iVES OENO One vine & wine

DOI: https://doi.org/10.20870/oeno-one.2025.59.2.8245

OPEN ACCESS

ORIGINAL RESEARCH ARTICLES — VITICULTURE

Canopy management through crop forcing impacts the metabolism of phenolic compounds in grapevine canes

António Teixeira[1*], Inês L. Cabral[2], Henrique Noronha[1], Manon Ferrier[3], Marin-Pierre Gémin[3], Joana Valente[4], Fernando Alves[4], Susana M. P. Carvalho[2], Jorge Queiroz[2], Arnaud Lanoue[3], Hernâni Gerós[1]

[1] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, Campus de Gualtar, University do Minho, 4710-057 Braga, Portugal
[2] GreenUPorto—Research Centre on Sustainable Agrifood Production/Inov4Agro & DGAOT, Faculty of Sciences, Campus de Vairão, University of Porto, Rua da Agrária 747, 4485-646 Vairão, Portugal
[3] EA2106 Biomolécules et Biotechnologies Végétales, Université de Tours, 37200 Tours, France
[4] Symington Family Estates, Vinhos SA, Travessa Barão de Forrester 86, 4431-901 Vila Nova de Gaia, Portugal

Article number: 8245

*correspondence:
antonio.teixeira@bio.uminho.pt

Associate editor:
Simone Castellarin

Received:
30 July 2024

Accepted:
10 April 2025

Published:
5 May 2025

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ABSTRACT

Despite extensive research on phenolic metabolism in grapevines, the effects of canopy management practices on the phenolic composition of woody canopy tissues remain insufficiently understood. This study investigates the impact of severe canopy management through crop forcing (CF) on the metabolic profile of grapevine canes cv. 'Touriga Nacional', building on previous evidence that CF alters the vine physiology. A targeted UPLC-MS analysis was used to identify treatment effects on 54 metabolites, including phenolic acids, stilbenoids, flavonols, and flavan-3-ols. PCA and OPLS-DA showed that woody canes from CF vines have distinct metabolic profiles, with stilbenoids increasing consistently. Notably, there was an increase of *E*-resveratrol (7-fold), *E*-piceatannol (7-fold), and *E*-ε-viniferin (7-fold), compounds that are recognised to play crucial roles in boosting the plant's defence mechanisms. On the other hand, flavan-3-ols like procyanidin B1 showed a consistent decrease in both vintages by up to 24 %. These findings suggest that CF promotes the accumulation of stilbenes and the reduction of flavonols and flavan-3-ols in grapevine canes.

KEYWORDS: phenylpropanoid metabolism, canopy management, stilbenoids, flavan-3-ols, metabolic profile

INTRODUCTION

The phenolic metabolism of grapevines has been extensively studied due to its significant role in wine quality. The biosynthesis of phenylpropanoids begins with the aromatic amino acid phenylalanine, which is synthesised in the plastids via the shikimate pathway. Enzymes like chalcone (CHS) and stilbene (STS) synthases play a crucial role in converting intermediates from the phenylpropanoid pathway into flavonoids and stilbenoids. These enzymes play a critical role in determining whether carbon flux goes towards flavonoid or stilbenoid synthesis, influenced by environmental, hormonal, and biotic signals (Billet *et al.,* 2023; Dao *et al.,* 2011; Matus, 2016; Noronha *et al.,* 2023; Teixeira *et al.,* 2013). Resveratrol and other stilbenoids are extensively studied plant compounds, renowned for their antioxidant, antibacterial, antifungal, cardioprotective, neuroprotective, anti-ageing, and anticancer properties (Biais *et al.,* 2017). Although most research on secondary metabolism in grapevines has focused on berries, it is important to note that key compounds such as *E*-resveratrol and other stilbenoids, including *E*-piceid and *E*-ε-viniferin, also accumulate in perennial tissues such as canes, perennial wood, roots, and winter buds (Billet *et al.,* 2021; Houillé *et al.,* 2015a; Houillé *et al.,* 2015b; Lambert *et al.,* 2013; Németh *et al.,* 2017; Noronha *et al.,* 2023). In winter-harvested canes, the accumulation of stilbenoids was influenced by storage temperatures after pruning (Houillé *et al.,* 2015b) and downy mildew infections during the growing season (Houillé, *et al.,* 2015a).

Based on the discoveries made in grapevine woody tissues, (Noronha *et al.,* 2021), a marked transcriptional reprogramming was observed in genes associated with secondary metabolic pathways following the induction of bud burst. More recently, we confirmed through UPLC-MS analysis that bud burst promoted the activation of the shikimate and phenylpropanoid pathways in these canes tissues (Noronha *et al.,* 2023). This transition is marked by the accumulation of significant metabolites, including ferulic acid, as well as the stilbenoids *E*-resveratrol, *E*-piceatannol, and *E*-ε-viniferin. The observed accumulation of stilbenoids correlated with the heightened expression of several *STS* genes and the transcription factor *VviMYB14*, which regulates the stilbene biosynthesis. The results from this study coupled with other supporting evidence (Ferrandino *et al.,* 2023; Houillé *et al.,* 2015b), suggest the idea that these compounds are produced when newly formed tissues require additional protection from stress, including potential attacks from pathogens. Beyond its scientific implications, this research holds significance in the field of by-product biorefinery, as grapevine woody tissues presently serve as a primary source for extracting biologically active phenolic compounds or producing extracts with antimicrobial properties (Billet *et al.,* 2018b; El Khawand *et al.,* 2020). This shows the potential role of alternative agricultural practices, including pruning techniques, aiming at stimulating the stilbenoid metabolism which could in turn produce woody biomass with increased levels of bioactive compounds.

