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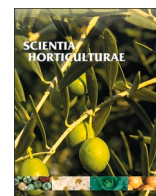
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Research Paper

Polyphenol profiling unveils time-dependent responses of grape berries to high temperature

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ABSTRACT

High temperature is a major abiotic stress that profoundly affects grape berry development and secondary metabolism. This study investigated the temporal dynamics of polyphenolic compounds in *Vitis vinifera* cv. Cabernet Sauvignon (CS) and Merlot berries subjected to berry-scale heat treatments (HT) under greenhouse conditions. HT was applied at both the herbaceous and ripening stages. Polyphenolic profiles were only marginally altered during the herbaceous stage, whereas a markedly broader response was observed during the ripening stage. In both cultivars, HT induced a characteristic “increase-then-decrease” pattern in polyphenol accumulation, with CS exhibiting an earlier onset of the induction and a more delayed decline phase than Merlot. Furthermore, transient variations in specific phenolic classes, particularly phenolic acids and flavanols, displayed distinct cultivar- and time-dependent signatures under HT. Taken together, these results reveal both conserved and cultivar-specific responses to heat stress and highlight the critical importance of high temporal resolution for capturing transient yet biologically meaningful changes in grape berry composition.

1. Introduction

Among fruit crops, *Vitis vinifera* ranks among the most economically significant species worldwide, owing to its extensive cultivated area and the global economic weight of the grape and wine industries (OIV, 2024). The economic value of grapes depends not only on yield but also on fruit quality, which encompasses intrinsic traits such as berry size, colour, firmness, and chemical composition as well as extrinsic factors including cultivar, geographical origin, and viticultural practices (Poni et al., 2018; Ribéreau-Gayon et al., 2021; Van Leeuwen, 2022). Understanding how environmental drivers, particularly temperature, affect these quality parameters is critical for sustaining grapevine production and ensuring the economic viability of viticulture under a changing climate.

Wine quality is fundamentally governed by the metabolic profile of the berries, encompassing soluble carbohydrates, organic acids,

flavonoids, amino acids and aromatic compounds. The relative abundance, composition, and balance of these metabolites exert a decisive influence on key enological attributes, including alcohol concentration, acidity and freshness, color, structure, flavor, aftertaste and aging potential of the wines (Poni et al., 2018; Van Leeuwen, 2022). The effects of high temperatures on wine characteristics are likely dependant on several parameters, including grapevine cultivar, development stage, stress severity (maximal temperature, stress duration, etc.) and wine making processes. However, some of the biochemical consequences of heat stress on grape berry have already been well described (Drappier et al., 2019; van Leeuwen et al., 2019). When berry ripening occurs under higher temperatures or during heat waves, fruit primary and secondary metabolism are deeply impacted (Venios et al., 2020). A loss of balance between sugar and acidity in grape juice, as well as changes in the aromatic character are frequently observed (Azuma et al., 2012; Sweetman et al., 2014; Bonada et al., 2015). A decrease in grape colour

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is also expected due to the inhibition of anthocyanin biosynthesis (Gouot et al., 2019). These effects of high temperature on berry metabolite contents usually result in a loss of wine typicity (De Orduna, 2010; van Leeuwen et al., 2019).

Cultural practices such as canopy management or irrigation adjustments only partially mitigate the negative effects of elevated temperature on berry development and composition. A longer-term strategy involves exploiting the broad genetic diversity within *Vitis vinifera* to select or breed cultivars capable of maintaining key quality traits under higher temperature regimes (Ollat et al., 2019). Among widely cultivated wine grape cultivars, Cabernet Sauvignon and Merlot are prominent. However, multiple studies show that Merlot tends to exhibit greater sensitivity to high temperature than Cabernet Sauvignon, particularly in the Bordeaux region (Duchêne and Schneider, 2005; Parker et al., 2011; van Leeuwen and Destrac-Irvine, 2017). These cultivar-specific thermal requirements for achieving both optimal maturity and desired fruit composition highlight the importance of understanding varietal responses to elevated temperatures as part of adaptation strategies. The basal defoliation commonly used around the fruiting zone to promote optimal ripening and prevent fungal infections (Alem et al., 2019; Poni et al., 2018), could also be questioned since exposing the bunches to direct sunlight can cause an increase in berry temperature beyond optimal physiological thresholds (Lecourieux et al., 2017; Ćirković et al., 2019; Cataldo et al., 2021). Despite advances in understanding temperature effects on grape berry metabolism, comprehensive metabolic profiling of polyphenols in grape berries exposed to controlled heat stress remains scarce. Polyphenols, defined by aromatic rings bearing one or more hydroxyl groups, constitute one of the most structurally diverse and functionally significant classes of secondary metabolites in grape berries (Šikuten et al., 2020). In addition to their central role in determining grape and wine quality through contributions to colour, astringency, and antioxidant potential (Merkytė et al., 2020), polyphenols are actively involved in plant defence and stress signalling (Xu and Wang, 2025). Transcriptomic studies have shown that elevated temperature can alter the expression of genes involved in phenylpropanoid and flavonoid biosynthesis, demonstrating that polyphenol metabolism is temperature responsive (Lecourieux et al., 2017). However, few studies have integrated these transcriptomic insights with detailed metabolite analyses, and direct comparisons between cultivars with contrasting thermotolerance are particularly limited. Importantly, as emphasized by Cataldo et al. (2023), monitoring phenolic dynamics before, during, and after heatwaves is a key research priority in the context of climate change. Our study directly responds to this need by characterizing grape berry polyphenol responses to HT across developmental stages.

To address this gap, we employed a controlled heat treatment system designed to increase cluster temperature from fruiting cuttings grown under greenhouse conditions (Lecourieux et al., 2017). Targeted analyses using UHPLC-QqQ and UHPLC-DAD enabled the quantification of 42 polyphenolic compounds across five major subclasses (phenolic acids, flavanols, flavonols, stilbenes, and anthocyanins) throughout key developmental stages. This approach provides a high-resolution overview of polyphenol dynamics in Merlot and Cabernet Sauvignon under both control and heat-treated conditions. Our results offer new insights into the metabolic remodelling of polyphenols in response to heat stress, revealing both shared and cultivar-specific thermoadaptive responses.

2. Materials and methods

2.1. Plant materials

Fruiting cuttings from *Vitis vinifera* L. cv Cabernet Sauvignon, clone #169 and *Vitis vinifera* L. cv Merlot, clone #343 were cultivated in a greenhouse using 0.5 L pots filled with a uniform mixture of perlite, sand, and vermiculite (1:1:1). All the cuttings were irrigated four times a day using a drip system with nutrient-enriched solutions. During growth

and development, each fruiting cutting carried one cluster and was maintained with 16 leaves following the established protocol (Ollat et al., 1998). The plants with a homogeneous cluster were selected for our experiments.

2.2. Heat treatment, temperature monitoring and sample collection

During experiments, all plants were cultivated in a cooling greenhouse maintained at a constant temperature of approximately 27 °C. In the control group, plants were exposed to fan-generated airflow to simulate natural, field-like conditions. For the heat treatment, grape clusters were heated using radiators to achieve the temperature differences typically observed in vineyards between sun-exposed and shaded berries, where direct solar exposure can raise cluster temperatures by more than 10 °C above ambient air temperature (Smart and Sinclair, 1976). To create a localized heat-stress condition (HT), a thick polystyrene foam board was used to minimize heat loss. Additionally, polystyrene insulation was applied to shield the leaves and roots from heat exposure, ensuring that only the grape clusters were subjected to HT.

HT was applied locally on clusters of Cabernet Sauvignon and Merlot fruiting cuttings at two developmental stages, namely middle-green and middle-ripening. HT was applied every day during 10 days from 8:00 a. m. to 4:00 pm. To mimic usual daytime temperature course of sunlight-exposed berries. To assess the efficiency of our experimental heating system, mesocarp temperatures of control and heat-treated berries were continuously monitored throughout the experiments, using copper-constantan thermocouples inserted into the berries, with data acquisition every 5 min facilitated by a Campbell datalogger (Campbell Scientific).

