

# Use of Olive Processing Waste to Support Vine Protection

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**Abstract.** The olive oil production chain generates large quantities of waste with a high polyphenolic content that can be valued for protection against fungal diseases in viticulture. In this work, we evaluated the effects of two different experimental extracts (olive mill wastewater and olive leaf decoction) against downy mildew, observing the possible impact on the vine physiology and grape quality at once. The experimental trial was set up in Arezzo (Tuscany, Italy), in a cv Merlot vineyard, where three different pest management strategies were compared for three years (2020-2022). During the vegetative season, downy mildew symptoms on leaves and clusters were monitored. Physiological measurements were carried out on the vines before and after the treatments. At harvest, yields were quantified, and the technological maturities and polyphenol contents of the grapes were assessed. According to the results, the antioxidant properties of the polyphenol extracts added to the phytosanitary treatments with significantly lower amounts of copper limited the infections caused by downy mildew without influencing the physiological and productive capacities of the vines and without altering the grape characteristics.

## 1 Introduction

For some years, the EU has been imposing increasingly stringent rules on agriculture, asking farmers to apply sustainable field management and implement circular economy policies [1].

The most valuable Mediterranean crops include viticulture and olive growing which, for various reasons, have a significantly high environmental impact. Grape cultivation, both for winemaking and fresh consumption, requires large quantities of pesticides [2], while olive oil production generates massive processing waste over periods limited to a few months every year [3]. The ideal intervention, therefore, would be to find an eco-friendly way to exploit the by-products of olive growing to reduce the use of agrochemicals in the vineyards.

Several recent studies have ascertained that olive mill wastes have effective antimicrobial and antifungal properties thanks to their considerable concentrations of phenolic compounds [4-5]. Therefore, this evidence suggests reusing these materials for a more sustainable, integrated pest management strategy within the farm production system, a potentially advantageous practice, considering that many farms grow both crops.

This study aimed to evaluate the possibility of using intra-company waste products from olive oil production (olive mill wastewater and olive leaf decoction) to control the spread of downy mildew in vineyards and reduce the quantities of copper applied in organic viticulture.

## 2 Materials and methods

### 2.1 Vineyard characteristics and experimental plan

The research activity was set up in a commercial Merlot (*Vitis vinifera* L.) vineyard, hosted by “Fattoria La Violla” farm in Castiglion Fibocchi (Arezzo, Tuscany, Italy - 43°31'30" N, 11°46'55" E). The vineyard is north-south oriented; the rows have a distance of 2.5 m x 0.7 m. The vines are trained on an upward-vertical-shoot-positioned trellis, with spur cordon pruning (10 buds/vine). No irrigation system is present.

The experimental trial occurred during three consecutive growing seasons (2020-2022). In each season, phytosanitary management was carried out according to weather conditions, following the provisions of a decision support system (DSS). The three treatments for the defense against downy mildew were ME 4, as the winery standard, with a copper dose of 5 g/L; ME 3, 50% of the copper dose and polyphenols; ME 2, 25% of the copper dose and polyphenols. ME 1, the untreated control, was added to assess the annual disease pressure of downy mildew within the vineyard (Table 1). The mixes to add in ME 3 and ME 2 treatments were prepared by combining olive mill wastewater (OMWW) and a decoction of olive leaves (OLD) obtained experimentally by the farm staff using intra-company waste from olive growing. The polyphenol concentrations of both products were adjusted to 1 g/L before composing the phytosanitary mixes.

Each treatment was applied on four adjacent rows of approximately 200 vines. The experimental measurements were always carried out in the central rows, keeping the first and fourth as buffer zones.

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**Table 1.** Composition of the phytosanitary mix applied in each treatment. OMWW = Olive Mill Wastewater; OLD = Olive Leaf Decoction.

