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ORIGINAL RESEARCH ARTICLE

VineyardFACE: Investigation of a moderate (+20 %) increase of ambient CO₂ concentration on berry ripening dynamics and fruit composition of Cabernet-Sauvignon

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ABSTRACT

Climate change and rising atmospheric carbon dioxide concentration are a concern for agriculture, including viticulture. Studies on elevated carbon dioxide have already been conducted on grapevines, mainly taking place in greenhouses using potted plants or using field-grown vines under instant and higher CO₂ enrichment, i.e., > 650 ppm. The VineyardFACE, located at Hochschule Geisenheim University, is an open field Free Air CO₂ Enrichment (FACE) experimental set-up designed to study the effects of elevated carbon dioxide using adapted, field-grown vines (Vitis vinifera L. cv. Cabernet-Sauvignon). As the carbon dioxide fumigation started in 2014, the long-term effects of elevated carbon dioxide treatment can be investigated on berry ripening parameters and fruit metabolic composition.

The present study investigates the effect on fruit composition under a moderate increase (+20 %; eCO₂) of carbon dioxide concentration, as predicted for 2050 on Cabernet-Sauvignon. Berry growth, ripening dynamics and composition were determined and primary (sugars, organic acids, amino acids) and secondary metabolites (anthocyanins) were analysed. Compared to previous results of the early adaptive phase of the vines (Wohlfahrt et al., 2020), our results show little effects of eCO₂ treatment on primary metabolites composition in berries. However, total anthocyanins concentration in berry skin was lower for eCO₂ treatment in the hot and dry season of 2020, although the ratio between anthocyanins derivatives did not differ.

KEYWORDS: Free Air CO₂ Enrichment, carbon dioxide, berry ripening, berry composition

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INTRODUCTION

Ongoing changes in global mean temperature, precipitations and continuously increasing carbon dioxide (CO₂) concentration are reshuffling conditions in which plants are growing. Indeed, atmospheric carbon dioxide concentration increases continuously due to anthropogenic emissions and currently reaches 410 ppm, while global surface temperature is already 1.09 °C higher in 2011–2020 compared to the 1850–1900 era (IPCC, 2021). Global surface temperature at the end of the century is predicted to increase by 1.0 °C to 1.8 °C (low greenhouse gas GHG emissions scenario), 2.1 °C to 3.5 °C (intermediate GHG emissions scenario) and by 3.3 °C to 5.7 °C (very high GHG emissions scenario; IPCC, 2021). Furthermore, according to the most pessimistic scenarios, rainfall variability should be amplified in the near future, and climate change is predicted to intensify the severity of wet and dry events (IPCC, 2021).

Crops are sensitive to environmental conditions, and grapevine is no exception to the rule. Berry oenological potential (i.e., its composition at harvest) is a complex trait that mainly results from genotypes (scions and rootstocks) and environmental interactions. Thus, climate change’s impact on grapevine fruit composition at harvest needs to be thoroughly studied to help the wine industry adapt to future climate conditions (Duchêne et al., 2010; Schultz, 2000). Numerous studies have already characterised how grapevine and wine characteristics are impacted by elevated temperature (Luchaire et al., 2017; Sadras et al., 2013), heat stress (Lecourieux et al., 2017), water deficit (Deluc et al., 2009) or UV-B (Martínez-Lüscher et al., 2013).

Studies concerning the impact of atmospheric carbon dioxide concentration increase are scarcer and it should be noticed that studying carbon dioxide concentration effects is technically more challenging. Thus, prior studies on the impact of carbon dioxide on Vitis vinifera L. were mostly conducted in greenhouses or enclosed (tunnel or chamber) systems, using potted plants (fructifying cuttings) (Arrizabalaga-Arriaza et al., 2020; Arrizabalaga-Arriaza et al., 2021; Martínez-Lüscher et al., 2016). However, the translation of the results obtained from greenhouses to vineyard-based studies can be biased, as reported by Poorter et al. (2016). FACE (Free Air Carbon dioxide Enrichment) systems are open field setups designed to create a CO₂ enriched atmosphere around crops and represent a more realistic experimental system. Several FACE systems have been installed already, whether on various herbaceous crops such as pea (Bourgault et al., 2016), wheat and rice (Cai et al., 2016), barley and maize (Erbs et al., 2015) or trees such as poplars (Gienlen and Ceulemans, 2001). According to literature reports on FACE based experiments, elevated CO₂ caused decreased stomatal conductance (Ainsworth and Rogers, 2007; Leakey et al., 2009) and increased light-saturated CO₂ uptake/net assimilation rate (Reddy et al., 2010) in C₃ plants. Consequently, the carboxylation efficiency of RuBisCo, compared to the oxygenation efficiency, was increased under elevated CO₂. An increase in leaf area was also observed in response to an increase in CO₂ atmospheric concentration (Ainsworth and Long, 2005).

