained over three growing seasons (2018-2020) in response to the adoption of a strategy based on a reduced use of copper products in an organic vineyard. Plant protection treatments have been strictly planned according to forecasting models for disease development and fungicides have been partially substituted with products improving plant resistance. Green manure, known for contributing to the health of the vineyard, was also adopted. Results suggest the effectiveness of the "Green Grapes" strategy under low downy mildew pressure. Furthermore, no declines in grape quality have been observed; on the contrary, the synergic effect of green manure and substances beneficial to plants improved yield. An overall positive influence on the edaphic biodiversity was also observed.

1 Introduction

According to 2016 ISTAT (Italian national statistical Institute) data, most (26%) of fungicides distributed in Italy is used for grapevine protection [1]. Indeed, viticulture is one of the most widespread agricultural sectors in Mediterranean countries where infections caused by fungal pathogens are one of the main causes of production losses. Up to 70% of the total quantity of Plant Protection Products (PPP) applied to vines are copper and sulphur-based products [1] with high effectiveness to prevent downy and powdery mildew, respectively. However, copper is known for its persistence in soils and therefore in the environment resulting in impaired vineyard ecological balance, also by reducing edaphic biodiversity that is crucial for maintaining soil fertility [2]. For those reasons, the use of copper in agriculture is limited (28 kg/ha for 7 years) by 1981 (Commission Implementing Regulation (EU) 2018/1981 of 13 December 2018).

Nevertheless, copper-based fungicides represent to date the only category of phytosanitary products allowed in organic viticulture that can effectively counteract downy mildew attacks [3].

The application of models that analyse the disease development and forecast infection risk can strongly help in reducing the use of plant protection products when implemented in Decision Support Systems (DSSs).

Moreover, several products, containing microorganisms, bacterial derivatives or botanical pesticides, have been proven to counterbalance the effects of pathogens in grapevines [4-9]; these products exhibit different modes of action [10], such as pathogen parasitism, production of toxic compounds and competition for nutrients or space. Moreover, several of these substances are also known to act as elicitors, activating the plant endogenous mechanisms involved in the resistance against plant pathogens [8, 9, 11-13]; some evidence for their efficacy in downy mildew control has also been obtained [14-17]. A defense induction activity can be performed also as a side effect of other products as they contribute to vine health and therefore in general to vine active response. Indeed, such substances can stimulate the physiological processes involved in the absorption and assimilation of nutrients, with an overall invigorating effect [18-21]. Plant nutritional status can be improved even by an adequate soil management; in particular, green manure plays fertiliser function and can significantly increase the organic matter content as well as nutrient availability in soil [22-28].

Life Green Grapes is a demonstrative project aimed to

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increase the sustainability of viticulture throughout the supply chain: from the vine nursery to the table. To reach this goal, strategies were set up based on a reduced use of agrochemicals. Agronomical practices contributing to support the vine in its defence activity such as two soil management practices: green manure and permanent cover crop were also adopted [29-30] and combined with organic plant protection management.

2 Materials and methods

The "Green Grapes" strategy was applied for three years (2018-2020) in an organic vineyard owned by "Castello di Gabbiano - Beringer Blass Italia" farm, within the DOCG Chianti Classico area (San Casciano in Val di Pesa, Florence, Italy).

The 10-year-old vineyard of the cv. Sangiovese (*V. vinifera*), grafted on 110R rootstock, was planted with a spacing of 0.7 m (between vines) × 2.6 m (between rows). Vines were trained on a vertical shoot positioning and spur-pruned with a single cordon system, at 70 cm above ground with 4 spurs (8 buds per vine).

The vines were not irrigated and were grown using standard cultural practices. Soil horizons present a clay texture with the following characteristics: clay 40%; silt 35%; sand 25%.

Two different soil management strategies were applied: "Green manure" (M) and "Permanent cover crop" (P) [31-33]. Green manure consists of growing specific crops (35% Vicia faba, 25% Hordeum vulgare, 20% Avena sativa, 10% Vicia sativa, 5% Sinapis alba, 5% Trifolium incarnatum) in all the inter-rows. The crops were sown in autumn and cut and buried in early spring, during flowering period, with a mechanical tillage that also removes any weeds. The permanent cover crop was natural and mostly represented by grasses.