Prior to treatment, total soluble solids content (TSS) was quantified using a digital refractometer (Atago, Tokyo). At the herbaceous stage, TSS values were 3.51 ± 0.34 for CS and 3.68 ± 0.49 for Merlot. Whereas at the ripening stage, the values were 18.36 ± 2.11 for CS and 18.61 ± 2.34 for Merlot. For both cultivars, 16 plants were assigned to the control group and 16 plants to the heat treatment group, establishing four biological replicates per condition. Berry samples were collected after different treatment durations, comprising 30 min (30min), 1 h (1h), 2 h (2h), 4 h (4h), 1 day (1d), 2 days (2d), 5 days (5d), and 10 days (10d). Five berries were collected from each plant at each time point of treatment and pooled to minimize sample variation. Berries were immediately frozen in liquid nitrogen. Seeds were removed and the remaining tissues were ground in liquid nitrogen.

2.3. Anthocyanin extraction and analysis

A total of $70 \text{ mg} \pm 5\%$ of ground, lyophilized powder (ripening stage) was extracted with 500 μL of methanol containing 0.1% formic acid (v/v). The extracts were filtered through a 0.2 μm polypropylene syringe filter (Pall Gelman Corp., Ann Arbor, MI, USA) prior to chromatographic analysis. Separation of individual anthocyanins was conducted following the protocol described with minor modifications (Arrizabalaga-Arriazu et al., 2021). Briefly, chromatographic separation was achieved using a Synchronis C18 column ($2.1 \times 100 \text{ mm}$, 1.7 μm ; Thermo Fisher Scientific, Waltham, MA, USA) on a Vanquish UHPLC system equipped with a Vanquish DAD CG detector (Thermo Electron SAS, Waltham, MA, USA). Detection was performed at 520 nm. Chromatographic data were processed using Chromeleon software (version 7.2.10; Thermo Electron SAS, USA), and quantification was based on peak area integration. All anthocyanins were quantified at 520 nm using malvidin-3-O-glucoside (Extrasynthese, Genay, France) as an external standard. The concentrations of individual anthocyanin are expressed in $\text{mg} \cdot \text{kg}^{-1}$ dry weight (DW) as equivalents of malvidin-3-O-glucoside.

2.4. Non-pigmented polyphenol extraction and analysis

Approximately 10 mg $\pm$ 2 % of ground, lyophilized samples were transferred into 1.1 ml micronic tube (MP32033L, Micronic, Lelystad, Netherlands) and randomized onto a 96-micronic rack (MPW51001BC6, Micronic, Lelystad, Netherlands). Sample extraction was performed using an automated method developed at Bordeaux Metabolome Facility. The extraction process was employed with two sequential solvents: 300 μ l of 80% ethanol containing 0.1% formic acid (v/v) in the first step, followed by 300 μ l of 50% ethanol in the second step. The resulting supernatants were retained for subsequent analysis (Luna et al., 2020).

Non-anthocyanin polyphenols were analyzed using UHPLC-HRMS as described previously (Mairata et al., 2025). Samples (1 μ L) were injected into a Luna Ω Omega Polar C18 column (50 mm $\times$ 2.1 mm, 1.6 μ m; Phenomenex) maintained at 40 $^{\circ}$ C. Chromatographic separation was performed with a gradient of water and acetonitrile, both containing 0.1% formic acid, at 0.5 mL/min: 1–36% B (0–7.5 min), 36–95% B (7.5–8.5 min), held at 95% B (8.5–10 min), followed by re-equilibration at 1% B (10–12 min). Mass spectrometry was carried out in negative electrospray ionization mode with a spray voltage of 3000 V, using standard source parameters (sheath gas: 45 a.u.; auxiliary gas: 15 a.u.; capillary temperature: 320 $^{\circ}$ C; probe heater temperature: 250 $^{\circ}$ C; S-lens RF level: 100). Full MS scans (m/z 70–1050) were acquired at 35,000 resolution (FWHM), with data-dependent MS/MS fragmentation for the top two ions. Quantification used calibration curves from 59 polyphenol standards (0.03–16.95 mg/L). Data processing and peak integration were performed using Xcalibur software (version 4.6.67.17; Thermo Scientific). Results are expressed as mg $\cdot$ kg $^{-1}$ dry weight (DW).

2.5. Statistics

All statistical analyses were performed using R (version 4.4.3). The Shapiro-Wilk test was used to assess the normality of each dataset, and Levene's test was conducted to evaluate the homogeneity of variances between groups. For comparisons between heat-treated and control groups, unpaired *t*-tests were conducted. *p*-values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) method, with adjusted *p*-values < 0.05 considered statistically significant. Data visualizations were generated using the *ggplot* package for chord correlation plots, *ggplot2* package for bar plots and volcano plots, and *ComplexHeatmap* package for heatmaps.

3. Results

3.1. Heat treatment induces marked increases in mesocarp temperature during herbaceous and ripening stages

At the herbaceous stage, the initiation of heat treatment (HT) led to a pronounced increase in mesocarp temperature in both cultivars (Fig. S1). In Cabernet Sauvignon (CS), the mean mesocarp temperature over the 10-day treatment period was 22.16 $\pm$ 2.89 $^{\circ}$ C in control berries compared with 34.18 $\pm$ 4.98 $^{\circ}$ C under HT, representing an average increase of 12.02 $^{\circ}$ C and reaching a maximum of 41.09 $^{\circ}$ C. Merlot berries showed a similar response, with mean temperatures of 22.17 $\pm$ 2.87 $^{\circ}$ C in controls and 33.70 $\pm$ 5.14 $^{\circ}$ C under HT, corresponding to an average increase of 11.53 $^{\circ}$ C and a maximum temperature of 43.66 $^{\circ}$ C (Fig. S1). During ripening, HT also resulted in a consistent elevation of berry temperatures. In CS, mean mesocarp temperature was 25.96 $\pm$ 1.81 $^{\circ}$ C in controls and 35.52 $\pm$ 3.39 $^{\circ}$ C under HT, a difference of 9.56 $^{\circ}$ C with a maximum value of 42.37 $^{\circ}$ C. In Merlot, mean temperatures were 26.27 $\pm$ 1.85 $^{\circ}$ C in controls and 34.83 $\pm$ 3.49 $^{\circ}$ C under HT, reflecting an average increase of 8.56 $^{\circ}$ C and a maximum temperature of 41.75 $^{\circ}$ C (Fig. S1).

3.2. Polyphenol profiles in Cabernet Sauvignon and Merlot berries under control conditions

To determine the main polyphenol composition, 42 compounds were quantified in CS and Merlot berries under control conditions. These compounds were grouped into four subclasses based on their chemical structure: phenolic acids, flavanols, flavonols, and stilbenes. As anthocyanin biosynthesis begins at veraison, anthocyanin content was not assessed until the ripening stage (Table S1). For each sample, the total amount of each of the four subclasses was quantified, and the total polyphenol content was calculated by summing these subclasses, excluding anthocyanins (Fig. 1; Supplementary data 1).

Under control conditions at the herbaceous stage, phenolic acids were the predominant subclass in both cultivars, followed by flavanols, flavonols, and stilbenes (Fig. 1). CS exhibited a significantly higher sum of polyphenol content (average: 9067.71 mg $\cdot$ kg $^{-1}$ DW) than Merlot (7355.05 mg $\cdot$ kg $^{-1}$ DW). This difference was largely attributable to phenolic acids (CS: 7522.03 mg $\cdot$ kg $^{-1}$ DW; Merlot: 4995.90 mg $\cdot$ kg $^{-1}$ DW). In contrast, Merlot berries contained higher levels of flavanols (2093.13 mg $\cdot$ kg $^{-1}$ DW) than CS (1305.28 mg $\cdot$ kg $^{-1}$ DW), while no significant difference was detected in flavonol contents between cultivars. Stilbenes were present at similarly low levels in both cultivars at this stage (Fig. 1J).

