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The effects of a moderate grape temperature increase on berry secondary metabolites

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ABSTRACT

Context and purpose of the study: Like in other wine producing regions around the world, Bordeaux vineyards already experience the effects of climate change. Recent trends as well as model outputs for the future strongly support an increase of average and extreme temperatures. For the maturation period, this increase will by far exceed mean atmospheric temperature increase, as the ripening period will occur earlier in hotter climatic conditions. Therefore, a detrimental secondary metabolism response is expected in grape berries, and of particular concern are the impacts on phenolics and aromas and aroma precursors. The effects of high temperatures on secondary metabolism control have been partly characterized for phenolics, however mostly in artificial growing conditions, while little is known with respect to aromas. A better understanding of how high temperatures influence grape berry secondary metabolites could help vineyard growers to adapt to climate change and maintain wine quality.
Material and methods: A two-year field study was carried out in 2015 and 2016 in a vineyard in Bordeaux, France. Two treatments, heated (H) and control (C), were applied to two varieties, Cabernet-Sauvignon and Sauvignon blanc, from fruit-set to maturity. Field heating was achieved by a very local greenhouse effect applied to the bottom of the rows, by enclosing most of the underlying soil surface by polycarbonate shields. As the training system was vertically trellised, the heated volume surrounded most of the bunches but did not disturb most of the leaves in the canopy. This simple and robust setup allowed an increase of berry temperature by about +1.5°C in mean value, up to +5°C at times during clear sky days. This moderate increase of temperature was indicative of the predicted future climatic conditions for the mid-21st century. Berry samples were collected at 4 time points from bunch closure to maturity for each cultivar and treatment. Primary and secondary metabolites were measured in whole berries or skins.
Results and conclusions: With this moderate temperature increase, primary metabolite content in berries did not change significantly. In H samples, anthocyanins were reduced and tannins increased before veraison, and both decreased thereafter. H samples also exhibited lower concentrations of some amino acids, especially alanine, serine and phenylalanine. IBMP (2-methoxy-3-isobutylpyrazine) concentrations were also reduced in H samples of Cabernet-Sauvignon, in both seasons, especially at bunch closure stage, but the differences diminished at full maturity. For thiol 3-sulfanyl hexanol precursors, H samples again exhibited much lower concentrations for both varieties, with weak differences at early stages that increased at later stages (up to -70% decline at maturity in 2015 for Sauvignon blanc). These results demonstrate the potential negative impact of elevated temperature on polyphenols and aroma quality of grape berries.
Significance and impact of the study: For viticulture to adapt to new climatic conditions, the negative impacts of high temperature on secondary metabolites and aromas, and therefore on wine quality, need to be contemplated. Thus, already established or new vineyard plantings must prepare and consider practices able to mitigate these impacts, for instance practices that increase bunch shading.

KEYWORDS

grapevine, climate change, secondary metabolism, polyphenols, aromas

INTRODUCTION

The quantity and quality of grape production largely depends on genotype, environmental conditions and viticultural practices (van Leeuwen *et al.*, 2004; Deluc *et al.*, 2007). Among environmental factors, temperature plays a key role in regulating grapevine phenology (Parker *et al.*, 2011) and berry composition (Coombe, 1992; Bergqvist *et al.*, 2001; Tomasi *et al.*, 2011). Meanwhile, like in other wine producing regions around the world, Bordeaux vineyards already experience the effects of climate change (Duchêne and Schneider, 2005; Jones *et al.*, 2005). Recent trends as well as model projections strongly support an increase of average and extreme temperatures. In France, mean temperature is projected to rise by 0.6 to 1.3°C by the mid-21st century (2021-2050) (Ouzeau *et al.*, 2014). For the maturation period, this increase will by far exceed mean atmospheric temperature increase, as ripening will occur earlier in the season, under hotter conditions (Pieri, 2010; Pieri and Lebon, 2014). Therefore, global warming is a real challenge to viticulture and the grape industry, not only because it may influence grape quality and production, but also because it may threaten the sustainability of viticulture in hot regions (Keller, 2010; Hannah *et al.,* 2013; Duchêne *et al.*, 2014).

In general terms, higher temperature accelerates phenological development and decreases organic acid content while increasing sugar content (Coombe, 1992; Jones and Davis, 2000). It is also known empirically to have detrimental effects on phenolics and aromas and aroma precursors (Tonietto and Carbonneau, 2004; Duchêne and Schneider, 2005; Jones *et al.*, 2005; Mori *et al.*, 2007; Azuma *et al.*, 2012; Sweetman *et al.*, 2014; Bonada *et al.*, 2015). However, recent studies focused on higher temperature effects, with higher temperatures applied under controlled conditions and tight temperature control, showed that the temperature effects might be more complex. For example, Sweetman *et al.* (2014) showed that elevated night temperature before veraison could increase the concentration of malic acid. Moreover, Lecourieux *et al.* (2017) demonstrated that higher temperature before veraison could delay veraison by two weeks.