The impact of CO₂ atmospheric concentration on grapevine vegetative growth and berry composition has also been investigated by Bindi et al. (2001) using a FACE system, with different carbon dioxide concentrations (550 ppm, 700 ppm compared to ambient), on cv. Sangiovese. Vegetative growth, as well as fruit fresh and dry mass, were significantly increased by elevated CO₂ concentrations. Sugars and organic acid concentrations increased during berry development and ripening, but at maturity, these effects tended to disappear (Bindi et al., 2001). However, in these experiments, the treatment was applied instantaneously as a “shock” of CO₂, i.e. by suddenly rising local CO₂ to the desired level, which will not mimic realistic climate change scenarios, in which CO₂ increases gradually by approximately 2 ppm per year (IPCC, 2021). Therefore, such results must be discussed with caution since vines did not face a long-term acclimation towards gradual changes in carbon dioxide.

Using the VineyardFACE system described by Wohlfahrt et al. (2018), vegetative growth, leaf gas exchanges and yield parameters of cvs. Riesling and Cabernet-Sauvignon were compared under elevated and ambient CO₂ conditions. A significant increase in net assimilation rate, transpiration rate, stomatal conductance and water use efficiency was reported during the early years of grapevine adaptation, i.e., up to six years after planting (Wohlfahrt et al., 2018). Conversely, other results in the literature rather reported a decrease in stomatal conductance (Ainsworth and Rogers, 2007), although some authors agree with the fact that depending on weather conditions, an increase in stomatal conductance can occur under enriched CO₂ conditions (Purcell et al., 2018). Further studies showed that elevated CO₂ altered bunch parameters, increased single berry weight as well as malic acid content (Wohlfahrt et al., 2020). Moreover, minor differences in galacturonic acid for Cabernet-Sauvignon wines, pH or volatile acidity for Riesling were observed (Wohlfahrt et al., 2021). However, fruit quality at ripeness was globally not affected by elevated CO₂. Indeed, sugar concentration did not differ at harvest (Wohlfahrt et al., 2018). No negative impact of elevated CO₂ treatment was reported on must and wine composition for the years 2014 to 2016, and no difference occurred in total anthocyanin concentration in young wines of Cabernet-Sauvignon (Wohlfahrt et al., 2021).

In previous studies of the VineyardFACE, young grapevines adapted to the fumigation from an early stage of their development, and vegetative growth, as well as berry composition, was extensively studied. However, the long-term effect of elevated CO₂ has hardly been studied yet, as recently highlighted by Clemens et al. (2022). In VineyardFACE, vines have been well established since 2014, and the duration of the fumigation allows us to study grape berry parameters and composition of well-adapted vines using Cabernet-Sauvignon under near future (i.e. mid-century, 2050) forecasted scenarios.
MATERIALS AND METHODS

1. Experimental set-up
VineyardFACE is an experimental setup located at Hochschule Geisenheim University (49° 59’ N, 7° 57’ E; Rheingau, Germany) with a total area of 0.5 ha planted in 2012 with Cabernet-Sauvignon (clone 170, grafted on rootstock 161-49 Coudrec). Six Free Air Carbon dioxide Enrichment (FACE) rings surround the vineyard, three with ambient CO₂ levels (~410 ppm, aCO₂, “A” rings) and three with elevated CO₂ levels (aCO₂ plus 20%; eCO₂, “E” rings). These rings are built with 36 towers, with a built-in blower creating an airstream and emitters releasing carbon dioxide (Wohlfahrt et al., 2018). Each ring consists of seven rows, with the inner five rows used for sampling and rings A1-E1, A2-E2, A3-E3 are defined as experimental blocks.