Crop forcing is a viticultural practice involving the severe removal of certain parts of growing shoots to induce bud regrowth and delay technical maturation. This approach involves trimming the growing shoots to five nodes and manually eliminating all summer laterals, leaves, and clusters to induce the bud break of the dormant buds developed in the current season. We previously showed that CF shifted the timing of harvest in cv. 'Touriga Nacional' from September to October/November, accompanied by an increase in key phenolics like flavan-3-ols and a significant reduction in both yield and pruning weight (Cabral *et al.,* 2023). Building on these findings, we hypothesise that strong interventions in the grapevine canopy may also induce noticeable shifts in the production of secondary compounds in grapevine canes. Thus, in this study, we investigated this hypothesis by assessing the changes in the specialised metabolic profile of grapevine canes when plants undergo CF using a targeted analysis by UPLC-MS.

MATERIALS AND METHODS

1. Canes sampling

Pruned canes from *Vitis vinifera* cv. 'Touriga Nacional' were sourced from experimental trials conducted during the 2019 and 2020 seasons in a commercial vineyard situated in the Douro region of Portugal (41º 14' 36" N, 7º 06' 55" W). Grapevines were subjected to crop forcing practice as described by Cabral *et al.* (2023). The vines were planted in 2014 and grafted onto 196-17Cl rootstock. The vineyard was arranged in a west-southwest to east-northeast orientation, with a spacing of 2.2 meters between rows and 1.0 meter between plants within each row. The vines were trained on a vertical trellis system at a planting density of 4,546 plants per hectare and pruned uniformly using a unilateral Royat cordon with approximately 10 buds per vine. A randomised block design was implemented across eight adjacent rows. Eight plants per treatment were randomly selected and winter pruned manually, leaving four to five spurs and two buds per spur after all leaves had fallen; crop forcing was applied at two stages (Figure 1A): 15 days after cap fall complete (CF1, stage 26, according to the E-L modified scale; Coombe (1995) and 30 days after cap fall complete (CF2). CF1 was performed on the 6th of June 2019 and the 2nd of June 2020, while CF2 was performed on the 25th of June 2019 and the 16th of June 2020. The control grapevines were only for the winter pruning. In both study seasons, experiments were conducted on the same vines.

Canes were collected from vines subjected to three treatments: control (CF0), involving only winter pruning, and two crop-forcing treatments (CF1 and CF2). Crop forcing consisted of hedging growing shoots to five nodes and manually removing all summer laterals, leaves, and clusters to induce budbreak of dormant buds developed during the current season. Each treatment had four replicates, with eight plants per replicate. The canes were sampled in January at winter pruning. The woody material used for subsequent analysis was randomly chosen from the internodes of canes originating

