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New bio-protector for combating heat and light stress across different grape cultivars and environments

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ABSTRACT

Direct sunlight on berries during ripening enhances flavonoid accumulation in the skin, acting as a defence against UV damage and lipid peroxidation. However, these flavonoids, particularly quercetins, influence bitterness, astringency, and deposit formation in wines. This study evaluated the effectiveness of a new bio-protector (NBP), composed of β-carotene and α-pinene, in reducing flavonol levels in grape skins across cultivars and locations, applied by spraying the basal part of the canopy, completely wetting all leaves and grapes. In 2023, trials were conducted in non-irrigated vineyards with Sangiovese and Trebbiano Toscano in Umbria, and Grillo and Carricante in Sicily. In 2024, Merlot and Grenache in South Australia were included. Thirty vines per vineyard were treated with NBP (2 % in water) at veraison (BBCH 81), while 30 control vines received water. In two vineyards, basal leaf removal was performed a day before NBP application. Gas exchange measurements, yield components, grape composition, and berry phenolic and carotenoid profiles were recorded. NBP-treated leaves showed reduced photosynthesis, transpiration, and stomatal conductance but no impact on yield, cluster weight, or berry weight. NBP delayed technological ripening, reducing sugar accumulation and increasing acidity. Flavonol profiles revealed quercetin-3-O-glucoside (60–74 %) and quercetin-3-O-glucuronide (28–38 %) as dominant, with Sicilian cultivars showing 10 times lower flavonol levels than central Italian cultivars. Basal leaf removal increased quercetin and kaempferol by three–four times. NBP significantly reduced flavonol accumulation in berry skins across cultivars and sites, delaying ripening without causing dehydration or sunburn damage. This could reduce wine bitterness and astringency and allow later harvests, especially in red cultivars. Exogenous β-carotene application did not increase berry skin carotenoids, but the correlation between flavonol accumulation and irradiance was confirmed. NBP's potential to mitigate the impact of rising temperatures on grape and wine quality is promising.

KEYWORDS: flavonols, quercetin, β-carotene, leaf removal

INTRODUCTION

Globally, 2023 was the hottest year on record, with the average air temperature rising 1.32 °C above pre-industrial levels (Schmidt *et al.*, 2024). The author highlights that the planet warmed 0.2 °C more last year than climate scientists had anticipated, given all the known factors. This supports the urgent need for more comprehensive and accurate climatic data. Consequently, the planet is now dangerously close, albeit temporarily, to exceeding the 1.5 °C threshold above pre-industrial levels established by the 2015 Paris Agreement (COP21) on climate change. This situation cannot be changed in a few years; therefore, it is crucial to develop new strategies to preserve, year by year, woody crops and physiological behaviour. Regarding grapes, the main concerns relate to yield, grape composition, bud differentiation, and the storage of reserves. In newly established vineyards, some strategic decisions are gaining relevance to address changing environmental conditions. These include: 1) the implementation of irrigation systems in areas where water is available; 2) prioritising sites at higher elevations above the sea level; 3) avoiding steep parcels with southern exposition to minimise excessive sun exposure; 4) selecting cultivars and rootstocks that demonstrate greater tolerance to dry conditions; and 5) adopting alternative training systems, such as sprawl canopies instead of the traditional Vertical Shoot Positioning (VSP), to protect grapes from direct sunlight and excessive radiation in warm regions (de la Fuente *et al.*, 2016; de la Fuente *et al.*, 2024). In productive vineyards, the adoption of innovative practices can be vital (Palliotti *et al.*, 2013; Palliotti *et al.*, 2014). Among these, the use of biostimulants and/or bioprotectants represents a new and interesting development. In recent decades, research in this area has validated the effectiveness of various natural substances in helping plants cope with challenges linked to rising temperatures and lack of rainfall (Tombesi *et al.*, 2021; Frioni *et al.*, 2020; Del Zozzo *et al.*, 2024). The rise in air temperature, the solar irradiance, and ultraviolet A and B radiations (Herman *et al.*, 2010), increases the cuticular transpiration from the grapes, leading to water loss, and berry shrivelling and sunburn damage, respectively. Furthermore, excessive fruit exposure may increase phenol concentrations beyond desirable levels (Smart *et al.*, 1990). This condition, expressed as brown or necrotic spots on grape skins and, in severe cases, complete desiccation, is becoming increasingly prevalent worldwide (Gambetta *et al.*, 2021). In Italy, sunburn damage appeared at severe levels from around 2010, having been relatively rare before this. By 2013, it was considered a catastrophic event and included in multi-risk insurance policies (European Parliament and Council of the European Union, 2013; Regulation (EU) N. 1305/2013). It is well documented that the direct sunlight on berry skins improves flavonoid biosynthesis and accumulation (Downey *et al.*, 2004; Feng *et al.*, 2015; Pastore *et al.*, 2017). While flavonoid biosynthesis is a natural defensive response to protect berries against UV radiation and lipid peroxidation (Möhle *et al.*, 1985; Mariani *et al.*, 2008; Keller *et al.*, 2010), the balance between beneficial and excessive sun exposure is critical, especially in warm climates. In cooler viticultural regions, enhanced sun exposure is often desirable. However, in warm Mediterranean climates, it can increase flavonol concentrations beyond optimal levels, contributing to excessive bitterness and astringency in the final wine. This serves as a defensive response by plants to environmental stresses, offering protection against UV radiation and lipid peroxidation (Möhle *et al.*, 1985; Mariani *et al.*, 2008; Keller *et al.*, 2010). Friedel *et al.* (2016) found that exposure to sunlight for just eight hours significantly enhanced flavonol biosynthesis in grape berries, with a four-fold increase in the expression of flavonol synthase (VvFLS1) and glycosyltransferase genes (VvGT5 and VvGT6) at the cluster level. Within this context, another photoprotective mechanism involves the cycling of β-carotene and xanthophyll. Under intense light exposure before veraison, berries produce significant amounts of β-carotene, which serves as a precursor for the synthesis of violaxanthin and lutein. These compounds are then used in the xanthophyll cycle to facilitate the dissipation of excessive energy. The greater level of berry β-carotene, violaxanthin and lutein at veraison, the more effective the associated non-photochemical quenching, potentially reducing enzymatic browning (Gambetta *et al.*, 2021). Recent studies have shown that basal leaf removal, which exposes clusters to direct sunlight, significantly increases the flavonol content in the berry skin (Feng *et al.*, 2015; Allegro *et al.*, 2019). Price *et al.* (1995) found that Pinot noir berry skins exposed to full sunlight contained 10 times more quercetin-*O*-glucoside compared to those grown in shaded conditions. However, this practice is generally not recommended for warm climates. Similarly, in Sangiovese wine, intense basal leaf removal at flowering resulted in higher levels of total flavonols and quercetin glycoside derivatives compared to undefoliated vines (Romboli *et al.*, 2018). Among flavonols, quercetin is the most abundant in grape skin. It appears as a yellow, crystalline solid that is insoluble in water, slightly soluble in alcohol and is characterised by a distinctly bitter and astringent taste (Weast, 1979). In the grape skin, flavonols are bound to various sugars (glycosides), with quercetin-3-*O*-glucoside and quercetin-3-*O*-glucuronide being the most abundant (Cheynier & Rigaud, 1986). Other minor flavonols such as kaempferol, laricitin, myricetin, syringetin and isorhamnetin can also be detected (Mattivi *et al.*, 2006). In wine, flavonols significantly influence bitterness and astringency (Allegro *et al.*, 2019; Preys *et al.*, 2006; Ferrer-Gallego *et al.*, 2014). In red wines, they also play a crucial role in co-pigmentation and colour stability (Boulton, 2001). During fermentation, the hydrolysis of flavonols in their glycoside forms begins, leading to the production of aglycones. These aglycones can develop yellow precipitates and deposits, which may accumulate in barrels during ageing and bottles during the refinement. In recent decades, a significant quality issue has emerged: the accumulation of high levels of quercetin in wines. It has occurred in bottles of premium Sangiovese wine, the most widely cultivated red grape cultivar in Italy, covering