2. Berry sampling and processing
Berries were collected in seasons 2019, 2020 and 2021 and sampled from E/L 33-34 onwards (Coome, 1995). Véraison’s progress was assessed by the percentage of individual berries starting to change colour. Between 14 and 18 berries were picked per sampling day (Supplementary Table 1) and selected randomly from the inner row of each ring (half of the berries from the eastern side of the canopy and half from the west), put in pre-chilled Falcon tubes, immediately frozen in liquid nitrogen and stored in freezers at –80 °C until processing. Berries were counted and weighted. Pulp, skin and seeds were separated, and all compartments were weighed. Pulp and skins were reduced into a fine powder using an MM400 grinder (Retsch, Haan, Germany) under liquid nitrogen. Relative skin and seed mass, expressed in percentage, was calculated by dividing respectively skin fresh weight and seed fresh weight by berry fresh weight. Seed and ground powders were then stored in a –20 °C freezer for further analysis.

3. Berry volume calculation
Equatorial and polar diameters were measured on each frozen berry from samples 2020 and 2021 with a digital calliper. A formula for the volume of a spheroid was applied to calculate berry volume, according to Arrizabalaga-Arriazu et al. (2021).

\[ V = \frac{4}{3} \pi r_1^2 r_2 \]

\( r_1 \) equatorial radius, \( r_2 \) polar radius.

4. Primary metabolites analysis
Primary metabolites in berries (sugars, organic acids and amino acids) were extracted and their contents were determined as previously described by Arrizabalaga-Arriazu et al. (2021). Briefly, pulp frozen powder (250 mg ± 10 %) was successively hot extracted with ethanol 80 % (v/v), ethanol 50 % (v/v) and Milli-Q water (80 °C for 15 min). Supernatants of all three extractions were combined, dried (Speed Vac System ISS110, Savant) then resuspended in Milli-Q water. Extracts were filtered through a 0.45 µm filter (Millipore) and stored in a –20 °C freezer for further analysis.

Sugar analysis was performed using an Alphal-4 (CHRIST, Osterode, Germany), were extracted using 500 or 750 µL methanol acidified with 0.1 % HCl (v/v). Extracts were filtered into U-HPLC vials through a 0.2 µm porosity filter (Millex-GS Syringe filter unit, Millipore) and then analysed according to Arrizabalaga-Arriazu et al. (2021). Individual amino acid quantification was determined after filtered extracts derivatisation with 6-aminoquinolyl-N-hydroxy-succinimidyl-carbamate AQ (AccQ-Tag derivatisation reagent, Waters, Milford, MA, USA) according to Arrizabalaga-Arriazu et al., 2021. The software used to acquire the data and integrate the peaks is Chroméleon version 7.1 (ThermoScientific).

5. Anthocyanins profiling
Freeze-dried skin powder (20–30 mg), frozen initially at 100 mg ± 10 % (Alph1-4, CHRIST, Osterode, Germany), were extracted using 500 or 750 µL methanol acidified with 0.1 % HCl (v/v). Extracts were filtered into U-HPLC vials through a 0.2 µm porosity filter (Millex-GS Syringe filter unit, Millipore) and then analysed according to Arrizabalaga-Arriazu et al. (2021). The malvidin-3-glucoside standard was used to quantify anthocyanin concentration.

6. Berry must analyses
During the season 2020 and 2021, forty berries from the three inner rows were taken, twenty from each side of the row, for each ring. Berries were crushed and pressed (Longarone 85, QSh System GmbH, Norderstedt, Germany), then samples were centrifuged at 7830 rpm for 5 min (5430R, Eppendorf AG, Hamburg, Germany). For N-OPA analysis, 500 µL of the sample was added to 500 µL of Milli-Q water in Eppendorf tubes, according to a method described by Wohlfahrt et al. (2020). For Oenofoss™ measurements, 1 mL of sample was added in Eppendorf tubes and then centrifuged.