More specifically, the effects of high temperature on secondary metabolites, which largely influence berry and wine quality, are less researched. A few studies have mainly focused on the phenylpropanoid biosynthetic pathway or some aspects of aromatic compounds (Jeandet *et al.*, 2010; Peña-Gallego *et al.*, 2012). A loss of anthocyanins in red-wine grapes under high temperature was reported (Mori *et al.*, 2007; Pieri *et al.*, 2016). Conversely, tannins were less affected by temperature (Cohen *et al.*, 2008). Lower 2-methoxy-3-isobutylpyrazine (IBMP, a green pepper aroma) content in grape berry has been associated with a warmer vintage (Allen and Lacey, 1993; Falcão *et al.*, 2007). Defoliation associated with a higher berry temperature only slightly, but not significantly, modified the precursors of thiols in Sauvignon blanc berries (Sivilotti *et al.*, 2017).

In grapes, free amino acids are the major nitrogenous compounds. Phenylalanine and other branched chain amino acids can be metabolized into the precursors of phenylpropanoids and volatile compounds and therefore influence grape flavors (Guan *et al.*, 2017). They can also influence wine flavors, because of their role as precursors for the synthesis of aromatic compounds, such as isoamyl-acetate (Marcy *et al.*, 1981; Conde *et al.*, 2007). However, higher temperature is known to affect the biosynthesis of amino acids in many plants (Kaplan *et al.*, 2004; Yamakawa and Hakata, 2010). A significant increase of 7 amino acids (Thr, Arg, Tyr, Phe, Cys, Lys and GABA) was observed after a heat treatment (+8°C) was applied to grapevine bunches at veraison and ripening stages under greenhouse conditions (Lecourieux *et al.*, 2017). In field-grown Riesling, negative correlations were observed between the accumulation of amino acids and higher temperatures after leaf removal (Friedel *et al.*, 2015). However, similar amino acid concentrations in Merlot berries were found under vineyard and leaf removal conditions associated with higher temperature and solar radiation, where south-exposed berries were compared to north-exposed berries (Pieri *et al.*, 2016).

Methoxypyrazines (MPs) are a group of nitrogen heterocycle compounds responsible for the "green, herbaceous, or vegetative" aromas of Sauvignon blanc and Cabernet-Sauvignon varieties. Five MPs were identified in grape berries and wines but three of them are highly odorous with very low odor thresholds in water: 2-methoxy-3-isobutylpyrazine (IBMP), 2-

methoxy-3-isopropylpyrazine (IPMP) and 2-methoxy-3-sec-butylpyrazine (SBMP) (Allen and Lacey, 1998; Darriet *et al.*, 2012). IBMP is the major MP component in grape berries, juice and wine. An excessive level of IBMP concentration (≥15 ng/L) in wine has a negative effect as an herbaceous off-flavor (Roujou de Boubée *et al.*, 2000), whereas a concentration near its detection threshold can be considered pleasant as a varietal aroma in Sauvignon blanc wines (Allen *et al.*, 1991). IBMP concentration in wine is highly correlated with its concentration in berries at harvest (Darriet *et al.*, 2012). MPs were shown to accumulate in grape berries before veraison (Darriet *et al.*, 2012) and high temperature was suspected to reduce IBMP level (Lacey *et al.*, 1991; Marais *et al.*, 1999; Falcão *et al.*, 2007). However, these results were often obtained from large-scale comparisons where the temperature effect was blurred by other environmental factors.

In summary, most of the knowledge about the consequences of a temperature increase on grape berry secondary metabolites was established from large-scale observations or from experimental studies in artificial conditions, often after a rather abrupt step change of temperature associated with heat-shock stress. Hence, these results could be questionable regarding the effects of long-term and progressive increase of temperature in the context of climate change and plant secondary metabolism acclimation. Whether a modest but long-term temperature elevation in actual vineyard conditions influences secondary metabolites remains unclear.

Moreover, an adverse secondary metabolism response to higher temperature is expected in grape berries, with concerns about the impact on phenolics, aromas and aroma precursors. The secondary metabolism control by higher temperatures has been partly characterized for phenolics, whereas it is still mostly unknown with respect to aromas.

To overcome the previously described limitations, a few in-field experimental studies were carried out by applying an artificial local greenhouse effect to the bottom of plants (Sadras and Soar, 2009; Sadras and Moran, 2012). This passive open-top heating system increased bunch temperature by about 1-3°C, which is consistent with the projected warming, with little bias on solar radiation interception, temperature diurnal cycle or other microclimate factors.

The aim of this study was to investigate the response of some grape berry secondary metabolites to higher temperature in vineyard conditions, with a moderate temperature increase throughout the growing - ripening period. A passive open-top heating system was established in-field for two representative red and white varieties of the Bordeaux wine region, Cabernet-Sauvignon and Sauvignon blanc. Berry primary and secondary metabolites were analyzed for their responses to temperature increase.