At the ripening stage, phenolic acids remained the most abundant subclass, followed by flavanols. Stilbenes showed a marked increase compared to the herbaceous stage (Fig. 1). The sum of polyphenols decreased in both cultivars relative to the herbaceous stage, primarily due to reductions in phenolic acids, flavanols, and flavonols, despite increased accumulation of stilbenes and anthocyanins (Fig. 1). Merlot displayed a higher sum of polyphenols (1400.91 mg $\cdot$ kg $^{-1}$ DW) than CS (1044.81 mg $\cdot$ kg $^{-1}$ DW). CS still contained slightly higher levels of phenolic acids (615.70 mg $\cdot$ kg $^{-1}$ DW vs. 379.85 mg $\cdot$ kg $^{-1}$ DW in Merlot), whereas Merlot showed higher concentrations of flavanols (379.85 mg $\cdot$ kg $^{-1}$ DW vs. 216.27 mg $\cdot$ kg $^{-1}$ DW) and flavonols (79.87 mg $\cdot$ kg $^{-1}$ DW vs. 65.27 mg $\cdot$ kg $^{-1}$ DW) (Fig. 1D, F, G). Stilbene accumulation was substantially higher in Merlot than in CS (514.68 mg $\cdot$ kg $^{-1}$ DW vs. 147.58 mg $\cdot$ kg $^{-1}$ DW) (Fig. 1H).

Anthocyanins, quantified only at the ripening stage, were significantly more abundant in CS (4280 mg $\cdot$ kg $^{-1}$ DW) than in Merlot (2660 mg $\cdot$ kg $^{-1}$ DW) (Fig. 1K). These anthocyanins were classified into five structural groups according to their aglycone backbone: the Delphinidin (Dp) groups included Delphinidin-3-O-glucoside (Dp-3-glc), Delphinidin-3-O-(6"-acetyl)-glucoside (Dp-3-glc-ac) and Delphinidin-3-O-(6"-p-coumaroyl)-glucoside (Dp-3-glc-cou). The Malvidin (Mv) group included Malvidin-3-O-glucoside (Mv-3-glc), Malvidin-3-O-(6"-acetyl)-glucoside (Mv-3-glc-ac), Malvidin-3-O-(6"-p-coumaroyl)-glucoside (Mv-3-glc-cou) and *cis*-Malvidin-3-O-(6"-*cis*-p-coumaroyl)-glucoside (*cis*-Mv-3-glc-cou). The Petunidin (Pt) group included Petunidin-3-O-glucoside (Pt-3-glc), Petunidin-3-O-(6"-acetyl)-glucoside (Pt-3-glc-ac) and Petunidin-3-O-(6"-p-coumaroyl)-glucoside (Pt-3-glc-cou). The cyanidin (Cy) group included Cyanidin-3-O-glucoside (Cy-3-glc), Cyanidin-3-O-(6"-acetyl)-glucoside (Cy-3-glc-ac), Cyanidin-3-O-(6"-p-coumaroyl)-glucoside (Cy-3-glc-cou). The Peonidin (Pn) group included Peonidin-3-O-glucoside (Pn-3-glc), Peonidin-3-O-(6"-acetyl)-glucoside (Pn-3-glc-ac), Peonidin-3-O-(6"-p-coumaroyl)-glucoside (Pn-3-glc-cou), *cis*-Peonidin-3-O-(6"-*cis*-p-coumaroyl)-glucoside (*cis*-Pn-3-glc-cou). Mv (CS: 56.49%; Merlot: 54.84%) was the most abundant in both cultivars. Cy was the least abundant (CS: 4.29%; Merlot: 5.31%). Dp, Pn and Pt were present at comparable levels (Fig. S2; Supplementary data 2).

3.3. Heat treatment alters polyphenol profiles in Cabernet Sauvignon and Merlot berries

To characterize time-course variations in polyphenol content under heat stress, the number of significantly affected polyphenols was determined at different time points across cultivars and developmental

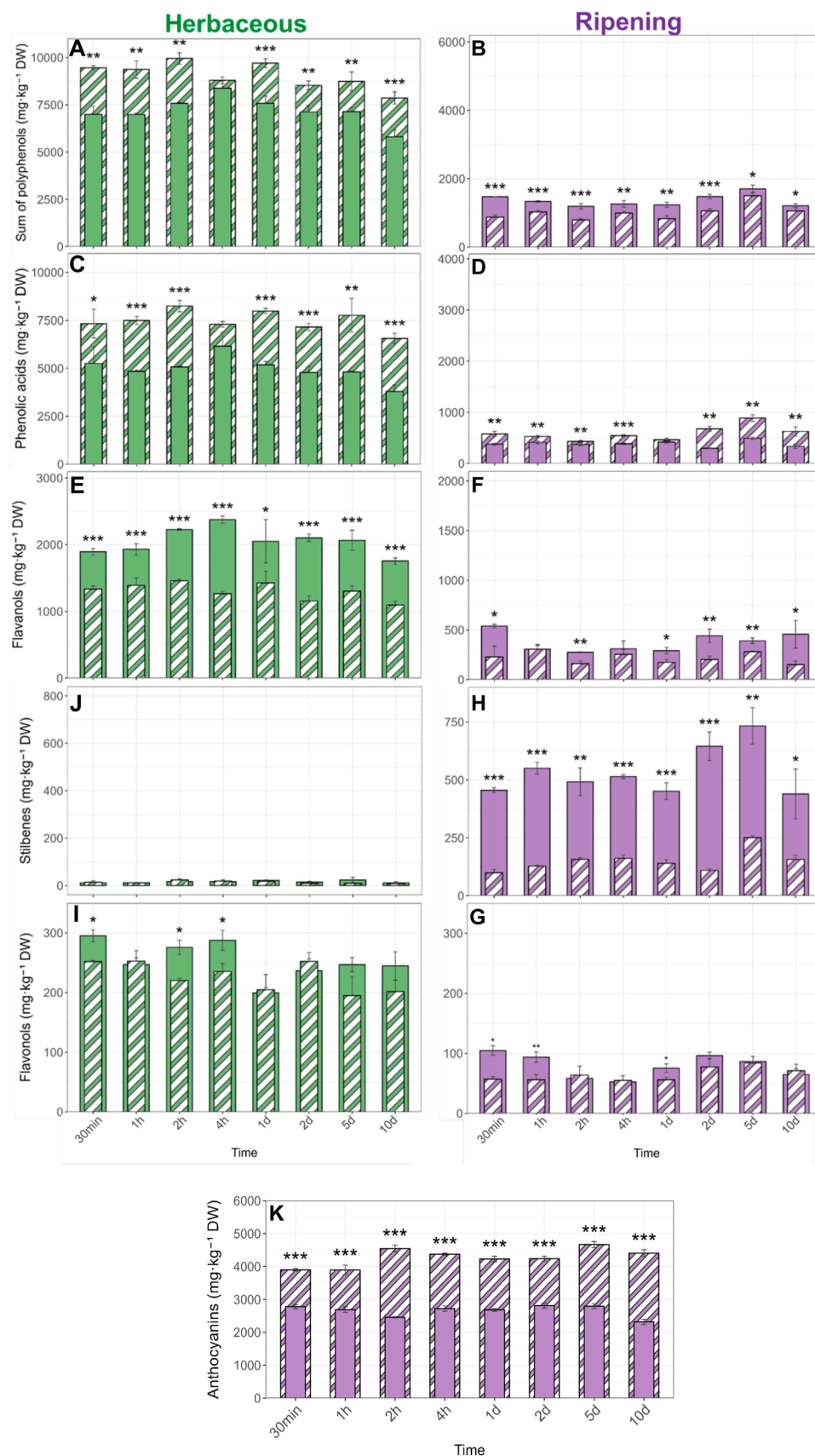


Fig. 1. Sum of polyphenol content (A, B), phenolic acids (C, D), flavanols (E, F), stilbenes (J, H), and flavonols (I, G) in grape berries (mg·kg⁻¹ DW) at the herbaceous and ripening stage, sum of anthocyanins (mg·kg⁻¹ DW) at the ripening stage (K). Statistical significance was determined using unpaired *t*-test: $p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***, and $p > 0.05$ is considered not significant (no symbol), with Benjamini-Hochberg false discovery rate (FDR) correction applied.

stages.