Treatment	Mix composition
ME 4	Winery standard: Copper (5 g/L)
ME 3	Copper (50% ME 4) + OMWW (1 g/L polyphenols) + OLD (1 g/L polyphenols)
ME 2	Copper (25% ME 4) + OMWW (1 g/L polyphenols) + OLD (1 g/L polyphenols)
ME 1	Untreated control

## 2.2 Weather data

The weather data were collected relying on an automated weather station managed by the Tuscany region ([www.sir.toscana.it](http://www.sir.toscana.it)) located near the vineyard, from the beginning of January 2020 to the end of December 2022. The following parameters were recorded: daily maximum temperature (T Max, °C), daily minimum temperature (T Min, °C), and annual rainfall (mm). For each vegetative season, the sum of days with T Max above 35 °C and above 40 °C was determined. Moreover, growing degree days (GDDs) on a 10 °C-based temperature were calculated in the period 1 April – 31 October, according to Amerine and Winkler [6].

## 2.3 Health status of vine organs

During the three years of experimentation (2020-2022), the downy mildew attacks were monitored from leaf development to ripening, considering the infection reference points at BBCH 63 for leaves and BBCH 73 for clusters. The disease incidence was assessed as the percentage of leaves/clusters affected by symptoms (%) on 100 leaves and 100 clusters in four random replicates per treatment, out of approximately 200 vines. The severity of the attack was evaluated following the indications provided by the EPPO guidelines, based on the extension of the leaf surface/berries in the cluster affected by symptoms [7] as five classes (0 = 0%, 1 = max 5%, 2 = 5-25%, 3 = 25-50%, 4 = more than 50%).

## 2.4 Leaf gas exchange and chlorophyll fluorescence

The effect of the treatments on the physiology of the vine leaves (gas exchange and photosynthetic efficiency) was evaluated in three phenological phases for each vegetative season (BBCH 53, BBCH 65, BBCH 75) [8]. The measurements were performed before (T0), 24 (T24)

and/or 48 (T48) hours after the treatments, between 10:00 and 12:00. Leaf gas exchanges were detected as net photosynthesis (A), transpiration rate (E), stomatal conductance (gs), and water use efficiency was calculated (WUE, as A/E) on six adult leaves of the cluster area per treatment, using an infrared gas analyzer (Ciras3-PP Systems) equipped with a leaf chamber. CO<sub>2</sub> flow was set at 400 ppm, relative humidity to ambient level, saturating light at 1500 μmol m<sup>-2</sup> s<sup>-1</sup>.

The chlorophyll fluorescence was measured using a Handy Pea chlorophyll fluorimeter (Hansatech Instruments) on eighteen adult leaves of the cluster area per treatment. The leaves were dark-adapted for 30 minutes with proper plastic clips and then subjected to a saturating light pulse (duration 1 s, intensity 3000 μmol/m<sup>2</sup>/s, wavelength 650 nm) to collect the data. The parameters considered were the following: minimum fluorescence (F0), maximum photochemical efficiency (quantum yield) of photosystem II (Fv/Fm), and performance index (PI).

## 2.5 Yield components and grape quality

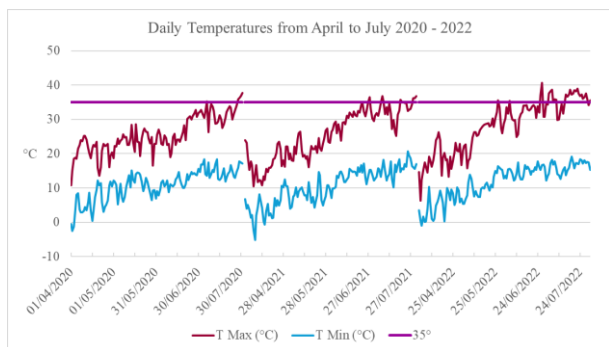
Grape production and quality were assessed for three consecutive vintages (2020-2022). At the harvest, the main production traits were determined: yield/vine (kg), cluster number/vine, cluster weight (g), and berry weight (g). Subsequently, three replicates of 150 berries were collected and pressed to assess grape technological maturity (sugar content, titratable acidity, pH) according to the OIV methodology [9]. Additional grape samples (three replicates of 150 berries) were used to evaluate berry phenolic content. The extraction of the polyphenolic fraction from berry skin was performed according to Di Stefano and Craverio [10]. The total polyphenol content (mg/kg) was determined using the Folin-Ciocalteu method, whereas the determination of anthocyanins and flavonoids (mg/kg) was carried out spectrophotometrically as described by Di Stefano and co-workers, with some revisions [11-12].