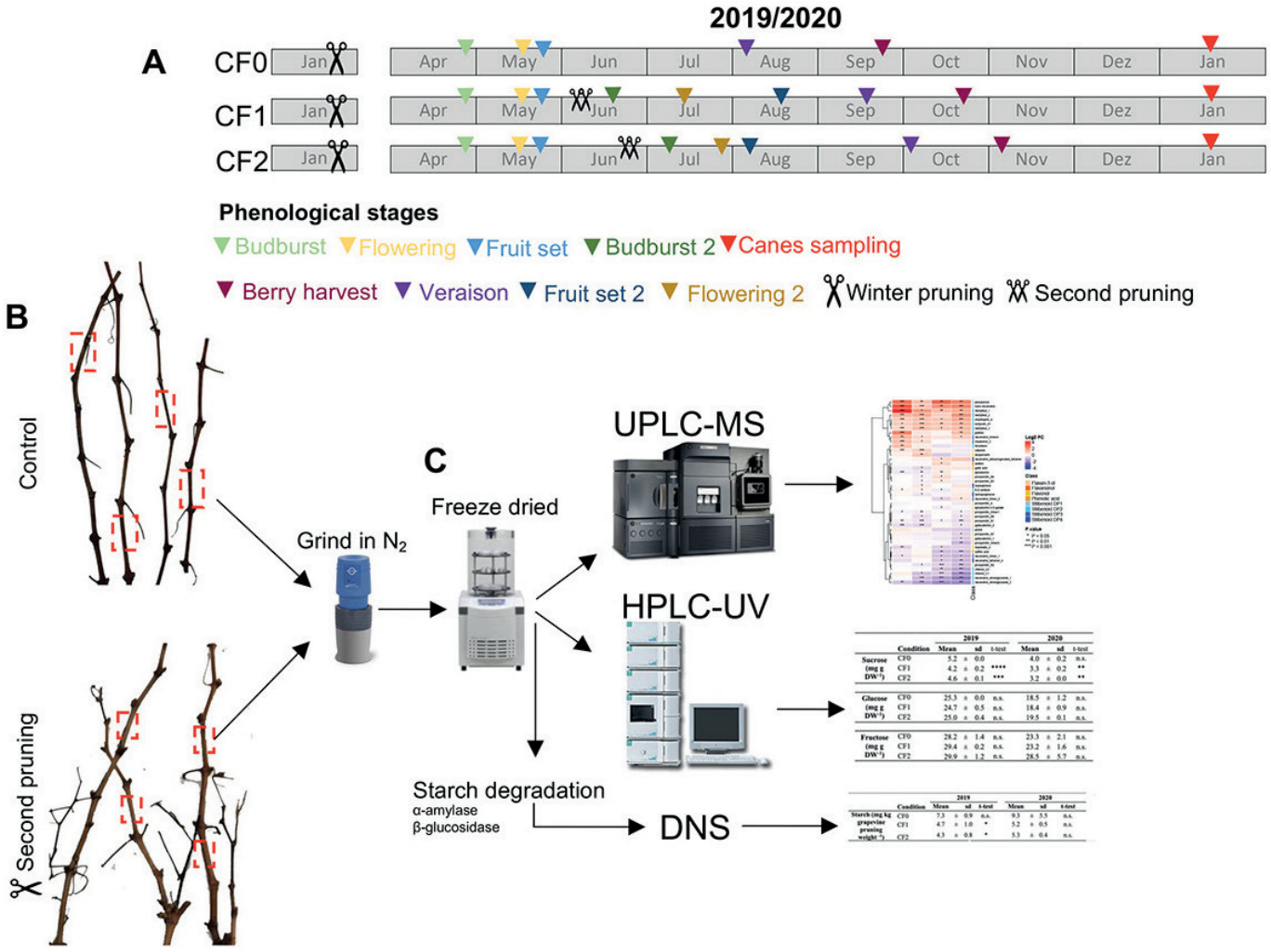


FIGURE 1. Schematic representation of the grapevine phenological stages, crop forcing application dates, and sampling time (A), red dashed squares represent internode sections used for downstream analysis (B) and analysis of primary and secondary metabolites in the seasons 2019 and 2020 (C). The different phenological stages dates are indicated by coloured arrowheads. The scissors symbol indicates the dates of crop forcing (CF1 and CF2) and the control (CF0) consists of grapevines without crop forcing. The terms DP1, DP2, DP3, and DP4 denote the Degree of Polymerization in stilbenoids, corresponding to monomers, dimers, trimers, and tetramers, respectively. The dates of the canes sampling were represented by a red arrowhead.

from eight individual plants per replicate. After removing the bark, the internodes were ground into a fine powder using an IKA A11 basic analytical mill and stored at –80 °C. The powdered canes were then freeze-dried for five days using a Christ Alpha 2-4 LD Plus lyophilizer, preparing them for subsequent biochemical and metabolomics analysis.

2. Starch and sugar measurements

The starch storage in grapevine canes was quantified using the method developed by Smith and Zeeman (2006). Briefly, lyophilized cane tissues (Figures 1B and 1C) weighing 50 mg were subjected to three extractions with 5 mL of ethanol to eliminate soluble sugars and the resulting starch grains were gelatinised through autoclaving. Subsequently, α-amylase (AMY; 1U, Sigma–Aldrich) and β-glucosidase (10U, Sigma–Aldrich) were employed to enzymatically degrade the starch in a medium containing 200 mM sodium acetate (pH 5.5). The glucose content was then quantified using the DNS method (Miller, 1959).

To extract sugars, we followed a previously described procedure by Eyéghé-Bickong *et al.* (2012) with modifications from Teixeira *et al.* (2020). Briefly, 50 mg of lyophilized cane powder tissues were thoroughly mixed with 1 mL of ultrapure H_2O by vortexing. The mixture was then combined with an equal volume of chloroform, followed by vortexing for 5 minutes and incubation at 50 °C for 30 minutes with continuous shaking. After incubation, the samples underwent centrifugation at 14,500 × g for 10 minutes at 4 °C, and the resulting supernatant was carefully collected. The supernatant obtained was filtered through PTFE 0.2 µm filters and the sugars were quantified using HPLC-RI with a Rezex RCM-Monosaccharide Ca^{2+} (8 %) column from Phenomenex. The analysis was performed at a temperature of 40 °C and a flow rate of 0.5 mL min^{-1}, with water as the

mobile phase. The sugar concentration in each sample was calculated by comparing the peak area and retention time with standard sample curves (Teixeira *et al.,* 2020).

3. UPLC-MS-based metabolic profiling

The methods employed for metabolome profiling of grapevine canes were adapted from established and validated procedures from prior studies (Billet *et al.,* 2018a; Noronha *et al.,* 2023). In a concise procedure, 50 mg of dried powder was extracted in 1 mL of ethanol/water (60/40; v/v) through vigorous shaking for 30 minutes at 83 °C using a Thermomixer Comfort (Eppendorf AG, Hamburg, Germany). The resulting mixture was then centrifuged at 18,000 × g for 5 minutes. The supernatant underwent a 5-fold dilution in 80 % (v/v) methanol and was subsequently stored at –20 °C for subsequent analyses. The mass spectrometric system comprises an ACQUITY UPLC H-Class system and a XevoTM TQD triple-quadrupole tandem mass spectrometer equipped with an electrospray ionization (ESI) interface (Waters Corp. in Milford, MA, USA). The chromatographic conditions were the same as previously described. (Billet *et al.,* 2018a; Noronha *et al.,* 2023). Analyte identification relied on a combination of retention times, m/z values, and UV spectra, cross-referenced with commercial standards, internally purified compounds, or information from the literature when authentic standards were not at hand. The complete description of analyte identification can be seen in Martins *et al.* (2020) and the present ID numbers are; gallic acid, caffeic acid, *E*-resveratrol, catechin, *E*-piceatannol, epicatechin, gallocatechin 1, *E*-piceid, vitisinol C1, epicatechin 3-*O*-gallate, astilbin, pallidol, *Z*-ε-viniferin, *E*-ε-viniferin, ω-viniferin, δ-viniferin, isoquercetin, ampelopsin A, scirpusin A1, scirpusin A2, restrytisol 1, restrytisol 2, restrytisol 3, quercetin 3-*O*-glucuronide, procyanidin B1, procyanidin B3, procyanidin B4, procyanidin B2, procyanidin dimer 5, procyanidin dimer 6, resveratrol dimer glycoside 1, resveratrol dimer glycoside 2, resveratrol trimer 1, resveratrol trimer 2, *E*-miyabenol C, resveratrol trimer 3, resveratrol trimer 4, procyanidin C1, procyanidin trimer 2, resveratrol dehydrogenated tetramer, hopeaphenol, isohopeaphenol, resveratrol tetramer 1, resveratrol tetramer 2, resveratrol tetramer 3, *E*/*Z*-vitisin B, viniferol E, gallocatechin 2, vitisinol C2, scirpusin A3, procyanidin A1, resveratrol tetramer 4, resveratrol trimer 5, procyanidin dimer 7. Extraction and UPLC-MS analyses were conducted in quadruplicates.