52,000 hectares of vineyards (approximately 8 % of the total area in the country). A voluminous yellow precipitate of aglycon quercetin has been detected in these wines, making them non-marketable (Lanati *et al.*, 2014). While this precipitation is primarily an issue for red wines due to extended skin contact during maceration, high flavonol levels in white grapes can also negatively impact wine quality by increasing bitterness and the potential for browning (Somers & Ziemelis, 1985; Macheix *et al.*, 1991). To address the issue of increased flavonol levels in grapes, which can lead to phenolic precipitation in wine, this study aimed to evaluate the efficiency of a new bioprotector (NBP), composed of a mix of β-carotene and α-pinene, in reducing the flavonol content in grape berry skins. The study was conducted across different grapevine cultivars (cvs.) and environments, including central and southern Italy and South Australia.

MATERIALS AND METHODS

1. Plant material, site location, and experimental layout

The trials were carried out in non-irrigated vineyards in Umbria (central Italy) and Sicily (southern Italy) in 2023 and in Adelaide (South Australia) in 2024.

1.1. Umbria region

Red cv. Sangiovese and white cv. Trebbiano Toscano grapes, both grafted onto 420 A rootstock, were used in an 18-year-old vineyard located near Perugia (42° 57' N 12° 24' E, elevation 405 m a.s.l.), with N–S row orientation. Both cvs. were trained to a vertically shoot-positioned, spur-pruned cordon trellis with a bud-load of about 8–10 nodes per vine. Vine spacing was 2.5 m × 1.0 m (inter- and intra-row, 4,000 vines per hectare), and the cordon was trained 0.9 m above ground with three pairs of surmounting catch wires for a canopy wall extending about 1.2 m above the permanent cordon. Pest management was carried out according to local standard practices, and shoots were mechanically trimmed when most of them started to outgrow the top wire.

1.2. Sicily region

Two autochthonous white cvs. Grillo and Carricante, both grafted onto 140 Ru rootstock, were used in a 12-year-old vineyard located on the Etna volcano (37° 41' N 15° 9' E, elevation 252 m a.s.l.). Both cultivars were trained to vertical-shoot positioned, spur pruned permanent cordon at 0.7 m from the soil with a bud-load of about 8–10 buds per vine. Vine spacing was 2.5 m × 1.2 m (inter- and intra-row) (3,330 vines per hectare).

1.3. South Australia

Two red grape varieties were used in this study. The first cultivar, Merlot, grafted onto Schwarzmann rootstock, was sourced from a 17-year-old vineyard located at the Coombe vineyard of the University of Adelaide, Waite Campus (34.97° S 138.63° E). Vines were trained to a vertical-shoot positioned, spur-pruned permanent cordon system at 0.9 m from the ground with a bud-load of about 55–65 per vine. Vine spacing was 3 m × 1.5 m (inter- and intra-row) (2,222 vines per hectare). The second cultivar, Grenache, included fourteen 3-year-old potted (6 L volume) vines grown on their own roots in a greenhouse at the Waite Campus, University of Adelaide, Australia. Each vine was pruned to retain 3–4 spurs with 1 bud each, and all shoots were oriented upright using stakes.

1.4. NBP treatment

In all vineyards and cultivars included in the experiment, three uniform rows were selected to establish a complete randomised block design, with each row serving as a block. Ten vines within each block were randomly assigned to NBP treatment, while another 10 vines were sprayed with water and used as controls (C). The NBP treatment was applied once, just before veraison at the BBCH 81 growth stage (beginning of ripening). Following the supplier's recommendations based on previous trials, a 2 % NBP solution was prepared in water and gently stirred slowly to form an emulsion. Using a portable electric pump, the solution was sprayed onto all the clusters and leaves in the basal part of the canopy. NBP is a formulation based on a terpenic polymer matrix, derived from distilled conifer resins, containing β-carotene (~0.015 %) and α-pinene (~88 %) as active components. In the vineyards of Sangiovese (Umbria region, Italy) and Merlot (South Australia), defoliation treatments were organised on an additional five vines per block. Basal leaves were removed up to the 6th or 7th node from the base of the shoots, the day before NBP application. For the Grenache potted vines in South Australia, seven vines were treated with NBP at a 2 % concentration at full veraison, while the remaining seven vines were sprayed with water and used as controls (C). At all experimental sites, weather conditions were monitored using an automatic meteorological station located near the vineyards to ensure consistent environmental data collection and record daily maximum and minimum air temperatures and rainfall. Data were subsequently used to calculate the accumulated heat, expressed as growing degree days (GDD), with a base of 10 °C from 1 April to 30 September.

2. Gas exchange measurements

2.1. Umbria region

Five weeks after NBP treatment, single-leaf gas exchange measurements were taken on clear days for both NBP and C vines. In the morning (09:00 to 10:00), readings were recorded on the east side of the canopy, and in the afternoon (15:00 to 16:00), measurements were taken on the west side. These measurements were performed using a portable, open system, LCA-3 infrared gas analyser (ADC Bio Scientific Ltd., Herts, UK). The system was equipped with a broad leaf chamber featuring a 6.25 cm^2 window, and all readings were taken at ambient relative humidity with an airflow adjusted to 350 mL min^{-1}. For each of the three replicates per treatment, one fully expanded leaf was selected from each of the 10 vines (totalling 10 leaves per replicate). The selected leaves were located at nodes 8 to 10 above the distal cluster on a primary shoot and were selected under saturating

light conditions (PAR > 1,500 µmol photons m^{-2} s^{-1}). Net photosynthesis (Pn), transpiration rate (E) and stomatal conductance (g_s) were calculated from the inlet and outlet CO_2 and H_2O relative concentrations. Simultaneously with the gas exchange readings, leaf temperature was measured using an infrared thermometer (Model TM909L9, Assi-control, Varese, Italy).

2.2. Sicily region

Gas exchange measurements (Pn and E) were conducted in four weeks and nine weeks after the treatment, between 12:00 and 14:00, using an LCA-4 infrared gas analyser (ADC Bio Scientific Ltd., Herts, UK). On the same dates and on the same plants, midday leaf water potential and chlorophyll fluorescence analysis were performed. Leaf water potential was measured using a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA), and chlorophyll fluorescence was analysed with a portable continuous excitation fluorimeter (Handy-PEA, Hansatech Institute Ltd., Norfolk, UK). For all measurements, 10 middle leaves per treatment and per measurement date were sampled.

2.3. South Australia

Gas exchange measurements were conducted on leaves at the 8th to 10th node, 10 and 24 days after NBP spraying, on both undefoliated and defoliated vines, between 12:00 and 14:00. A LI-600 Porometer/Fluorometer (Li-COR, Lincoln, Nebraska, USA) was used for measurements. Ten leaves per treatment and measurement date were used.

The differences in the timing, frequency, and specific parameters of gas exchange measurements across the sites were due to the multi-centre nature of the study. These variations reflect the specific logistical constraints, available instruments, and standard operating protocols of the collaborating research teams at each location. However, in all cases, the Pn measurements were done at saturation light.