## 3 Results and discussion

### 3.1 Weather data evaluation and annual phytosanitary management

The three seasons in which the experimental trial was carried out had quite different weather trends (Fig. 1 and Table 2). The years 2020 and 2021 had similar temperatures as GDDs and the number of days with temperatures above 35 °C (none above 40 °C). In 2021, there was a case of late frost between 7 and 8 April (T min -5.1 °C) that caused problems of sprouted tips freezing with consequences on the production of the current year, but also on the following one. The year 2022 was characterized by higher temperatures, higher accumulation of GDDs (about 300 more than in previous

years), and double the number of days with temperatures above 35 °C (one above 40 °C), with respect to 2020 and 2021. The precipitation pattern was similar between 2020 and 2022; 2021 was drier, with approximately 200 mm less than the other two years. Given the climatic differences, the phytosanitary treatments varied between years, in number, and period, as suggested by the DSS used: 10 in 2020 (first treatment on April 27, the last on July 21), 11 in 2021 (first treatment on May 18, the last on August 08), and 8 in 2022 (first treatment on May 09, the last on July 18).



**Fig. 1.** Daily temperatures (°C) of the period April-July for the years 2020-2022.

**Table 2.** Bioclimatic indices calculated for the three years considered.

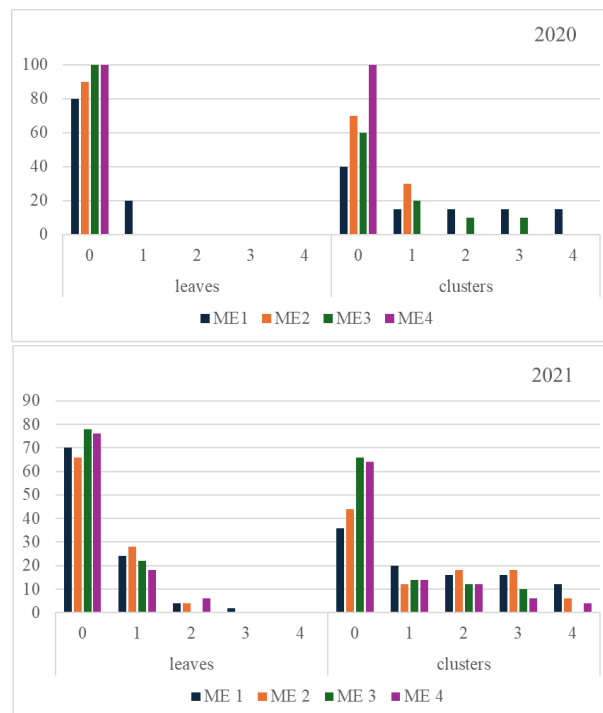
	2020	2021	2022
Number of days with temperature ≥ 35 °C	16	18	32
Days ≥ 40 °C	0	0	1
GDDs (1 April – 31 October)	1850	1881	2178
Annual rainfall (mm)	1022	842	1044

### 3.2 Health status of vine organs

The data analysis of the phytosanitary monitoring carried out on leaves (BBCH 63) and clusters (BBCH 73) highlighted statistically significant differences between the experimental treatments (Figure 2). In 2022, the weather conditions were unfavorable for the downy mildew, and no damage was detected during the field surveys, not even in the untreated control ME 1.

In 2020, the leaves were healthy in all treatments, with few cases in the untreated control and low severity of the attack. In 2021, the disease was more widespread but with low incidence (mainly class 1). On the contrary, the clusters were considerably affected by

*Plasmoparaviticola* in both years 2020 and 2021 with different levels of damage, but either way, the fungal infection was kept under control by the treatments. It is important to note that no significant differences were found between the winery standard phytosanitary management and the one with reduced use of copper (50%) with the addition of polyphenols (ME 3).



**Fig. 2.** Health status of leaves (BBCH 63) and clusters (BBCH 73) in 2020 (top) and 2021 (bottom) of the vines subjected to experimental treatments. Classes of severity of *Plasmoparaviticola* infection on leaves and clusters: 0 = 0%, 1 = max 5%, 2 = 5-25%, 3 = 25-50%, 4 = more than 50%.