4. Data mining

The relative quantification of metabolites was performed using the selected ion monitoring (SIM) mode, and the data were integrated using the QuanLynx 4.1 subroutine. To create a quality control sample (QC), a composite pool of all individual samples was prepared. These QC samples were subsequently injected independently and randomly, irrespective of their treatment conditions. At the onset of the sample set, three QC samples were injected, followed by the injection of one QC sample every eight samples to monitor potential analytical drifts. The analysis of QC samples employed Principal Component Analysis to assess the reproducibility of the UPLC-MS method (Fiehn, 2008).

5. Statistical analysis

Principal Component Analysis was obtained using SIMCA P+ version 12.0 (Umetrics AB, Umeå, Sweden), and heatmapping was performed with the R ComplexHeatmap package (v1.18.1) on Bioconductor v3.9 after log values transformation. The statistical significance between crop forcing treatments was assessed through the ANOVA test. Bar plots depicting the mean values ± standard deviation (SD) of four biological replicates were generated using Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

As previously described, the pruning weight was markedly lower in CF vines during both seasons, with a more pronounced effect observed in 2020 (92 %) (Cabral *et al.,* 2023). A picture of the corresponding canes is depicted in the schematic representation of Figure 1B. This resulted in a 41 % reduction in the total starch content per woody pruning weight in canes from the 2019 season and a 92 % reduction in canes from 2020, highlighting the differences between vintages (Table 1). The concentration of soluble sugars (sucrose, fructose, and glucose) showed that only sucrose experienced major reductions (up to 20 %) after crop forcing (Table 1).

Targeted metabolomics analysis using UPLC–MS allowed the detection and identification of 54 metabolites, including 2 phenolic acids, 18 flavonoids, 3 monomeric stilbenoids (DP1), 16 dimeric stilbenoids (DP2), 6 trimeric stilbenoids (DP3), 9 tetrameric stilbenoids (DP4), (Table S1). The unsupervised PCA score plot, based on the first two principal components (PC), explained 43 % of the variance and effectively differentiated the metabolic profiles of grapevine canes under crop forcing (Figure S1). The season emerged as the primary factor driving the variation in cane metabolites (reflected in PC1). Additionally, the crop-forcing treatment was identified as the secondary driving force. Data were then separated into two sets according to the vintages and a supervised OPLS-DA with "crop forcing" as the dependent variable was performed. OPLS-DA score plots provided clear evidence of differentiation based on the CF treatment in both the 2019 (Figure 2A) and 2020 (Figure 2C) vintages (up to 37 % in the *x-axis*).

The prominent group of metabolites responsible for distinguishing samples treated with CF1, CF2, and CF0 are depicted in Figures 2B and 2D. In the 2019 vintage, most stilbenoid DP1-4 were projected on PC1 negative showing an over-accumulation under CF1 and CF2 conditions, whereas flavan-3-ols were mainly projected on PC1 positive suggesting their decrease under CF1 and CF2 conditions (Figure 2B). In the 2020 vintage, a noticeable pattern of separation between CF1 and CF2 canes emerged, with flavan-3-ols playing a pivotal role in distinguishing both CF0 and CF1 from CF2. The results are illustrated by the VIP plot showing the most important metabolite features identified by OPLS-DA (Figure S2). These findings highlight the influence of flavan-3-ols in differentiating between the crop-forcing

TABLE 1. Quantification of starch, sucrose, glucose, and fructose in grapevine canes subjected to crop-forcing second pruning (CF1 and CF2) across two consecutive vintages (2019 and 2020). CF0 represents the control group. CF1 and CF2 represent crop forcing treatments applied at different time points: CF1 corresponds to 15 days after the complete cap fall (stage 26, according to the modified E-L scale; Coombe, 1995), and CF2 corresponds to 30 days after the complete cap fall. The abbreviations used are as follows: pruning weight (PW) and dry weight (DW). Data are presented as mean ± standard error (n = 8). Statistical analysis was performed using one-way ANOVA, followed by Tukey's post-hoc test. Different letters indicate statistically significant differences between groups ($p < 0.05$).

		2019				2020			
	Condition	Mean		sd	AOV	Mean		sd	AOV
Starch per woody PW (mg)	CF0	7.3	±	0.9	a	9.3	±	5.5	a
	CF1	4.7	±	1.0	b	5.2	±	0.5	a
	CF2	4.3	±	0.8	bc	5.3	±	0.4	a
Sucrose (mg g DW^{-1})	CF0	5.2	±	0.0	a	4.0	±	0.2	a
	CF1	4.2	±	0.2	b	3.3	±	0.2	b
	CF2	4.6	±	0.1	c	3.2	±	0.0	bc
Glucose (mg g DW^{-1})	CF0	25.3	±	0.0	a	18.5	±	1.2	a
	CF1	24.7	±	0.5	a	18.4	±	0.9	a
	CF2	25.0	±	0.4	a	19.5	±	0.1	a
Fructose (mg g DW^{-1})	CF0	28.2	±	1.4	a	23.3	±	2.1	a
	CF1	29.4	±	0.2	a	23.2	±	1.6	a
	CF2	29.9	±	1.2	a	28.5	±	5.7	a

treatments within the 2020 vintage (Figure 2D). Although differences were observed between the two vintages, an increase in stilbenoids was associated with treatment CF2 in both years.