MATERIALS AND METHODS

1. Location, vine material and experimental set-up

The experimental study was conducted in 2015 and 2016 in an experimental vineyard, "VitAdapt", in Bordeaux, France (44° 46' 46'' N, 00° 34' 01'' W) with two *Vitis vinifera* varieties: cv. Sauvignon blanc (SB) and Cabernet-Sauvignon (CS). The grapevines were 8 years old, spur pruned, with distances of 1.6 m between rows and 1 m between plants. Vineyard management was the same for both varieties and followed the local standards of a traditional Bordeaux vertically trellised training system. The vineyard is planted on flat land with no significant soil variations. Experiments were conducted on two blocks of about 30 vines (5 plants × 6 rows) for each variety; one block was control (C); the bunch zones of the second block were moderately heated (H) from fruit-set to harvest. Each block was separated from the other by at least one buffer row.

A passive open-top heating system was used to increase temperature continuously from fruit-set to maturity. This system followed the main principles of Sadras and Soar (2009) with a few minor modifications in order to adapt to the denser training system and less dense canopy used in Bordeaux. This system was made of modular rectangular polycarbonate panels (100 cm high × 300 cm width); two panels were installed obliquely (at about 45°) on each of the two sides of the row for each series of 5 adjacent vines; the top and two sides were kept open. The panels enclosed a volume bounded by the soil surface and covered most of the bunches since bunches were located at the bottom of the canopy. However, most of the leaves were not contained within this heating system, therefore

global plant function and source-sink ratios were assumed to remain unchanged. Moreover, the panels were transparent enough (>90% transmission ratio for visible light) and thus solar radiation load on bunches was not significantly reduced.

Treatments were applied to five or six replicates (in order to avoid vines showing esca symptoms and get the same number of useful vines for sampling) of five vines each and the same vines were used in both years of the study. To avoid boundary effects, measurements and sample collections were only made on the central three vines within each treatment replicate. Soil-, bunch zone air- and berry- temperatures and bunch zone relative humidity were recorded continuously at 20 min intervals using temperature and humidity probes (Vaisala HMP155 air temperature and humidity probes, copper-constantan thermocouples for berry and soil temperature) and stored by a data logger (Campbell Sci. CR1000).

2. Metabolite quantification

One hundred fresh berries were randomly sampled from each replicate at four different berry developmental stages: bunch closure (BC), mid-veraison (MV), mid-ripening (MR) and ripening (R). All samples were ground into powder in liquid nitrogen using a ball grinder MM200 (Retsch, Haan, Germany), and stored at -80°C for later analysis. For anthocyanin and tannin quantifications in CS, some berries were peeled after freezing and seeds separated from flesh.

2.1 Sugars and organic acids

An aliquot of 500 mg of ground whole berry sample was extracted with ethanol (80% and 50%), dried in a Speed-Vac, and re-dissolved in 2.5 mL de-ionized water. Glucose and fructose contents were measured enzymatically with an automated micro-plate reader (Elx800UV, Biotek Instruments Inc., Winooski, VT, USA). Malic acid was determined with an automated colorimetric method using the TRAACS 800 autoanalyzer (Bran-Luebbe) by using an enzymatic kit (R-Biopharm, Darmstadt, Germany).

2.2 Free amino acids

Amino acids were extracted as for soluble sugars and organic acids, and were determined by using HPLC (Waters, Milford, MA, USA) after derivation with 6-aminoquinolyl-N-hydroxysuccinimidyl-carbamate (AccQ-Fluor Reagent Kit, Waters). All the amino acids were identified and quantified with external chemical standards purchased from Sigma (St Louis, MO, USA).

2.3 Anthocyanin quantification

An aliquot of 500 mg of ground berry skin sample was freeze-dried for 72 h and the dried powder (50 mg) was extracted in 1.0 mL methanol containing 0.1% HCl (v/v). Extracts were filtered through a 0.45-µm polypropylene syringe filter (Pall Gelman Corp., Ann Arbor, MI, USA) for HPLC analysis. Each individual anthocyanin was analyzed with HPLC as described in Soubeyrand *et al.* (2014). Quantification was carried out by peak area integration at 520 nm. The concentration of individual anthocyanins was calculated in milligrams per gram (mg/g) of dry skin weight (DW) using malvidin 3-O-glucoside (Extrasynthese, Genay, France) as an external standard.

2.4 Tannin quantification

An aliquot (1 g) of fine powder of skins or seeds was extracted using 40 mL methanol containing 0.1% HCl. This solution was stirred for 3 h at 20°C. The extract was filtered through 20-µm PTFE filters. Monomeric-dimeric tannins were analyzed with HPLC as described in Cholet *et al.* (2014). 600 µL of phenolic extract, desiccated under nitrogen flow, plus 200 µL of reagent (0.2 N methanol-HCl + ascorbic acid + phloroglucinol) were mixed and incubated at 50°C for 20 min. The reaction was stopped with 200 µL of sodium acetate. This extract was filtered and placed in a 2-mL HPLC sealed vial. 10 µL were injected into the HPLC for analysis [column, 250 × 4.6 µm, 5 µm, ODS (Beckman, Roissy Charles de Gaulle, France): precolumn, 10 × 4.6 mm, 5 µm, BDS C18 (Thermo Hypersil); flow rate, 1 mL/min; solvent A, water/acetic acid (19:1 v/v); solvent B, MeOH/acetic acid (19:1 v/v); gradient, 5% B from 0 to 30 min, 20% B from 30 to 55 min, 40% B from 55 to 60 min, 90% B from 60 to75 min, 5% B from 75 to 80 min; injection volume, 10 µL; detection wavelength, 280 nm].

