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ORIGINAL RESEARCH ARTICLE

Thiol precursors and amino acids profile of white interspecific hybrid *Vitis* varieties and impact of foliar urea and sulfur supplementation on the concentration of thiol precursors in *Vitis* sp. Vidal berries

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ABSTRACT

Thiols are a group of highly potent aroma compounds found in many *Vitis vinifera* wines. In berries, thiols occur as precursors formed from a thiol attached to an amino moiety (e.g., cysteine and glutathione). Foliar sulfur and urea supplementation has been found to increase thiol precursors in *V. vinifera* berries. However, such data are still scarce for interspecific hybrid *Vitis* varieties, despite a growing interest in these cultivars. In this study, eight thiol precursors, 21 amino acids and glutathione (GSH) of seven white hybrid *Vitis* varieties collected in Quebec, Canada, were quantified by liquid chromatography with tandem mass spectrometry. Then, the impact of foliar sulfur and urea supplementation on thiol precursors of fully mature berries was tested on the *Vitis* sp. Vidal, using a complete randomised block design. Among the analysed thiol precursors, only the precursors of 3-mercaptohexan-1-ol (3MH) were detected, with glutathione-3MH (20-711 µg/kg berry fresh weight) as the main precursor in most studied varieties. Vidal samples had the highest concentrations of glutathione-3MH (313 µg/kg berry fresh weight) and GSH (17 mg/L in juice), but a large variability was found among the biological replicates. The amino acids proline, arginine, glutamine and alanine were the main amino acids found in Vidal, Frontenac blanc, Seyval blanc and Saint-Pepin respectively. Correlation analysis showed no significant relationship between thiol precursor and glutathione/amino acid content in berries. Foliar urea and sulfur supplementation had no impact on thiol precursor concentration in Vidal. However, a trend in a higher content of thiol precursors in berries treated with urea and sulfur compared to the control was observed on one of the two experimental sites.

KEYWORDS: interspecific hybrid grape, cool climate, northern winemaking, thiol precursor, amino acid, glutathione, aroma, urea, sulfur, foliage spraying

INTRODUCTION

Wine varietal aroma relates to a large range of compounds found in grape berries as precursors that are released during winemaking. Among those, glycosylated aroma precursors are possibly the most extensively studied group in *Vitis vinifera* and other *Vitis* sp. (González-Barreiro *et al.*, 2015; Lee *et al.*, 2016; Ghaste *et al.*, 2015). Meanwhile, despite their significance in terms of wine aroma, volatile sulfur compounds such as thiols and their precursors have received less attention. Varietal thiols, such as 3-mercaptohexan-1-ol (3MH) and 4-mercapto-4-methylpentan-2-one (4MMP), are known for their contribution to the characteristic bouquet of Sauvignon blanc wines and other *V. vinifera* varieties (Rauhut and Kiene, 2019; Capone *et al.*, 2015; Piano *et al.*, 2015). Their presence has also been reported in wines from other *Vitis* varieties, including the interspecific hybrid Cayuga grown mainly in the State of New York for wine production (Musumeci *et al.*, 2015).

Thiol precursors are present as non-volatile and odourless cysteinylated, dipeptide and glutathionylated precursors in berries (e.g., cysteine-3MH, cysteine-4MMP, glutathione-3MH, glutathione-3MH-aldehyde, glutathione-4MMP, cysteinyl-glycine-3MH and γ-glutamyl-cysteine-3MH) and are further released into volatile and odorous compounds by certain yeast strains (*Saccharomyces* sp.) during alcoholic fermentation (Bonnaffoux *et al.*, 2017; Santiago and Gardner, 2015). In addition, C_6-compounds, such as hexenal and hexenol, can also contribute to the formation of 3MH during alcoholic fermentation (Harsch *et al*., 2013, Araujo *et al*., 2017; Thibon *et al*., 2016). Thibon *et al.* (2016) identified S-3-(hexanal)-glutathione as an additional precursor of 3MH.

The concentration of thiol precursors has been investigated in red and white *Vitis vinifera* varieties, such as Cabernet-Sauvignon, Sauvignon blanc, petit Manseng, Riesling and others (Roland *et al*., 2011; Fracassetti *et al*., 2018; Roman *et al*., 2017), as well as in red and white fungus-resistant interspecific *Vitis* varieties, such as Leon Millot (red), Baco noir (red), Villard blanc (white), Villaris (white) and Floreal (white), among others (Dournes *et al*., 2020; Nicolini *et al*., 2020). Glutathione-3MH is the main thiol precursor reported in interspecific hybrid grapes thus far, with levels ranging from 4.4 to 1141 µg/kg berry fresh weight (FW), whereas cysteine-3MH is second, with concentrations ranging from 0.3 to 136 µg/kg berry FW (Nicolini *et al*., 2020). The concentration of thiol precursors increases in berry with maturation, but the final concentration is influenced by grapevine variety, soil composition, climate and cultural practices (Capone *et al*., 2011; Dufourcq, 2018; Roland *et al*., 2010b).