At the herbaceous stage, polyphenol contents were less affected by HT when compared to the ripening stage (Figs. S4, 2, Fig. 3). In CS, 13 polyphenols showed significant increases under HT over time (Fig. S4A). These included one flavanol (procyanidin B4) at 1 h, four flavonols (kaempferol-3-glucoside, quercetin 3-D-galactoside, quercetin-3-rutinoside, quercetin-3-rhamnoside) at 2 h, and five flavanols (epicatechin, epicatechin-3-O-gallate, epigallocatechin-3-O-gallate, procyanidin B2, procyanidin B4), two stilbenes (*cis*- and *trans*-piceid) and one flavanol (procyanidin B2) after 2d HT. Conversely, six polyphenols decreased significantly under HT, comprising four flavonols (quercetin 3-D-galactoside, quercetin-3-glucoside, quercetin-3-rutinoside) at 2d and one flavanol (epigallocatechin-3-O-gallate) at 10d. In Merlot, 16 polyphenols showed significant accumulation under HT, predominantly at 1d (six compounds) and 10d (nine compounds), as detailed in Fig. S4B. No significant reduction in polyphenol content was detected in Merlot at this stage.

At the ripening stage, polyphenol profiles were markedly influenced by HT in both cultivars (Figs. 2 and 3). In CS, 72 polyphenols exhibited significant accumulation in response to HT (Fig. 2A). Of these, 50 compounds accumulated predominantly after short-term HT (within 4 h), while 22 showed significant increases after long-term HT (from 1 to 10d). By contrast, 98 identified polyphenols displayed significant reductions across all HT durations, with 67 of these decreases observed after prolonged exposure ($\geq 2d$), particularly at 5 and 10 days. Most of the decreasing compounds belonged to anthocyanin, stilbene and flavanol classes (Fig. 2B). Similarly, in Merlot, 73 polyphenols significantly accumulated under HT, with 53 of these increases occurring after short-term exposure (Fig. 3A). Conversely, 116 polyphenols significantly decreased across all time points, 86 of which declined under long-term HT. As in CS, most of the reduced compounds were anthocyanins, stilbenes and flavonols (Fig. 3B). Further details are provided in Supplementary data 3.

3.4. Time-course classification of polyphenols in Cabernet Sauvignon and Merlot berries under heat treatment at the ripening stage

Due to the limited significant changes in polyphenol content at the herbaceous stage under HT, our analysis focused on the ripening stage. To systematically evaluate polyphenols responses to HT at the ripening stage, unpaired *t*-tests with FDR correction were performed for each metabolite at each sampling time. For each metabolite, the frequency of statistically significant changes ($p < 0.05$) was compiled, producing a significance-time matrix (Fig. 4). Based on the persistence of significance across time points, polyphenols were categorized into two classes: "Wide significant", significant at four or more time points and "Transient", significant at three or fewer time points.

In both cultivars, most "Wide significant" metabolites were anthocyanins (15 in CS and 14 in Merlot). Among these, Pn-3-glc-ac (significant at 2d, 5d, and 10d) and *cis*-Pn-3-glc-cou (2 h, 4 h, 5d) were classified "Transient" in CS but "Wide significant" in Merlot. Reversely, Mv-3-glc and Pt-3-glc-cou were "Wide significant" in CS but only "Transient" in Merlot (30 min, 1 h, 1d) (Fig. 4). Stilbenes were mainly classified as "Wide significant" in both cultivars. Exceptions were observed for *trans*-piceid which was "Transient" in CS (30 min, 1 h, 5d) and in Merlot (5d, 10d), and for ϵ -viniferin which was categorized as "Wide significant" in Merlot only (Fig. 4). Among flavonols, quercetin 3-glucuronide was transiently significant in both cultivars (1d, 5d) whereas quercetin 3-rutinoside was "Wide significant" only in Merlot. Flavanols and phenolic acids were mostly "Transient" in both cultivars, except for catechin, gallic acid, and epicatechin-3-O-gallate, which were classified as "Wide significant" in Merlot (Fig. 4).

We next analyzed the kinetic trends of "Wide significant" metabolites in both cultivars (Fig. 5). Integrating these profiles with the global trends (Fig. S3) revealed that most compounds showed an initial accumulation under short-term HT followed by a decrease under prolonged exposure.

Overall, the transition from increase to decrease contents was observed between 1d and 2d HT. For some compounds, the transient accumulation under HT was more pronounced in CS than in Merlot as soon as 30 min (Fig. 5). Within these dynamics, Mv-3-glc-cou and *cis*-Mv-3-glc-cou exhibited contrasting pattern in CS, showing a decrease after short-term HT followed by an increase under long-term HT. A similar trend was observed for Mv-3-glc-ac and Mv-3-glc-cou in Merlot. Mv-3-glc showed a continuous decline across all time points in CS. *cis*-resveratrol and piceatannol decreased after short-term HT in CS but increased in Merlot. Overall, these results highlight cultivar-specific differences in both the extent and pattern of polyphenol modulation under HT at ripening.

4. Discussion

4.1. Baseline polyphenol profiles reveal stage- and cultivar-specific patterns prior to heat treatment

Clear differences in polyphenol profiles were observed between CS and Merlot under control conditions at both developmental stages (Fig. 1). A marked decline in several polyphenol classes, particularly phenolic acids, flavanols, and flavonols, was detected when comparing the herbaceous to the ripening stage (Fig. 1D, F, G). Similar developmental trends have been reported in other fruit species, such as *Citrus sinensis* (Hou et al., 2021), *Mespilus germanica* (Gruz et al., 2011) and *Punica granatum* (Han et al., 2015). One well-established explanation is the so-called dilution effect: as berries enlarge during maturation, the concentration of phenolic compounds expressed on a fresh weight (FW) basis can be reduced due to a dilution effect (Zhao et al., 2015). In apples, for instance, phenolic concentration per gram FW weight declined while the total phenolic content per fruit remained constant or increased (Awad et al., 2000). In the same study, leucoanthocyanidin reductase (LAR) activity, a key enzyme in flavonoid catechin biosynthesis, also declined on a fresh weight basis but increased when calculated per fruit. Although in the present study, polyphenols were normalized on a dry weight (DW) basis to minimize the influence of water content, a dilution effect may still occur as other non-phenolic dry components, such as sugars and minerals, accumulate during ripening (Coombe and McCarthy, 2000). In addition to dilution, enzymatic oxidation may contribute to the decrease in phenolic levels. Indeed, polyphenol oxidase (PPO) activity has been shown to increase during maturation in several fruit species promoting the degradation of phenolic compounds (Mayer, 2006; Yoruk and Marshall, 2003). In grape berries, higher polyphenol levels at the herbaceous stage have similarly been associated with lower PPO activity and reduced dilution effects early in development (Adams, 2006; Downey et al., 2003; Kennedy et al., 2002). Altogether, these findings highlight that both biochemical and physiological factors should be considered when interpreting polyphenol dynamics during berry development.

A pronounced accumulation of stilbenes was observed at the ripening stage in both cultivars (Fig. 1H, J), consistent with previous reports showing that stilbenes accumulation initiates at veraison and increases sharply thereafter in grape berry skins (Gatto et al., 2008; Wang et al., 2016). Transcriptomic studies have shown that genes involved in the stilbene pathway including stilbene synthase (STS), phenylalanine ammonia-lyase (PAL), and 4-coumarate-CoA ligase (4CL), displayed enhanced expression from veraison to ripening, particularly in cultivars with high resveratrol content (Gatto et al., 2008), supporting the interpretation that transcriptional regulation drives these metabolic changes. Likewise, the onset of anthocyanin biosynthesis at veraison has been well documented (Boss et al., 1996; Jaakola, 2013), coinciding with the upregulation of several structural and regulatory genes involved in anthocyanin biosynthesis and transport. These genes include glutathione S-transferase (GST4), UDP-glucose:flavonoid 3-O-glucosyl transferase (UGT), MYBA1 transcription factor, and flavonoid 3',5'-hydroxylase (F3'5'H) (Massonnet et al., 2017).

