



ORIGINAL RESEARCH ARTICLE

# Canopy management through crop forcing impacts grapevine cv. 'Touriga Nacional' performance, ripening and berry metabolomics profile

Inês L. Cabral<sup>1</sup>, António Teixeira<sup>2</sup>, Manon Ferrier<sup>3</sup>, Arnaud Lanoue<sup>3</sup>, Joana Valente<sup>4</sup>, Frank S. Rogerson<sup>4</sup>, Fernando Alves<sup>4</sup>, Susana M. P. Carvalho<sup>1</sup>, Hernâni Gerós<sup>2,\*</sup> and Jorge Queiroz<sup>1</sup>

<sup>1</sup> 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

<sup>2</sup> Centre of Molecular and Environmental Biology (CBMA), Department of Biology, Campus de Gualtar, University do Minho, 4710-057 Braga, Portugal

<sup>3</sup> EA2106 Biomolécules et Biotechnologies Végétales, Université de Tours, 37200 Tours, France

<sup>4</sup> Symington Family Estates, Vinhos SA, Travessa Barão de Forrester 86, 4431-901 Vila Nova de Gaia, Portugal



\*correspondence:  
geros@bio.uminho.pt

Associate editor:  
Markus Rienth



Received:  
22 July 2022

Accepted:  
10 December 2022

Published:  
13 January 2023



This article is published under  
the **Creative Commons  
licence (CC BY 4.0)**.

Use of all or part of the content  
of this article must mention  
the authors, the year of  
publication, the title,  
the name of the journal,  
the volume, the pages  
and the DOI in compliance with  
the information given above.

## ABSTRACT

Climate changes are speeding up the maturation of grapes in numerous areas of the world, including in the Mediterranean basin, but warmer temperatures often uncouple technical and phenolic maturity, resulting in unbalanced wines. We tested the efficacy of crop forcing (CF) in delaying the maturation of cv. 'Touriga Nacional' vines of the Douro Region, and their impacts on plant performance, berry quality attributes and metabolome were also evaluated. In two consecutive seasons (2019 and 2020), CF was conducted 15 (CF1) and 30 (CF2) days after fruit set by hedging growing shoots to five nodes and removing summer laterals, leaves and clusters. Results showed that while CF2 delayed ripening up to 51 days till first autumn rainfall, which compromised optimal sugar ripeness, CF1 delayed the technical maturation by one month, but both treatments severely impacted the production, mainly CF1, which reduced grapevine yield up to 90 %. The effect of CF in protecting vines against drought stress was not evident, judging by the values of leaf pre-dawn water potential measured along both seasons. CF1 and CF2 resulted in berries with lower pH and higher titratable acidity than controls, while total phenolics content increased by up to 48 % in 2020. A UPLC–MS-based targeted metabolomic analysis showed that CF increased the relative abundance of key metabolites like flavan-3-ols (i.e., catechin gallate increased by up to 661 %), trihydroxylated anthocyanins (i.e., delphinidin-3-O-glucoside increased by up to 656 % after CF2) and stilbenes (resveratrol increased by up to 700 % after CF2) with potential positive impacts in wine quality.

**KEYWORDS:** berry quality, metabolomics, crop forcing, berry phenolics, phenolic maturity, *Vitis vinifera* L.

## INTRODUCTION

Over a period of more than a century, there has been a 1 °C increase in mean land surface temperature, and climate projections for the end of the 21st century forecast further increases in temperature, depending on the mitigation measures adopted (IPCC, 2014; Martínez-Lüscher *et al.*, 2016). These changes in climate result in the occurrence of extreme weather events, including heat waves and periods of extreme drought (Ramos, 2017; Fraga *et al.*, 2018). Numerous highly important wine regions in the world are located in areas of the planet highly vulnerable to climate change. This is the case of the Mediterranean basin, including the Douro Demarcated Region (DDR), with models for this region predicting significant thermal increases and decreases in rainfall occurrence (Jones and Alves, 2012; IPCC, 2014; Fraga *et al.*, 2016; Costa *et al.*, 2019).

Different studies have addressed the effect of drought on grapevine vigour, yield and fruit quality (Matthews *et al.*, 1987; Romero *et al.*, 2014; Chaves *et al.*, 2010; Dayer *et al.*, 2013; Teixeira *et al.*, 2013; Gerós *et al.*, 2015; Cabral *et al.*, 2022). It is well known that water deficit impairs vine growth and decreases yield, but moderate drought may enhance berry colour and improve grape and wine quality (Chaves *et al.*, 2010). These responses, however, depend on a number of different factors, including the cultivar, crop load, vineyard age, soil type, phenological stage or canopy development (Ojeda *et al.*, 2002; Cook *et al.*, 2015; Hochberg *et al.*, 2015; Cabral *et al.*, 2022). Temperature impacts vine phenology, including the dates of bud break, flowering and veraison, but also the content of the berry in sugars and organic acids (Iland *et al.*, 2002; Sadras and Moran, 2013; Rienh *et al.*, 2016), secondary compounds, including anthocyanins (Mori *et al.*, 2007; Degu *et al.*, 2016; Poni *et al.*, 2020), and aromatic compounds (Schultz, 2000; Lebon, 2002). Thus, increasing temperatures shortens the time from anthesis until maturation (Keller and Tarara, 2010; Martínez-Lüscher *et al.*, 2016), uncoupling technical and phenolic maturity, which results in berries with higher sugar concentration and lower acidity (Rienh *et al.*, 2016), lower anthocyanins, tannins, and total phenolic concentration, with negative effects on grape and wine aroma and flavour, especially in red grape varieties (Palliotti *et al.*, 2014).

Different management strategies have been tested to mitigate the above-mentioned climate change effects on fruit maturation, including the displacement of vineyards for cooler regions, the selection of better-adapted cultivars, clones or accessions, or the implementation of new viticultural practices (Poni *et al.*, 2018; Martínez-Lüscher *et al.*, 2016). Some of these viticultural practices, including the application of chemical compounds, such as auxins (Böttcher *et al.*, 2011), late winter pruning (Zheng *et al.*, 2017a; Moran *et al.*, 2017), shoot trimming (Martínez de Toda *et al.*, 2014; Zheng *et al.*, 2017b; Santesteban *et al.*, 2017), and minimal pruning (Clingeffer, 2010; Zheng *et al.*, 2017c; Molitor *et al.*, 2019), have shown a positive delay in sugar

ripeness up to 10 to 15 days when performed individually, or even 15 and 45 days when combined.

Forcing bud regrowth (crop forcing) is still a poorly explored viticulture practice that consists of removing growing shoot parts, including tips, summer laterals, clusters and leaves, leaving several nodes, which delays maturation for up to two months (Dry, 1987; Gu *et al.*, 2012; Lavado *et al.*, 2019; Martínez de Toda *et al.*, 2019; Pou *et al.*, 2019; Prats-Llinàs *et al.*, 2020). The crop forcing releases the control exerted by the shoot apex and the summer laterals over the outgrowth of the dormant buds that normally produce the inflorescences in the upcoming season (Dry, 1987; Fang *et al.*, 2000; Lavee and May, 1997), enabling the regrowth of new vegetative and reproductive structures (Cline, 1991; Cline, 1994; Martínez-Moreno *et al.*, 2019).

In cv. ‘Cabernet-Sauvignon’ and ‘Tempranillo’ vines, crop forcing in June shifted fruit ripening from the hot (July and August) to the cool season (October through early November). Smaller and more acidic berries (due to increments of both tartaric and malic acids) were produced, with higher contents of total anthocyanins, tannins, and total phenolics (Gu *et al.*, 2012; Martínez-Moreno *et al.*, 2019). In another study, berries of cv. ‘Tempranillo’ accumulated significantly higher total polyphenols and anthocyanins in response to crop forcing, and wines were notably rich in tannins, but the cluster’s weight was compromised, and, therefore, the yield, although a higher number of clusters was produced (Lavado *et al.*, 2019). Results also showed that yield reduction was less pronounced when crop forcing was applied later in the season. Yield reduction was also observed in other studies (Gu *et al.*, 2012; Martínez de Toda *et al.*, 2019), whose intensity depended on the time of crop forcing application (Martínez de Toda *et al.*, 2019).

A bud-forcing technique aimed at obtaining two crops per season was explored in Pinot Noir vines grown in pots (Poni *et al.*, 2021). The primary crop was maintained, but a second, late-ripening crop was ensured through the release of dormancy of the auxiliary buds. The technique proved feasible to warrant two crops in the same season with different features.

Forcing vines to regrowth is an aggressive technique (Lavado *et al.*, 2019), whose advantages need to be carefully validated for each wine region, growing season and cultivar. The present study was implemented in the context of the European project, VISCA (Vineyards Integrated Smart Climate Application, <https://visca.eu/>), which aimed to make European wine industries resilient to climate changes through the application and optimisation of techniques like crop forcing, shoot trimming and irrigation assisted by medium-long term forecast models. In Douro Demarcated Region (DDR), the three main grapevine varieties, ‘Touriga Nacional’, ‘Touriga Franca’ and ‘Tinta Roriz’ (‘Tempranillo’), are cultivated during part of the growing season in marginal weather conditions for agricultural production (low precipitation and high radiation and temperature) that tend to worsen in the future (Gouveia *et al.*, 2011). Models predict

advances in budburst of up to 14 days and in flowering and veraison of up to 10 days in ‘Touriga Nacional’ cultivated at low elevations along the Douro River in a time window from 2051–2080 (Reis *et al.*, 2022). Although this cultivar is considered adapted to warm climates, with a high capacity for heat dissipation and for withstanding high light intensities (Carvalho *et al.*, 2016), elevated temperatures tend to increase the pH and sugar content of the berry and decrease berry weight, titratable acidity and anthocyanins (Costa *et al.*, 2020). Thus, in the present study, we hypothesise that crop forcing may delay the maturation and prevent this uncoupling of technical and phenolic maturity in cv. ‘Touriga Nacional’. Moreover, we aim to perform a metabolic berry analysis to characterise in detail the modifications of the secondary metabolism in response to crop forcing in this cultivar.

## MATERIALS AND METHODS

### 1. Field conditions and experimental site

The experimental trial was conducted during the 2019 and 2020 seasons in a commercial vineyard with sandy loam soil located in the Douro Superior sub-region, Portugal (41°14′36″N, 7°06′55″W), at an altitude of about 140 m. The cultivar ‘Touriga Nacional’ (*Vitis vinifera* L.) used in the study was planted in 2014 and grafted in 196-17Cl rootstock. Plants were oriented in west southwest to east/northeast, spaced at 2.2 m between rows, 1.0 m along the row and trained on a vertical trellis (4546 plants/ha), uniformly pruned on a unilateral Royat cordon, ca. 10 buds per vine. Eight adjacent rows were selected to implement a randomised block design. Plants were manually winter pruned, when all the leaves had fallen, to four to five spurs and two buds per spur.