### 3.3 Leaf gas exchange and photosynthetic efficiency

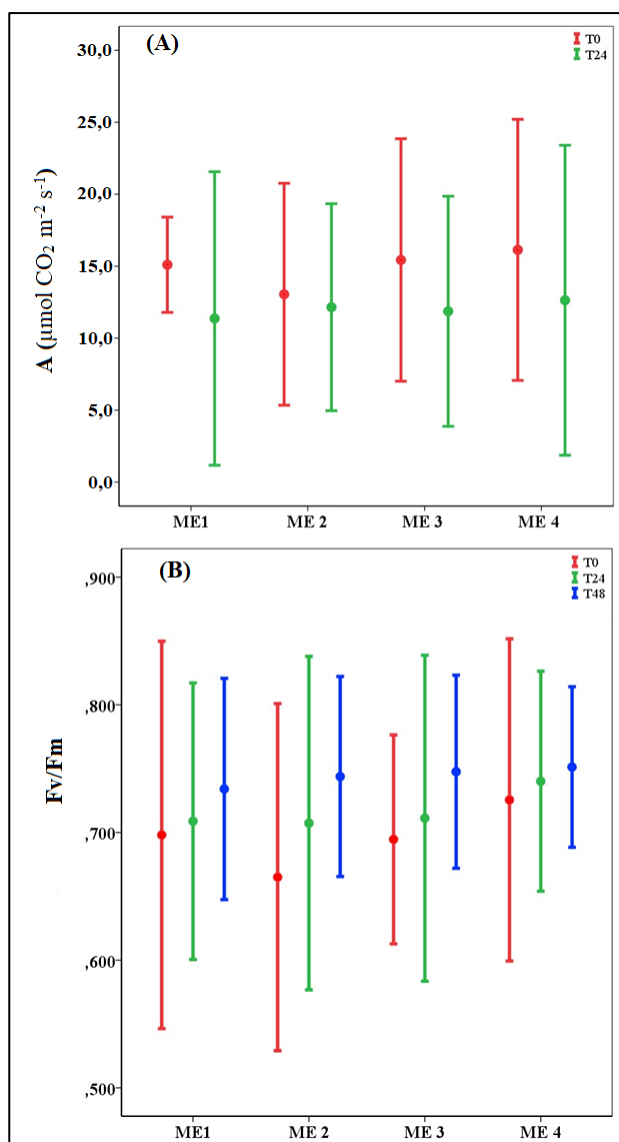
The measurement of physiological parameters on the vines belonging to the four experimental treatments carried out in different phenological phases gave univocal results in the three years under investigation. In fact, comparing the values of gas exchange and photosynthetic efficiency, no significant differences were detected between treatments. This is why the indicative results of one season (2021) are reported (Figure 3). This result denotes that no physiological alterations ever arose in the leaves due to the high concentrations of polyphenols in the products used.

### 3.4 Grape production and quality

The harvest in the three years considered took place on 14 September 2020, 9 September 2021, and 5 September 2022. The increasingly early dates confirm the general

trend dictated by environmental conditions linked to climate change that wine companies must comply with to organize cellar operations.

In general, there was no significant influence on the yield components attributable to the phytosanitary treatments (Table 3). The supply of polyphenols, therefore, had no consequences on the vine production and the quantitative characteristics of clusters and berries. A significant effect of the vintage emerged on grape production and the number of clusters by comparing 2022 with the previous years. The only appreciable differences among treatments emerged in 2021, with a higher yield per vine and cluster number in ME 4 and ME 3 treatments compared to ME 1. This result is a direct consequence of the health conditions of the Merlot cluster, which, as previously indicated, were more protected by the winery standard treatment and the one with 50% copper added with OMWW and OLD polyphenols.



**Fig. 3.** A: net photosynthesis (A) at timings T0 and T24 recorded at BBCH 75 (15-16 July 2021). B: maximum photochemical efficiency of photosystem II (Fv/Fm) at timings T0, T24, and T48 detected at BBCH 65 (26-28 May 2021). Data subjected to one-way ANOVA; no significant differences emerged.