3. Yield and grape composition

Grapes from the Umbria region were harvested on 12 September 2023, while grapes from both cultivars in the Sicily region were harvested on 20 September 2023 and in South Australia on 27 February 2024. In all vineyards, grapes from the 10 vines in each of the three replicates per treatment (30 vines per treatment) were individually harvested, and the number of clusters per vine as well as the crop weight were recorded. The average cluster weight was then calculated. At harvest, three samples of 250 berries per treatment (750 berries in total) were randomly collected, and their fresh weight was recorded. After crushing, the soluble solids content (°Brix) in the must was measured using a temperature-compensating refractometer. Titratable acidity was determined by titration with 0.1 N NaOH to an end point of pH 8.2 and expressed as g/L of tartaric acid equivalent. The must pH was measured using a PHM82 standard pH meter (Radiometer, Copenhagen, Denmark). Total anthocyanin and phenolic contents, expressed as mg/cm^2 skin, were determined on the skin of 50 berries per treatment following the methods of Ough and Amerine (1988) and Slinkard and Singleton (1977), respectively. The phenolic composition of the berry skin, expressed as mg/kg of berry skin, was measured by high-performance liquid chromatography (HPLC) according to Ritchey and Waterhouse (1999). For flavonol extraction, three biological replicates were prepared from the berry skin powder. For each replicate, 5 g of powder was suspended in 50 mL of an ethanol:water (70:30, *v/v*) extraction buffer. The mixture was incubated for 24 hours at 4 °C in the dark with continuous agitation. Following maceration, solid debris was pelleted by centrifugation at 5,000 × g for 10 minutes, and the clear supernatant was collected for subsequent analysis. A 10 mL juice sample was filtered using syringe filters (0.22 µm pore size, 33 mm diameter, Sigma-Aldrich, Italy) and then diluted 1:20 in ultrapure water (Milli Q-Water® system, Merck Millipore, Burlington, MA, USA) for analysis. A 1 mL aliquot of diluted juice was transferred into a 2 mL amber glass vial with a crew cap for analysis. The HPLC system used was a Dionex (Dionex Corporation, Sunnyvale, CA, USA), controlled by Chromeleon software (version 6.50). The system was equipped with a P680 quaternary pump, a manual injector (Rheodyne) with a 20 µL loop, a TCC-100 thermostatic oven, and a PDA 100 detector (Photodiode Array Detector). A C-18 column (Dionex Acclaim® 120 C18, 5 µm, 4.6 × 250 mm) was maintained at 40 °C, with a mobile phase flow rate of 0.5 mL/min. The mobile phases used were solvent A = 50 mM dihydrogen ammonium phosphate, adjusted to pH 2.8 with orthophosphoric acid; solvent B = 20 % solvent A with 80 % acetonitrile; and solvent C = 0.2 M orthophosphoric acid adjusted to pH 1.5 with NaOH. For the qualitative and quantitative analysis of individual polyphenols, a 5-point calibration curve (0.1–200 mg/L) was prepared for each molecule (Sigma-Aldrich, Milan, Italy). Grape carotenoids were analysed following the method described by Crupi *et al.* (2012). Carotenoid extraction was performed separately on three biological replicates of fresh berries. All procedures were carried out on ice under dim yellow light to prevent light- and temperature-induced degradation. For each replicate, a 50 g sample of fresh berries was homogenised using an Ultraturrax, spiked with 200 µL of β-apo-8-carotenal (183.2 mg/L) as an internal standard, and then diluted with 40 mL of water. The extraction was performed three times using a diethyl ether:hexane (1:1, *v/v*) solution containing 0.1 % BHT as an antioxidant. The first extraction used 40 mL of the solvent, followed by two subsequent extractions with 20 mL each, with the mixture being agitated for 30 minutes each time. The combined organic phases were filtered, evaporated to dryness under vacuum, and the final residue was resuspended in 1 mL of acetone:hexane (1:1, *v/v*) for HPLC analysis. Carotenoids were identified by comparing their UV-visible spectra with those of commercially available standards: β-carotene (Sigma 95 %, synthetic, C-9750), lutein (Sigma 70 %, from alfalfa), and neoxanthin (0234.1) from CaroteNature GmbH (Erlenauweg 17, 3110 Münsingen, Switzerland).

4. Statistical analysis

Experimental data were statistically analysed using one-way ANOVA (SigmaStat software package; Systat Software, Inc., San Jose, CA, USA). Mean comparisons were performed using Student's *t* test at a significance level of $P < 0.05$. One-way ANOVA was chosen because the main objective of this work was to assess the effect of the NBP treatment within each year and grape cultivar, rather than to evaluate the influence of meteorological factors.

RESULTS AND DISCUSSION

1. Meteorological data

1.1. Umbria region

The summer of 2023 was characterised by high daily maximum temperatures, with peaks reaching 28 °C in May, 36.3 °C in June, 38.3 °C in July and August, and 33.2 °C in September (Figure 1A). The accumulated heat totalled 2087 GDD, with 522 GDD in July (25 % of the total) and 473 GDD in August (23 %). There were 25 days with air temperatures exceeding 35 °C, and 26 nights with air temperatures above 20 °C, classified as tropical nights (Vincent *et al.*, 2005). Total rainfall during the growing season amounted to 370.2 mm, with the majority concentrated in May (105 mm) and June (125 mm). Aside from two intense rain events in early July and August, bringing 65.8 and 57.6 mm, respectively, the remainder of the season was very dry.

1.2. Sicily region

The summer of 2023 was warmer compared to that of the Umbria region, with daily maximum air temperatures reaching peaks of 27 °C in May, 36.5 °C in June, 46.5 °C in July, 37.0 °C in August, and 35.0 °C in September (Figure 1B). The total cumulative GDD was 2374, with about 24 % of the total in July (572 GDD) and 22 % in August (513 GDD). There were 22 days with air temperatures exceeding 35 °C and 50 nights with temperatures higher than 20 °C, classified as tropical nights. Total rainfall during the season was only 150 mm, primarily concentrated in May (68 mm) and September (52 mm). Aside from June, which received approximately 30 mm of rain, the rest of the summer, including July and August and the first week of September, was characterised by a lack of rainfall.

1.3. South Australia

The daily maximum air temperatures peaked at 39.7 °C in November and 40.1 °C in January (Figure 1C). The cumulative GDD from 1 September to 28 February, based on a 10 °C threshold, was 1531, with January contributing 24 % (370 GDD) and February 23 % (355 GDD). There were 8 days with air temperatures exceeding 35 °C and only 7 nights with air temperatures above 20 °C (tropical nights). Total rainfall for the period amounted to 245 mm, with the majority falling in November (67 mm), December (67 mm) and January (55 mm). Notably, no rainfall was recorded in February.

Based on the cumulative Growing Degree Days (GDD) and total rainfall for the whole season, the environment in Eastern Sicily was found to be the hottest and driest, while South Australia was the coolest. Photosynthetic efficiency is highly sensitive to temperature variations, particularly maximum air temperatures. When temperatures exceed 35 °C, especially over two–three consecutive days, stomatal closure significantly reduces photosynthesis (Pn), and excitation energy exceeds the capacity for CO_2 assimilation, leading to overreduction of the photochemical components (Osmond, 1994). Prolonged stress conditions also impair repair mechanisms, resulting in photoinhibition, chlorosis and necrosis as leaves are unable to dissipate the excess energy (Palliotti & Poni, 2015). Furthermore, nighttime temperatures above 20 °C hinder carbohydrate export from leaves, promoting end-product feedback downregulation, which reduces Pn the following day (Tombesi *et al.*, 2019), while increasing nighttime respiration (Palliotti *et al.*, 2005). Under such conditions, the lack of water compounds the negative effects of high temperatures and radiation, intensifying these physiological stresses. In both Sicily vineyards, physiological performance was significantly limited during the summer, and the total sugar accumulation in the clusters at harvest was very low.