The vineyard was managed with two protection protocols: the farm organic strategy (BIO) and the strategy with reduction of copper distribution and addition of products with a plant defence inducing activity, such sea weeds extracts, yeast extracts, plant extracts (GG). In particular, the GG protocol followed the DSS indication, in relation to the infection risk reports.

An agro-meteorological station was installed close to the vineyard and phytosanitary treatments were scheduled relying on the indication given by the DSS Vite.net® (Horta Srl, Piacenza, Italy).

In order to evaluate the effect of the strategies used on the containment of downy mildew, the vineyard was divided into 2 plot each with an area of approximately 10,000 m^2: BIO (organic plant protection management) and GG strategy (organic plant protection management with copper reduction). Each plot was divided into two subtreatment: "Green manure" and "Permanent cover crop". From each subtreatment, 4 replicates composed of 20 plants each were chosen in a randomized manner.

Downy mildew development was constantly monitored during the trial. According to the European and Mediterranean Plant Protection Organisation (EPPO) protocols, 100 randomly-chosen samples (leaves or clusters) were monitored for each of the four replicates within the two disease management strategies.

Yield components (clusters/vine and cluster weight) grape composition (technological and phenolic maturity) were recorded over the three years; the vine balance was assessed by the Ravaz index.

Statistical analysis was performed by SPSS24®; "year", "protocol" and "soil conduction" were the factors; mean values were separated by Duncan Test with $P<0.05$.

To evaluate soil microarthropods, soil samplings were carried out in October before the beginning of the trial and at the end of the three years. for each soil management treatment, three soil samples were collected using a special 10 cm^3 corer for mesofauna sampling. Microarthropods were extracted using modified Berlese-Tullgren funnels following the standard methodology [34]. The edaphic microarthropod communities were characterized using the QBS-ar index according to [34] in order to evaluate the soil biological quality by organism adaptation to the edaphic habitat.

The total soil copper content was determined by microwave digestion in a 3:1 HCl/HNO_3 solution (aqua regia), followed by flame atomic absorption spectroscopy.

Microbial diversity was determined before the beginning of the whole three-years trial and at the end of the three years by a high-throughput sequencing approach with Miseq Illumina technology (IGA Technology Services, Udine, Italy), targeting the 16S DNA ribosomal genes with primers 515F and 806R.

Total DNA was extracted from 0.5 g of soil samples using FastDNA SPIN Kit for soil (MP Biomedicals, Irvine, CA, USA), following the manufacturer's instructions. Data were analyzed by Qiime2 standard pipeline (https://qiime2.org), which also provides significance test Kruskal–Wallis one-way analysis of variance.

3 Results and discussion

Weather data show a substantial similarity regarding temperature trends over the 3 years; the first year showed the highest Winkler index (4,251) respect 2019 and 2020 (4,009 and 3,883 respectively). The second year was the most rainy with 626 mm (April-October), respect 2018 and 2020 (461 and 419 mm respectively) (Fig. 1).

Two out of three years (2018 and 2020) under GG management displayed higher downy mildew incidence both on leaves and on clusters. On the contrary, in 2019 the incidence of downy mildew in both disease control strategies was lower (2% BIO and 3.5% GG strategy) and no yield losses were observed (Tab. 1 and 2).

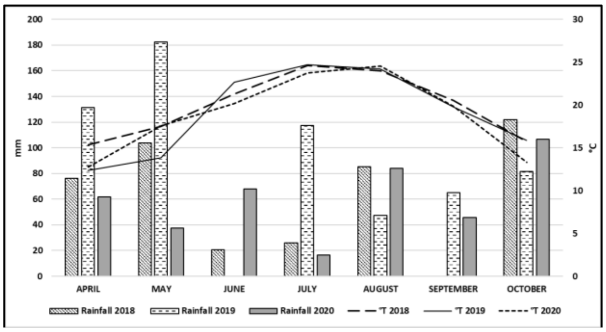

Fig. 1. Vineyard Microclimate: monthly total rainfall (mm), and mean temperature (°C) from April to October for the three-year period 2018-20.

Table 1. Downy mildew severity (%) on leaves and clusters of grapevines subjected to different plant protection management. Different letters show significant differences at Duncan test (P<0.05).