7. Gas exchange measurements
In both seasons (2019 and 2020), the gas exchange measurements were performed from June to October using an open gas exchange measurement system (GFS-3000, Walz GmbH, Germany). Three grapevines (Vitis vinifera L., cv. Cabernet-Sauvignon) per ring and one fully developed and sun-exposed leaf per plant were measured (in total nine per treatment) between 8.30 a.m. and latest until 2 p.m. to avoid shading conditions on the leaf surface due to the row orientation in the VineyardFACE. To simulate
the surrounding light conditions, a LED light source was used. Net assimilation rate $A$, transpiration rate $E$ and stomatal conductance $g_s$ were calculated in response to the predominant environmental conditions without extra cooling of the leaf chamber. A buffer tank was used to keep the surrounding CO$_2$ concentration stable.

**8. Statistical analyses**

Statistical analyses were performed with RStudio (version 4.1.2.). The packages *cowplot*, *tidyverse* and *ggplot2* were used in RScripts. Datasets were subjected to two-way ANOVA to verify the effects of time (DOY), treatment and their interaction. When parameters were measured for two years, two-way ANOVA was performed on combined datasets for each developmental stage to verify treatment and vintage effect.

**RESULTS**

1. **Net assimilation rate**

The net assimilation rate $A$, averaged over the season, was 16.6% higher under elevated CO$_2$ (eCO$_2$) concentration in 2019 with 14.19 µmol.m$^{-2}$.s$^{-1}$ compared to 12.17 µmol.m$^{-2}$.s$^{-1}$ under ambient CO$_2$ (aCO$_2$; Table 1). This effect was even more pronounced in 2020, with an increase of approximately 31% compared to ambient conditions.

2. **Total acidity and total soluble solids**

Total acidity in must decreased during berry development, ranging from 39.37 ± 2.95 g.L$^{-1}$ to 9.33 ± 1.14 g.L$^{-1}$ for aCO$_2$ at the green stage and from 40.16 ± 1.65 g.L$^{-1}$ to 9.81 ± 0.6 g.L$^{-1}$ for eCO$_2$ at maturity in 2020 (Figure 1A).

<table>
<thead>
<tr>
<th>treatment</th>
<th>Net assimilation rate A (µmol m$^{-2}$s$^{-1}$)</th>
<th>2019</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCO$_2$</td>
<td>12.17</td>
<td>12.77</td>
<td></td>
</tr>
<tr>
<td>eCO$_2$</td>
<td>14.19</td>
<td>16.72</td>
<td></td>
</tr>
<tr>
<td>% to aCO$_2$</td>
<td>116.64</td>
<td>130.91</td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 1.** Total acidity (g.L$^{-1}$), Cabernet-Sauvignon, year 2020 A) year 2021 B) and TSS (°Brix), year 2020 C) year 2021 D) under ambient carbon dioxide treatment/aCO$_2$ (open circles) and elevated carbon dioxide treatment/eCO$_2$ (grey triangles) treatment.
No significant difference in total acidity was observed between the two CO$_2$ treatments (Supplementary Table 2). Interaction between treatment and day of year was not significant. Statistical analyses performed on both years, for each sampling date, demonstrated a clear vintage effect but no treatment nor year vs. treatment interaction effects.

Total soluble solids (TSS) showed no difference between the two treatments throughout each season (Figure 1B). Due to particular weather conditions in 2021, Cabernet-Sauvignon did not reach 22 °Brix but nevertheless was harvested at the end of October. For each developmental stage, the vintage effect was significant for TSS (Supplementary Table 3).

3. Berry volume and berry weight evolution

Berry volume increased in 2020 from 877.4 ± 68.3 to 1513.6 ± 123.1 mm$^3$ for aCO$_2$ and from 927.4 ± 55.5 to 1493 ± 47.6 mm$^3$ for eCO$_2$ (72.5 % and 60.9 %, respectively, Figure 2B). In 2021, berry volume ranged from 939.9 ± 16.4 to 1473.5 ± 92.1 for aCO$_2$ and from 974.4 ± 59.6 to 1541.2 ± 43.7 for eCO$_2$ (56.7 % and 58.2 %, respectively). Indeed, there was a trend of higher berry volume for both years. Although berry volume was significantly impacted by sampling day as expected, neither treatment nor interaction between day of year and treatment did demonstrate significant differences (Supplementary Table 4). However, by combining datasets both years and for each stage of development, there was no vintage effect but a treatment effect at 25 % véraison.