Figure 3 illustrates the phenylpropanoid pathway and heatmap representations of the significant changes in metabolite levels after CF treatments compared to control. Stilbenoids, the downstream metabolites of the STS enzyme, seem to over-accumulate under CF treatments (Figure 3). For example, piceatannol showed an increase of up to 7- and 2-fold after CF1 and CF2 in the 2019 vintage, and up to 8- and 6-fold after CF1 and CF2 in the 2020 vintage (Figure 4A). Similarly, *E*-viniferin and *E*-resveratrol also increased (Figures 3 and 4D). However, certain stilbenoids, such as vitisinol C2 or resveratrol dimerglycoside 2, decreased in both CF treatments and vintages (Figures 3, 4F and 4G).

Among the fourteen identified flavan-3-ols (the downstream metabolites of the CHS enzyme), a consistent pattern of decrease was observed in both vintages (Figures 3 and 4, Table S1). For instance, procyanidin B1 slightly decreased by up to 28 % in the 2019 and 2020 vintages (Figure 4D). In contrast, procyanidins B3 and B4, epicatechin, and astilbin displayed a generally decreased trend in 2019 but increased in the 2020 vintage (Figure 3). Gallic acid exhibited a similar pattern, while caffeic acid, the second identified phenolic acid, showed a decrease only in the 2020 vintage (Figure 3). In terms of flavonols, isoquercetin showed an increase of 2-fold in a single condition (CF2) in the 2019 vintage (Figure 3).

DISCUSSION

Crop Forcing is a viticultural practice involving the removal of certain parts of growing shoots to induce bud regrowth, which can influence the timing of maturation and the organoleptic properties of grape berries (Cabral *et al.,* 2023; Dry, 1987; Lavado *et al.,* 2019; Martinez De Toda, 2021; Pou *et al.,* 2019; Prats-Llinàs *et al.,* 2020). In our previous study, we demonstrated that CF has a significant impact on key metabolites in grape berries and highlighted a marked reduction in pruning weight (Cabral *et al.,* 2023). As shown in the present study, CF also affected starch levels accumulated in canes across both vintages. Other studies indicated that CF can delay phenology, lower yield, and delay grape ripening, which may also affect the accumulation of phenolic compounds in grapevine canes (Cabral *et al.,* 2023; Lavado Rodas *et al.,* 2023; Martínez-Moreno, 2019). The observed reduction in sucrose concentration in the shoots was likely due to the late ripening of the grapes on these