2.5 Thiol precursors (Cys-3SH, Glut-3SH and Glut-3SH-Al)

An aliquot of 500 mg of ground whole berry sample was extracted in 1.5 mL methanol containing 0.1% HCl (v/v) and added 50 µL of 0.1 µg/L internal standard solution containing a deuterated form of the glutathionylated S-conjugate ((3-S-hexan-1-ol)-glutathione-d3). Extracts were centrifuged and subsequently evaporated using a RapidVap Vertex Dry Evaporator (Labconco, Kansas City, MO, USA). The residues were filtered through a 0.45-µm membrane before being analyzed by C18-RP-UHPLC-HRMS (Thermo Scientific, Illkirch, France).

The separation was performed on a Syncronis aQ column (100 × 2.1 mm i.d., 1.7 µm, Thermo Scientific, Bremen, Germany) with a flow rate of 300 µL/min of solvent A (0.1% aqueous formic acid) and solvent B (0.1% formic acid in acetonitrile). The gradient for solvent B was as follows: 0 min, 9%; 0.8 min, 9%; 5 min, 40%; 5.2 min, 90%. The column was equilibrated with 9% of solvent B for 1 min prior to an injection. The ion source was operated in the positive ion mode at 3.5 kV. The vaporizer temperature of the source was set at 300°C, the capillary temperature at 350°C, the nitrogen sheath gas at 80, and the auxiliary and sweep gas at 5 (arbitrary units). A mass range of 100-500 was acquired in full scan MS mode. The resolution setting was 25000 (m/Δm, fwhm at m/z 400). To quantify the metabolites in samples, standards were prepared at the same time as the berry samples, by adding 50 µg/L of the internal standard to solutions of the synthetized metabolites (mix of 1 mL water and 1 mL grape juice).

2.6 IBMP

IBMP was quantified in whole grape berry samples by a stable isotope dilution assay using headspace solid phase micro-extraction coupled to a gas chromatograph and a mass spectrometer (SIDA-SPME-GC-MS) adapted from Guillaumie *et al.* (2013). Sample preparation involved weighing 1 g of ground frozen berries dissolved in 6 mL of de-ionized water into 20-mL SPME vials along with 4 g of sodium chloride (NaCl). An internal standard, [2H3]-IBMP, was also added to yield a final concentration of 100 ng/L. Samples were submitted to agitation (500 rpm for 5 s, stop for 2 s) for 5 min at 50 °C and then extraction with the SPME fiber for 40 min at 50°C. A three-phase divinylbenzene/carboxen/polydimethylsiloxane fiber (DVB/CAR/PDMS 50/30 µm thickness, 24 gauge, Supelco Bellefonte, PA, USA) was inserted, and the vial was agitated at 500 rpm for 40 min at 50°C. SPME injection was then implemented in splitless mode for 10 min with a desorption temperature of 240°C. Automated GC-MS analysis was carried out on a 6890 N gas chromatograph (Agilent Technologies) equipped with a Combi PAL autosampler (CTC Analytics). The GC was coupled to an HP 5973N mass selective detector (Agilent Technologies) functioning in electron impact mode at 70 eV. The analyses were performed on a Carbowax 20 M capillary column (BP20, 50 m, 0.25 mm internal diameter, 0.2-µm film thickness, Scientific Glass Engineering). Helium N60 (Air Liquide) was used as a carrier gas at a flow rate of 0.9 mL/min. The temperature program was as follows: initial hold for 5 min at 45°C, followed by a 3°C/min ramp to 140°C and then a ramp at 30°C/min to 240°C, and a 10 min hold. The injector port was at 240°C. During the elution of the methoxypyrazine, the GC-MS was switched to single-ion monitoring mode and tuned to measure m/z values of 127, 94, and 154 for 2H3-IBMP and 124, 94, and 151 for IBMP. Data processing was carried out by MSD Chemstation software (5973n Data Analysis, Agilent Technologies). Results were reported on a per weight basis of ng per kg of fresh weight of berries.