2. Physiological data

In the Umbria region, as found on Trebbiano vines (data not shown), five weeks after NBP treatment, the sprayed Sangiovese leaves showed a significant reduction in Pn compared to unsprayed leaves, precisely –27 % in the morning and –20 % in the afternoon (Table 1). Consequently, leaf transpiration rate (E) and stomatal conductance (g_s) were also reduced, while leaf temperature remained relatively unchanged. These results were expected, as the compound α-pinene, a terpenic polymer, forms a thin, transparent, and flexible film on leaf surfaces. This film inhibits gas exchange by limiting CO_2 uptake and water vapour loss through the stomata (Palliotti *et al.*, 2013).

In Sicily, on both measurement dates, unlike in the Umbria region, the NBP treatment on cv. Carricante vines did not affect Pn, g_s, midday leaf water potential or the Fv/Fm ratio (Table 2). However, in cv. Grillo, Pn and g_s were significantly higher on 9 August (Table 2). Despite these differences, the photochemical efficiency of PSII remained unaffected in both cultivars and across both measurement dates, as shown by the Fv/Fm ratios consistently above 0.65, with no significant variations observed.

In South Australia, 10 days after NBP spraying, treated Merlot leaves showed a significant reduction in g_s and E values compared to C vines, while leaf vapour pressure deficit (VPD_{leaf}) and leaf temperature remained unchanged (Table 3). As expected, Φ_{PSII}, which reflects the proportion of light absorbed by photosystem II used in biochemistry, decreased by approximately 31 %. Twenty-four days after NBP treatment, none of the analysed parameters differed from C vines (Table 3). Interestingly, at both measurement dates, g_s, E, and Φ_{PSII} values in leaves from defoliated vines

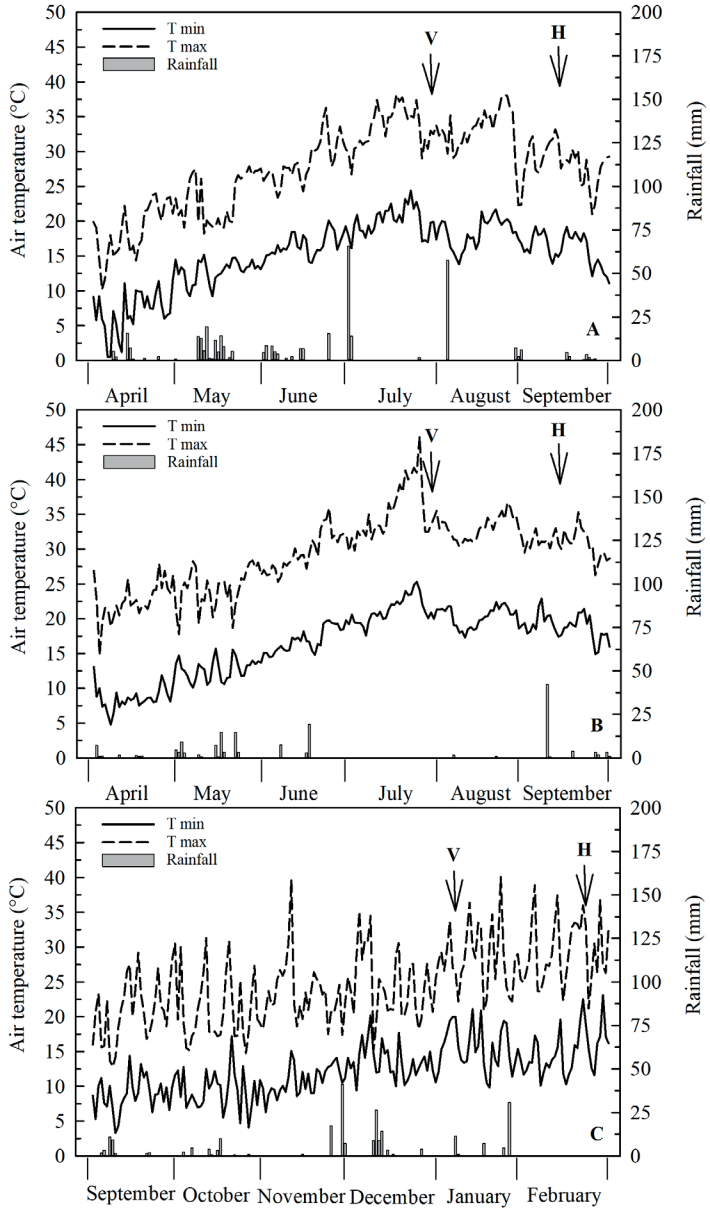

FIGURE 1. Seasonal trends of maximum and minimum air temperatures and rainfall recorded in vineyard sites across different regions: (A) from April to September 2023 in Sangiovese and Trebbiano Toscano vineyards located in Umbria region (Deruta, Perugia); (B) from April to September 2023 in Grillo and Carricante vineyards located in Sicily region (Etna volcano, Catania); and (C) from September 2023 to February 2024 in Merlot and Grenache vineyards located in Adelaide (South Australia). V = veraison; H = harvest.

TABLE 1. Net photosynthesis (Pn), transpiration rate (E), stomatal conductance (g_s), and mean leaf temperatures (T° leaf) were recorded five weeks after NBP spraying (August, 11) in mid-morning and mid-afternoon on fully expanded leaves from primary shoots of Sangiovese vines (Umbria region).

Treatment	Time	Pn (μmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	g_s (mmol H_2O m^{-2} s^{-1})	T° leaf (°C)
Control	10.00–11.00	10.2 ± 0.5 a	2.48 ± 0.2 a	242 ± 11 a	30.4 ± 0.34 a
NBP		7.3 ± 0.8 b	2.14 ± 0.2 a	210 ± 19 a	29.2 ± 0.35 a
Control	15.00–16.00	8.6 ± 1.2 a	3.08 ± 0.3 a	190 ± 14 a	35.4 ± 0.16 a
NBP		6.1 ± 0.7 b	2.63 ± 0.2 a	162 ± 12 b	35.9 ± 0.37 a

Data are presented as mean ± Standard Deviation (SD) (n = 10). Different letters indicate statistically significant differences between treatments by Student's t test ($P < 0.05$ level).

TABLE 2. Net photosynthesis (Pn), stomatal conductance (g_s), midday leaf water potential, and maximum quantum yield of PSII (Fv/Fm) were recorded monthly after NBP spraying on fully expanded leaves from primary shoots of the cv. Carricante and Grillo vines (Sicily region).

Data of measurement	Treatment	Pn (µmol CO_2 m^{-2} s^{-1})	g_s (mmol H_2O m^{-2} s^{-1})	Midday leaf water potential (MPa)	Fv/Fm (arbitrary unit)
CARRICANTE					
August, 9 (four weeks after treatment)	Control	5.3 ± 1.3 a	130 ± 26 a	–1.35 ± 0.13 a	0.66 ± 0.07 a
	NBP	4.2 ± 1.5 a	80 ± 25 b	–1.32 ± 0.22 a	0.69 ± 0.08 a
September, 13 (nine weeks after treatment)	Control	3.3 ± 0.6 a	70 ± 20 a	–1.45 ± 0.21 a	0.68 ± 0.09 a
	NBP	2.3 ± 1.8 a	52 ± 18 b	–1.36 ± 0.21 a	0.65 ± 0.05 a
GRILLO					
August, 9 (four weeks after treatment)	Control	5.4 ± 0.4 a	42 ± 6 a	–1.59 ± 0.16 a	0.65 ± 0.08 a
	NBP	3.7 ± 0.5 b	32 ± 3 b	–1.57 ± 0.12 a	0.67 ± 0.07 a
September, 13 (nine weeks after treatment)	Control	2.1 ± 0.8 a	32 ± 3 a	–1.64 ± 0.05 a	0.75 ± 0.08 a
	NBP	2.0 ± 0.6 a	30 ± 2 a	–1.70 ± 0.06 a	0.79 ± 0.07 a

Data are presented as mean ± SD (n = 10). Different letters indicate statistically significant differences between treatments by Student's t test ($P < 0.05$ level).