The experiment considered three treatments, a control (CF0) with vines grown under conventional practices (only winter pruning) and two crop forcing dates (CF1 and CF2), with four replicates per treatment and five plants per replicate. The crop forcing consisted of hedging the growing shoots to five nodes and removing all the summer laterals, leaves and clusters manually to force the bud break of the dormant bud developed in the current season. Crop forcing was applied 15 days after the fruit set (stage 26–cap-fall complete, according to E-L modified scale) corresponding to CF1 and 30 days after fruit set, which corresponded to CF2 treatment (Figure 1A,B). CF1 was performed on 6th June 2019 and 2nd June 2020, whereas CF2 was performed on 25th June 2019 and 16th June 2020. In both seasons of the study, the experiments were performed in the same vines. Winter pruning and removal of leaves and clusters were performed manually. Desuckering was performed more often in crop-forced vines.

### 2. Meteorological data, predawn leaf water potential, phenological stages and vegetative growth

Meteorological data were obtained from an automatic weather station (ADCON, Kempten, Germany). Precipitation (P), maximum temperature (Tmax), minimum temperature (Tmin), average temperature (Tavg), insolation (Insol) and

evapotranspiration (Etp) were registered. Growing Degree Days (GDD) were also calculated in both vintages following the Winkler (1974) and McMaster and Wilhelm (1997) method:  $GDD = ((T_{max} + T_{min})/2) - T_{base}$ , where base temperature ( $T_{base}$ ) is 10 °C, and is calculated over the growing season (April–October). The ripening season was considered to be from veraison until November because crop forcing delayed the harvest.

Pre-dawn leaf water potential ( $\Psi_{pd}$ ) was measured 2 h before sunrise with a Schölander pressure chamber (PMS Instruments Co., Model 600, Corvallis, OR, USA) (Schölander *et al.*, 1965). The measurements were carried out in eight plants per treatment (using one well-exposed and fully expanded leaf per plant) every 15 d, from fruit set until harvest.

Phenological stages, including budbreak, fruit set, veraison and harvest, were recorded when 50 % of the plants exhibited each stage, according to the modified E-L system (Coombe, 1995). At harvest, the total leaf area was registered using the method developed by Lopes and Pinto (2005). At the dormancy stage, the number of shoots was recorded, vines were pruned, and pruning mass (kg/vine) was determined for each vine of each replication (32 vines per treatment) (Figure 1A,B).

The budburst rate was calculated by dividing the number of shoots of each vine by the number of buds, and the total fertility index was estimated from the ratio between the number of clusters and the total number of shoots per plant.

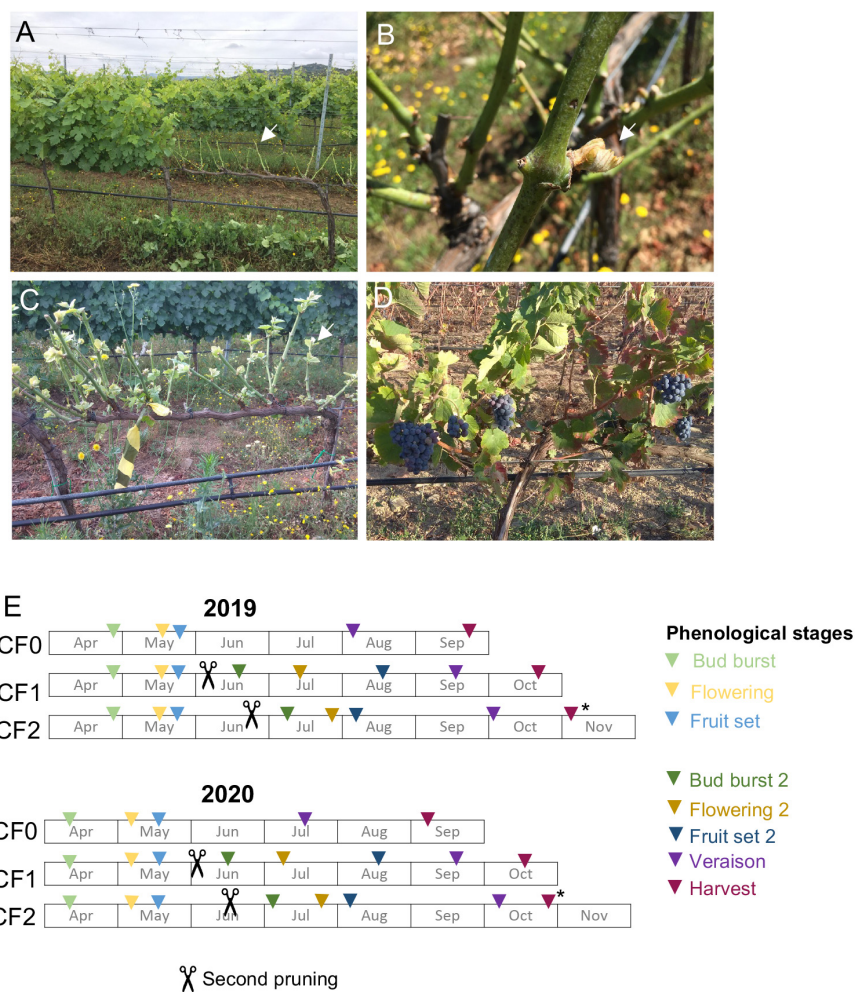
### 3. Yield and berry composition

To evaluate the plant yield at harvest, the number of clusters was counted, and cluster weight (g) was measured in each vine. Average cluster weight was calculated from the ratio between the total cluster weight and the number of grapevines under study.

Ten berries were collected from each plant, being selected from different cluster positions. The berries were crushed, and several biochemical quality parameters were determined: pH, total soluble solids (TSS; °Brix), total acidity (g L<sup>-1</sup>), malic acid (g L<sup>-1</sup>), total phenolics (Absorption Unit—A.U.) and yeast assimilable nitrogen (YAN; mg L<sup>-1</sup>) using OenoFoss™ (FOSS Analytical, Hilleroed, Denmark) according to manufacturer’s protocol equipment and by official methods of the *Organisation Internationale de la Vigne et du Vin* (OIV, 2021).

### 4. UPLC-MS-based metabolic profiling

Methods for metabolic profiling of grape berries were adapted from previous studies (Billet *et al.*, 2018; Martins *et al.*, 2020). For sample preparation, 50 mg of berry dry weight was extracted using 1 mL of 80 % (v/v) methanol. After 30 min of sonication, samples were macerated overnight at 4 °C in the dark and centrifuged at 18,000 × g for 10 min. The supernatant was diluted 5-fold in 80 % (v/v) methanol and stored at –20 °C prior to further analyses. UPLC-MS was performed using an ACQUITY™ Ultra Performance Liquid Chromatography system coupled to a photodiode array detector (PDA) and a Xevo TQD mass spectrometer



**FIGURE 1.** Time of crop forcing in cv. 'Touriga Nacional'.

Photographs of grapevines subjected to crop forcing (A), grapevine shoot with a second bud burst (B), inflorescence elongating (C) and harvest (D). (E) recorded dates of different phenological stages indicated by coloured arrow heads. The scissors symbol indicates the dates of crop forcing (CF1 and CF2). Asterisk (\*) indicate harvest before technical mature stage.

(Waters, Milford, MA) equipped with an electrospray ionisation (ESI) source controlled by Masslynx 4.1 software (Waters, Milford, MA). Analyte separation was achieved by using a Waters Acquity HSS T3 C18 column (150 × 2.1 mm, 1.8 μm) with a flow rate of 0.4 mL min<sup>-1</sup> at 55 °C. The injection volume was 5 μL. The mobile phase consisted of solvent A (0.1 % formic acid in water) and solvent B (0.1 % formic acid in acetonitrile). Chromatographic separation was achieved using an 18-min linear gradient from 5 to 50 % solvent B. MS detection was performed in both positive and negative modes. The capillary voltage was 3000 V, and sample cone voltages were 30 and 50 V. The cone and desolvation gas flow rates were 60 and 800 Lh<sup>-1</sup>. Identification of analytes was based on retention times, m/z values, and UV spectra and by comparison with commercial standards, own purified compounds or data from literature when no authentic standards were available. The complete description of analyte identification can be seen in Martins *et al.* (2020) and the present ID numbers are; L-proline (m1), L-leucine (m2), L-isoleucine (m3), phenylalanine (m4), L-tyrosine (m5), L-tryptophan (m6), cyanidin-3-*O*-

glucoside (m7), peonidin-3-*O*-glucoside (m8), delphinidin-3-*O*-glucoside (m9), petunidin-3-*O*-glucoside (m10), cyanidin-3-*O*-(6-*O*-acetyl)-glucoside (m11), malvidin-3-*O*-glucoside (m12), petunidin-3-*O*-(6-*O*-acetyl)-glucoside (m13), malvidin-3-*O*-(6-*O*-acetyl)-glucoside (m14), petunidin-3-*O*-(6-*p*-coumaroyl)-glucoside (m15), malvidin-3-*O*-(6-*p*-coumaroyl)-glucoside (m16), malvidin-3,5-*O*-diglucoside (m17), gallic acid (m18), citric acid (m19), *t*-resveratrol (m20), piceatannol (m21), catechin (m22), epicatechin (m23), coumaric acid (m24), caffeic acid (m25), ferulic acid (m26), piceid (m27), catechin gallate (m28), kaempferol-3-*O*-glucoside (m29), pallidol (m30), *t*-ε-viniferin (m31), quercetin-3-*O*-glucoside (m32), quercetin-3-*O*-glucuronide (m33), myricetin-hexoside1 (m34), myricetin glucoside (m35), quercetin derivative (m36), procyanidin B1 (m37), procyanidin B2 (m38), procyanidin B3 (m39), procyanidin B4 (m40), kaempferol-3-*O*-rutinoside (m41), procyanidin gallate1 (m42), procyanidin C1 (m43), procyanidin gallate2 (m44), procyanidin trimer2 (m45). Extraction and UPLC-MS analyses were performed in quadruplicates.

## 5. Data mining

UPLC-MS analyses were achieved using selected ion monitoring (SIM) mode and the resulting SIM chromatograms were integrated using the subroutine QuanLynx 4.1 for data mining. A pool of all samples was prepared to obtain a quality control sample (QC), and the samples were randomly injected independently from treatment conditions. Three QC samples were injected at the beginning of the sample set, and one QC sample was injected every 8 samples to check for potential analytical drifts. QC samples were analysed by Principal Component Analysis to evaluate the reproducibility of the UPLC-MS method (Fiehn *et al.*, 2008).