In grapevine, thiol precursor biosynthesis occurs in berries and has been shown to be closely associated with the biosynthesis of amino acids and glutathione (Chen *et al.*, 2019), which is further related to the nitrogen status of grapevine (Choné *et al.*, 2006; Helwi *et al.*, 2016). In the vineyard, nitrogen and sulfur supplementation may enhance thiol precursors in berries. For instance, soil nitrogen supply has been shown to enhance the synthesis of cysteinylated precursors and glutathione in grapes; in some cases, it also promoted vegetative growth and/or plant susceptibility to pathogens (Choné *et al.*, 2006; Helwi *et al.*, 2016). In trials on white *Vitis vinifera* varieties, foliar nitrogen supplementation (in the form of urea, 10 to 20 kg/Ha) along with sulfur (5 to 10 kg/ha) during veraison increased thiol concentration in wines by up to five times, depending on the variety and growing conditions, with limited impact on grapevine vigour (Buica *et al*., 2016; Dufourcq *et al*., 2010; Dufourcq *et al*., 2009). Foliar supply has several benefits compared to soil fertilisation: (i) a faster assimilation of the urea is achieved through the leaf cuticle due to its small molecular weight, non-ionic nature and high solubility, (ii) a smaller amount of nitrogen is required, because losses are limited, and (iii) it has a lower impact on vine vigour (Witte *et al*., 2002). The impact of nitrogen and sulfur on the concentration of thiol precursors of interspecific hybrid varieties has been little investigated thus far. Many interspecific hybrids, especially those adapted to a northern climate, show a higher vigour and noticeable biochemical differences with *V. vinifera* varieties, including, among others, a tendency to accumulate higher levels of nitrogen in berries (Pedneault and Provost, 2016).

The objective of the current study was to screen the thiol precursor content of seven white hybrid *Vitis* varieties grown in Eastern Canada for wine production, and evaluate the impact of foliar urea and sulfur sprays starting at mid-veraison on grapevine physiology and thiol precursor in fully mature berries. In addition, amino acids and glutathione were analysed, and their relationship with berry thiol precursor concentration was studied.

MATERIALS AND METHODS

1. Screening of thiol precursors in interspecific hybrid *Vitis* sp. varieties

Samples (3 kg per sample) from seven white hybrid varieties (Adalmiina, Frontenac blanc, L'Acadie blanc, Louise Swenson, Saint-Pepin, Seyval blanc and Vidal; 1 to 6 biological replicates per variety for a total of 23 samples) were collected during the 2016 season from ten vineyards located in the province of Quebec, Canada. Grape clusters were hand-picked randomly at commercial harvest, based on the total soluble solid content usually targeted at maturity for each variety (Dubé and Turcotte, 2014), and then immediately frozen at -30 °C until analysis.

2. Chemical analyses

2.1. Sugar analysis

Glucose and fructose from grape juice were quantified as described by Nicolle *et al*. (2021). Briefly, the grapes were thawed overnight at 4 °C and then manually destemmed. Next, the grape juice was manually extracted using food-grade polyethylene bags and centrifuged at 10 000 rpm at 4 °C for 15 min, diluted 10-fold and then filtered through 0.45 µm PTFE syringe filters (25 mm diam., Silicycle, Quebec, Canada) prior to HPLC analysis. The separation was achieved on a HPLC system (Waters, Millipore Corp., Milford, MA, USA) equipped with a refractive index detector (Hitachi model L-7490, Foster City, CA, USA), using a Waters Sugar Pack-I column (6.5 mm x 300 mm) from Waters (Millipore Corp., Milford, MA, USA).

2.2. Organic acid analysis

The concentrations of tartaric acid and malic acid from the grape juice were quantified as described by Serre *et al.* (2016). Briefly, the grapes were thawed overnight at 4 °C and then manually destemmed. Next, the grape juice was manually extracted using food-grade polyethylene bags, centrifuged at 10 000 rpm at 4 °C for 15 min and then filtered through 0.45 µm PTFE syringe filters (25 mm diam., Silicycle, Quebec, Canada). The acids were then extracted by solid phase extraction (SPE) using C18 cartridges (non-end-capped 6 mL, 500 mg, Silicycle, Québec City, QC, Canada) before being separated on a HPLC Agilent Technologies 1100 (Santa Clara, CA, USA) equipped with a UV-DAD detector, using a Synergi 4 µm Hydro-RP column (4.6 mm x 250 mm) from Phenomenex (Torrance, CA, USA).