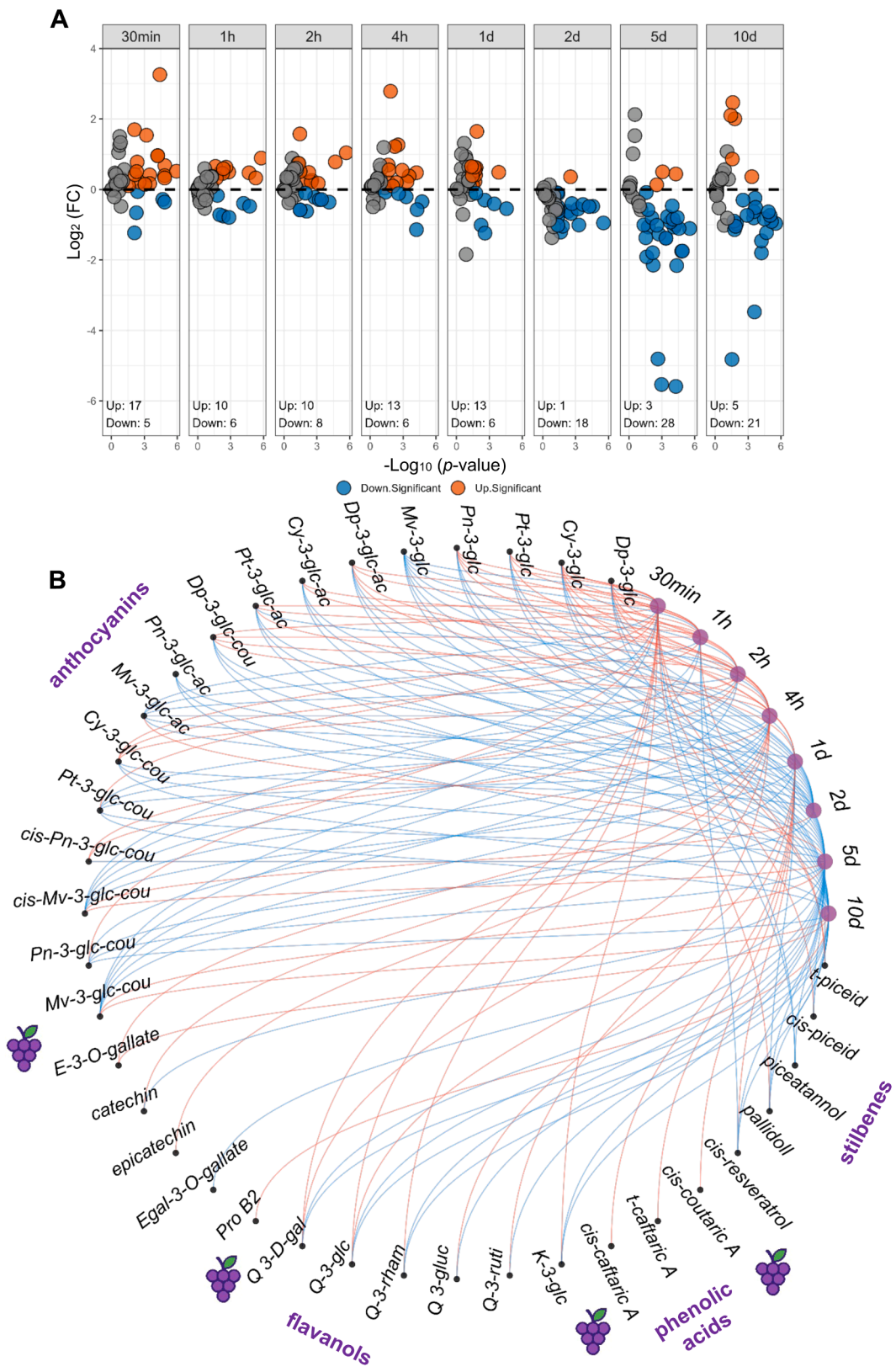


Fig. 2. Volcano plots showing the polyphenol response to heat treatment at different time points, 30 min (30min), 1 h (1h), 2 h (2h), 4 h (4h), 1 day (1d), 2 days (2d), 5 days (5d), and 10 days (10d) at the ripening stage in Cabernet Sauvignon (A). The x-axis represents the Log_2 fold change (Log_2FC) between heat-treated and control samples, and the y-axis shows the $-\text{Log}_{10}(p)$ value. Polyphenols significantly accumulated after heat treatment are highlighted in red, while those significantly reduced are shown in blue (unpaired t -test, $p < 0.05$, FDR). (B) Chord diagrams illustrating the polyphenol groups across different time points. Only significant changes are connected by lines: polyphenols significantly accumulated after heat treatment are linked by red lines, and those significantly reduced are linked by blue lines.