## 6. Statistical analysis

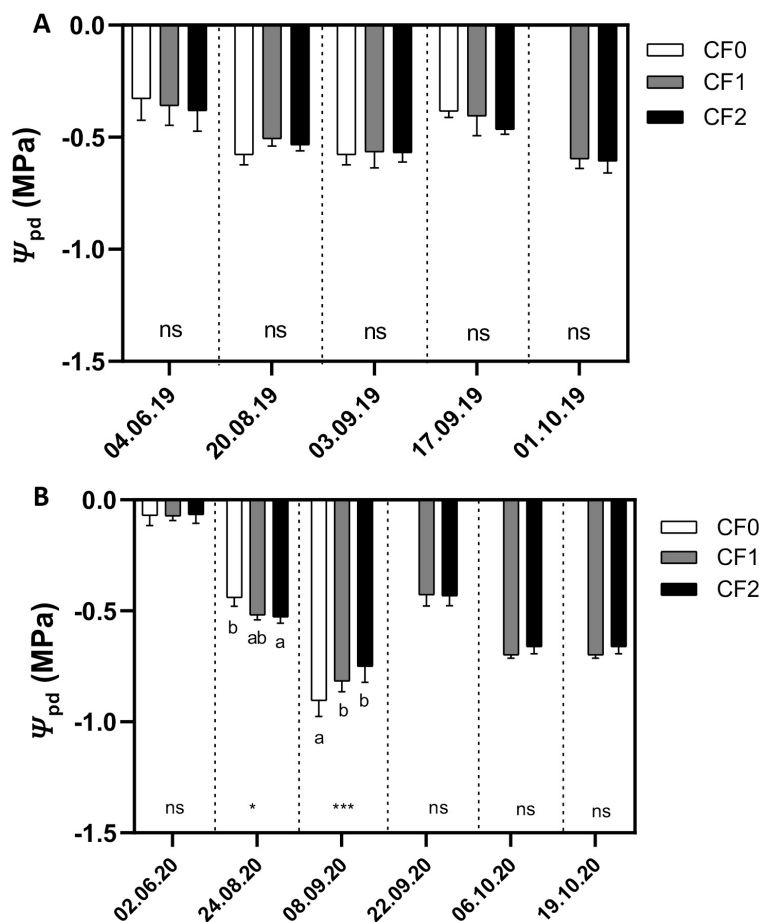
All data are presented as mean values  $\pm$  standard deviation (SD) of four biological replicates in each assay. One-way ANOVA, using Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA), was performed for data analysis. The Principal Component Analysis was made using SIMCA P+ version 12.0 (Umetrics AB, Umeå, Sweden) and Heatmap metabolomics was performed with the ComplexHeatmap package (v1.18.1) on Bioconductor v3.9 after values normalisation by using the R centre and scale functions.

## RESULTS

### 1. Evolution of phenological stages and pre-dawn water potential in 'Touriga Nacional' after Crop Forcing

The season of 2019 in Douro Valley was characterised by low levels of precipitation throughout the year (Figure S1). The highest monthly average maximum temperature was observed in July (34.7 °C), and the lowest was in January (1.1 °C). In the 2020 season, precipitation was similar to the previous year. The highest monthly average maximum temperature was 41 °C measured in July and August, while in January, a below-zero minimum temperature (−2.2 °C) was registered (Figure S1A). In both seasons, potential evapotranspiration (ETp) showed an inverse correlation with precipitation and a positive correlation with temperature and radiation (Figure S1).

A representative vine subjected to a second pruning is shown in Figure 1A. New vegetative and reproductive structures developed mainly from the apical and sub-apical dormant buds on the trimmed shoots, while the more basal nodes stayed dormant (acrotony) (Figure 1B,C). Figure 1D shows a representative result of the number and size of bunches per vine at the mature stage.



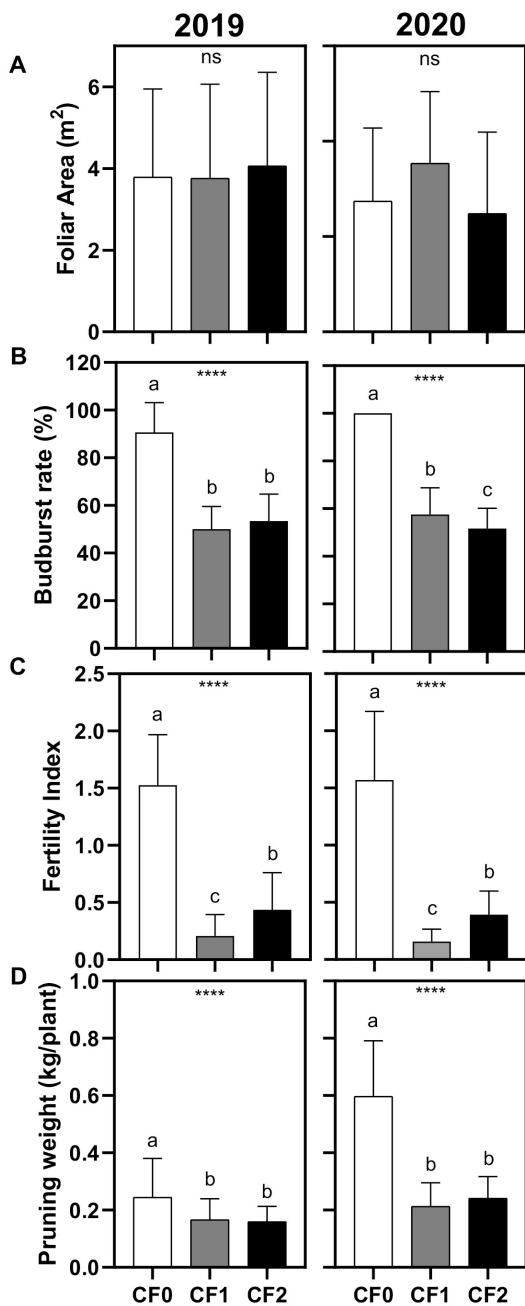
**FIGURE 2.** Predawn leaf water potential ( $\Psi_{pd}$ ) of grape cv. 'Touriga Nacional'.

(A) 2019 and (B) 2020 growing seasons. Crop forcing conditions: CF0 = plants with no crop forcing; CF1 = plants submitted to CF 15 days after fruit set; CF2 = plants submitted to CF 30 days after fruit set. Results represent mean  $\pm$  SD of four replicates. Asterisks indicate ANOVA statistical differences: \* $p \leq 0.05$ ; \*\*\* $p \leq 0.001$ . Lowercase letters indicate differences between treatments.

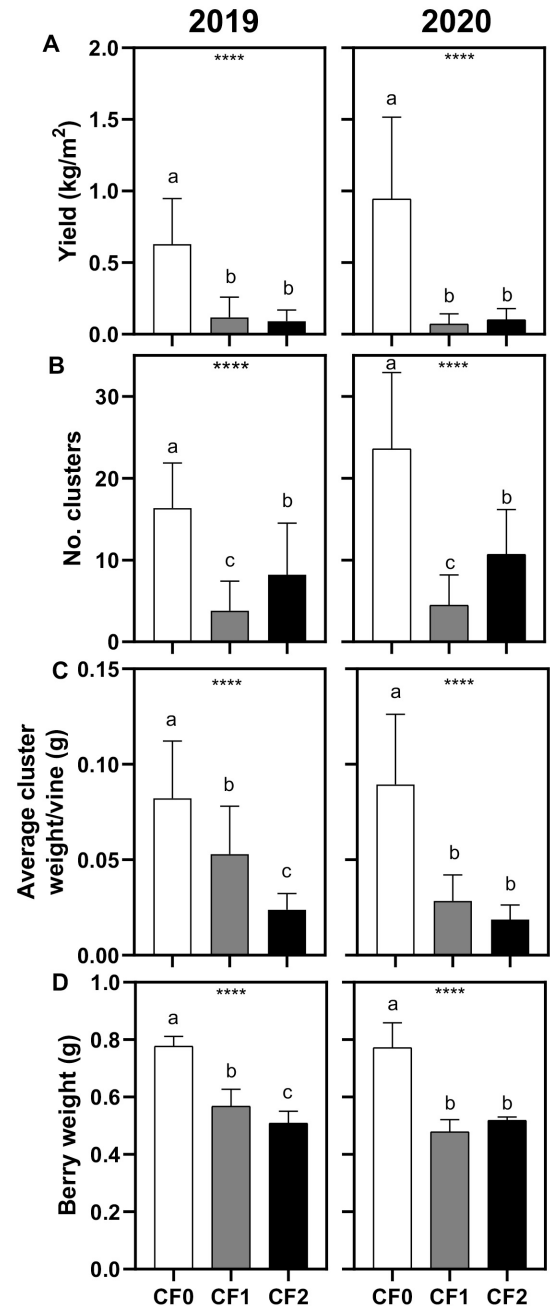
Compared with control plants (CF0), CF1 and CF2 in cv. ‘Touriga Nacional’ resulted in a delay in all phenological events in both seasons, as follows (in 2019 and 2020, respectively): second budburst in 48 and 66 days in CF1, and 70 and 74 days in CF2, and harvest in 27 and 41 days in CF1, and 41 and 51 days in CF2 (Figure 1E). GDD

calculated in both vintages from April until October were as follows: 2201.7 °C (2019) and 2335.9 °C (2020); thus, 2020 was warmer than the previous season.

Differences in GDD between veraison and harvest were also significant in both seasons and followed the trend CF0 > CF1 > CF2: in CF0, 708.75 (2019) and 867.50 °C (2020); in CF1,



**FIGURE 3.** Effect of crop forcing on grapevine vegetative growth. (A) leaf area; (B) budburst rate; (C) total fertility index and (D) pruning weight, in 2019 and 2020 vintages of grape cv. ‘Touriga Nacional’ in different crop forcing conditions. Pruning conditions: CF0 = plants with no crop forcing; CF1 = plants submitted to CF 15 days after fruit set; CF2 = plants submitted to CF 30 days after fruit set. Results represent mean ± SD of four replicates. Asterisks indicate ANOVA statistical differences: \*\*\*\*p ≤ 0.0001; n.s. = non-significant. Lowercase letters indicate differences between treatments.