**Table 3.** Yield components assessed at the harvest on Merlot grapes. Data subjected to one-way ANOVA (\*\* = p<0.005; \* = p<0.05; ns = not significant) and LSD post-hoc test. The values in red flanked by different letters indicate statistically significant differences. Lines 1-6-11: average values for the single vintages.

	Yield/vine (kg)	Clusters/vine (n)	Cluster weight (g)	Berry weight (g)
2020	<b>1,47 a</b>	<b>10,54 a</b>	<b>142,18</b>	<b>1,15</b>
ME 1	0,91 a	10	90,10 a	1,09
ME 2	1,25 ab	11,17	117,66 a	1,13
ME 3	1,72 bc	10,33	171,26 b	1,22
ME 4	2,01 c	10,67	189,69 b	1,18
2021	<b>1,38 a</b>	<b>11,17 a</b>	<b>121,31</b>	<b>1,19</b>
ME 1	0,92	9,33	98,07	1,21
ME 2	1,77	10	158,34	1,05
ME 3	1,14	10,83	105,61	1,23
ME 4	1,71	14,5	123,24	1,26
2022	<b>2,06 b</b>	<b>14,35 b</b>	<b>145,61</b>	<b>1,16</b>
ME 1	2,27	13,75	165,15	1,21
ME 2	2,41	15,08	166,58	1,26
ME 3	1,66	14,5	113,57	1,03
ME 4	1,91	14,08	137,13	1,15
Year	*	**	ns	ns
Treatment	ns	ns	ns	ns

No differences were observed in the chemical characteristics of the must obtained from the grapes harvested from the vines subjected to the experimental treatments (Table 4). The only result to highlight is a difference in the sugar content that was higher in 2020 compared to the following vintages, when the accumulation of solutes in the berries probably stopped in the last stages of ripening, especially in 2022 due to the high temperatures of the ripening period.

**Table 4.** Must characteristics at harvest of Merlot grapes. Data subjected to one-way ANOVA (\*\*\*) = p<0.001; ns = not significant) and LSD post-hoc test. The values in red flanked by different letters indicate statistically significant differences. Lines 1-6-11: average values for the single vintages.

	Sugar content °Brix	Titrateable acidity (g/L)	pH
2020	<b>25,5 b</b>	<b>5,14</b>	<b>3,51</b>
ME 1	25,3	4,88	3,57
ME 2	25,3	5	3,49
ME 3	25,8	5,18	3,5
ME 4	25,6	5,5	3,48
2021	<b>23,8 a</b>	<b>5,21</b>	<b>3,45</b>
ME 1	24,8	4,86	3,44
ME 2	24,8	4,63	3,57
ME 3	23,4	5,44	3,4
ME 4	24,2	5,92	3,38
2022	<b>22,8 a</b>	<b>5,1</b>	<b>3,47</b>
ME 1	22,2	5,47	3,4
ME 2	22,6	5,37	3,43
ME 3	23	4,75	3,51
ME 4	23,2	4,82	3,55
Year	***	ns	ns
Treatment	ns	ns	ns

The concentration of skin phenolic substances was generally homogeneous among the experimental treatments, even if in 2021, higher concentrations of total polyphenols were found in the treatment ME 2 and ME 3 which included OMWW and OLD polyphenols, that possibly impacted on the total concentrations of skin anthocyanins and polyphenols (Table 5).

**Table 5.** Berry skin phenolic content of Merlot grapes. Data subjected to one-way ANOVA (\*\*\* =  $p < 0.001$ ; ns = not significant) and LSD post-hoc test. The values in red flanked by different letters indicate statistically significant differences. Lines 1-6-11: average values for the single vintages.

	Total anthocyanins (mg/kg)	Total flavonoids (mg/kg)	Total polyphenols (mg/kg)
2020	<b>1020 b</b>	<b>3154</b>	<b>3056 b</b>
ME 1	1009	3311	3212
ME 2	1053	3231	3202
ME 3	1037	3110	2990
ME 4	983	2964	2819
2021	<b>1130 b</b>	<b>3016</b>	<b>2660 a</b>
ME 1	1106	3001	2303 a
ME 2	1011	2832	3001 b
ME 3	1335	3068	2917 b
ME 4	1070	3165	2419 a
2022	<b>683 a</b>	<b>3086</b>	<b>3091 b</b>
ME 1	741	3176	3161
ME 2	716	3097	3090
ME 3	628	3161	3156
ME 4	647	2911	2958
Year	***	ns	***
Treatment	ns	ns	ns

## 4 Conclusions

The research allowed us to test the effectiveness against downy mildew of the polyphenols contained in intra-company olive processing waste (olive mill wastewater and olive leaf decoction) added to a conventional

phytosanitary treatment based on copper (5g/L), but with reduced concentrations.