TABLE 3. Stomatal conductance (g_s), transpiration rate (E), leaf vapour pressure deficit (VPD), leaf temperature (T°) and quantum yield of fluorescence (ΦPSII) recorded 10 and 24 days after NBP spraying on fully expanded leaves from the central part of primary shoots control and subjected to basal leaf removal in Merlot vines (South Australia). Data are presented as mean ± SD (n = 10). For each treatment, different letters indicate statistically significant differences between treatments by Student's t test ($P < 0.05$ level).

Data of measurement	Treatments	g_s (mmol H_2O m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	VPD leaf (kPa)	T° leaf (°C)	Φ_{PSII}
February, 12 (10 days after NBP-treatment)	Control	140 ± 18 a	4.42 ± 0.49 a	3.71 ± 0.20 a	34.3 ± 0.71 a	0.57 ± 0.09 a
	NBP	90 ± 21 b	3.38 ± 0.32 b	3.68 ± 0.49 a	33.8 ± 1.78 a	0.39 ± 0.05 a
	Control defoliated	180 ± 10 a	6.66 ± 0.50 a	3.85 ± 0.23 a	35.2 ± 0.74 a	0.40 ± 0.09 a
	NBP defoliated	190 ± 40 a	6.34 ± 0.91 a	3.53 ± 0.15 a	33.9 ± 0.53 a	0.49 ± 0.02 a
February, 27 (24 days after NBP-treatment)	Control	108 ± 12 a	5.24 ± 1.33 a	4.36 ± 0.28 a	35.4 ± 1.07 a	0.23 ± 0.04 a
	NBP	100 ± 18 a	5.28 ± 1.30 a	4.36 ± 0.28 a	33.4 ± 0.89 a	0.27 ± 0.03 a
	Control defoliated	162 ± 22 a	6.08 ± 1.31 a	4.53 ± 0.15 a	36.5 ± 0.55 a	0.32 ± 0.04 a
	NBP defoliated	170 ± 20 a	6.05 ± 0.56 a	4.73 ± 0.19 a	37.6 ± 0.46 a	0.38 ± 0.06 a

were higher compared to those from undefoliated ones. This finding is likely due to the absence of basal leaves, which allows the remaining medial and apical leaves to make more efficient use of the available water. Across the three study sites, the NBP treatment demonstrated antitranspirant activity, attributed to its polymeric fraction α-pinene. The magnitude of reductions in leaf Pn, E and g_s was more pronounced when g_s exceeded 100 mmol m^{-2} s^{-1} (*e.g.*, in Umbria and cv. Carricante on 9 August in Sicily). Conversely, when g_s below 100 mmol m^{-2} s^{-1} (*e.g.*, in cv. Grillo), or in the presence of defoliation-induced compensatory effects (*e.g.*, defoliated vines in South Australia), the observed impacts were minimal or non-existent. In this context, NBP could represent a useful tool for reducing vineyard water consumption and/or modulating photosynthetic activity under non-limiting conditions, with no adverse effects observed even under subsequent summer stress events.

3. Vine performance and berry composition

At harvest, no symptoms of sunburn damage were visually observed in any of the cultivars and treatments, including on the grapes of the defoliated vines.

3.1. Vine yield

Across cultivars and sites, the NBP treatment did not significantly alter yield components. In Sangiovese, application at the onset of veraison, whether in defoliated or

undefoliated vines, had no effect on yield per vine, cluster weight, or berry weight (Tables 4, 5, 6, 7 and 8). Likewise, in Trebbiano, NBP application did not influence yield or its components, nor did it affect soluble solids, titratable acidity, malic acid, or must pH (Table 5). Similar results were observed in the Sicilian cultivars Grillo and Carricante, where neither cluster nor berry weight was affected by the treatment (Table 6). In Merlot, irrespective of defoliation, NBP application did not modify yield per vine, cluster weight, or berry weight compared with control vines (Table 7). Finally, in Grenache, the treatment had no impact on yield per vine, cluster weight, or berry weight (Table 8). Overall, these findings indicate that, despite its effects on ripening dynamics and phenolic composition, NBP application consistently preserved yield potential across environments and cultivars.

3.2. Grape composition

In Sangiovese, no significant differences were observed between NBP-treated and control vines in undefoliated conditions for soluble solids, titratable acidity, malic acid, must pH, anthocyanin, and polyphenol contents, whereas in defoliated vines the treatment delayed ripening, with lower soluble solids (19.2 *vs* 22.4 °Brix) and anthocyanin levels (0.317 *vs* 0.422 mg/cm^2 skin); compared with defoliated controls, must from treated vines also showed a reduction of 1.4 °Brix and an increase of 0.59 g/L in malic acid (Table 4). Similarly, in Trebbiano, NBP application did not affect soluble solids, titratable acidity, malic acid, or must pH (Table 5). In the Sicilian cultivars, Carricante showed a significant decrease only in total phenols, while Grillo displayed a significant reduction in soluble solids (–1.4 °Brix) together with an increase in titratable acidity (+1.22 g/L), whereas must pH remained unaffected (Table 6).

TABLE 4. Yield component, grape composition, phenolic and carotenoid profiles in berry skin recorded at harvest in Sangiovese vines control and treated with NBP and vines subjected to basal leaf removal and sprayed with NBP or not treated in 2023 (Umbria region). For each parameter, letters indicate statistically significant differences ($P < 0.05$) by Student's *t* test between the Control and NBP treatments within the same defoliation condition (*i.e.*, within the undefoliated group or within the defoliated group).

	Control	NBP	Defoliated control	Defoliated NBP
Cluster per vine (n°)	10.2 a	10.8 a	11.0 a	10.6 a
Yield (kg/vine)	3.15 a	3.26 a	3.39 a	3.24 a
Cluster weight (g)	312 a	304 a	316 a	310 a
Berry weight (g)	2.39 a	2.28 a	2.36 a	2.40 a
Total soluble solids (°Brix)	21.8 a	22.4 a	20.6 a	19.2 b
Titratable acidity (g/L)	6.0 a	6.2 a	5.7 a	5.9 a
Malic acid (g/L)	1.83 a	1.70 a	0.79 b	1.38 a
Must pH	3.28 a	3.29 a	3.25 a	3.24 a
Total anthocyanins (mg/cm^2 skin)	0.411 a	0.422 a	0.331 a	0.317 a
Total phenols (mg/cm^2 skin)	0.842 a	0.818 a	0.992 a	0.960 a
Phenolic composition of the berry skin (mg/kg of skin) Data are means ± SD (n = 3)				
Quercetin-3-*O*-glucoside	757.3 ± 3.7 a	659.8 ± 6.4 b	1,105 ± 44.4 a	771.8 ± 2.7 b
Quercetin-3-*O*-glucuronide	339.4 ± 2.7 a	265.6 ± 5.9 b	968.2 ± 14.8 a	516.8 ± 18.9 b
Kaempferol-3-*O*-glucoside	20.1 ± 0.93 a	16.0 ± 0.78 b	79.9 ± 2.33 a	57.4 ± 1.38 b
Myricetin	n.d.	n.d.	0.52 ± 0.10 a	0.32 ± 0.09 b
Epicatechins	24.5 ± 0.5 a	24.0 ± 0.4 a	33.7 ± 2.2 a	34.5 ± 1.1 a
Catechins	49.0 ± 0.3 a	46.6 ± 0.1 a	60.5 ± 2.4 a	64.2 ± 1.3 a
Polydatins	106.0 ± 7.7 a	93.2 ± 2.1 b	198.1 ± 10.2 a	178.9 ± 11.8 a
Resveratrol	n.d.	n.d.	n.d.	n.d.
β-carotene (mg/g)	0.151 ± 0.04 a	0.142 ± 0.04 a	0.095 ± 0.01 a	0.099 ± 0.02 a
Lutein (mg/g)	n.d.	n.d.	n.d.	n.d.

n.d. = not detected

For each treatment, different letters indicate statistically significant differences between treatments by Student's *t* test ($P < 0.05$ level).