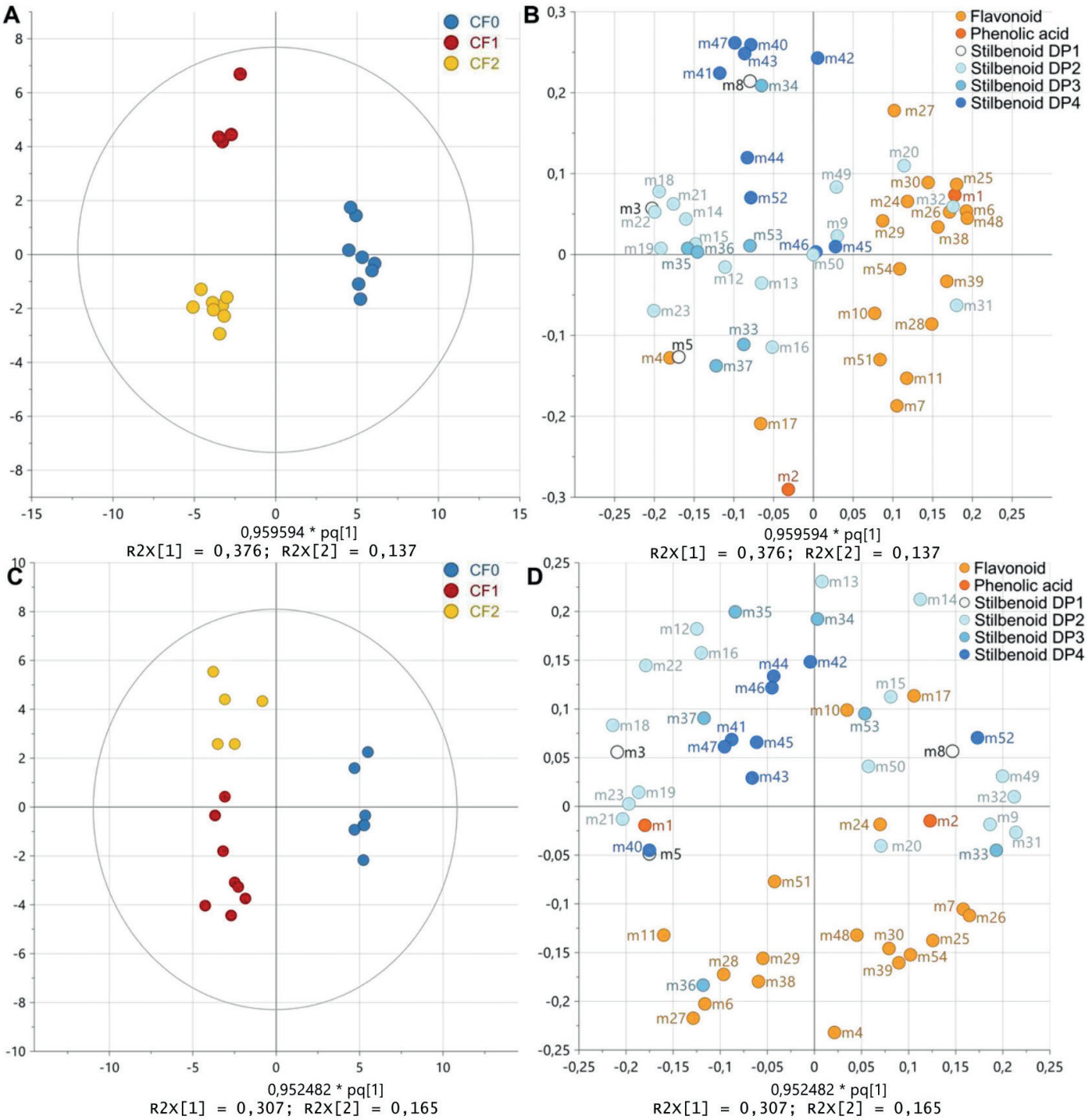


FIGURE 2. Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) with crop forcing as dependent variables on metabolomic data from grapevine canes of cv. 'Touriga Nacional' in the vintages of 2019 (A and B), with an overall variability of 50 % in the first two dimensions and 2020 (C and D), with an overall variability of 47 % in the first two dimensions. Observations in the score plots were coloured according to the sample treatments (CF0-blue, CF1-red and CF2-yellow). Variables in loading plots were numbered according to the metabolite category. The terms DP1, DP2, DP3, and DP4 denote the Degree of Polymerization in stilbenoids, corresponding to monomers, dimers, trimers, and tetramers, respectively.

shoots. Once the grapes had fully matured, the time available for assimilate accumulation in the shoots was relatively short. An early study on cv. Gamay Fréaux var. Teinturier cell suspensions reported that the application of sucrose slightly increased stilbene production (Larronde *et al.*, 1998). More recently, it was demonstrated that increasing sucrose concentrations in cv. Barbera consistently promoted stilbene production (Ferri, 2011). According to a study conducted by Noronha *et al.* (2021), there is an increase in the expression of genes associated with sugar metabolism and sugar transporters before the spring bud burst, which may explain the mobilisation of sugar that occurs following CF.