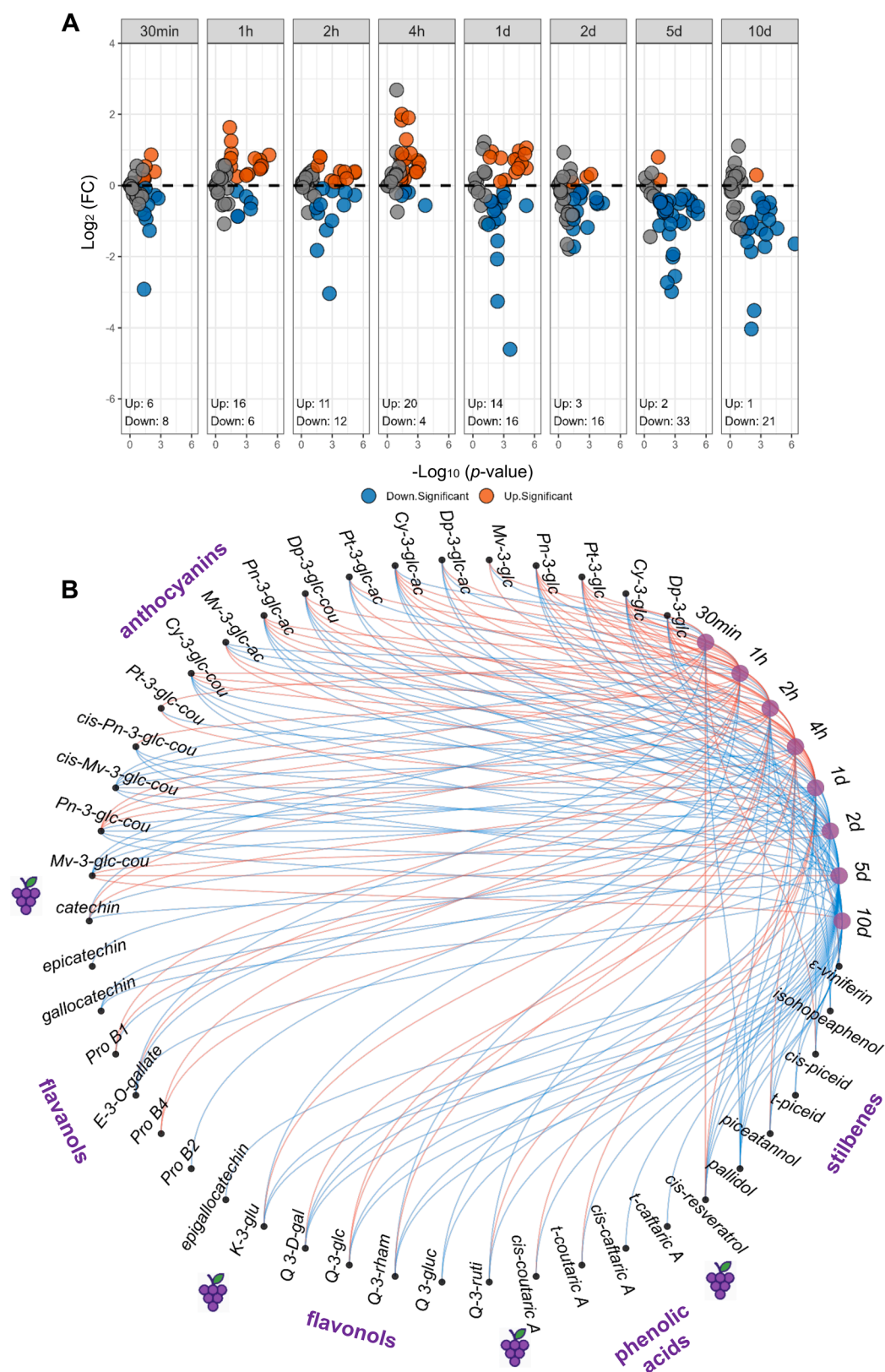


Fig. 3. Volcano plots showing the polyphenol response to heat treatment at different time points, 30 min (30min), 1 h (1h), 2 h (2h), 4 h (4h), 1 day (1d), 2 days (2d), 5 days (5d), and 10 days (10d) at the ripening stage in Merlot (A). The x-axis represents the Log_2 fold change (Log_2FC) between heat-treated and control samples, and the y-axis shows the $-\text{Log}_{10}(p)$ value. Polyphenols significantly accumulated after heat treatment are highlighted in red, while those significantly reduced are shown in blue (unpaired t -test, $p < 0.05$, FDR). (B) Chord diagrams illustrating the polyphenol groups across different time points. Only significant changes are connected by lines: polyphenols significantly accumulated after heat treatment are linked by red lines, and those significantly reduced are linked by blue lines.

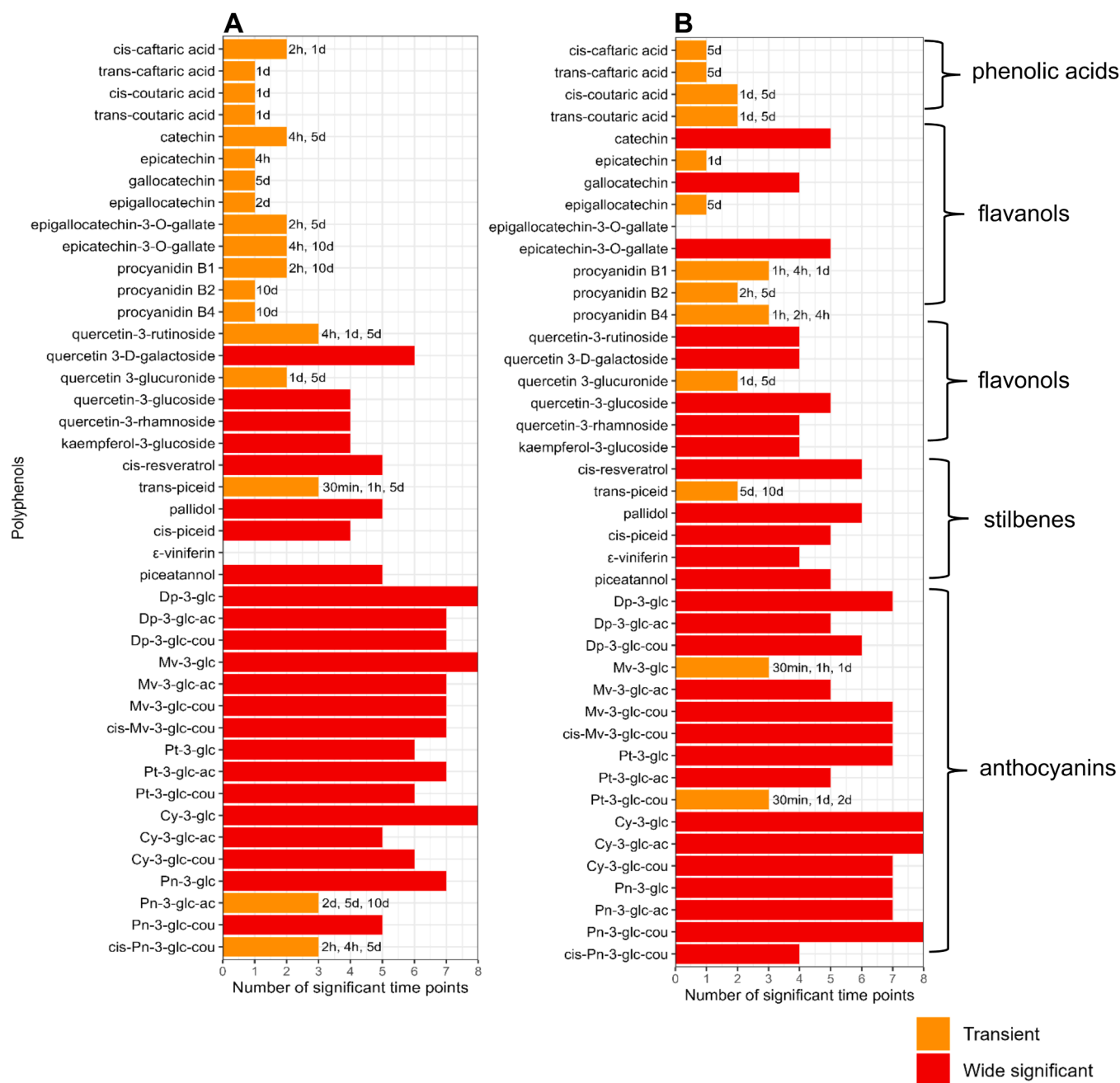


Fig. 4. Number of significant time points (out of eight) at which the ratio of heat-treated to control levels was statistically significant (unpaired *t*-test, $p < 0.05$, FDR), for 42 polyphenols at the ripening stage in Cabernet Sauvignon (A) and Merlot (B). Polyphenols were categorized based on their temporal significance profiles as follows: Wide significant: significant in more than 4 time points (≥ 4), Transient: significant in fewer than 3 time points (≤ 3).

Under control conditions, the two cultivars exhibited distinct polyphenolic profiles. Stilbene contents were substantially higher in Merlot than in CS at both stages (Fig. 1H, J), consistent with previous surveys highlighting genotype-dependent stilbene diversity (Gatto et al., 2008). In contrast, total anthocyanin levels were significantly higher in CS than in Merlot (Fig. 1K), aligning with reported genotype-linked variation in anthocyanin composition (Ortega-Regules et al., 2006). In addition, Mv was the most dominant anthocyanin group in both cultivars, consistent with previous studies conducted under Bordeaux vineyard conditions (Lorrain et al., 2011). Phenolic acids were more abundant in CS, whereas Merlot contained higher flavanols concentrations across both stages (Fig. 1D, F). Such cultivar-specific patterns in phenolic acids and flavanols have also been reported in comparative analyses of grape varieties (Mané et al., 2007).

Altogether, these baseline differences provide a comprehensive framework for interpreting how each cultivar's polyphenol network may be reprogrammed subsequent high-temperature stress.