TABLE 5. Yield component, grape composition, phenolic and carotenoid profiles in berry skin recorded at harvest in Trebbiano Toscano vines treated with NBP or Control in 2023 (Umbria region).

	Control	NBP
Cluster per vine (n°)	13.5 a	13.0 a
Yield (kg/vine)	3.05 a	3.34 a
Cluster weight (g)	226 a	257 a
Berry weight (g)	1.50 a	1.47 a
Total soluble solids (°Brix)	22.1 a	21.4 a
Titratable acidity (g/L)	4.0 a	4.5 a
Malic acid (g/L)	0.72 a	0.88 b
Must pH	3.40 a	3.38 a
Phenolic composition of the berry skin (mg/kg of skin) Data are means ± SD (n = 3)		
Quercetin-3-*O*-glucoside	659.4 ± 13 a	560.6 ± 4.0 b
Quercetin-3-*O*-glucuronide	193.7 ± 0.3 a	125.3 ± 3.3 b
Kaempferol-3-*O*-glucoside	32.6 ± 1.7 a	15.4 ± 1.0 b
Myricetin	n.d.	n.d.
Epicatechins	15.0 ± 0.3 a	12.5 ± 0.7 a
Catechins	52.5 ± 2.3 a	55.2 ± 4.9 a
Polydatins	376.8 ± 17.6 a	359.0 ± 9.6 a
Resveratrol	n.d.	n.d.
β-carotene (mg/g berry skin)	0.294 ± 0.14 a	0.312 ± 0.19 a
Lutein (mg/g berry skin)	1.024 ± 0.78 a	2.088 ± 0.62 b

n.d. = not detected

For each treatment, different letters indicate statistically significant differences between treatments by Student's t test ($P < 0.05$ level).

In Merlot, no significant differences were found in must pH, anthocyanin content, or total phenols, although NBP spraying reduced soluble solids in both undefoliated and defoliated vines and maintained higher titratable acidity in undefoliated vines (Table 7). Finally, in Grenache, NBP treatment did not affect must pH but led to a consistent reduction in soluble solids (–3.3 °Brix) and an increase in titratable acidity (+1.9 g/L) (Table 8). Overall, these results indicate that, although the effects varied among cultivars, the most consistent impact of NBP was the reduction of sugar accumulation and the preservation of acidity, leading to a delay in technological ripening under different growing conditions. In both Sicilian vineyards, the total sugar accumulation in clusters at harvest was significantly lower compared to the other sites. Untreated cvs. Grillo and Carricante accumulated approximately 197 and 330 g of sugar per vine, respectively, compared to 687 g/vine for Sangiovese and 674 g/vine for Trebbiano. These differences are likely due to the combination of limited rainfall and consistently high daytime and nighttime temperatures, which impaired Pn during the day and elevated nighttime respiration, thereby reducing overall sugar accumulation.

3.3. Phenolic profile of berry skin

In Sangiovese, NBP treatment compared with control vines led to a significant reduction in specific flavonols in the berry skin, namely quercetin-3-*O*-glucoside (–13 %), quercetin-3-*O*-glucuronide (–22 %), and kaempferol (–20 %), while myricetin was not detected (Table 4). Conversely, epicatechins, catechins, polydatins (a precursor of resveratrol), and β-carotene showed no significant differences between treatments. Basal leaf removal at veraison greatly increased flavonol accumulation: compared with undefoliated vines, quercetin-3-*O*-glucoside increased by 46 %, quercetin-3-*O*-glucuronide by 185 %, and kaempferol by 297 %. Moreover, myricetin was detected in defoliated controls (0.52 mg/kg skin), whereas it was absent in undefoliated vines. In the present study, defoliation also increased epicatechins, catechins, and polydatins compared with undefoliated vines, but when NBP was applied to defoliated vines, all major flavonols were significantly reduced relative to defoliated controls (–30 % for quercetin-3-*O*-glucoside, –46 % for quercetin-3-*O*-glucuronide, –29 % for kaempferol, and –38 % for myricetin). Across all treatments, resveratrol and lutein were not detected, and NBP did not influence

TABLE 6. Yield component, grape composition, phenolic and carotenoid profiles in berry skin recorded at harvest in cvs. Grillo and Carricante vines treated with NBP or Control in 2023 (Sicily region).

	GRILLO		CARRICANTE	
	Control	**NBP**	**Control**	**NBP**
Cluster per vine (n°)	6.2 b	8.6 a	11.7 a	10.3 a
Yield (kg/vine)	0.780 b	1.037 a	1.428 a	1.360 a
Cluster weight (g)	125.4 a	120.6 a	122.1 a	132.3 a
Berry weight (g)	1.98 a	2.11 a	2.26 a	2.13 a
Total soluble solids (°Brix)	25.3 a	23.9 b	23.1 a	22.5 a
Titratable acidity (g/L)	4.05 b	5.27 a	4.29 a	4.54 a
Must pH	3.54 a	3.37 a	3.58 a	3.42 a
Total phenols (mg/L gallic acid)	277.4 b	364.9 a	286.8 b	365.1 a
Phenolic composition of the berry skin (mg/kg of skin) Data are means ± SD (n = 3)				
Quercetin-3-*O*-glucoside	60.3 ± 0.1 a	55.9 ± 3.0 b	107.0 ± 0.5 a	91.3 ± 1.1 b
Quercetin-3-*O*-glucuronide	26.3 ± 0.3 a	22.9 ± 1.1 b	34.1 ± 0.6 a	32.6 ± 0.5 a
Kaempferol-3-*O*-glucoside	7.33 ± 0.2 a	4.71 ± 0.4 b	3.56 ± 0.2 a	2.07 ± 0.1 b
Myricetin	n.d.	n.d.	n.d.	n.d.
Epicatechins	n.d.	n.d.	n.d.	n.d.
Catechins	0.4 ± 0.03 a	0.4 ± 0.03 a	0.7 ± 0.1 a	0.6 ± 0 a
Polydatins	n.d.	n.d.	n.d.	1.0 ± 0.1
Resveratrol	n.d.	n.d.	n.d.	n.d.
β-carotene (mg/g berry skin)	0.701 ± 0.1	0.616 ± 0.06	0.386 ± 0.22	0.421 ± 0.05
Lutein (mg/g berry skin)	n.d.	n.d.	n.d.	n.d.

n.d. = not detected

For each treatment, different letters indicate statistically significant differences between treatments by Student's *t* test ($P < 0.05$ level).