The results collected in three years of experimentation (2020-2022) demonstrated that the incidence of downy mildew was kept under control by using polyphenol-based treatments and lower contents of copper in ME 2, but, above all, ME 3 with comparable protection to the winery standard treatment ME 4 (Cu at 5 g/L). The treatments based on different mixes of copper and polyphenols did not affect the physiological and productive performance of the vines, nor the technological characteristics of the must or the berry skin polyphenols.

In conclusion, the data presented demonstrate that it is possible to exploit the properties of waste products for sustainable integrated pest management, boosting the circular economy, lowering environmental impact, and with minimal costs for both an olive and a wine-growing farm.

The authors want to thank Dr. Alceo Orsini, Dr. Donato Ciofini, and the owners of “Fattoria La Violla” farm in Castiglion Fibocchi (Arezzo, Tuscany, Italy) that kindly financed and hosted the experimental trials.

## References

1. J. Onofre, *Chapter 24 - European wine policy framework—The path toward sustainability* (Academic Press, 2022)
2. R. Perria, A. Ciofini, W.A. Petrucci, M.E.M. D’Arcangelo, P. Valentini, P. Storchi, G. Carella, A. Pacetti, L. Mugnai, A study on the efficiency of sustainable wine grape vineyard management strategies. *Agronomy*. **12**(2), 1–17 (2022)
3. F. Sciubba, L. Chronopoulou, D. Pizzichini, V. Lionetti, Fontana, C., R. Aromolo, S. Socciarelli, et al., Olive mill wastes: a source of bioactive molecules for plant growth and protection against pathogens. *Biology*. **9**(12), 1–20 (2020)
4. S. Dermeche, M. Nadour, C. Larroche, F. Moulati-Mati, P. Michaud, Olive mill wastes: Biochemical characterizations and valorization strategies. *Process Biochem*. **48**(10), 1532–1552 (2013)
5. V.G. Kontogianni, I.P. Gerothanassis, Phenolic compounds and antioxidant activity of olive leaf extracts. *Nat. Prod. Res.* **26**(2), 186–189 (2011)
6. M.A. Amerine, A.J. Winkler, Composition and quality of musts and wines of California grapes. *Hilgardia*, **15**, 493–673 (1944)
7. European and Mediterranean Plant Protection Organisation, EPPO standards for the efficacy evaluation of plant protection products. Available online: <https://pp1.eppo.int/standards/PP1-031-3>
8. D.H. Lorenz, K.W. Eichhorn, H. Bleiholder, R. Klose, U. Meier, E. Weber, Growth stages of the grapevine: phenological growth stages of the

- grapevine (*Vitis vinifera* L. *ssp. vinifera*) - Codes and descriptions according to the extended BBCH scale. Aust. J. Grape Wine Res. **1(2)**, 100-103 (1995)
9. International Organisation of Vine and Wine - OIV. Second Edition of the OIV descriptor list for grape varieties and *Vitis* species. [www.oiv.int/public/medias/2274/code-2e-edition-finale.pdf](http://www.oiv.int/public/medias/2274/code-2e-edition-finale.pdf)
  10. R. Di Stefano, M.C. Cravero, Metodi per lo studio dei polifenoli dell'uva. Riv. Vitic. Enol. **44**, 37-45 (1991)
  11. R. Di Stefano, S. Guidoni, La determinazione dei polifenoli totali nei mosti e nei vini. Vignevini. **16**, 47-52 (1989)
  12. A. Asproudi, A. Ferrandino, F. Bonello, E. Vaudano, M. Pollon, M. Petrozziello, Key norisoprenoid compounds in wines from early-harvested grapes in view of climate change. Food Chem. **268**, 143–152 (2018)