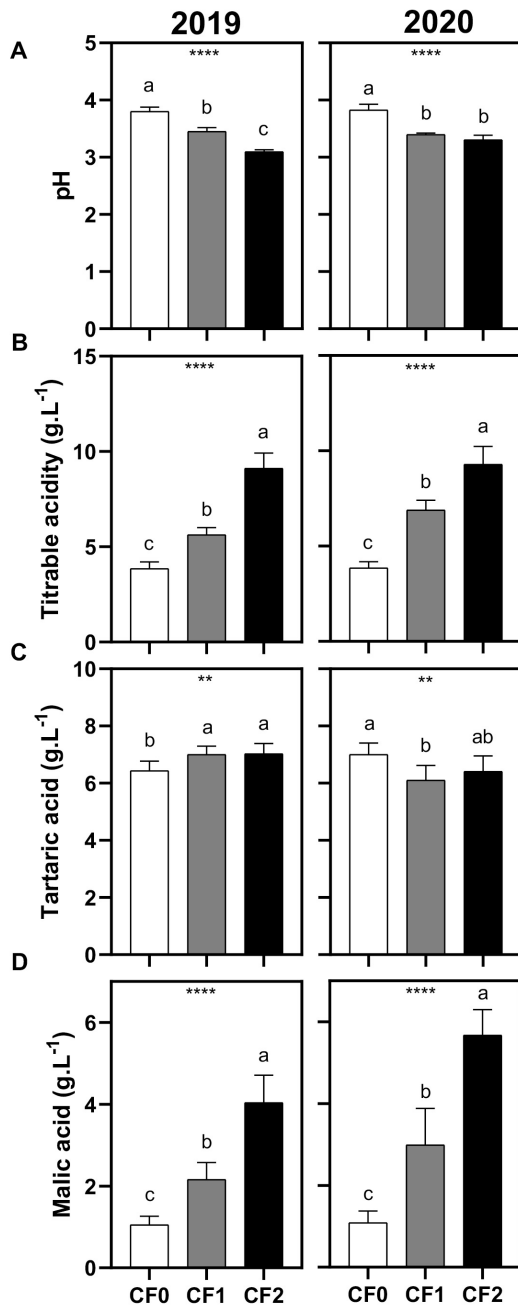


**FIGURE 4.** Effect of crop forcing on grapevine productivity. (A) yield; (B) number of clusters per plant; (C) average cluster weight per plant and (D) berry weight, in 2019 and 2020 vintages of grape cv. ‘Touriga Nacional’. Crop forcing conditions: CF0 = plants with no crop forcing; CF1 = plants submitted to CF 15 days after fruit set; CF2 = plants submitted to CF 30 days after fruit set. Results represent mean ± SD of four replicates. Asterisks indicate ANOVA statistical differences: \*\*\*\*p ≤ 0.0001; Lowercase letters indicate differences between treatments.

321.60 (2019) and 224.10 °C (2020); and in CF2, 247.15 (2019) and 132.00 °C (2020).

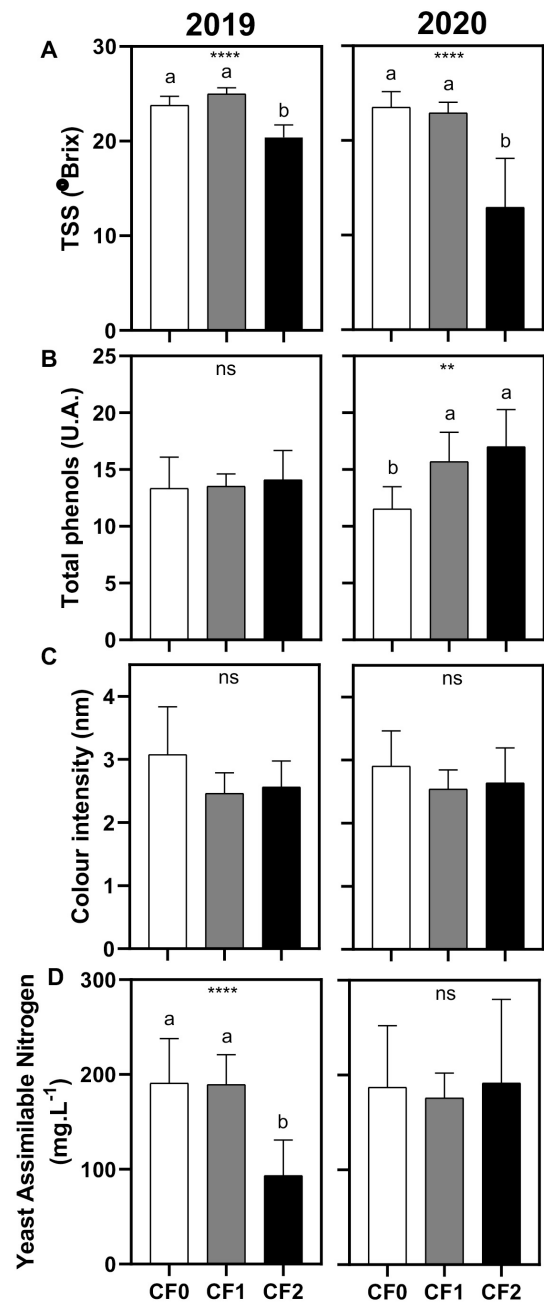
Pre-dawn leaf water potential was measured in both seasons, from the beginning of June until harvest, and no significant differences were observed between control plants and the ones subjected to CF treatments (Figure 2).

The only exception was observed in 2020 when pre-dawn leaf water potential in response to CF decreased by up to 15 % in August and increased by up to 13 % in September. Still, vines were under higher stressful conditions in 2020 than in 2019, judging from the values of GDD.



**FIGURE 5.** Berry acids of grape cv. 'Touriga Nacional' during maturation until harvest.

(A) pH; (B) Titratable acidity; (C) Tartaric acid and (D) Malic acid in 2019 and 2020 vintages of grape cv. 'Touriga Nacional' in different pruning conditions. Crop forcing conditions: CF0 = plants with no crop forcing; CF1 = plants submitted to CF 15 days after fruit set; CF2 = plants submitted to CF 30 days after fruit set. Results represent mean  $\pm$  SD of four replicates. Asterisks indicate ANOVA statistical differences: \*\* $p \leq 0.01$ ; \*\*\*\* $p \leq 0.0001$ ; n.s. = non-significant. Lowercase letters indicate differences between treatments.



**FIGURE 6.** Berry quality attributes of grape cv. 'Touriga Nacional' during maturation until harvest.

(A) Total Soluble Solids (TSS); (B) Total phenols; (C) Colour intensity and (D) Yeast Assimilable Nitrogen in 2019 and 2020 vintages of grape cv. 'Touriga Nacional' in different pruning conditions. Crop forcing conditions: CF0 = plants with no crop forcing; CF1 = plants submitted to CF 15 days after fruit set; CF2 = plants submitted to CF 30 days after fruit set. Results represent mean  $\pm$  SD of four replicates. Asterisks indicate ANOVA statistical differences: \*\* $p \leq 0.01$ ; \*\*\*\* $p \leq 0.0001$ ; n.s. = non-significant. Lowercase letters indicate differences between treatments.

## 2. Grapevine yield, vigour and berry quality attributes after crop forcing

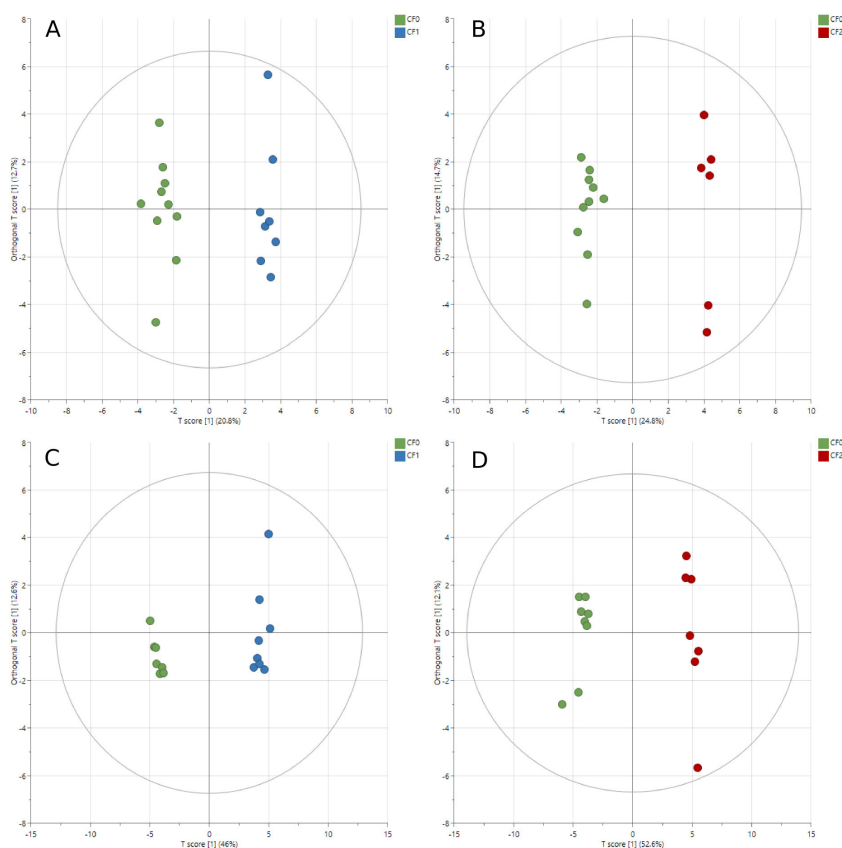
Both CF1 and CF2 induced a substantial increase in the number of leaves (data not shown), but no differences were observed in the total leaf area at harvest compared with controls (Figure 3A) because the leaf size was substantially decreased in plants subjected to CF (data not shown). Differences in the primary leaf area were only observed in 2020 between CF1 (2.59 m<sup>2</sup>) and CF0 (1.52 m<sup>2</sup>) (Figure S2A).

Figure 3B shows that second pruning decreased the bud burst rate in both seasons by up to 94 %, but while no significant differences were observed between CF1 and CF2 in 2019, CF2 induced a higher decrease in bud burst rate than CF1 in 2020. Crop forcing also negatively impacted the fertility index in both seasons by up to 800 % in 2020, but the fertility index was higher in vines subjected to CF2 than in CF1 (Figure 3C).

Crop forcing treatments also induced an increase of up to 114 % in the number of shoots and by up to 393 % in the number of buds in both growing seasons (Figure 3). On the contrary, the pruning weight was significantly lower in CF vines in both seasons, although more evident in 2020 (Figure 3D). The yield (kg/m<sup>2</sup>) of vines subjected to CF substantially decreased in both seasons by up to 92 % (Figure 4A). Values of yield in kg per linear meter are shown in Figure S2D.

The number of clusters decreased by up to 81 % after CF1 and by up to 55 % after CF2 (Figure 4B). Thus, in both seasons, CF2 treatment resulted in a higher number of clusters than CF1. Average cluster weight per plant also registered a significant reduction in CF (Figure 4C), with CF2 246 % and 379 % and CF1 55 % and 214 % lower compared to CF0 in both years of the experiment. Figure 4C shows a negative impact of CF2 treatment stronger than CF1, especially in 2019. The cluster size, measured at harvest, was significantly lower after CF treatments in both seasons (up to 50 %) (Figure S2E), and berry weight also decreased by up to 38 % (Figure 4D).