Year	Protocol	Leaves	Clusters
2018	BIO	2.32 ± 0.47 a	0.91 ± 0.29 a
	GG	4.94 ± 1.83 b	5.20 ± 2.26 b
2019	BIO	0.33 ± 0.33 a	0.00 -
	GG	1.00 ± 0.37 b	0.00 -
2020	BIO	3.38 ± 0.45 a	1.49 ± 0.10 a
	GG	9.13 ± 1.65 b	17.98 ± 1.44 b

Table 2. Plant productivity (kg and clusters per vine) of grapevines managed with different plant protection protocols (GG or BIO) and soil management (M or P). Different letters show significant differences at Duncan test (P<0.05).

Year	Protection	Soil	yield kg/vine	cluster weight g	TSS °Brix	TA (g/l)
2018	GG	M	1.7 bc	218 bcd	25.4 fg	5.3 ab
		P	1.2 ab	157 a	25.8 g	5.5 abc
	BIO	M	1.3 ab	254 d	24.1 cde	5.6 abcd
		P	1.5 ab	223 cd	24.8 efg	5.4 ab
2019	GG	M	1.7 bc	187 abc	22.3 a	5.9 bcde
		P	1.4 ab	167 ab	22.5 a	6.2 e
	BIO	M	2.2 c	236 cd	22.8 ab	6.2 de
		P	1.6 bc	187 abc	23.0 ab	6.0 cde
2020	GG	M	1.7 bc	181 abc	23.5 abcd	5.3 ab
		P	0.9 a	140 a	24.5 def	5.2 ab
	BIO	M	1.2 ab	186 abc	24.1 cde	5.1 a
		P	1.5 ab	138 a	24 bcde	5.3 ab

In 2018, the combination protocol x soil management showed no significant difference for yield while cluster was higher for the BIO protocol (M and P managed) and there was a difference for P<0.05 only respect GG-P (Tab. 2). In the next year only the trials GG-P and BIO-M showed significant difference for yield and cluster weight. In the last year only GG-M and GG-P showed significant difference for grapevine production (1.7 and 0.9 kg/vine, respectively). In the same year, grapes did not show significant differences for titratable acidity while TSS differed only in 2018 between GG-M (25.4) and GG-P (25.8) against BIO-M (24.1).

In both years, the analytical characterisation of the main organic acids in juice (tartaric, malic, shikimic and citric acid) showed a general similarity among the different strategies.

Furthermore, the "Green Grapes" management had negligible effect also on the phenolic potential of productions (Tab. 3): no significant differences emerged from the GG protocol application, respect the Bio one, for total and extractable anthocyanins for each year.

Finally, pruning weight and the Ravaz index did not differ among treatments (Tab. 4).

Table 3. Phenolic maturity indices (±SD) of grapes subjected to different plant protection management. Data expressed as mg/kg of malvidin- 3 glucoside equivalents. Different letters show significant differences at Duncan test (P<0.05).

Anthocyanins	Protocol	2018	2019	2020
Total	BIO	1560 ± 298 b	1396 ± 291 ab	1645 ±203 b
	GG	1610 ± 211 b	1256 ± 315 a	1592 ±145 b
Extractable	BIO	785 ± 123 b	641 ± 108 a	823 ±80 b
	GG	820 ±80 b	574 ± 70 a	826 ±77 b

Table 4. Plant vigour of grapevines managed with different treatment protocols (GG or BIO) and soil management (M, P) over two years (2019-20).

Year	Soil management	Treatment management	Pruned wood g/vine	Ravaz Index
2019	P	GG	300	4.7
		BIO	337	5.0
	M	GG	363	4.6
		BIO	407	5.4
2020	P	GG	250	3.5
		BIO	230	6.6
	M	GG	270	6.5
		BIO	430	2.7

Overall, GG management allowed a 25% reduction of copper distribution. The GG-M copper content in soil remained similar to the initial value while in the BIO protocol an increase has been observed (Tab. 5).

Bacterial diversity indices showed no significant differences among treatments and soil management. However, GG management increased the *Proteobacteria*/*Acidobacteria* ratio in all plots (Tab.6), independently to the soil management, suggesting an improvement of soil nutritional status. This result confirms the key role of *Proteobacteria* in soil nutrient cycling in Sangiovese vineyards from Tuscany, as recently reported [35].

Table 5. Copper distribution (kg/ha) in vineyard for each season/disease control strategy and relative copper concentration in the soil (20 cm) at the beginning and at the end of the study (2020).