RESULTS AND DISCUSSION

1. Temperature increase

A mean increase of bunch zone air temperature of about +1.5°C (H vs. C) was recorded over the whole experiment duration from fruit-set to harvest time (Figure 1). However temperature elevation was higher during the daytime, and especially during clear-sky days. Berry temperature rise varied in the same range and similarly could reach +5°C at some times during clear-sky days. The open-top system also maintained daily temperature dynamics, slightly increased air relative humidity and increased vapor pressure deficit inside the enclosure (data not shown). Soil temperature was not noticeably affected; neither were soil water content and plant water status, as measured at MV, MR and R by predawn and midday water potential (data not shown). Neither berry weight nor sugar content were influenced by the heating treatment for the two seasons and the two varieties assessed (Table 1), which is a strong indication

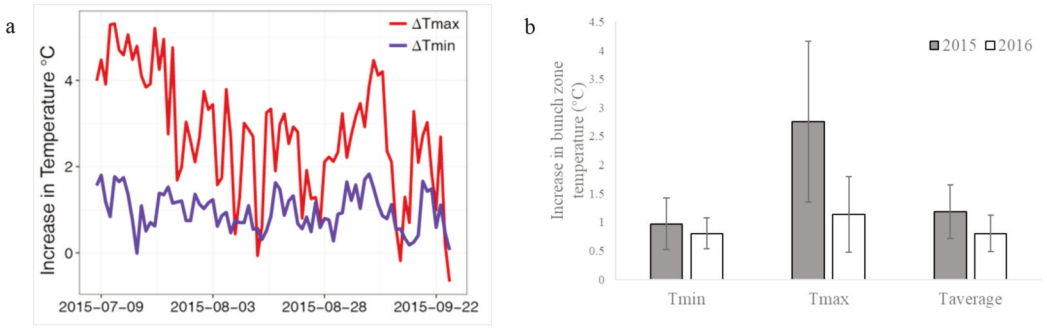

FIGURE 1. Time course of minimum and maximum air temperature increase in 2015 (a) and measured increase of minimum, maximum and average air temperatures in bunch zone of heated treatment (H) vs. control (C) during berry development in 2015 and 2016 (b).
Error bars indicate standard deviation.

TABLE 1 . Effect of elevated temperature on berry weight, sugars and malic acid content measured at bunch closure (BC) and mid-ripening (MR) stages in 2016.

	Bunch closure				Mid-ripening			
	Berry weight g	Glucose	Fructose mg/g (FW)	Malic acid	Berry weight g	Gluctose	Fructose mg/g (FW)	Malic acid
Cabemet-Sauvignon								
Control	0.6 [a]	3.17[a]	0.7 [a]	17.12 [a]	1.17 [a]	71.16 [a]	67.19 [a]	4.28 [a]
Heated	0.52 [a]	2.63 [a]	0.78 [a]	15.89 [a]	1.06 [a]	74.39 [a]	70.7 [b]	3.42 [b]
Sauvignon blanc								
Control	0.66 [a]	4.83 [a]	0.51 [a]	16.99 [a]	1.28 [a]	79.89 [a]	79.11 [a]	3.52 [a]
Heated	0.71 [a]	5.22 [a]	0.49 [a]	17.57 [a]	1.29 [a]	77.51 [a]	77.29 [a]	3.02 [a]

Values are the mean of five replicates. Different letters for the same parameter indicate statistically significant differences as determined by Student's t test (p value < 0.05). FW, fresh weight.

the whole plant behavior and berry growth were not disturbed.

2. Metabolism

2.1 Sugars and organic acids

Very few differences were obtained for primary metabolites (Table 1). A significantly lower concentration of malic acid and a higher concentration of fructose in CS berries were found in 2016 at MR stage only. Again, this result suggests that the heating treatment did not disturb the whole plant function and the main fluxes of sugars towards the berries were preserved. However, the heating treatment might have slightly influenced metabolism of sugars and acids in the berries, a very likely effect of higher temperature; this effect was considered small enough and with no direct consequence for secondary metabolism.

2.2 Amino acids

Few significant differences were obtained for measurements of amino acid content in the berries (Figure 2). The time-course pattern of total amino acid concentration was similar in all cases, with a strong increase from BC to R (Figure 2). The total amino acids only showed a significantly lower concentration in H berries at R stage with SB in 2015. Comparing the two vintages, very few differences were observed in 2016 whereas a clear trend of lower amino acid concentration in H berries was observed consistently at nearly all stages and for both varieties in 2015. This result could be linked to a slightly higher temperature increase along 2015 season (Figure 1).

The ratio of each amino acid family was fairly conserved during berry development and the glutamate family was consistently predominant, along with proline; no response of proline to heat