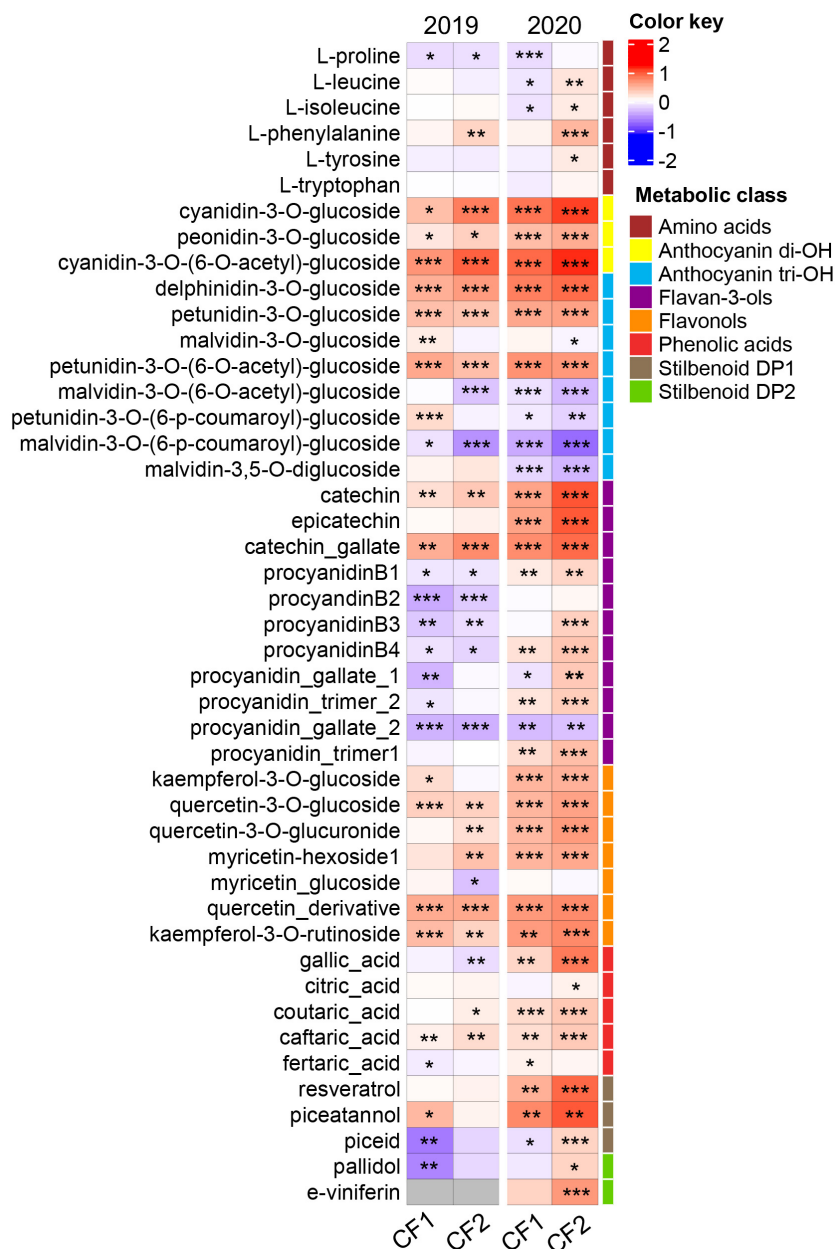
CF treatments induced significant modifications in berry quality attributes at harvest in both seasons (Figures 5 and 6): a decrease in the pH of up 0.7 units (18 %) (Figure 5A; differences among CF treatments being observed only in 2019), associated with an increase in the titratable acidity, more evident after CF2, with a ca. 140 % increase over the control in both seasons (Figure 5B). Tartaric acid was variable among seasons, showing higher values in CF in 2019, whereas in 2020, CF1 registered the least content (Figure 5C). The content in malic acid (Figure 5D) increased substantially in response to CF in both seasons: by up to 176 % after CF1 and by up to 421 % after CF2, while tartaric acid slightly increased only in 2019 by ca. 9 % in response to CF1 or CF2. Total soluble solids (TSS) showed no differences in berries



**FIGURE 7.** Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA).

Supervised classification using OPLS-DA with crop forcing (CF1 and CF2) as dependent variables on metabolomic data from grape berries cv. 'Touriga Nacional' at harvest in the vintages of 2019 (A and B) and 2020 (C and D). Variables in the score plots were coloured according to the treatment (CF0-green, CF1-blue and CF2-red).





**FIGURE 8.** Heatmap representation of metabolome of cv. 'Touriga Nacional' berries.

Modifications observed in amino acids, anthocyanins, flavan-3-ols, flavonols, phenolic acids and stilbenoids in berries from vines cv. 'Touriga Nacional', subjected to crop forcing. Each row represents a different metabolite and each column represents the crop forcing treatments in two consecutive years. Values were expressed as the logarithm of the ratio between treatments and the control ( $\log_{10}(CF1/CF0)$ ;  $\log_{10}(CF2/CF0)$ ) and presented in virtual colours as indicated by the colour key, in which the offset was determined by the average values found within the four biological replicates of each sample type. Metabolites were labelled according to their metabolic class. Asterisks indicate statistical significance between crop forcing treatment and the respective control within each sample year following Student's t-test: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$ .

from vines subjected to CF1 in both seasons, but a substantial decrease by 14 % and 45 % was observed after CF2 in 2019 and 2020, respectively (Figure 6A), where oenological maturation was not accomplished, since the TSS did not reach the optimal conditions (22–24 °Brix). In 2020, total phenols increased by up to 48 % after CF1 and CF2 treatments, but in 2019 no differences were observed (Figure 6B). Noteworthy, this increase did not translate in the colour intensity of the must that was similar in control and CF-treated plants in both seasons (Figure 6C). CF2 treatment induced a significant reduction by 51 % in yeast assimilable nitrogen (YAN) in

2019 but this effect was not observed in 2020, while CF1 did not change YAN in both seasons (Figure 6D).

### 3. Grape berry metabolome under crop-forcing treatments

Targeted metabolomics identified a total of 45 metabolites (Table S1), including 6 amino acids, 5 phenolic acids, 5 stilbenoids, 7 flavonols, 11 flavan-3-ols and 11 anthocyanins. Unsupervised PCA analysis showed that the vintage has a stronger effect on variability than the crop-forcing treatments. The effect of crop forcing treatments

on grape berries metabolites was particularly evident when a supervised OPLS-DA analysis was performed, showing score plots with a clear separation of treatments versus control (Figure 7 and Figure S3). This separation was driven by the metabolites projected in the PC1 positive quadrant of the vintage of 2019 (flavan-3-ols and anthocyanins di-OH on CF0 vs CF1; anthocyanins di-OH, anthocyanins tri-OH and amino acids on CF0 vs CF2) and vintage of 2020 (amino acids, flavonols, phenolic acids and flavan-3-ols on CF0 vs CF1; anthocyanins tri-OH, anthocyanins di-OH and flavan-3-ols, flavonols and stilbenoid DP1 on CF0 vs CF2).

A heatmap representation of each metabolite change after CF treatments compared with controls is shown in Figure 8. Results showed that forcing buds to regrowth clearly and consistently changed the biosynthesis of grape berries' secondary metabolites in two consecutive vintages. Most of the identified phenolic acids showed an increase in both treatments and vintages. For instance, caftaric acid increased by 24 % and 63 % after CF1 and CF2 in 2019 and by 67 % and 115 % after CF1 and CF2 in 2020. Flavonols detected showed an even greater increase in grapes from treated vines. This is the case of quercetin-3-*O*-glucoside, that increased by 99 % and 185 % after CF1 and CF2 in 2019 and 95 % and 278 % after CF1 and CF2 in 2020 (Figure 8). The eleven flavan-3-ols identified showed a similar pattern, with substantial increases being observed in 2020, particularly after CF2 (*e.g.* catechin gallate increased by 661 %, while procyanidins' increased only in the 2020 vintage after CF1 and CF2, but decreased in 2019 (Figure 8)). The three anthocyanin di-OH identified showed also a strong increase in grapes from vines subjected to CF. This is the case of cyanidin-3-*O*-glucoside, that increased by 155 % and 580 % after CF1 and CF2 in 2019 and 506 % and 1073 % after CF1 and CF2 in 2020. Interestingly, non-acylated forms of anthocyanins tri-OH revealed a strong increase in response to CF (*e.g.*, delphinidin-3-*O*-glucoside increased by 215 % and 503 % after CF1 and CF2 in 2019 and 315 % and 656 % after CF1 and CF2 in 2020), while most of the acylated (except the acylated petunidin) forms showed a consistent decrease, particularly in CF1 and CF2 of 2020 season. CF treatments affected amino acid levels mostly in the 2020 vintage in response to CF2, but L-phenylalanine revealed a significant increase after CF2 in both vintages. CF treatments affected stilbenoids, particularly in the 2020 vintage, when resveratrol increased by 214 % and 700 % after CF1 and CF2, respectively (Figure 8).

## DISCUSSION

### 1. Crop forcing delays the vegetative cycle and alters production in cv. 'Touriga Nacional'

Previous reports have carefully addressed the potential advantages but also the limits and limitations of crop-forcing (Pommer, 2006; Martínez-Moreno *et al.*, 2019; Lavado *et al.*, 2019; Poni *et al.*, 2021). Our results agree with previous reports in 'Cabernet-Sauvignon', 'Chardonnay', 'Tempranillo' and 'Maturana Tinta' cultivars, showing that

the crop forcing after fruit set shifted fruit ripening and consequently harvest stage to the October to early-November period, which has been reported as the main hallmark of crop forcing (Gu *et al.*, 2012; Lavado *et al.*, 2019; Martínez de Toda *et al.*, 2019; Martínez-Moreno *et al.*, 2019; Pou *et al.*, 2019; Tian and Gu, 2019; Prats-Llinàs *et al.*, 2020). The majority of these studies also report a strong decrease in the vine yield, much like it was observed in the present study in 'Touriga Nacional', where yield reduction after CF1 and CF2 was accompanied by a decrease in the number of clusters, cluster size (cluster length) and berry weight. The number of clusters produced by vines subjected to CF1 were lower than after CF2, most likely because the buds that produce the inflorescences in the upcoming season (Lavee and May, 1997) were forced to break when they were still developing and were then less fertile (Lavado *et al.*, 2019). Flower disorder, when initiated flowers do not develop, turning into tendrils due source to sink limitation (Bessis, 1967), may also have accounted for the observed reduction.

CF2 was applied later in the season when the buds of the new shoots already differentiated well, as previously observed (Smith and Holzzapfel, 2009; Sadras and Moran, 2013; Prats-Llinàs *et al.*, 2020). Apical dominance, the control exerted by the shoot apex or summer laterals (and/or leaves) over the outgrowth of the lateral latent buds, is thought to play a primary role in the paradormancy of these buds, which normally do not grow out until the following season after over-wintering (Cline and Deppong, 1999; He, 2008; Lavee and May, 1997). However, the observed strong reduction in plant yield after crop forcing can also be related to a reduced source-sink, due to a lower number of fully expanded photosynthetically active leaves in CF conditions (Gu, 2012; Martínez de Toda, 2019) and with different meteorological conditions observed when maturation is postponed till October or November (Martínez-Moreno *et al.*, 2019).

The development of secondary (smaller) leaves after CF may have accounted for the observed similar values of total leaf area between treatments in both seasons. As the total leaf area in plants subjected to crop forcing and control plants did not change, the overall available surface area for the loss of water through transpiration was similar (Hochberg *et al.*, 2017), thus, explaining why leaf pre-dawn water potential was similar between treatments at the end of the season.