β-carotene levels. However, basal leaf removal decreased β-carotene by 32–37 % compared with undefoliated vines, consistent with Tevini (1996), who showed that excessive solar and UV-B radiation can inhibit carotenoid accumulation. In conclusion, in Sangiovese subjected to basal defoliation, NBP application delayed ripening and decreased flavonol accumulation. In Trebbiano, berries from NBP-treated vines also showed a significant reduction in flavonols compared to controls, including quercetin-3-*O*-glucoside (–15 %), quercetin-3-*O*-glucuronide (–36 %), and kaempferol (–54 %), while myricetin was not detected in either treatment (Table 5). Levels of epicatechins, catechins, polydatins, and β-carotene showed little variation between treatments. Interestingly, the lutein content in berries from NBP-treated vines was doubled relative to controls. This increase could be considered positive, since lutein, along with other carotenoids, is a precursor of norisoprenoid compounds that contribute to the aromatic complexity of wines (Razungles *et al.,* 1993). In summary, while NBP treatment did not affect yield or ripening parameters in Trebbiano, it significantly altered the phenolic profile by reducing flavonol concentrations and simultaneously enhancing lutein levels, with potential positive implications for wine aroma development. In the Sicilian cultivars Grillo and Carricante, the berry skin phenolic profile was also strongly influenced by NBP (Table 6). Both cultivars showed significant decreases in quercetin content, including both glucoside and glucuronide forms, as well as kaempferol, while myricetin was not detected. Catechins showed no appreciable variation, and epicatechins, polydatins, resveratrol, and lutein were not detected in either treatment. Interestingly, in Carricante, NBP led to a modest increase of about 10 % in β-carotene content in the berry skin. Overall, the low quercetin content observed in the Sicilian cultivars may be linked to the region's very high air temperatures, which are likely to promote flavonol degradation and suppress their synthesis, as previously reported by Keller and Hrazdina (1998). Alternatively, these cultivars may be particularly well adapted to high temperatures and rely on other, yet unidentified, mechanisms to protect berry skins from UV radiation and heat stress. Supporting this, Mattivi *et al.* (2006) found similarly low quercetin concentrations in two other Sicilian white grape

TABLE 7. Yield component, grape composition and phenolic profile in berry skin recorded at harvest in Merlot vines control and treated with NBP and vines subjected to basal leaf removal and sprayed with NBP or not treated in 2024 (South Australia). For each parameter, letters indicate statistically significant differences ($P < 0.05$) by Student's *t* test between the Control and NBP treatments within the same defoliation condition (*i.e.*, within the undefoliated group or within the defoliated group).

	Control	NBP	Defoliated control	Defoliated NBP
Cluster per vine (n°)	65 a	61 a	55 a	62 a
Yield (kg/vine)	10.0 a	10.6 a	8.5 a	8.3 a
Cluster weight (g)	155 a	170 a	155 a	160 a
Berry weight (g)	1.58 a	1.50 a	1.34 a	1.44 a
Total soluble solids (°Brix)	27.0 a	25.3 b	26.1 a	24.0 b
Titratable acidity (g/L)	4.6 b	5.7 a	4.9 a	5.1 a
Must pH	3.88 a	3.83 a	3.81 a	3.77 a
Total anthocyanins (mg/cm² skin)	0.349 a	0.320 a	0.324 a	0.333 a
Total phenols (mg/cm² skin)	0.704 a	0.670 a	0.705 a	0.695 a
Phenolic composition of the berry skin (mg/kg of skin) Data are means ± SD ($n = 3$)				
Quercetin-3-*O*-glucoside	353.1 ± 13.3 a	261.2 ± 6.5 a	582.6 ± 22.6 a	368.2 ± 11.5 a
Quercetin-3-*O*-glucuronide	225.4 ± 7.5 a	140.8 ± 4.0 a	308.3 ± 10.2 a	194.6 ± 9.9 a
Kaempferol-3-*O*-glucoside	17.5 ± 0.6 a	12.4 ± 0.3 a	33.9 ± 0.8 a	16.2 ± 1.5 a
Myricetin	n.d.	n.d.	n.d.	n.d.
Epicatechins	19.5 ± 0.9 a	16.5 ± 1.9 a	22.8 ± 1.4 a	19.5 ± 0.9 a
Catechins	33.3 ± 0.7 a	23.1 ± 1.2 a	43.7 ± 4.4 a	33.3 ± 0.7 a
Polydatins	36.0 ± 0.3 a	29.3 ± 0.5 a	59.7 ± 1.9 a	44.5 ± 2.1 a
Resveratrol	0.15 ± 0.09 b	0.56 ± 0.06 a	0.14 ± 0.07 b	0.40 ± 0.03 a

n.d. = not detected

varieties, Inzolia and Catarratto (1.6–3.2 mg/kg of berry), compared with an average of 11.5 mg/kg across 25 other white cultivars. In Merlot, the phenolic profile of the berry skin showed a significant decrease in both quercetin and kaempferol levels in NBP-treated vines, consistent with findings from Italian vineyards across all cultivars studied, while myricetin was not detected in any treatments (Table 7). In accordance with previous results from the Umbria trial, basal leaf removal in Merlot significantly increased all flavonol compounds in the berry skin, highlighting the direct influence of sun exposure in stimulating these metabolites, which likely serve a protective function against UV radiation. In Grenache, NBP treatment did not impact yield per vine, cluster weight, berry weight, or must pH, but it led to a significant reduction in soluble solids (–3.3 °Brix) and an increase in titratable acidity (+1.9 g/L) (Table 8). Moreover, NBP significantly reduced flavonol content in Grenache berry skins, with decreases of 33 % in quercetin-3-*O*-glucoside, 31 % in quercetin-3-*O*-glucuronide, and 37 % in kaempferol, while myricetin was not detected (Table 8). Catechin and epicatechin concentrations were also lower in treated vines, whereas resveratrol content increased by approximately 29 %. These results highlight the complex effects of NBP spraying on phenolic composition, which vary across compounds but consistently point to a reduction in flavonol accumulation across cultivars and growing conditions.

In all three analysed sites, the main flavonols in the berry skins of cvs. Sangiovese, Trebbiano Toscano, Grillo, Carricante, Merlot and Grenache were quercetin-3-*O*-glucoside (ranging from 60 % to 74 %) and quercetin-3-*O*-glucuronide (ranging from 28 % to 38 %), with kaempferol contributing a smaller proportion (2 % to 8 %). Myricetin was not detected in any cultivar. This predominance of quercetins aligns with previous findings in cultivars such as Shiraz and Chardonnay (Downey *et al.*, 2003) Pinot noir (Price *et al.*, 1995), Cinsault (Cheynier & Rigaud, 1986) and a range of other cultivars including Sauvignon blanc, Moscatel, Gewürztraminer, Riesling, Viognier, Cabernet-Sauvignon, Shiraz and Cencibel (Montealegre *et al.*, 2006). A study conducted in northern Italy prior to the pronounced effects of global warming, examining an ampelographic collection of 91 *Vitis vinifera* cultivars, also observed similar

TABLE 8. Yield component, grape composition and phenolic profile in berry skin recorded at harvest in potted vines of cv. Grenache treated with NBP in 2024 (South Australia).