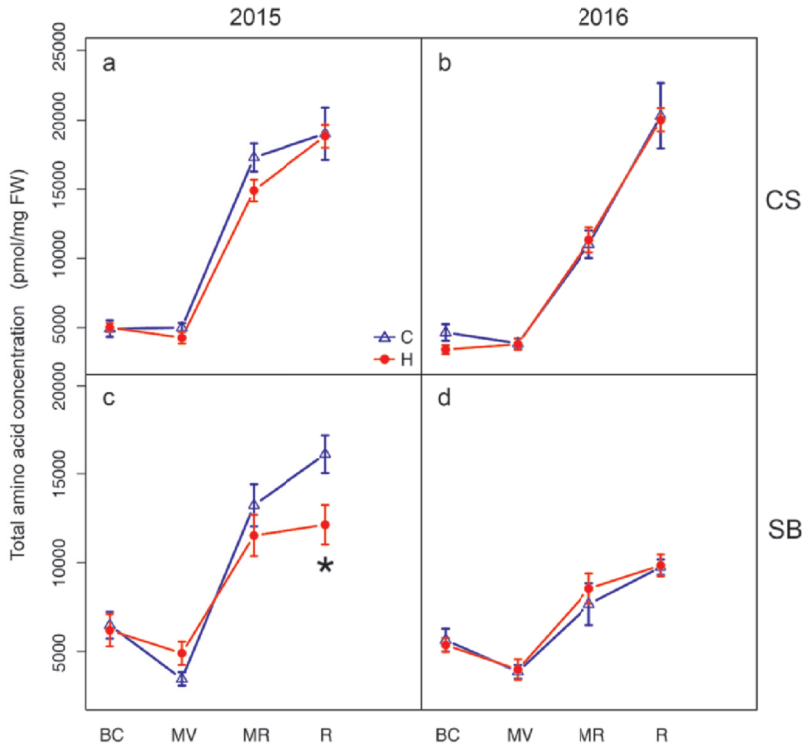

FIGURE 2. Measured total amino acid concentrations in whole berries of Cabernet-Sauvignon (CS) (top) and Sauvignon blanc (SB) (bottom) in 2015 (left) and 2016 (right). Heated treatment (H) vs. control (C) values. Bunch closure (BC), mid-veraison (MV), mid-ripening (MR) and ripe (R) stages.
(Each point is the mean of five replicates. Error bars indicate standard error. * indicates significant difference between treatments (independent t-test, $p<0.05$).

treatment was observed here whereas the abundance of amino acids from the glutamate family was reduced with H in CS and no clear trend in SB. This result contradicts previous studies where increases of glutamate family amino acids were observed in response to heating in Shiraz berries (Sweetman *et al.*, 2014), in Arabidopsis (Kaplan *et al.*, 2004) and in rice (Yamakawa and Hakata, 2010). However, the present study was conducted in-field and the intensity and duration of warming was very different. H samples also exhibited lower concentrations of some amino acids, especially alanine, serine and phenylalanine. The latter is a precursor for phenylpropanoid biosynthesis, hence a lower concentration under elevated temperature may be explained by consumption and anthocyanin loss under higher temperature (Mori *et al.*, 2007; Pieri *et al.*, 2016). Finally, methionine and cysteine, precursors of sulfur volatile compounds in wine (Moreira *et al.*, 2002), showed different responses to temperature; higher methionine and lower cysteine concentrations were obtained in heated berries.

2.3 Anthocyanin and tannin content in CS berries

Anthocyanins are the major pigment compounds in grapes of most red cultivars. A total of 13 anthocyanins were identified in CS berry skin at harvest. All anthocyanins identified were monoglucosides, including malvidin, peonidin, petunidin, delphinidin and cyanidin derivatives, with -3-O-glucoside as the main derivatives and 3-acylated derivatives. Malvidin, peonidin and petunidin were the only detected coumaroyl derivatives (data not shown).

Total anthocyanin content was clearly reduced in H berry skins (Table 2), confirming previous studies which demonstrated a negative effect of higher temperature on anthocyanin concentration (Mori *et al.*, 2007; Lecourieux *et al.*, 2017). According to the number of substituents on the B-ring of the anthocyanin, cyanidin and peonidin and their derivatives have two hydroxylated

TABLE 2. Measured effects of elevated temperature on total anthocyanins and ratio of 3'-substituted anthocyanins to 3',5'-substituted anthocyanins in Cabernet-Sauvignon berry skin at harvest.

	Total anthocyanins	3'-substituted anthocyanins	3',5'-substituted anthocyanins	F3'H/F3',5'H
		mg/g DW		
Control	23.46±3.62[a]	6.25±1.15[a]	17.21±3.06[a]	0.36±0.03[a]
Heated	16.91±1.77[b]	5.20±0.26[a]	11.71±1.61[b]	0.45±0.04[b]

Different letters for the same parameter indicate statistically significant differences between treatment and control as determined by Student's t test (p value < 0.05). DW, dry weight.

TABLE 3. Measured effects of elevated temperature on tannin monomers and oligomers in Cabernet-Sauvignon berry seeds and skin at harvest.

	B1	B3	C	B2	B4	EC
CS seeds			mg/g FW			
Control	0.14±0.04[a]	0.56±0.11[a]	2.10±0.40[a]	0.28±0.02[a]	0.14±0.05[a]	1.43±0.14[a]
Heated	0.11±0.01[a]	0.56±0.03[a]	2.00±0.26[a]	0.28±0.03[a]	0.13±0.02[a]	1.35±0.08[a]
CS skin						
Control	n.d.	0.03[a]	0.09±0.01[a]	0.02[a]	0.02[a]	0.04[a]
Heated	n.d.	0.03[a]	0.08±0.03[a]	0.02[a]	0.02[a]	0.04[a]

Different letters for the same parameter indicate statistically significant differences between treatment and control as determined by Student's t test (p value < 0.05). FW, fresh weight; n.d., not detected; C: (+)-catechin; EC: (-)-epicatechin); B1: (-)-epicatechin-(4β-8)-(+)-catechin; B2: (-)-epicatechin-(4β-8)-(-)-epicatechin; B3: (+)-catechin-(4a-8)-(+)-catechin; and B4: (+)-catechin-(4a-8)-(-)-epicatechin.

groups and are called 3'-substituted anthocyanins. Delphinidin, petunidin and malvidin and their derivatives have three hydroxylated groups and are called 3',5'-substituted anthocyanins. With elevated temperature, the decrease of total anthocyanin levels was due to the decrease in 3',5'-substituted anthocyanins (Table 2).