The observed decrease in pruning weight in plants subjected to CF, more evident in 2020, is apparently in contradiction with the higher number of shoots in response to the crop forcing. Most likely, the amount of stored starch in grapevine canes is also compromised after crop forcing, which is a good topic for future studies, as these reserves are of pivotal importance for winter acclimation (Noronha *et al.*, 2021a) and bud burst in the upcoming season (Noronha *et al.*, 2021b). Thus, the need for longer-term studies and detailed analysis of the implications of these extreme canopy management strategies on carbohydrate reserves, bud fertility and future yield are still needed, as recently discussed (Santesteban, 2022).

## 2. Crop forcing changes berry qualitative traits in 'Touriga Nacional'

As aforementioned, an important consequence of global warming in grapevine cultivation is the uncoupling of technical and phenolic maturation. In 2019 and 2020, the desired sugar concentration, between 23 and 25 °Brix, was attained in September in the control plants and in October in grapevines subjected to CF1; therefore, CF1 effectively delayed the technical maturation by one month. However, grapes from plants subjected to CF2 were harvested before optimal sugar ripeness was attained, in early November (2019) and late October (2020), to prevent the loss of the harvest, due to the increase in rot incidence, by the autumn rainfall. Indeed, the risks of delaying the ripening to Autumn, especially in CF2, may compromise the optimum ripening (lower temperatures, precipitation) and the production of high-quality wines, as observed before (Prats-Llinàs *et al.*, 2020).

The delay in the harvest in 'Touriga Nacional' promoted by CF was also reflected in the increased acidity of the grape juice, which correlated with increased levels of malic acid upon CF1 and CF2 treatments, which is in agreement with previous observations in other cultivars (Gu *et al.*, 2012; Palliotti *et al.*, 2014; Martínez-Moreno *et al.*, 2019). Most likely, the cooler conditions occurring during the ripening of plants subjected to CF slowed down the degradation of malic acid, which is normally stimulated at high temperatures (Buttrose *et al.*, 1971; Ford, 2012).

As suggested before (Dry, 1987; Lavado *et al.*, 2019), the reduction in bud fertility after crop forcing may explain the production of smaller berries. Other studies have shown that these smaller berries have higher contents in secondary compounds. This is the case of 'Cabernet-Sauvignon' and 'Tempranillo' cultivars, where higher contents of total anthocyanins, tannins and total phenolics were measured at harvest (Gu *et al.*, 2012, Martínez-Moreno *et al.*, 2019; Lavado *et al.*, 2019; Tian and Gu, 2019). However, in the present study in 'Touriga Nacional', the effect of crop forcing in the maturation delay was not so obvious in the stimulation of the synthesis of secondary compounds, although important qualitative changes were observed. Indeed, total phenolics at harvest only increased after crop forcing in the 2020 season, and this increase was not accompanied by an increment in the colour intensity of the musts. It is known that temperature accumulation plays an important role in the synthesis of phenolics. Maximum phenolics accumulation occurs when the daily temperature is between 15 and 25 °C and the night temperature is between 10 and 20 °C (Kliever and Torres, 1972; Azuma *et al.*, 2012). In the present study, the observed lower cumulative temperature (GDD) from veraison to harvest after CF1 and CF2, when compared to CF0, could counteract the expected increase in total phenolics in response to CF. During October and November, monthly minimum temperatures did not reach 10 °C, which could impair phenolic synthesis. Besides TSS and total acidity, phenolic content is important to schedule

harvest date because it determines astringency, ageing, colour and body of the wine (Hidalgo, 1999).

## 3. Crop forcing modifies the metabolomic profile of 'Touriga Nacional' berries

Metabolomic analysis showed that despite total phenols only increasing after CF2 in 2020 and no differences were observed between treatments and control regarding pigments, important qualitative changes in key metabolites were observed in plants subjected to CF. Indeed, results from the targeted metabolomics analysis clearly discriminated between CF treatments, with specific variations in each polyphenol class highly consistent among vintages.

Amino acids are particularly prone to variations triggered by exogenous stimuli (Martins *et al.*, 2014) and, in the present study, also suffered important changes in response to CF in 'Touriga Nacional'. The amino acids content in berries may account for up to 90 % of the yeast assimilable nitrogen (YAN) in grape juice, which is responsible for the success of fermentation, fermentation rate and aromatic profile of the wine (Carrau *et al.*, 2008). Thus, the consistent decrease observed after CF1 treatments may negatively impact wine quality.

The management of the canopy substantially affected stilbenoid production (stilbenoids DP1 and DP2), particularly CF2 in 2020, which goes in agreement with previous observations that stilbenes production can be induced by abiotic stress or mechanical injuries (Bavaresco *et al.*, 1997; Bavaresco and Fregoni, 2001; Pezet *et al.*, 2004).

The increase in catechin, epicatechin and catechin gallate was generally observed after CF1 and CF2 in both seasons and may have important repercussions in wine quality as they are the most abundant flavan-3-ols in the berry and well known for their high antioxidant capacity, besides conferring bitterness and astringency to wines (reviewed by Teixeira *et al.*, 2013). Conversely, there was a consistent decrease in procyanidins after CF1 and CF2, particularly in 2019, which are built from flavan-3-ols (+)-catechin and (-)-epicatechin (Bittner *et al.*, 2013). The low temperatures that occurred later in the season may explain, at least in part, this observation because it was shown low diurnal temperatures reduce the degree of polymerisation of proanthocyanidin (Cohen *et al.*, 2008).

It was observed that flavonoid biosynthesis-related genes in grape skins are differentially regulated by temperature and light conditions (Azuma *et al.*, 2012), which could explain our results showing that canopy management through CF1 and CF2 positively affected the content in flavonols in grapes from 'Touriga Nacional' whose maturation occurred later in the season, at lower temperatures. CF clusters also had a higher incidence of radiation since they were more exposed to direct sunlight. Although, flavonols are known to contribute to wine colour as co-pigments (Asen *et al.*, 1972, Boulton, 2001) and act as internal regulators, antioxidants and UV screeners, among others (Kaffarnik *et al.*, 2005; Agati *et al.*, 2013; Martinez *et al.*, 2016).

Anthocyanins are the main compounds involved in the colour of red grapes; in *Vitis vinifera* L., the monoglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin but also their acetyl-, *p*-coumaroyl-, and caffeoyl derivatives are present in quantities varying among varieties, climate, and viticulture practices (Crupi *et al.*, 2012, Coletta *et al.*, 2013). Our results showed that although no differences were observed between must colour from grapes harvested from treated vines and control, di-OH and tri-OH anthocyanins substantially increased after CF, while acetylated and *p*-coumaroylated anthocyanins decreased. Trihydroxylated anthocyanins (delphinidin, petunidin, and malvidin-3-glucosides) were described to be more stable in wines than dihydroxylated ones (cyanidin and peonidin-3-glucosides) (Hernández-Jiménez *et al.*, 2013). Grape anthocyanin profile changes during ripening, with an increase in the proportion of malvidin 3-glucoside and its *p*-coumaroyl derivatives at the late ripening stages (25 and 28 °Brix) (Guidoni and Hunter, 2012). The lower maturation degree (lower °Brix) reached by grapes under CF2 treatments may have accounted for the observed lower levels of acetylated and *p*-coumaroylated anthocyanins measured in juices.

## CONCLUSION

Increasing temperatures during the growing season tend to damage wine typicity since early veraison dates lead to earlier maturation of the grapes. These grapes tend to have higher sugar content, less acidity and phenolics. Our results showed that crop forcing (CF) shifted fruit ripening in ‘Touriga Nacional’ cultivated in the Douro region for up to 1.5 months, from September up to October/November. This delay in sugar ripeness was accompanied by a substantial increase in total phenols in 2020 and malic acid in both seasons. Thus, CF may have positive impacts on sugar/acid balance of the wine and be useful to synchronise technical and phenolic maturity, depending on the vintage. However, new refinements of the technique are still necessary because yield potential was severely compromised through a strong reduction in bunch number and bunch weight. A metabolomic analysis showed that CF increases the relative abundance of key phenolics like flavan-3-ols, well known for their high antioxidant capacity besides conferring bitterness and astringency to wines, or trihydroxylated anthocyanins, more stable than dihydroxylated ones in wines, but their potential benefits in wine quality deserve further research and attention of the winemakers.

## ACKNOWLEDGEMENTS

This research was funded by the VISCA project (Vineyards’ Integrated Smart Climate Application), funded by European Union’s Horizon 2020 research and innovation programme under grant agreement no. 730253. The Région-Centre Val de Loire (France) supported this work under the grant agreement to Project VITI’ACTIF. 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, CCDR-N (Norte Portugal Regional Coordination and Development Commission) and European Funds (FEDER/POCI/COMPETE2020) through the project AgriFoodXXI (NORTE-01-0145-FEDER-000041) and the research projects BerryPlastid (PTDC/BIA-FBT/28165/2017 and POCI-01-0145-FEDER-028165), MitiVineDrought (PTDC/BIA-FBT/30341/2017 and POCI-01-0145-FEDER-030341), and the research project GrapeMicrobiota (PTDC/BAA-AGR/2691/2020). A.T. was supported by a post-doctoral researcher contract/position within the project “BerryPlastid”. This work also benefited from the networking activities within the European COST Action CA 17111 INTEGRAPE, the CoLAB VINES & WINES, and the CoLAB 4FOOD—Collaborative Laboratory for Innovation in the Food Industry.