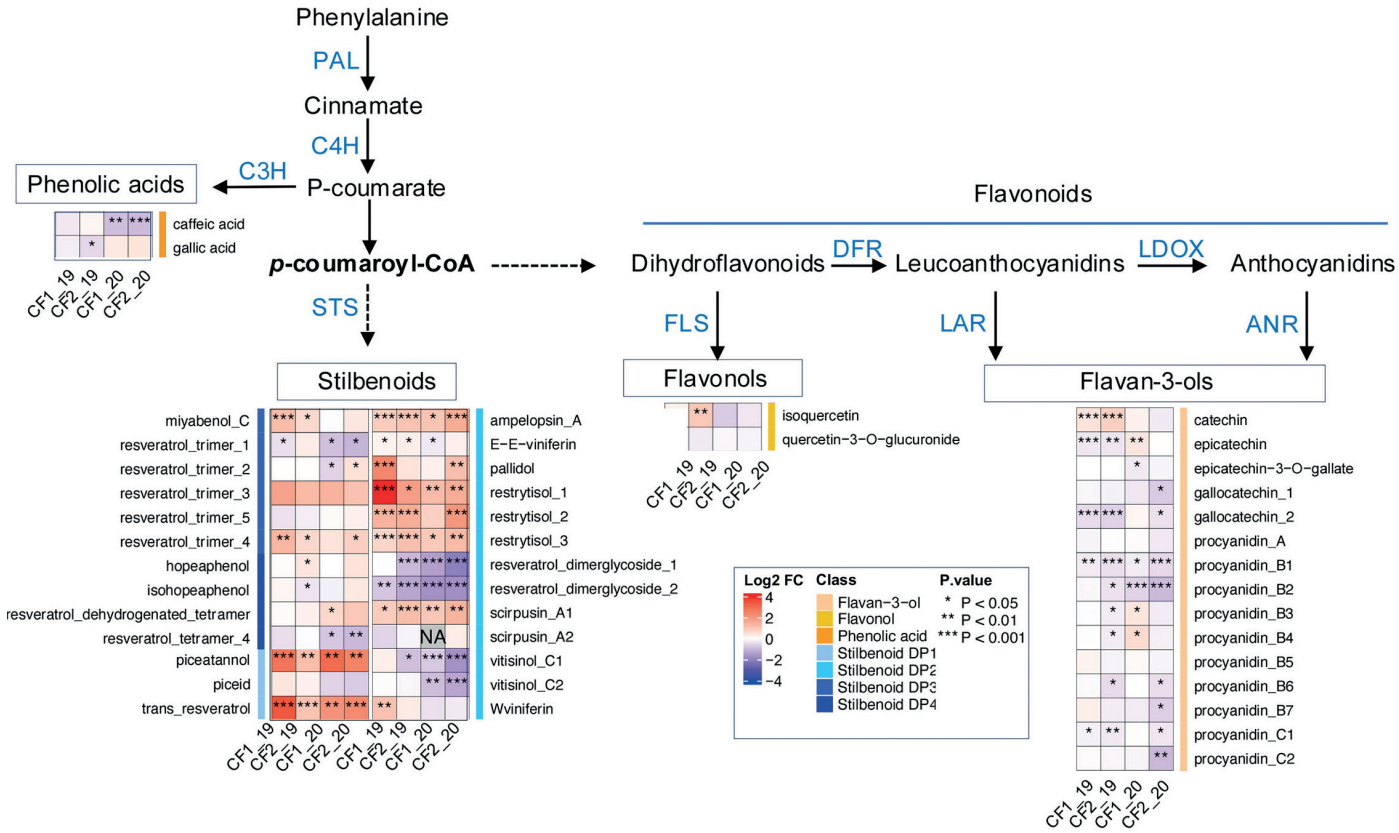


FIGURE 3. Metabolite changes in secondary metabolites in grapevine canes of cv. 'Touriga Nacional' in the vintages of 2019 and 2020. Heatmaps show the Log_2 of Fold Change to the control canes of phenolic acids, monomeric stilbenoids (Degree of Polymerization 1-DP1), dimers stilbenoids (DP2), trimers stilbenoids (DP3), tetrameric stilbenoids (DP4), flavonols and flavan-3-ols. PAL, henylalanine ammonia-lyase (PAL); C4H, cinnamate 4-monooxygenase; C3H, coumarate 3-hydroxylase; STS, stilbene synthase; FLS, flavonol synthase; DFR, dihydroflavonol 4-reductase; LAR, leucoanthocyanidin reductase; LDOX, leucocyanidin oxygenase; ANR, anthocyanidin reductase. Asterisks indicate statistical significance between crop forcing treatment and the respective control within each sample year following Student's t-test: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

The present study showed CF as a strong cause of variation in the cane's secondary metabolites with an increase of most stilbenoids and a general decrease of flavonols, which are in line with results from grape berries from the same CF-treated grapevines (Cabral *et al.,* 2023). This antagonism between flavonoids and stilbenoid metabolic branches was already observed following downy mildew-infected leaves (Billet *et al.,* 2020) and under terroir influence in grape canes (Billet *et al.,* 2023). A crosstalk of transcriptional, metabolic and hormonal signals controlling stress defence responses was observed in berries and vegetative organs (Ferrandino *et al.,* 2023). These include a stress-induced synthesis of defence compounds, hormonal changes affecting metabolic pathways, transcriptional reprogramming upregulating genes in the phenylpropanoid pathway, a strategic metabolic shift towards stilbenoids, resource reallocation to prioritise certain metabolites, and the amplification of environmental stress effects (Ferrandino *et al.,* 2023).

Among stilbenoids, *E*-piceatannol and *E*-resveratrol, revealed a consistent increase in almost all experimental conditions, particularly when vines were subjected to CF1. In grapevines, *E*-resveratrol is accumulated in the exocarp, seeds, and infected leaves, while *E*-piceid and *E*-ε-viniferin were more prominent in perennial tissues like canes (Billet *et al.,* 2020; Gatto *et al.,* 2008; Németh *et al.,* 2017). Other stilbenoids, including ampelopsin A, *E*-miyabenol C, *Z*/*E*-vitisin B, hopeaphenol, and isohopeaphenol, were also found to accumulate in grapevine canes, with their levels being influenced by genotype, storage temperatures, and downy mildew infections (Billet *et al.,* 2021; Houillé *et al.,* 2015a; Houillé *et al.,* 2015b; Lambert *et al.,* 2013). However, the results suggested that CF may not stimulate the glycosylation or polymerisation of resveratrol as resveratrol dimer glycoside or resveratrol trimer/tetramer did not increase.

Interestingly, fluorescence analysis showed that vascular tissues were the major site of stilbenoid accumulation in grape canes (Houillé *et al.,* 2015a), and their presence has been reported in xylem sap of vines following infection by esca and Pierce's disease (Bruno & Sparapano, 2006). Also, it has been shown that the STS enzyme, along with its stilbenoid product, is located in the cell wall of grapevines (Bellow *et al.,* 2012; Fornara *et al.,* 2008). The cell wall serves as a protective barrier that fungal pathogens need to overcome to access intracellular