Single berry weight increased throughout berry development, with berries in 2020 weighing 0.89 ± 0.03 g for aCO$_2$ and 0.91 ± 0.03 g for eCO$_2$ at green stages and 1.62 ± 0.11 g for aCO$_2$ and 1.62 ± 0.09 g for eCO$_2$ at maturity (Figure 2A, Supplementary Table 5). Berry weight under eCO$_2$ seemed to be slightly higher than for berries under aCO$_2$. However, this trend seemed to be alleviated around harvest time for both seasons 2020 and 2021.

4. Skin and seeds mass

Relative skin mass did not differ between the treatments for 2020 (Table 2). Seed mass reported to berry weight seemed to be decreased under elevated CO$_2$ treatment compared to ambient, and during ripening, the difference was alleviated. The difference between the two treatments was, however, not significant.

5. Primary metabolites

5.1. Sugars

Sugar concentration was expressed as glucose, fructose and total sugars (sum of glucose and fructose). In 2019, total sugar concentration increased from green stages to maturity from 4.2 ± 0.004 mg.g$^{-1}$ to 149.05 ± 19.3 mg.g$^{-1}$ for aCO$_2$ and from 4.17 ± 0.19 mg.g$^{-1}$ to 154.63 mg.g$^{-1}$ for eCO$_2$, compared to 2020 where it increased from 5.48 ± 1.23 mg.g$^{-1}$ to 157 ± 16 mg.g$^{-1}$ for aCO$_2$ and from 5.69 ± 0.68 mg.g$^{-1}$ to 162.03 ± 10.35 mg.g$^{-1}$ for eCO$_2$ (Figure 3). Sugar concentration seemed to be more affected by the vintage effect than by the treatment effect when combining both years 2019 and 2020.
However, at 75% véraison, a significant treatment effect was noticeable for fructose, visible for both years (Supplementary Table 6).

### 5.2 Organic acids

Malic and tartaric acid concentrations decreased during ripening as maturation progressed. Indeed, malic acid decreased by 80.61% for aCO₂ and 83.4% for eCO₂ in 2019, while in 2020, it decreased by 87.3% for aCO₂ and by 85.2% for eCO₂ (Figure 4A). Tartaric acid in 2019 decreased by 50.3% for aCO₂ and by 49.6% for eCO₂ compared to 2020, when it decreased by 48.4% for aCO₂ and 42.02% for eCO₂ (Figure 4B). As for total acidity, no significant difference was demonstrated between the two treatments for both malic and tartaric acids (Supplementary Table 7). The vintage effect was indeed more predominant at some stages, namely at 25% véraison and 50% véraison.

### 5.3 Amino acids content and composition

In 2019, amino acid content did increase along berry development, from 2.4 ± 0.35 nmol.mg⁻¹ to 6.4 ± 2.29 nmol.mg⁻¹ for aCO₂ and from 1.9 ± 0.58 nmol.mg⁻¹ to 8.11 nmol.mg⁻¹ for eCO₂ (Figure 4A). In comparison amino acids content was steadier or even decreased in 2020, ranging from 4.41 ± 2.23 nmol.mg⁻¹ to 6.81 ± 3.16 nmol.mg⁻¹ for aCO₂ and from 4.50 ± 1.16 nmol.mg⁻¹ to 2.89 ± 1.39 nmol.mg⁻¹ for eCO₂ (Figure 4B). Combining both years datasets, total amino acid content demonstrated a vintage effect at early

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**TABLE 2.** Relative skin and seed mass (%) for Cabernet-Sauvignon, year 2020, ambient aCO₂ or elevated CO₂.