## REFERENCES

- Agati, G., Brunetti, C., Di Ferdinando, M., Ferrini, F., Pollastri, S., & Tattini, M. (2013). Functional roles of flavonoids in photoprotection: new evidence, lessons from the past. *Plant Physiology and Biochemistry*, 72, 35-45. <https://doi.org/10.1016/j.plaphy.2013.03.014>
- Asen, S., Stewart, R. N., & Norris, K. H. (1972). Copigmentation of anthocyanins in plant tissues and its effect on color. *Phytochemistry*, 11(3), 1139-1144. [https://doi.org/10.1016/S0031-9422\(00\)88467-8](https://doi.org/10.1016/S0031-9422(00)88467-8)
- Azuma, A., Yakushiji, H., Koshita, Y., & Kobayashi, S. (2012). Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta*, 236(4), 1067-1080. <https://doi.org/10.1007/s00425-012-1650-x>
- Bavaresco, L., & Fregoni, C. (2001). Physiological role and molecular aspects of grapevine stilbenic compounds. In *Molecular biology & biotechnology of the grapevine* (pp. 153-182). Springer, Dordrecht.
- Bavaresco, L., Petegolli, D., Cantù, E., Fregoni, M., & Chiusa, G. (1997). Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. *Vitis*, 36(2), 77-83.
- Bessis, R. (1967). A propos de l’existence et de la nature du filage des grappes chez la vigne. *Comptes rendus de l’Académie d’agriculture de France*, 1429-1436.
- Billet, K., Houillé, B., Dugé de Bernonville, T., Besseau, S., Oudin, A., Courdavault, V., Delanoue, G., Guérin, L., Clastre, M., Giglioni-Guivarc’h, N., Lanoue, A. (2018). Field-based metabolomics of *Vitis vinifera* L. stems provides new insights for genotype discrimination and polyphenol metabolism structuring. *Frontiers in plant science*, 9, 798. <https://doi.org/10.3389/fpls.2018.00798>
- Bittner, L. K., A Schonbichler, S., K Bonn, G., & W Huck, C. (2013). Near infrared spectroscopy (NIRS) as a tool to analyze phenolic compounds in plants. *Current Analytical Chemistry*, 9(3), 417-423.
- Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American journal of enology and viticulture*, 52(2), 67-87. <https://www.ajevonline.org/content/52/2/67.short>
- Buttrose, M. S., Hale, C. R., & Kliewer, W. M. (1971). Effect of temperature on the composition of Cabernet-Sauvignon berries. *American Journal of Enology and Viticulture*, 22(2), 71-75. <https://www.ajevonline.org/content/22/2/71.short>

- Cabral, I. L., Teixeira, A., Lanoue, A., Unlubayir, M., Munsch, T., Valente, J., ... & Queiroz, J. (2022). Impact of Deficit Irrigation on Grapevine cv. 'Touriga Nacional' during Three Seasons in Douro Region: An Agronomical and Metabolomics Approach. *Plants*, 11(6), 732. <https://doi.org/10.3390/plants11060732>
- Carrau, F. M., Medina, K., Farina, L., Boido, E., Henschke, P. A., & Dellacassa, E. (2008). Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts: effects of yeast assimilable nitrogen on two model strains. *FEMS yeast research*, 8(7), 1196-1207. <https://doi.org/10.1111/j.1567-1364.2008.00412.x>
- Carvalho, L. C., Coito, J. L., Gonçalves, E. F., Chaves, M. M., & Amâncio, S. (2016). Differential physiological response of the grapevine varieties Touriga Nacional and Trincadeira to combined heat, drought and light stresses. *Plant Biology*, 18, 101-111. <https://doi.org/10.1111/plb.12410>
- Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, A. P., ... & Lopes, C. M. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of botany*, 105(5), 661-676. <https://doi.org/10.1093/aob/mcq030>
- Cline, M. G., & Deppong, D. O. (1999). The role of apical dominance in paradormancy of temperate woody plants: a reappraisal. *Journal of plant physiology*, 155(3), 350-356. [https://doi.org/10.1016/S0176-1617\(99\)80116-3](https://doi.org/10.1016/S0176-1617(99)80116-3)
- Cline, M. G. (1991). Apical dominance. *The Botanical Review*, 57(4), 318-358.
- Cline, M. G. (1994). The role of hormones in apical dominance. New approaches to an old problem in plant development. *Physiologia plantarum*, 90(1), 230-237. <https://doi.org/10.1111/j.1399-3054.1994.tb02216.x>
- Clingeffer, P. R. (2010). Plant management research: status and what it can offer to address challenges and limitations. *Australian Journal of Grape and Wine Research*, 16, 25-32. <https://doi.org/10.1111/j.1755-0238.2009.00075.x>
- Cohen, S. D., Tarara, J. M., & Kennedy, J. A. (2008). Assessing the impact of temperature on grape phenolic metabolism. *Analytica chimica acta*, 621(1), 57-67. <https://doi.org/10.1016/j.aca.2007.11.029>
- Coletta, A., Trani, A., Faccia, M., Punzi, R., Dipalmo, T., Crupi, P., ... & Gambacorta, G. (2013). Influence of viticultural practices and winemaking technologies on phenolic composition and sensory characteristics of Negroamaro red wines. *International journal of food science & technology*, 48(11), 2215-2227. <https://doi.org/10.1111/ijfs.12207>
- Cook, M. G., Zhang, Y., Nelson, C. J., Gambetta, G., Kennedy, J. A., & Kurtural, S. K. (2015). Anthocyanin composition of Merlot is ameliorated by light microclimate and irrigation in central California. *American Journal of Enology and Viticulture*, 66(3), 266-278. <https://doi.org/10.5344/ajev.2015.15006>
- Coombe, B. G. (1995). Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages. *Australian journal of grape and wine research*, 1(2), 104-110. <https://doi.org/10.1111/j.1755-0238.1995.tb00086.x>
- Costa, J. M., Egipto, R., Lopes, C. M., & Chaves, M. M. (2020). Canopy and soil thermal patterns to support management of irrigated vineyards. In Proceedings VIII Congresso Ibérico de Ciências Horticolas, 7-10 Junho 2017. Actas Portuguesas de Horticultura 30. APH.
- Crupi, P., Coletta, A., Anna Milella, R., Perniola, R., Gasparro, M., Genghi, R., & Antonacci, D. (2012). HPLC-DAD-ESI-MS Analysis of Flavonoid Compounds in 5 Seedless Table Grapes Grown in Apulian Region. *Journal of Food Science*, 77(2), C174-C181. <https://doi.org/10.1111/j.1750-3841.2011.02555.x>
- Dayer, S., Prieto, J. A., Galat, E., & Perez Peña, J. (2013). Carbohydrate reserve status of Malbec grapevines after several years of regulated deficit irrigation and crop load regulation. *Australian Journal of Grape and Wine Research*, 19(3), 422-430. <https://doi.org/10.1111/ajgw.12044>
- Degu, A., Ayenew, B., Cramer, G. R., & Fait, A. (2016). Polyphenolic responses of grapevine berries to light, temperature, oxidative stress, abscisic acid and jasmonic acid show specific developmental-dependent degrees of metabolic resilience to perturbation. *Food chemistry*, 212, 828-836. <https://doi.org/10.1016/j.foodchem.2016.05.164>
- Dry, P. (1987). How to grow 'cool climate' grapes in hot regions. *Austr Grapegrow. Winemak*, 283, 25-26.
- Fang, J. G., Cai, B. H., & Wei, Y. Y. (2000). Utilization and limitations of multiple cropping in grapevines. *Sino-Overseas Grapevine Wine*, 2000, 36-37.
- Fiehn, O., Wohlgemuth, G., Scholz, M., Kind, T., Lee, D. Y., Lu, Y., ... & Nikolau, B. (2008). Quality control for plant metabolomics: reporting MSI-compliant studies. *The Plant Journal*, 53(4), 691-704. <https://doi.org/10.1111/j.1365-313X.2007.03387.x>
- Ford, C. M. (2012). The biochemistry of organic acids in the grape. *The Biochemistry of the grape berry*, 67-88.
- Fraga, H., de Cortázar Atauri, I. G., & Santos, J. A. (2018). Viticultural irrigation demands under climate change scenarios in Portugal. *Agricultural water management*, 196, 66-74. <https://doi.org/10.1016/j.agwat.2017.10.023>
- Fraga, H., Santos, J. A., Moutinho-Pereira, J., Carlos, C., Silvestre, J., Eiras-Dias, J., ... & Malheiro, A. C. (2016). Statistical modelling of grapevine phenology in Portuguese wine regions: observed trends and climate change projections. *The Journal of Agricultural Science*, 154(5), 795-811. <https://doi.org/10.1017/S0021859615000933>
- Gerós, H., Chaves, M. M., Gil, H. M., & Delrot, S. (2015). Grapevine in a changing environment: a molecular and ecophysiological perspective. John Wiley & Sons.
- Gouveia, C., Liberato, M. L. R., DaCamara, C. C., Trigo, R. M., & Ramos, A. M. (2011). Modelling past and future wine production in the Portuguese Douro Valley. *Climate Research*, 48(2-3), 349-362.
- Gu, S., S. D. Jacobs, B. S. McCarthy, and H. L. Gohil. "Forcing vine regrowth and shifting fruit ripening in a warm region to enhance fruit quality in 'Cabernet-Sauvignon' grapevine (*Vitis vinifera* L.)." *The Journal of Horticultural Science and Biotechnology*, 87, no. 4 (2012): 287-292. <https://doi.org/10.1080/14620316.2012.11512866>
- Guidoni, S., & Hunter, J. J. (2012). Anthocyanin profile in berry skins and fermenting must/wine, as affected by grape ripeness level of *Vitis vinifera* cv. Shiraz/R99. *European Food Research and Technology*, 235(3), 397-408. <https://doi.org/10.1007/s00217-012-1744-5>
- He, D. (2008). Exploring mechanisms involved in grapevine bud paradormancy. South Dakota State University.
- Hernández-Jiménez, A., Gil-Muñoz, R., Ruiz-García, Y., López-Roca, J. M., Martínez-Cutillas, A., & Gómez-Plaza, E. (2013). Evaluating the polyphenol profile in three segregating grape (*Vitis vinifera* L.) populations. *Journal of Analytical Methods in Chemistry*, 2013. <https://doi.org/10.1155/2013/572896>
- Hidalgo, L. (1999). Tratado de Viticultura General. Mundi Prens Ed.
- Hochberg, U., Bonel, A. G., David-Schwartz, R., Degu, A., Fait, A., Cochard, H., ... & Herrera, J. C. (2017). Grapevine acclimation to water deficit: the adjustment of stomatal and hydraulic conductance