In contrast to anthocyanins, tannin content was not influenced by the H treatment (Table 3). Tannins are a group of flavan-3-ols including monomers, oligomers and polymers, and are also called condensed tannins or proanthocyanidins. Due to their contribution to both astringency and bitterness properties in grapes and hence wine, they are important for quality. Here, two procyanidins [C: (+)-catechin and EC: (-)-epicatechin] and flavan-3-ol dimers [B1: (-)-epicatechin-(4β-8)-(+)-catechin; B2: (-)-epicatechin-(4β-8)-(-)-epicatechin; B3: (+)-catechin-(4a-8)-(+)-catechin; and B4: (+)-catechin-(4a-8)-(-)-epicatechin] were identified and quantified at harvest in both seeds and skins of CS berries.

Neither seed nor skin tannin contents were affected by the H treatment (Table 3). A similar result was found in Sangiovese and Muscat Hamburg berry skin as well as in Merlot seeds for quite similar treatments (Cohen *et al.*, 2008; Carbonell-Bejerano *et al.*, 2013; Pastore *et al.*, 2017). Seeds had a much higher tannin concentration than the skins (Table 3), which is in agreement with previous studies (Cohen *et al.*, 2008). Although the final tannin concentrations were not changed by the increased temperature, the concentrations at MV stage were significantly higher in H berries, which is consistent with an earlier study (Cohen *et al.*, 2008).

2.4 Thiol (3SH) precursors (Cys-3SH, Glut-3SH and Glut-3SH-Al)

Volatile thiols are a group of sulfur containing alcohols that induce a variety of aromas. Depending on their concentration and proportions, they contribute positively or negatively to wine flavor. Among them, 3-sulfanylhexan-1-ol (3SH) is a major aromatic compound found in CS and SB wine, which result in a smell of grapefruit or passion fruit (Darriet *et al.*, 2012). 3SH is not present itself in berries and musts, but its odorless precursor forms are (Darriet *et al.*, 2012). The precursors of 3SH in grape juice include S-3-(hexan-1-al)-glutathione (Glut-3SH-Al), S-3-(hexan-1-ol)-

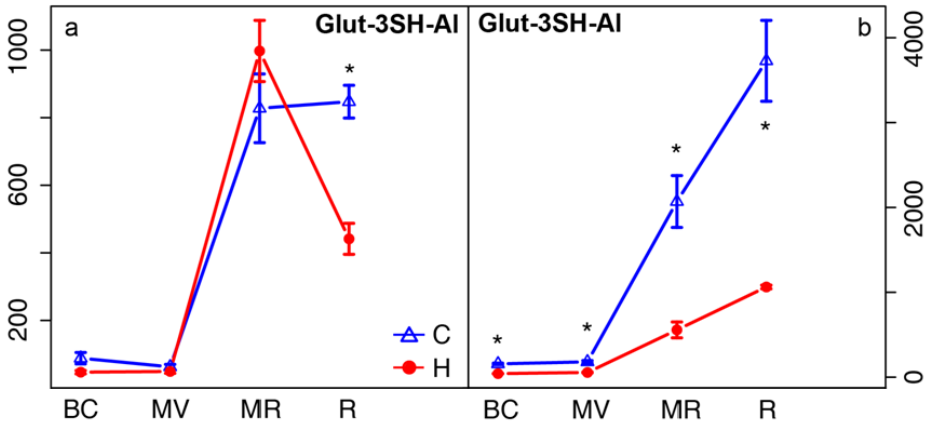

FIGURE 3. Measured concentrations of Glut-3SH-Al (µg/kg FW) in control (C, blue) and heated (H, red) whole berries of Cabernet-Sauvignon (CS) (a) and Sauvignon blanc (SB) (b) in 2015. Bunch closure (BC), mid-veraison (MV), mid-ripening (MR) and ripe (R) stages.
Each point is the mean of five replicates and error bars indicate standard error. * indicates significant difference between treatments (independent t-test, $p<0.05$).

glutathione (Glut-3SH) and S-3-(hexan-1-ol)-L-cysteine (Cys-3SH) (Tominaga *et al.*, 1998; Thibon *et al.*, 2016). The biosynthesis pathway of these precursors is not well understood. Here, the accumulation of glutathione and three 3SH precursors during ripening in CS and SB grape berries was investigated.