<table>
<thead>
<tr>
<th></th>
<th>Treatment\DOY</th>
<th>217</th>
<th>227</th>
<th>241</th>
<th>269</th>
<th>286</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative skin mass</td>
<td>aCO₂</td>
<td>10.98 ± 1.14</td>
<td>9.50 ± 0.22</td>
<td>9.14 ± 0.54</td>
<td>10.34 ± 0.12</td>
<td>10.52 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>eCO₂</td>
<td>10.48 ± 0.17</td>
<td>9.72 ± 0.3</td>
<td>9.46 ± 0.24</td>
<td>10.16 ± 0.18</td>
<td>11.02 ± 1.44</td>
</tr>
<tr>
<td>Relative seed mass</td>
<td>aCO₂</td>
<td>9.83 ± 0.70</td>
<td>9.41 ± 0.90</td>
<td>6.72 ± 0.61</td>
<td>4.25 ± 0.21</td>
<td>3.87 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>eCO₂</td>
<td>9.24 ± 0.49</td>
<td>8.46 ± 0.68</td>
<td>6.60 ± 0.64</td>
<td>4.28 ± 0.12</td>
<td>3.95 ± 0.19</td>
</tr>
</tbody>
</table>

**FIGURE 3.** Sugars for year 2019 and 2020 expressed as Glucose (A), Fructose (B) and total sugars (C) for Cabernet-Sauvignon under ambient carbon dioxide treatment/aCO₂ (open circles) and elevated carbon dioxide treatment/eCO₂ treatment (grey triangles) ** p < 0.001, * p < 0.01, n.s. not significant.
FIGURE 4. Organic acids 2020; malic (A) and tartaric acid (B) for Cabernet-Sauvignon, year 2019 and 2020, ambient carbon dioxide treatment/aCO$_2$ (open circles) and elevated carbon dioxide treatment/eCO$_2$ (grey triangles) treatment, ** $p < 0.001$, * $p < 0.01$, n.s. not significant.

FIGURE 5. Total amino acids expressed in nmol.mg$^{-1}$, for Cabernet-Sauvignon, year 2019 (A) and 2020 (B), ambient carbon dioxide treatment/aCO$_2$ (white bars) or elevated carbon dioxide treatment/eCO$_2$ (grey bars) treatment and (C) amino acids composition (Cabernet-Sauvignon) in 2020 for each DOY.
stages (Supplementary Table 8). However, during ripening, neither vintage nor treatment effects were significant. In 2020, the alpha-ketoglutarate amino-acid derivatives (Pro, Arg, Gln, Glu, GABA, His) were the most abundant, followed by the aspartate pathway derivatives (Thr, Asp, Asn, Ile, Met, Lys) for the first three sampling stages (Figure 5C). Pyruvate derivatives (Ala, Val, Leu) abundance increased throughout berry development, ranging from 2.17 % to 6.81 % for aCO$_2$ and from 1.36 % to 5.32 % for eCO$_2$.

5.4. Anthocyanins
The concentration of anthocyanins in skin samples in 2019 did not significantly differ among treatments according to two-way ANOVA (Figure 6A). For 2019 and 2020, respectively, the sampling date effect was significant, but the treatment was not (supplementary table 9). However, there is a consistent trend in decreased anthocyanins concentration in berries under eCO$_2$ treatment (mostly in 2020), but not statistically significant. Neither anthocyanin composition nor di- to tri-hydroxylated forms ratio were modified in 2020 for the two treatments (Figure 6C).

However, when combining both year datasets for statistical analysis, a treatment effect was noticeable at 25 % véraison and 75 % véraison, although the vintage effect was still predominant.

According to Figure 6, a trend of higher di-hydroxylated anthocyanins (cyanidin and peonidin derivatives) compared to tri-hydroxylated anthocyanins (malvidin, petunidin and delphinidin) was noticeable in 2020 in samples under elevated CO$_2$ treatment but the difference tended to decline at maturity. Around véraison, the standard deviation was important because of berry heterogeneity in colours.

Malvidin derivatives were the most abundant derivatives in the samples at all developmental stages, with a percentage of malvidin derivatives being respectively 45.5 % for aCO$_2$ and 44.8 % for eCO$_2$ at maturity. Even if slight differences

FIGURE 6. Total anthocyanins for Cabernet-Sauvignon skins, (A) year 2019, (B) year 2020 and (C) ratio between di- and tri-hydroxylated anthocyanin forms at ambient carbon dioxide treatment/aCO$_2$ (open circles) and elevated carbon dioxide treatment/eCO$_2$ (grey triangles).
could occur during berry development between elevated and ambient CO₂ treatment, no major change in anthocyanins composition is noticeable in 2020 (Figure 7).