4.2. Common polyphenol responses to high temperature at the ripening stage

Although only a limited number of polyphenols showed significant changes under heat treatment (HT) at the herbaceous stage (Fig. S4), our previous transcriptomic analysis of CS berries revealed significant modulation of several phenylpropanoid pathway-related genes under comparable HT conditions (Lecourieux et al., 2017). This apparent discrepancy is not unexpected, as similar observations in tomato have demonstrated that global correlations between transcript and

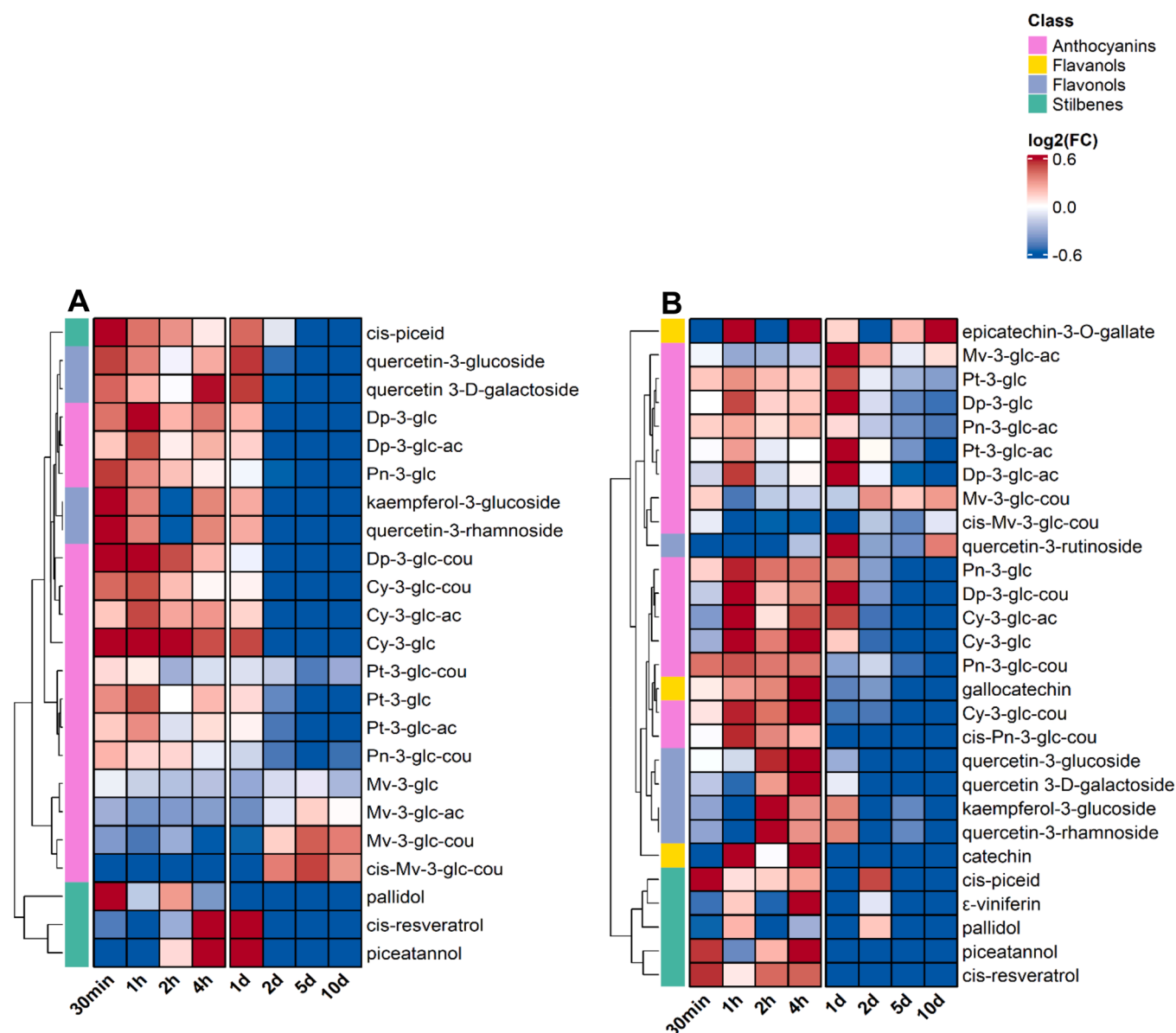


Fig. 5. Dendrogram clustering showing the kinetics of polyphenols classified as "wide significant" (i.e., significant at more than four time points) in Cabernet-Sauvignon (A) and Merlot (B) at the ripening stage. Values represent the log₂ fold change (FC) of heat-treated samples relative to controls. Statistical significance was determined using unpaired *t*-tests ($p < 0.05$), FDR.

metabolite levels are weak, strong correlation occur only for specific metabolite/transcript pairs, highlighting the importance of post-translational regulatory mechanisms in controlling metabolic outputs (Carrari et al., 2006). The same study further reported that despite an overall poor correlation, a subset of ripening-related metabolites and transcripts showed strong relationships, including hexose phosphates, cell wall-derived sugars, pigments, and tricarboxylic acid (TCA) cycle intermediates (Carrari et al., 2006). These findings underscore the importance of expanding metabolic profiling under stress conditions at the herbaceous stage to encompass a broader spectrum of both secondary and primary metabolites, which may reveal coordinated metabolic networks that remain undetected when focusing solely on specialized metabolites.

At the ripening stage, polyphenol contents in both cultivars followed a clear "rise-then-fall" pattern: they increased after short-term HT exposure but declined under prolonged HT (Figs. S3 and 5). This dynamic was consistent across the major polyphenol classes stilbenes, flavonols, and anthocyanins. Supporting this metabolic trend,

transcriptomic data from CS indicate that genes encoding phenylalanine ammonia-lyase (PAL) and cinnamate 4-hydroxylase (C4H), key entry points into phenylpropanoid metabolism, were upregulated after 1 day of HT but downregulated after 7 and/or 14 days of exposure (Lecourieux et al., 2017), mirroring the observed metabolite profiles.

Stilbenes, such as resveratrol and its derivatives, are well-established phytoalexins that accumulate in grapevine tissues in response to stress (e.g., fungal attack, UV irradiation, wounding) and are synthesized by STS enzymes in grapevine (Adrian et al., 1997; Jeandet et al., 1995). In our study, HT exposure triggered accumulation of several stilbenes within 1 day in both CS and Merlot berries. However, prolonged exposure (from 2 to 10d) led to a sharp decline in almost all detected stilbenes (Figs. S3 and 5). This pattern is consistent with transcriptomic data from CS berries, where most STS genes (39 of 48 putative STS) were strongly induced after 1 day of HT but markedly repressed after 7–14 days (Lecourieux et al., 2017), indicating that sustained HT inhibits stilbene biosynthesis at the transcriptional level. Altogether, these findings highlight STS genes as key regulators of stilbene biosynthesis under HT,

with their transcriptional response, and thus stilbene accumulation, being strongly dependent on the duration of heat exposure.

In our study, short-term HT enhanced flavonol accumulation in both cultivars, especially in CS. This observation is consistent with findings in *Camellia sinensis*, where integrative metabolomic and transcriptomic analyses revealed heat stress-induced flavonoid biosynthesis and upregulated flavonol synthase (FLS) genes in heat tolerant varieties (Huang et al., 2024). Similarly, the heat responsive transcription factor *MaHSF11* positively regulated flavonol biosynthesis and flavonoid B-ring hydroxylation in *Musa acuminata* (Naik et al., 2025). In grapevine, temperature and light strongly influence flavonoid pathway genes. *VvFLS1* expression and flavonol accumulation are enhanced by favorable light and temperature conditions and MYB transcription regulators such as MYBF1 directly control *VvFLS1* transcription (Czemmel et al., 2009). A recent study reported that overexpression of *VvFLS* strengthened the grapevine heat tolerance by increasing quercetin production and mitigating oxidative damages (Liu et al., 2025). Furthermore, flavonol biosynthetic genes in grape berries have been shown to respond dynamically to developmental and stress related cues (Loyola et al., 2016). Given their multiple hydroxyl groups that confer strong electron-donating capacity, flavonols are widely recognized as highly effective antioxidants and reductants (Daryanavard et al., 2023). Such properties likely underlie their protective role observed under short-term HT in our study, where enhanced flavonol accumulation may contribute to mitigation of heat-induced oxidative stress. Conversely, prolonged HT reduced flavonol content in our experimental conditions, consistent with the reported downregulation of *VvFLS1* expression in CS berries subjected to extended HT (Lecourieux et al., 2017). This indicates that sustained heat exposure suppresses flavonol biosynthesis through transcriptional repression. Furthermore, our results showed a decrease in flavanols, which correlates with the repression of *VvANR* and *VvLAR*, key genes in the proanthocyanidin branch, under unfavourable temperature and light conditions (Loyola et al., 2016). As flavanols contribute to plant defense and berry quality (especially astringency), their reduction under prolonged HT further highlights the significant impact of sustained heat on grape berry secondary metabolism. Taken together, these results indicate that flavonoid metabolism response to HT is dynamic and branch specific: short-term exposure promotes flavonol accumulation, probably as part of an antioxidant defense mechanism, while prolonged exposure inhibits both flavonol and flavanol biosynthesis by altering the transcription of key genes of the metabolic pathway. This heat-induced rise then fall in berry polyphenol content observed during ripening could reflect the phenomenon of hormesis, a biphasic adaptive response to various stimuli including temperature (Agathokleous et al., 2019; Erofeeva et al., 2022). Hormesis occurs when exposure to low doses of a stress results in a beneficial effect on the organism, as opposed to the harmful effects associated with higher doses of the same stress (Agathokleous et al., 2020). In the present study, the “dose effect” would refer to the heat stress duration rather than to an increase in temperature, since berries were daily exposed to a stable temperature increase (mean increase of about 12°C relative to control conditions, Supplementary Fig. 1).