- differs from petiole embolism vulnerability. *Planta*, 245(6), 1091-1104. <https://doi.org/10.1007/s00425-017-2662-3>
- Hochberg, U., Degu, A., Cramer, G. R., Rachmilevitch, S., & Fait, A. (2015). Cultivar specific metabolic changes in grapevines berry skins in relation to deficit irrigation and hydraulic behavior. *Plant Physiology and Biochemistry*, 88, 42-52. <https://doi.org/10.1016/j.plaphy.2015.01.006>
- Iland, P., Gago, P., & Humphrys, R. (2002). Australian wine: styles and tastes (Patrick Iland Wine Promotions: Campbelltown, SA, Australia)
- IPCC (2014). Climate Change (2014). Impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of working group II to the fifth assessment report of the intergovernmental Panel on Climate Change, 1132.
- Jones, G. V., & Alves, F. (2012). Impact of climate change on wine production: a global overview and regional assessment in the Douro Valley of Portugal. *International Journal of Global Warming*, 4(3-4), 383-406. <https://doi.org/10.1504/IJGW.2012.049448>
- Kaffarnik, F., Heller, W., Hertkorn, N., & Sandermann Jr, H. (2005). Flavonol 3-O-glycoside hydroxycinnamoyltransferases from Scots pine (*Pinus sylvestris* L.). *The FEBS journal*, 272(6), 1415-1424. <https://doi.org/10.1111/j.1742-4658.2005.04574.x>
- Kliwer, W.M., & Torres, R.E. (1972). Effect of controlled day and night temperatures on grape coloration. *American Journal of Enology and Viticulture*, 23(2), 71-77.
- Lavado, N., Uriarte, D., Mancha, L. A., Moreno, D., Valdés, E., & Prieto, M. H. (2019). Effect of forcing vine regrowth on 'Tempranillo' (*Vitis vinifera* L.) berry development and quality in Extremadura. *Vitis*, 58(Special Issue), 135-142. <https://doi.org/10.5073/vitis.2019.58.special-issue.135-142>
- Lavee, S., & May, P. (1997). Dormancy of grapevine buds-facts and speculation. *Australian Journal of grape and wine research*, 3(1), 31-46. <https://doi.org/10.1111/j.1755-0238.1997.tb00114.x>
- Lebon, E. (2002). Changements climatiques: quelles conséquences pour la viticulture. CR 6ième Rencontres Rhodaniennes, 31-36.
- Martínez de Toda, F., García, J., & Balda, P. (2019). Preliminary results on forcing vine regrowth to delay ripening to a cooler period. *Vitis*, 58, 17-22. <https://doi.org/10.5073/vitis.2019.58.17-22>
- Martínez de Toda, F., Sancha, J. C., Zheng, W., & Balda, P. (2014). Leaf area reduction by trimming, a growing technique to restore the anthocyanins: sugars ratio decoupled by the warming climate.
- Martínez-Lüscher, J., Kizildeniz, T., Vučetić, V., Dai, Z., Luedeling, E., van Leeuwen, C., ... & Delrot, S. (2016). Sensitivity of grapevine phenology to water availability, temperature and CO<sub>2</sub> concentration. *Frontiers in Environmental Science*, 4, 48. <https://doi.org/10.3389/fenvs.2016.00048>
- Martínez-Moreno, A., Sanz, F., Yeves, A., Gil-Muñoz, R., Martínez, V., Intrigliolo, D. S., & Buesa, I. (2019). Forcing bud growth by double-pruning as a technique to improve grape composition of *Vitis vinifera* L. cv. Tempranillo in a semi-arid Mediterranean climate. *Scientia Horticulturae*, 256, 108614. <https://doi.org/10.1016/j.scienta.2019.108614>
- Martinez, V., Mestre, T. C., Rubio, F., Girones-Vilaplana, A., Moreno, D. A., Mittler, R., & Rivero, R. M. (2016). Accumulation of flavonols over hydroxycinnamic acids favours oxidative damage protection under abiotic stress. *Frontiers in plant science*, 7, 838. <https://doi.org/10.1016/j.scienta.2019.108614>
- Martins, V., Billet, K., Garcia, A., Lanoue, A., & Gerós, H. (2020). Exogenous calcium deflects grape berry metabolism towards the production of more stilbenoids and less anthocyanins. *Food chemistry*, 313, 126123. <https://doi.org/10.1016/j.foodchem.2019.126123>
- Martins, V., Teixeira, A., Bassil, E., Blumwald, E., & Gerós, H. (2014). Metabolic changes of *Vitis vinifera* berries and leaves exposed to Bordeaux mixture. *Plant Physiology and Biochemistry*, 82, 270-278. <https://doi.org/10.1016/j.plaphy.2014.06.016>
- Matthews, M. A., Anderson, M. M., Schult, H. (1987). Phenologic and growth responses to early and late season. *Vitis*, 26, 147-160.
- McMaster, G. S., Wilhelm, W. W. (1997). Growing degree-days: one equation, two interpretations. *Agricultural and Forest Meteorology*, 87(4): 291-300. [https://doi.org/10.1016/S0168-1923\(97\)00027-0](https://doi.org/10.1016/S0168-1923(97)00027-0)
- Molitor, D., Schultz, M., Mannes, R., Pallez-Barthel, M., Hoffmann, L., & Beyer, M. (2019). Semi-minimal pruned hedge: A potential climate change adaptation strategy in viticulture. *Agronomy*, 9(4), 173. <https://doi.org/10.3390/agronomy9040173>
- Moran, M. A., Sadras, V. O., & Petrie, P. R. (2017). Late pruning and carry-over effects on phenology, yield components and berry traits in Shiraz. *Australian journal of grape and wine research*, 23(3), 390-398. <https://doi.org/10.1111/ajgw.12298>
- Noronha, H., Garcia, V., Silva, A., Delrot, S., Gallusci, P., & Gerós, H. (2021a). Molecular reprogramming in grapevine woody tissues at bud burst. *Plant Science*, 311, 110984. <https://doi.org/10.1016/j.plantsci.2021.110984>
- Noronha, H., Silva, A., Silva, T., Frusciant, S., Direccion, G., & Gerós, H. (2021b). VviRafS5 is a raffinose synthase involved in cold acclimation in grapevine woody tissues. *Frontiers in plant science*, 12. <https://doi.org/10.3389/fpls.2021.754537>
- OIV (2021). Compendium of international methods of wine and must analysis. <https://www.oiv.int/public/medias/7907/oiv-vol1-compendium-of-international-methods-of-analysis.pdf>
- Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A., & Deloire, A. (2002). Influence of pre-and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *American journal of Enology and Viticulture*, 53(4), 261-267.
- Pallioti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., & Poni, S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: A review. *Scientia Horticulturae*, 178, 43-54. <https://doi.org/10.1016/j.scienta.2014.07.039>
- Pezet, R., Gindro, K., Viret, O., & Spring, J. L. (2004). Glycosylation and oxidative dimerization of resveratrol are respectively associated to sensitivity and resistance of grapevine cultivars to downy mildew. *Physiological and Molecular Plant Pathology*, 65(6), 297-303. <https://doi.org/10.1016/j.pmpp.2005.03.002>
- Pommer, C.V. (2006). Double cropping of table grapes in Brazil. *Chronica Horticulturae*, 46, 22-25.
- Poni, S., Del Zozzo, F., Santelli, S., Gatti, M., Magnanini, E., Sabbatini, P., Frioni, T. (2021). Double cropping in *Vitis vinifera* L. cv. Pinot Noir: agronomical and physiological validation. *Australian Journal of Grape and Wine Research*, 27(4), 508-518. <https://doi.org/10.1111/ajgw.12507>
- Poni, S., Gatti, M., Tombesi, S., Squeri, C., Sabbatini, P., Lavado Rodas, N., & Frioni, T. (2020). Double cropping in *vitis vinifera* L. pinot noir: Myth or reality?. *Agronomy*, 10(6), 799. <https://doi.org/10.3390/agronomy10060799>
- Pou, A., Balda, P., Albacete, A., & Martínez de Toda, F. (2019). Forcing vine regrowth to delay ripening and its association to changes in the hormonal balance. *Vitis*, 58 (Special Issue), 95-101. <https://doi.org/10.5073/vitis.2019.58.special-issue.95-101>

- Prats-Llinàs, M. T., Nieto, H., DeJong, T. M., Girona, J., & Marsal, J. (2020). Using forced regrowth to manipulate Chardonnay grapevine (*Vitis vinifera* L.) development to evaluate phenological stage responses to temperature. *Scientia Horticulturae*, 262, 109065. <https://doi.org/10.1016/j.scienta.2019.109065>
- Reis, S., Martins, J., Gonçalves, F., Carlos, C., Santos, J.A. (2022). European grapevine moth and *Vitis vinifera* L. phenology in the Douro Region: (A)synchrony and climate scenarios. *Agronomy*, 12(1), 98. <https://doi.org/10.3390/agronomy12010098>
- Ramos, M. C. (2017). Projection of phenology response to climate change in rainfed vineyards in north-east Spain. *Agricultural and forest meteorology*, 247, 104-115. <https://doi.org/10.1016/j.agrformet.2017.07.022>
- Rienth, M., Torregrosa, L., Sarah, G., Ardisson, M., Brillouet, J. M., & Romieu, C. (2016). Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels their transcriptome. *BMC Plant Biology*, 16(1), 1-23. <https://doi.org/10.1186/s12870-016-0850-0>
- Romero, P., Pérez-Pérez, J. G., Del Amor, F. M., Martínez-Cutillas, A., Dodd, I. C., & Botía, P. (2014). Partial root zone drying exerts different physiological responses on field-grown grapevine (*Vitis vinifera* cv. Monastrell) in comparison to regulated deficit irrigation. *Functional Plant Biology*, 41(11), 1087-1106. <https://doi.org/10.1071/FP13276>
- Sadras, V. O., & Moran, M. A. (2013). Nonlinear effects of elevated temperature on grapevine phenology. *Agricultural and Forest Meteorology*, 173, 107-115. <https://doi.org/10.1016/j.agrformet.2012.10.003>
- Santesteban, L. (2022). Extreme canopy management for vineyard adaptation to climate change: is it a good idea? terclim2022. XIVth International Terroir Congress. 2nd ClimWine Symposium. July 3-8, 2022. Bordeaux, France. <https://terclim2022.symposium.inrae.fr/>
- Santesteban, L. G., Miranda, C., Urrestarazu, J., Loidi, M., & Royo, J. B. (2017). Severe trimming and enhanced competition of laterals as a tool to delay ripening in Tempranillo vineyards under semi-arid conditions. *OENO One*, 51(2), 191-203. <https://doi.org/10.20870/oeno-one.2017.51.2.1583>
- Schultz, H. (2000). Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. *Australian Journal of grape and wine research*, 6(1), 2-12. <https://doi.org/10.1111/j.1755-0238.2000.tb00156.x>
- Smith, J. P., & Holzapfel, B. P. (2009). Cumulative responses of Semillon grapevines to late season perturbation of carbohydrate reserve status. *American Journal of Enology and Viticulture*, 60(4), 461-470. <https://www.ajevonline.org/content/60/4/461.short>
- Teixeira, A., Eiras-Dias, J., Castellarin, S. D., & Gerós, H. (2013). Berry phenolics of grapevine under challenging environments. *International journal of molecular sciences*, 14(9), 18711-18739. <https://doi.org/10.3390/ijms140918711>
- Tian, T., & Gu, S. (2019). Improving fruit anthocyanins in 'Cabernet-Sauvignon' by shifting fruit ripening and irrigation reduction post veraison in warmer region. *Vitis*, 58(1), 23-31.
- Winkler, A.J. (1974). General Viticulture. University of California Press: Berkley, CA.
- Zheng, W., del Galdo, V., García, J., Balda, P., & de Toda, F. M. (2017). Use of minimal pruning to delay fruit maturity and improve berry composition under climate change. *American Journal of Enology and Viticulture*, 68(1), 136-140. <https://doi.org/10.5344/ajev.2016.16038>
- Zheng, W., García, J., Balda, P., & de Toda, F. M. (2017). Effects of late winter pruning at different phenological stages on vine yield components and berry composition in La Rioja, north-central Spain. *OENO One*, 51(4), 363-363. <https://doi.org/10.20870/oeno-one.2017.51.4.1863>
- Zheng, W., García, J., Balda, P., & Martínez de Toda, F. (2017). Effects of severe trimming after fruit set on the ripening process and the quality of grapes. *Vitis*, 56(1), 27-33.