Glut-3SH and Cys-3SH concentrations were near limits of quantification, and Glut-3SH was not detected in CS berries. Glut-3SH-Al content was about 1000-fold higher than Cys-3SH in all samples. Both compounds showed a similar accumulation profile with a marked increase from MV to R, in contrast with Glut-3SH. Glut-3SH-Al content was consistently much lower in H berries in SB, but only at R stage in CS (Figure 3). The concentrations were reduced by about -70% in SB and -50% in CS at maturity. For SB, this strong response to moderate heating differs from what was observed after leaf removal (Sivilotti *et al.*, 2017). Leaf removal might have a more transient effect during daytime than the more continuous heating investigated here. In CS, 3SH precursors seemed to peak around mid-ripening (MR) stage, before stabilizing, or decreasing in hotter conditions. Overall, moderate but long-term heating associated with climate change could be harmful for thiol aromas of these varieties.

2.5 IBMP

For both varieties, seasons and treatments, IBMP concentrations were similar (Figure 4). As expected, maximum values of IBMP were observed at BC stage, and then decreased until harvest. No significant difference was observed between C and H berries at harvest in both varieties. Only CS berries exhibited statistically lower IBMP concentrations under H conditions at BC, with a reduction of nearly -20%. IBMP content in both varieties at bunch closure was slightly higher in 2016 than in 2015, which could be linked to relatively lower temperature elevation in 2016 (Figure 1). Here, SB appeared quite insensitive to the H treatment, whereas CS was sensitive only at the earlier developmental stage. Since IBMP is usually not desirable in CS wines, long-term heating associated with climate change could have limited or potentially positive impact for MP aromas of CS.

CONCLUSION

In contrast with previous studies of the effects of temperature on secondary metabolism in grape, this study focused on seasonal effects, from fruit-set to harvest, of a moderate temperature rise in vineyard conditions. Cabernet-Sauvignon and Sauvignon blanc grape bunches in a Bordeaux vineyard were continuously heated by about +1.5°C, a temperature rise consistent with the projected global warming.

In summary, the heat treatment (i) did not greatly affect primary metabolites, such as sugars and organic acids; (ii) reduced amino acids and anthocyanins; (iii) did not greatly affect tannins; (iv) reduced in some cases IBMP, which could have a positive impact; and (v) most noteworthy,

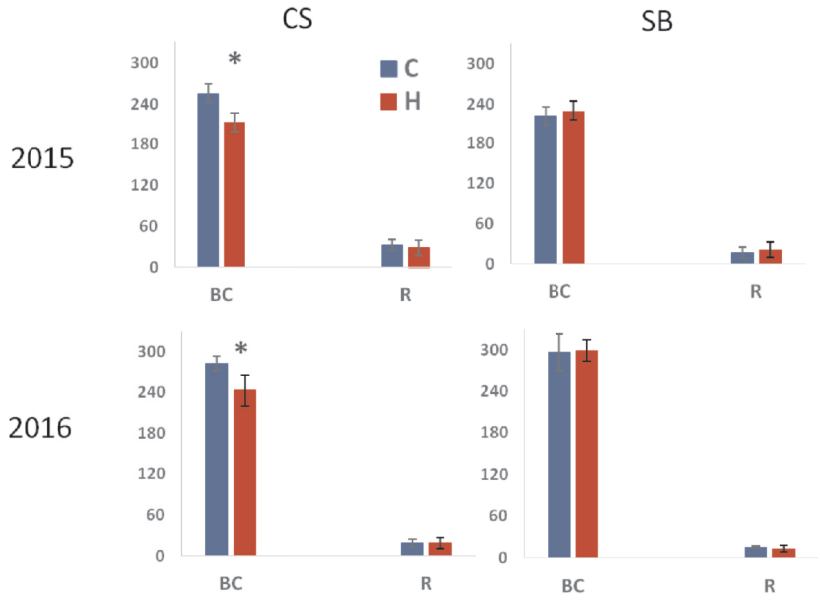

FIGURE 4. Measured IBMP concentrations (ng/g FW) in whole berries of Cabernet-Sauvignon (CS, left) and Sauvignon blanc (SB, right) berries in 2015 (top) and 2016 (bottom) at bunch closure (BC) and ripe (R) stages. Control (C) and heated treatment (H) values.
Each point is the mean of five replicates and error bars indicate standard error. * indicates significant difference between treatments (independent t-test, $p<0.05$).

significantly reduced some thiol-related aroma precursors.

These results therefore established and quantified the potentially negative impacts of climate change for polyphenols and aromas of grape and hence for wine quality. As a result, adaptation of viticulture to new conditions must take into account these foreseeable impacts of high temperature. Viticulture in temperate climate like the Bordeaux area must therefore adapt practices to avoid or mitigate higher temperature effects, although positive impacts could also appear in some cases. Potentially suitable practices include establishing new vineyards on north-exposed slopes (or higher in altitude if relevant) or all ways of favoring the shading of the bunches, either artificially or by the neighboring leaves; the latter could be achieved either by giving up leaf removal or by increasing vigor.

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