**DISCUSSION**

This study is unique since it has been performed in a vineyard located FACE setup (VineyardFACE) where vines have been treated by elevated CO₂ concentration for almost one decade from shortly after planting to mature vines. Hence, the results of the investigations can be interpreted as long-term acclimation responses of the vines. The net assimilation rate was higher under elevated CO₂ treatment for both the 2019 and 2020 seasons (Table 1). Similar physiological responses were reported in previous seasons (Wohlfahrt et al., 2018), resulting in enhanced vegetative growth.

Regarding reproductive plant biomass, berry volume did not differ significantly between ambient and elevated CO₂ treatment. However, when combining the years 2020 and 2021, there was a significant increase in berry volume at 25 % véraison and a trend of higher berry volume under elevated CO₂. Moreover, single berry weight demonstrated a clear trend of an increase under elevated CO₂ treatment for both seasons 2020 and 2021, a trend that was alleviated at maturity. Biomass increase in yield under eCO₂ for red cultivars such as Sangiovese was reported by Bindi et al. (2005), assuming a higher berry weight under eCO₂. For Cabernet-Sauvignon, single berry weight increased under elevated CO₂ on the same VineyardFACE experimental setup (Wohlfahrt et al., 2020) when the vines were younger. Berry size is reported to be related to berry and wine quality, particularly at véraison, which coincides with a simultaneous sugar accumulation and organic acid degradation (Chen et al., 2018). Sugars accumulated in berries are mostly glucose and fructose (Kliewer, 1966). Mature berries display the same amount of glucose and fructose, whereas, at the early stages, glucose is predominant. In this study, no difference was noticeable between the two treatments, neither for TSS in must nor in total sugars from frozen pulp powder, as it was shown for TSS at the beginning of VineyardFACE experiments (Wohlfahrt et al., 2020).

Organic acids start to accumulate in the early stages of grape berry development and are mainly represented by malic and tartaric acid (Kliewer, 1966). During ripening, malic acid is degraded by increased respiration rate (Conde et al., 2007), whereas tartaric acid concentrations decrease by dilution effect due to berry volume increase. Our results suggest no significant effect of elevated CO₂ on both malic and tartaric acids. The effect of temperature on organic acid and especially malic acid degradation has already been reviewed (Etienne et al., 2013). Using potted plants in greenhouses, Arrizabalaga-Arziaez et al. (2020) found that malic acid in berry decreased from mid-véraison onwards under elevated temperature, whereas under elevated CO₂ (700 ppm), malic acid increased at véraison but was significantly reduced at maturity. In VineyardFACE, malic acid degradation was slowed down under elevated CO₂ concentration for Cabernet-Sauvignon (Wohlfahrt et al., 2020). Our results suggest that this effect is alleviated by long-term acclimation of the vines.

Concerning berry composition, it was firstly reported that vines in FACE systems under elevated CO₂ were displaying a change in sugar and organic acid concentration, but this CO₂ effect did disappear at maturity (Bindi et al., 2005). In temperature gradient greenhouses, a mitigating role of elevated CO₂ was demonstrated on grapevine vegetative

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**FIGURE 7.** Anthocyanins derivatives composition (Cabernet-Sauvignon) of year 2020 under ambient carbon dioxide treatment/ aCO₂ and elevated carbon dioxide treatment/ eCO₂ treatment. Cy: cyanidin, Dp: delphinidin, Mv: malvidin, Pn: peonidin and Pt: petunidin
growth and yield when combined with elevated temperature and drought (Kizildeniz et al., 2015).

No effect of elevated CO\textsubscript{2} was found at maturity on total amino acid concentration, although CO\textsubscript{2} treatment reduced alpha-ketoglutarate derivatives in later stages. Moreover, phenylalanine content was significantly increased at véraison, and it was found that elevated CO\textsubscript{2} treatment decreased amino acid concentration at véraison and two weeks after mid-véraison (Arrizabalaga-Arriazu et al., 2020).