	Control	NBP
Cluster per vine (n°)	4.2 a	4.0 a
Yield (kg/vine)	0.352 a	0.322 a
Cluster weight (g)	78.6 a	80.5 a
Berry weight (g)	1.14 a	1.18 a
Total soluble solids (°Brix)	26.6 a	23.3 b
Titratable acidity (g/L)	5.2 b	7.1 a
Must pH	3.36 a	3.39 a
Phenolic composition of the berry skin (mg/kg of skin) Data are means ± SD (n = 3)		
Quercetin-3-*O*-glucoside	604.9 ± 30.9 a	406.0 ± 17.5 b
Quercetin-3-*O*-glucuronide	184.2 ± 9.9 a	126.9 ± 5.2 b
Kaempferol-3-*O*-glucoside	72.3 ± 0.3 a	45.7 ± 2.4 b
Myricetin	n.d.	n.d.
Epicatechins	46.0 ± 5.4 a	25.0 ± 1.7 b
Catechins	70.9 ± 1.5 a	46.4 ± 4.9 b
Polydatins	42.1 ± 0.3 a	35.3 ± 0.4 b
Resveratrol	0.85 ± 0.1 b	1.10 ± 0.1 a

n.d. = not detected

For each treatment, different letters indicate statistically significant differences between treatments by Student's t test ($P < 0.05$ level).

trends. Mattivi *et al.* (2006) reported that quercetin was the dominant flavonol in 44 % of the 64 red cultivars studied, followed by myricetin (37 %) and kaempferol (6.4 %). Among 27 white cultivars, quercetin was dominant in 81 %, followed by kaempferol in 17 %. In our study, total quercetins constituted 91 % to 98 % of the flavonols. This shift towards quercetin dominance could be attributed to the effects of global warming, as recent decades have seen dramatic increases in daytime and nighttime temperatures, along with greater thermal availability from bud burst to harvest. This is supported by GDD data, with central Italy recording peaks above 2080 GDD and experiencing 26 days with air temperatures exceeding 35 °C. In comparison, South Australia, where environmental conditions were cooler, generally exhibited lower flavonol content in berry skins than central Italy. These findings highlight the influence of regional climatic conditions, particularly temperature, on flavonol profiles in grape berry skins. The low flavonol content found in the two Sicilian cultivars compared to Sangiovese and Trebbiano from central Italy (on average about 10 times lower) could be attributed to the extremely high air temperatures in Sicily (2374 GDD and 25 days with air temperature exceeding 35 °C). Such conditions are known to accelerate flavonol degradation while simultaneously hindering their synthesis (Keller & Hrazdina, 1998). Another hypothesis is that these Sicilian varieties are highly acclimated to elevated temperatures during ripening. This adaptation could involve alternative, yet unidentified, mechanisms to protect the berry skin from UV radiation, suggesting a potential genetic influence. Supporting this hypothesis, Mattivi *et al.* (2006) reported similar findings in 27 white cultivars, where Catarratto and Inzolia, two autochthonous varieties from Sicily, had the lowest amount of quercetin, precisely 1.6 and 3.1 mg/kg of berry, respectively, against an average quercetin content of 12 mg/kg of berry found in the other 25 cultivars. This genetic predisposition, combined with the extreme climatic conditions in Sicily, likely contributes to the observed flavonol profiles. Transforming these data to align with our findings and assuming that the berry skin constitutes approximately 8 % of the berry's fresh weight, we estimated quercetin concentrations of 20 and 38 mg/kg in berry skin, which are three times lower than those observed in Grillo and Carricante in our study. At harvest, across all cultivars examined, the ratio of quercetin-3-*O*-glucoside (a more soluble compound that loses its glucoside during fermentation, becoming the free aglycone (Price *et al.*, 1995) to quercetin-3-*O*-glucuronide ranged from 2.2 to 4.5. This high ratio significantly increases the chromatic and physical instability of the resulting wines, potentially leading to the formation of viscous filaments and yellow deposits (Somers & Ziemelis, 1985). These challenges are particularly problematic in red grape varieties compared

to white ones, as the winemaking process for red wines often involves extended maceration, enhancing the transfer of flavonols from the berry skin to the wine. For example, in Italy, Sangiovese grapes, which are genetically rich in flavonols (Luciano *et al.*, 2025), have shown such issues. In this study, Sangiovese berries contained approximately 1,116 mg/g of flavonols in the skin, comprising 67 % quercetin-3-*O*-glucoside, 31 % quercetin-3-*O*-glucorunide, and 2 % kaempferol. Given that Sangiovese is widely used in the production of premium wines such as Brunello di Montalcino, Nobile di Montepulciano, and Chianti, there is a considerable risk of quercetin precipitate formation during barrel ageing or in bottle. A recent study by Luciano *et al.* (2025), which analysed 32 red wines from 17 grape varieties, found that quercetin concentrations exceeded the critical threshold of 5 mg/L in 25 samples, both at bottling and after eight months of ageing, with the highest levels observed in Sangiovese, Montepulciano and Nero di Troia. Moreover, quercetin-containing precipitates were detected in eight wines. This can cause undesirable colour changes and a decrease in the quality of the finished wine. In 2023, editors of *Wine Spectator* reviewed more than 9,200 wines and ranked "Brunello di Montalcino Argiano 2018" as the top wine of the year. This highlights the importance of managing flavonol stability in such high-value wines. In our study, the white cultivar Trebbiano had a high flavonol content of 885 mg/kg in berry skin, consisting of 74 % quercetin-3-*O*-glucoside, 22 % quercetin-3-*O*-glucuronide, and 4 % kaempferol. However, the risk of precipitate formation in Trebbiano wines is minimal due to the lack of maceration and the brief contact time between skin and must during winemaking. This reduces the transfer of flavonols into the wine, mitigating the risks observed in red wines. However, white grape browning reactions in grapes and wines may also occur (Macheix *et al.*, 1991). Regardless of cultivar, a positive relationship between irradiance and flavonols synthesis and accumulation in the berry skin was confirmed. Basal leaf removal applied at the beginning of veraison in cvs. Sangiovese and Merlot significantly increase the quercetins and kaempferol in the berry skin up to 3–4-fold compared to grapes of undefoliated vines. Our results are consistent with those of Allegro *et al.* (2019), who reported an increase in flavonols in cv. Grechetto Gentile after early defoliation. Considering the results above reported, all management practices able to cover the clusters during the ripening, which reduce the high temperature without limiting the light availability, are recommended, *i.e.*, kaolin spraying, no basal leaf removal, use of shading nets, etc.

CONCLUSION

Regardless of cultivar and site, the application of the new bio-protector NBP (β-carotene and α-pinene mixture) at the onset of veraison on the cluster zone of the canopy did not modify the yield but significantly reduced the accumulation of flavonols in the berry skin without inducing berry dehydration and sunburn damage. These suggest a reduction in the bitterness and the astringency of the resulting wine. At harvest, treated vines showed higher acidity and lower sugar content in the must, leading to wines with reduced alcohol levels. Interestingly, contrary to our initial hypothesis, the application of exogenous β-carotene did not increase its final content in berry skin at harvest.

Regardless of cultivar and site of cultivation, the application of the new bio-protector NBP (β-carotene and α-pinene mixture) at the onset of veraison on the cluster zone of the canopy consistently and significantly reduced the accumulation of flavonols in the berry skin across all tested cultivars and environments. This was achieved without negatively impacting yield components or inducing berry dehydration and sunburn damage, even under the high-exposure conditions of basal leaf removal. In several cultivars, the NBP treatment also delayed technological ripening, resulting in must with higher titratable acidity and lower sugar content, which is conducive to producing wines with lower alcohol levels. These results suggest a reduction in the potential bitterness and astringency of the resulting wine. Interestingly, contrary to our initial hypothesis, the application of exogenous β-carotene did not increase its final content in the berry skin at harvest. Overall, NBP shows significant promise as a practical tool for viticulturists to mitigate the effects of excessive heat and solar radiation, allowing for better management of the phenolic profile and ripening to improve final wine quality. These results derive from trials performed with a single NBP dosage, selected based on previous tests conducted by the company that supplied the product, and in one season per site; further studies across additional application rates and vintages will help to consolidate and extend these findings.

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