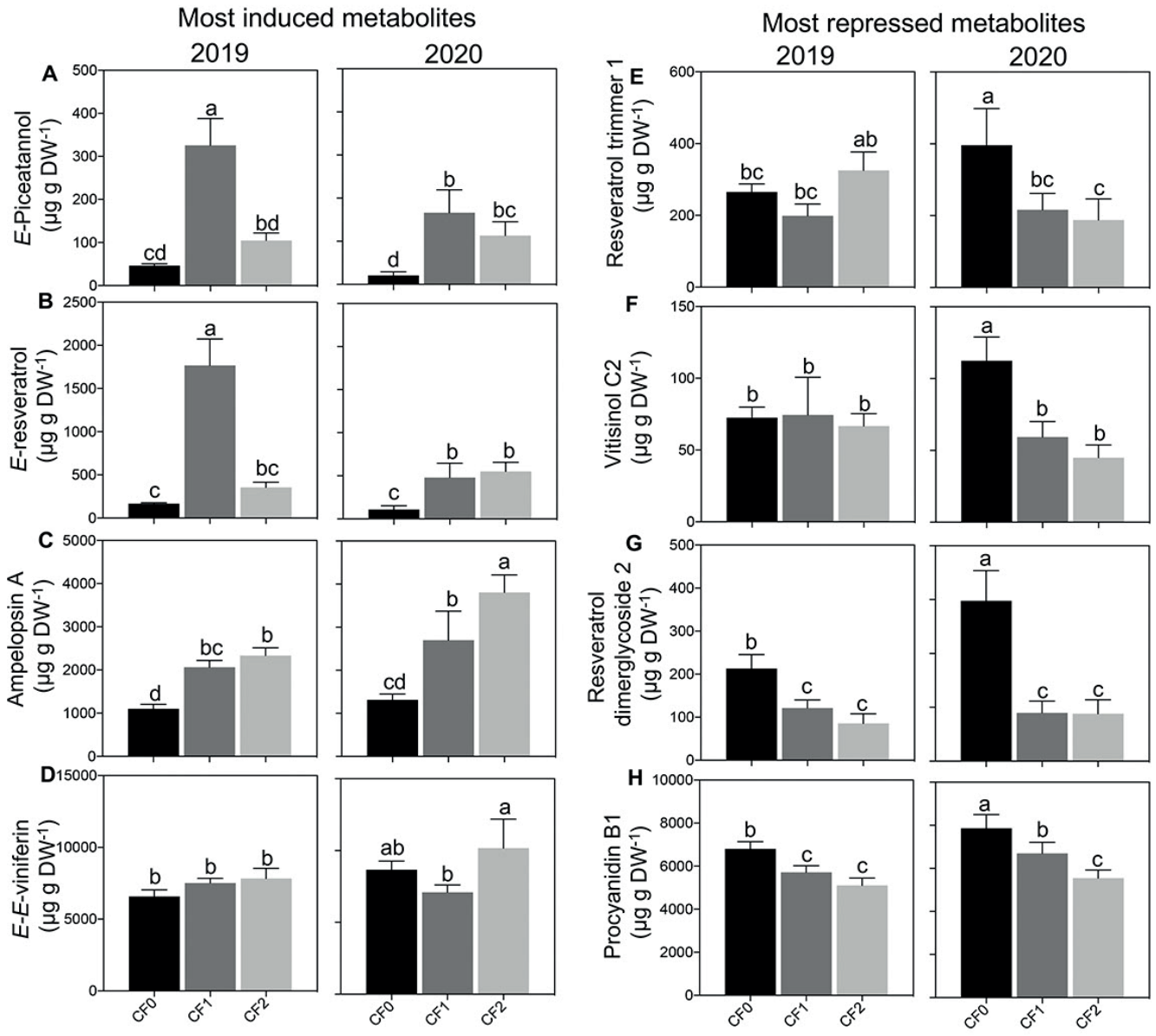


FIGURE 4. Most induced and repressed metabolites in grapevine canes of cv. 'Touriga Nacional', subjected to crop forcing in the vintages of 2019 and 2020. Letters indicate statistical significance between crop forcing treatment and the respective control.

components, and the deposition of lignin, as well as other phenolics, is acknowledged as a defence mechanism against such attacks (Ninkuu *et al.,* 2022). Consequently, the synthesis and accumulation of stilbenoids in the cell wall align with its antimicrobial and protective properties, potentially serving as an effective strategy to shield the plant from fungal diseases (Fornara *et al.,* 2008). As a whole, we cannot dismiss the hypothesis that the observed increase in stilbenoids in the fruits following crop forcing may result from their systemic accumulation after synthesis at the site of injury, followed by transportation through the xylem. Nonetheless, it cannot be ruled out that the observed accumulation of stilbenoids following CF in canes is due to the plant response to mechanical injury since previous work has shown that mechanical injury in canes rapidly induces *PAL* and *STS* expression levels, as well as *E*-resveratrol and *E*-piceatannol accumulation (Billet *et al.,* 2018b).

The present study showed that technical management approaches may impact the composition of biomolecules in perennial woody tissues like grape canes. Especially, a second forcing of the growing shoots in June resulted in a strong induction of *E*-resveratrol, *E*-piceatannol in grape canes pruned during winter. Since *E*-resveratrol, *E*-piceatannol are two well-studied compounds recognised a plethora of biological benefits on human health (Koh *et al.,* 2021), CF has the potential to increase the quality and composition of grape canes as raw material for the sustainable production of polyphenol-enriched extracts with nutraceuticals and cosmetics interests.

CONCLUSION AND PERSPECTIVES

Canopy management achieved through CF techniques induced a notable metabolic shift in grapevine canes. This transformation resulted in the heightened accumulation of stilbenoids, with a specific emphasis on *E*-resveratrol, *E*-piceatannol, and *E*-ε-viniferin, compounds that are recognised to play crucial roles in bolstering the plant's defence mechanisms. Besides their role in plant physiology, these findings have opened up new research avenues with important practical implications for science, agriculture, and biotechnology. For instance, exploring how different pruning techniques, ranging from timing to severity, affect the synthesis and accumulation of specific stilbenoids and other secondary metabolites in grapevine canes and how their biosynthetic pathways are modulated is of the utmost relevance. Also, taking into account the role of stilbenoids in plant defence, it could be important to assess if CF affects plant resilience against biotic and abiotic stresses, particularly in the face of changing environmental conditions and agricultural demands. Finally, canes, which are considered as vineyard by-products, from CF plants may be used in sustainable agricultural practices, such as biopesticides or biostimulants, to enhance crop protection and productivity.

ACKNOWLEDGEMENTS

This research was funded by the VISCA project (Vineyards' Integrated Smart Climate Application), funded by the European Union's Horizon 2020 research and innovation programme under grant agreement no. 730253. The authors also gratefully acknowledge the Région Centre-Val de Loire for funding the project INNOCOSM from 'Cosmétosciences Research & Development Ambition'. The authors wish to thank the C-Valo organisation for the grant to the LOCASARM project funded by FEDER and Région-Centre Val de Loire. The work was also supported by the "Contrato-Programa" UIDB/04050/2020, funded by Portuguese national funds through the FCT I.P. The work was also supported by FCT through the project GrapeMicrobiota (PTDC/BAA-AGR/2691/2020). This work also benefited from the networking activities within the European COST Action CA 17111 INTEGRAPE, the CoLAB VINES & WINES.

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