Anthocyanins are considered important metabolites in fruit, which are usually altered during wine ageing to form polymeric pigments. Elevated temperature decreased anthocyanins concentration (Spayd et al., 2002) and accumulation. Furthermore, genes for anthocyanins biosynthesis were down-regulated under high-temperature conditions (Mori et al., 2007). Indeed, elevated temperature conditions decreased anthocyanins concentration (Arrizabalaga-Arriazu et al., 2021). However, the effect of elevated CO\textsubscript{2} on anthocyanins remains less evident in the literature. Berry characteristics were described to be unaffected by elevated CO\textsubscript{2} in Open Top Chambers on cv. Touriga Franca, however, total anthocyanins and polyphenol concentrations in red wine were reported to decrease under elevated CO\textsubscript{2} treatment (Gonçalves et al., 2009). When elevated temperature and elevated CO\textsubscript{2} were combined, it was reported that total anthocyanins and malic acid declined (Salazar Parra et al., 2010). Studies applying different UV-B doses and two temperature/CO\textsubscript{2} regimes to grapevine fruit cuttings cv. Tempranillo demonstrated that anthocyanins concentration differed during berry development between the treatments. Indeed, their concentration was higher under elevated CO\textsubscript{2} and elevated temperature combined two weeks after véraison, but at maturity, the trend reversed (Martínez-Lüscher et al., 2016). When studying the two parameters independently, Arrizabalaga-Arriazu et al. (2020) found that elevated CO\textsubscript{2} treatment did increase anthocyanin concentrations at the onset of véraison and mid-véraison, whereas after mid-véraison, their concentration decreased, in a clone genotype-dependent manner. In the case of the current study, the decreased anthocyanin concentration could be due to increased single berry weight. Indeed, an increase in berry size could lead to a lower skin surface to berry volume ratio (Ojeda et al., 2002). Moreover, it was found that under carbon limitation induced by variation in source-to-sink ratio using Cabernet-Sauvignon the proportion of di-hydroxylated anthocyanins decreased (Bobeica et al., 2015; Wang et al., 2021). In our study, the di- to tri-hydroxylated anthocyanins ratio seemed to be higher, although not significantly, under elevated CO\textsubscript{2} treatment. To gain more knowledge, in the future experiment, the degradation rate of monomeric anthocyanins under eCO\textsubscript{2} conditions forming polymeric pigments will need to be investigated to better follow the ageing potential of the red wines.

In a recent study, the parameters of young wine, such as total anthocyanins and organic acids, were more affected by the vintage effect than the CO\textsubscript{2} treatment (Wohlfahrt et al., 2021). These results confirm that, even if photosynthesis is still enhanced by elevated CO\textsubscript{2} treatment, primary and secondary metabolites content of berries may not differ under scenarios of near-future atmospheric CO\textsubscript{2} conditions.

CONCLUSION

This study aims to evaluate the effects of 2050 atmospheric carbon dioxide conditions on the berry composition of cv. Cabernet-Sauvignon that has been grown under elevated CO\textsubscript{2} concentration for almost one decade. No major differences in primary metabolites were found under elevated carbon dioxide treatment in two recent seasons, although it was shown that photosynthesis and the net assimilation rate are still enhanced by elevated carbon dioxide. However, from the berry quality point of view, elevated carbon dioxide has little effect on berry ripening dynamics and fruit composition. Concerning at least atmospheric carbon dioxide concentration, the tipping point for grapevine seems to be not already crossed. However, climate change results in a combination of factors such as elevated temperature, drought in certain regions, and of course, elevated CO\textsubscript{2}. Further investigations focusing on combined environmental factors on primary metabolism intermediates, as well as aroma compounds, are part of ongoing studies within the VineyardFACE.

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REFERENCES


A Review.

Vitis vinifera

L. cv. ‘Cabernet-Sauvignon’

Volatile Composition, Phenolic

and anthocyanins to source-sink modulation in Cabernet-Sauvignon
and Sangiovese grapevines. Frontiers in Plant Science, 06.
https://doi.org/10.3389/fpls.2015.00382