Most anthocyanins increased in CS and Merlot berries after short-term HT, in agreement with reports in *Coleus lime* (*Plectranthus scutellarioides*), where anthocyanin induction within 48 h of abiotic stress was attributed to reactive oxygen species (ROS) production (Altangerel et al., 2017). This transient increase may contribute to protection against oxidative damage as anthocyanins are known to scavenge ROS under abiotic stress conditions (Naing and Kim, 2021). In our study, most anthocyanin contents significantly declined following prolonged HT exposure in both cultivars (Figs. S3 and 5). This reduction is well-documented in grape berries and has been linked to enhanced enzymatic vacuolar degradation, potentially involving laccase, polyphenol oxidase, class III peroxidase, and β -glucosidase, as well as the downregulation of key anthocyanin biosynthetic genes (Azuma et al., 2012; Cho et al., 2014; Lecourieux et al., 2017; Mori et al., 2005; Poudel

et al., 2020). Malvidin derivatives displayed a contrasted pattern. Their increased abundance after prolonged HT is consistent with studies showing that elevated temperature (up to 35 °C) decreases most anthocyanins while promoting the accumulation of highly methylated malvidin-based compounds (Mori et al., 2007). Moreover, acylated malvidin forms, including Mv-3-glc-cou and its *cis*-isomer *cis*-Mv-3-glc-cou continued to accumulate even after 10 days of HT in CS. This agrees with earlier reports (Mori et al., 2007; Tarara et al., 2008), indicating that acylated malvidin anthocyanins are more temperature-stable than their non-acylated counterparts. In Merlot, however, only Mv-3-glc-ac and Mv-3-glc-cou showed an increasing trend after 10 days of HT, while *cis*-Mv-3-glc-cou consistently declined under prolonged HT, which underscores the genotype-specific nature of anthocyanin stability and accumulation. Overall, these findings highlight that high temperature favors the retention and accumulation of more stable anthocyanin while repressing most other anthocyanin classes.

4.3. Divergent temporal responses to high temperature at the ripening stage

CS exhibited an earlier onset of biochemical responses to HT than Merlot, with significant accumulation of most polyphenols detected as early as 30 min after heat exposure. Moreover, CS maintained elevated levels of these compounds for a longer duration, with a delayed decline observed at 2 days, whereas in Merlot, polyphenol levels began to decline more rapidly, within 1 day. Previous studies addressing HT effects on grape berry development mainly focused on long-term exposures (Carrari et al., 2006; Lecourieux et al., 2017; Tarara et al., 2008), making our data on rapid responses particularly informative. Indeed, within 30 min of heat stress exposure, *Arabidopsis* showed rapid activation of heat shock transcription factor *HsfA1a* through redox-mediated sensing mechanisms (Liu et al., 2013). In plants, HT alters membrane fluidity, enabling cytosolic Ca^{2+} influx and phosphorylation events that activate NADPH oxidases (RBOHs) triggering an early burst of ROS. This initiates a signal transduction network involving calcium-binding proteins, mitogen-activated protein kinases (MAPKs), and heat shock factors (HSFs), which together drive broad transcriptomic, proteomic and metabolomic adjustments (Hendrix et al., 2023; Saidi et al., 2011). Importantly, these early signaling events converge on transcriptional regulators of secondary metabolism, such as MYB and bHLH factors, directly linking stress perception to metabolic reprogramming (Dubos et al., 2010; Hichri et al., 2011). Our results further suggest that heat-responsive metabolic pathways are activated in a cultivar-dependent manner, with CS exhibiting a more rapid sensing or signaling capacity under heat stress, coupled with a more sustained activation of these responses compared to Merlot. This provides a mechanistic basis for the rapid and cultivar-specific modulation of phenylpropanoid pathways observed in our study.

ϵ -viniferin displayed significant differences at most time points under HT in Merlot, but not in CS. In contrast, epigallocatechin-3-O-gallate exhibited a transient response in CS while remaining unchanged in Merlot (Fig. 4). This contrasting behavior may be explained by their initial baseline levels: ϵ -viniferin was nearly absent in CS, whereas epigallocatechin-3-O-gallate was almost undetectable in Merlot (Supplementary data 1). These results highlight the importance of initial metabolic states in shaping the magnitude and direction of stress-induced responses. Broadly, both cultivars exhibited transient and dynamic changes in phenolic acids and flavanols under HT, with CS showing particularly pronounced fluctuations (Fig. 4). The temporal profiles of these compounds suggest a time-dependent, cultivar-specific metabolic reprogramming in response to HT. Their transient accumulation may reflect their conversion into downstream metabolites or intermediates involved in stress-related processes such as oxidative coupling, lignin biosynthesis or cell wall remodeling. For instance, caffeic acid and coumaric acid, both hydroxycinnamoyl tartaric acid esters,

may undergo peroxidase-mediated oxidative coupling and become incorporated into lignin-like polymers, reinforcing the cell wall protective capacity under stress (El Modafar and El Boustani, 2001; El-Basyouni et al., 1963; Francoz et al., 2015; Mnich et al., 2020). Likewise, catechin, galocatechin, and epigallocatechin (flavan-3-ols), can serve as precursors for condensed tannins (proanthocyanidins) through laccase- or polyphenol oxidase-catalyzed polymerization, processes well documented in grape berries (Dixon et al., 2005; Kennedy et al., 2002). These condensed tannins are induced by both biotic and abiotic stresses and play critical roles in oxidative defense as observed in *Populus* spp. Leaves (Gourlay et al., 2022; Gourlay and Constabel, 2019). Altogether, the fate of these metabolites, whether polymerized or degraded, reflects a dynamic defense strategy in which phenolic compounds are deployed based on stress duration and cultivar.

5. Conclusion

This work provides an integrated view of polyphenol dynamics in grape berries exposed to HT, revealing both general and specific responses in each grape variety. By combining profiling and temporal analyses, we showed that CS and Merlot differ significantly in their constitutive polyphenolic signatures as well as in the timing and duration of their responses to heat. CS showed a faster accumulation but a delayed decline of phenylpropanoid metabolism, while Merlot showed a delayed accumulation and a faster decline of key metabolites. These results indicate that heat-induced remodelling of polyphenol metabolic pathways is not uniform, but depends on both genotype and stress duration. The transient increase in stilbenes, flavonols and anthocyanins during short-term exposure likely reflects an antioxidant and protective response, while prolonged heat suppresses most branches of the metabolic pathway, with significant accumulation of stable acylated anthocyanins. Overall, our work not only elucidates the dynamic nature of polyphenol metabolism under HT, but also highlights cultivar-dependent metabolic strategies that could be exploited in breeding and viticultural practices to improve berry resilience and maintain quality in a warming climate with increasingly frequent heat events.

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CRediT authorship contribution statement

Xi Zhan: Writing – original draft, Visualization, Investigation, Conceptualization. **Josep Valls-Fonayet:** Writing – review & editing, Supervision, Methodology. **Fatma Lecourieux:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Ghislaine Hilbert-Masson:** Writing – review & editing, Methodology. **Adrien Daudin:** Writing – review & editing, Methodology, Investigation. **Christel Renaud:** Writing – review & editing, Methodology. **Pierre Pétriacq:** Writing – review & editing, Supervision, Methodology, Conceptualization. **David Lecourieux:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2025.114599.

Data availability

Data will be made available on request.

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