Year	Soil management	Disease control strategy	Cu application (kg/ha)	Cu concentration in soil (ppm)
2017	-	-	-	95.4
2018	PM	BIO	6.8	-
		BIO	6.8	-
	PM	GG	5.9	-
		GG	5.9	-
2019	PM	BIO	4.1	-
		BIO	4.1	-
	PM	GG	2.6	-
		GG	2.6	-
2020	PM	BIO	4.8	111.0
		BIO	4.8	122.1
	PM	GG	3.2	114.4
		GG	3.2	94.4

Fifteen different taxa of microarthropods were identified in the soil. In general, only mites and springtails were prominent over the years while the other taxa showed a very low abundance. Concerning taxa distribution, in pre-trial, springtails were the dominant taxa and represented over 70% of the whole microarthropod population. The application of green manure increased the mites that reached 60% than 30% in 2017. In permanent grassing springtails remained the most abundant taxa.

QBS-ar index showed values under good soil quality in 2017, when soil conventional management was applied (tillage and mineral fertilization). Although these results should be interpreted within the limit of the short-term, after three years of application of grass covering and green manure all values were higher than 100 (Fig. 2). The highest values were found in the BIO-M and in the GG-P.

Despite the reduction of copper-based treatments, GG had no significant effects on soil copper concentration over the considered period, possibly due to the alkaline-calcareous nature of the soil which severely limited copper mobility, thus extending the time necessary for an appreciable removal from the soil/plant system.

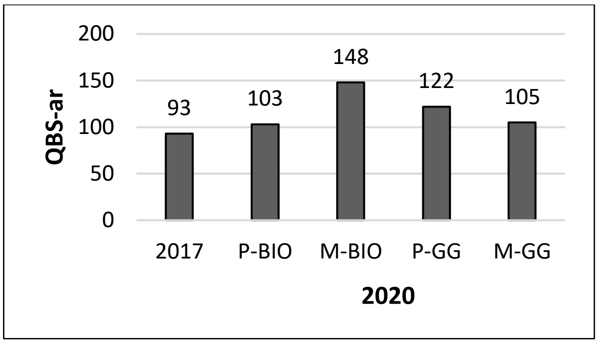

Fig. 2. Effect of different management on soil biological quality measured by QBS-ar, before treatment (2017) and at the end of the trial.

Table 6. Alpha diversity indices before (2017) and at the end of the tests (2020).

Index	*2017*	P-BIO	P-GG	M-BIO	M-GG
Taxa	*35.00*	34.00	32.00	35.00	35.00
Simpson	*0.85*	0.86	0.86	0.87	0.86
Shannon	*2.19*	2.23	2.23	2.24	2.20
Evennes	*0.25*	0.27	0.29	0.27	0.26
Chao 1	*35.00*	34.00	32.00	35.00	35.00
Ratio P/A*	*1.50*	1.63	1.82	1.51	1.80

* *Proteobacteria*/*Acidobacteria*.

4 Conclusions

In conclusion, the results obtained in 2018 and 2020 demonstrate the current ineffectiveness of copper reduction strategies to prevent downy mildew infections in organic vineyards; this is particularly true under climatic conditions leading to high pathogen pressure. On the other hand, the results show that, under lower pathogen pressure, the partial substitution of plant protection products with the use of products improving plant resistance response can represent a valid solution to minimize the use of copper in vineyard. Of course, the reduction of copper products must be reached through an appropriate use of disease forecasting models ensuring the accurate detection of the conditions strictly requiring copper-based treatments.

The use of products inducing natural plant defence showed its technical limits under high pathogen pressure highlighting the need of additional studies able to provide more accurate risk limits, beyond which the use of plant protection products it is necessary.

Therefore, the “Green Grapes” strategy could represent an effective instrument to comply with the current EU regulation limiting the use of copper products and, at the same time, allowing growers to have a further tool to better manage the amount of copper applied to crops on a 7-year basis.

Green manure tended to increase the vineyard productivity, suggesting its successful adoption for managing organic vineyards. However, the increased productivity of grapevines subjected to green manuring points out the need to fine-tune the overall vineyard management strategy to avoid potential declines in grape quality.

The reduced copper applications in GG management gave in some years a higher downy mildew severity on leaves and clusters but no significant yield loss. The study showed no significant difference between a vineyard conducted with permanent grass or green manure, as found by other authors [35-36].

The aim of a lower copper distribution and therefore of a reduced environmental impact has been reached and consequently, in particular, the final copper level remained similar to the pre-trial concentration when the GG-M protocol is applied.

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