2.3. Amino acid and glutathione analysis

Amino acids and glutathione (L-alanine, Ala; L-arginine, Arg; L-asparagine, Asn; L- aspartic acid, Asp; L-cysteine, Cys; γ-aminobutyric acid, GABA; L-glutamic acid, Gln; L-glutamine, Glu; L-glycine, Gly; glutathione, GSH; L-histidine, His; L-isoleucine, Ile; L-leucine, Leu; L-lysine, Lys; L-methionine, Met; L-phenylalanine, Phe; L-proline, Pro; L-serine, Ser; L-threonine, Thr; L-tryptophan, Trp; L-tyrosine, Tyr; L-valine, Val) from grape juice were analysed on an UPLC equipped with a triple quadrupole mass spectrometer (Thermo TSQ Quantiva, Thermo Scientific, Waltham, MA, USA). Grape juice was obtained from frozen berries as described by Roland *et al.* (2010a). The chromatographic separation was performed using an ACQUITY UPLC BEH Amide Column (2.1 mm × 100 mm, 1.7 µm particle size, Waters, Milford, MA, USA). Further technical aspects of the method are proprietary and were not divulged by the external laboratory (Nyseos, Montpellier, France). Analyses were performed in triplicates.

2.4. Thiol precursor analysis

Thiol precursors (cysteine-3MH, Cys-3MH; cysteine-4MMP, Cys-4MMP; glutathione-3MH, G-3MH; glutathione-4MMP, G-4MMP; cysteinyl-glycine-3MH, CysGly-3MH; cysteinyl-glycine-4MMP, CysGly-4MMP; γ-glutamyl-cysteine-3MH, γ-GluCys-3MH; γ-glutamyl-cysteine-4MMP, γ-GluCys-4MMP) from grape juice were quantified by Stable Isotope Dilution Assay (SIDA), using the method described by Bonnaffoux *et al.* (2017), on an UPLC chromatography system equipped with a triple quadrupole mass spectrometer (Thermo TSQ Quantiva, Thermo Scientific, Waltham, MA, USA) and fitted with a Hypersil gold AQ (1.9 µm, 100 mm × 2.1 mm; Thermo Scientific, Waltham, MA, USA). Grape juice was obtained from frozen berries as described by Roland *et al.* (2010a). Further technical aspects of the method are proprietary and were not divulged by the external laboratory (Nyseos, Montpellier, France). Analyses were performed in triplicates.

3. Foliar urea and sulfur supplementation and thiol accumulation in Vidal berries

3.1. Experimental design

The experiment was conducted during the 2017 season in two commercial vineyards located in Saint-Rémi and Dunham (QC, Canada; 45° 16′ 0″ N, 73° 37′ 0″ W and 45° 08′ 00″ N 72° 48′ 00″ W respectively). The Saint-Rémi site comprises a moderately drained calcareous sandy loam and is located at an elevation of about 40 m. The Dunham site comprises moderately drained sandy loam and is located at an elevation of 130 m. Both sites were planted at a density of 5 400 plants/ha (0.60 m x 3.0 m), which is a typical density for Vidal production in Quebec.

On each site, a factorial experimental design comprising three different amounts of added urea (0, 10, and 20 kg/ha per application), two different amounts of added sulfur (0 and 5 kg/ha per application) and three replications (blocks) for a total of 18 experimental units was implemented. The control was not treated with any urea or sulfur. The levels of urea and sulfur supplementation were determined based on the work of Dufourqc *et al*. (2009). Each experimental unit contained 10 plants. The blocks were established as follows: three guard rows at the end of each block, one guard row between each block, two guard plants between each experimental unit and five guard plants at the end of each experimental unit. Urea and sulfur treatments were applied as foliar spray twice: the first treatment was applied at mid-veraison (on 29 and 30 August 2017 on the Saint-Rémi and Dunham sites respectively) and the second treatment 11 days later (9 and 10 September 2017 on the Saint-Rémi and Dunham sites respectively). One kg per sample was harvested at commercial maturity (October 17 and 18, 2017, on Dunham and Saint-Rémi sites respectively). A hundred berries per sample were immediately processed for physicochemical analyses, while the remaining berries were immediately frozen at -30 °C until analysis.

3.2. Physiological parameters

The number of clusters per plant, the yield per plant and the height and width of the canopy (foliage) were measured on three plants per experimental unit. The average cluster weight, the exposed leaf area (SECV) and the ratio of exposed leaf area to fruit yield (SECV/PR) were calculated based on these measurements. Berry weight was estimated from 100 berries randomly selected from 15 representative clusters.

3.3. Physicochemical analysis of berries

The juice was manually extracted from 100 randomly selected fresh berries. Total soluble solids (TSS, °Brix), pH, and titratable acidity (g/L tartaric acid eq.) were measured as described by Amerine and Ough (1974). Thiol precursors were analysed from frozen berries, as described in Section 2.4.

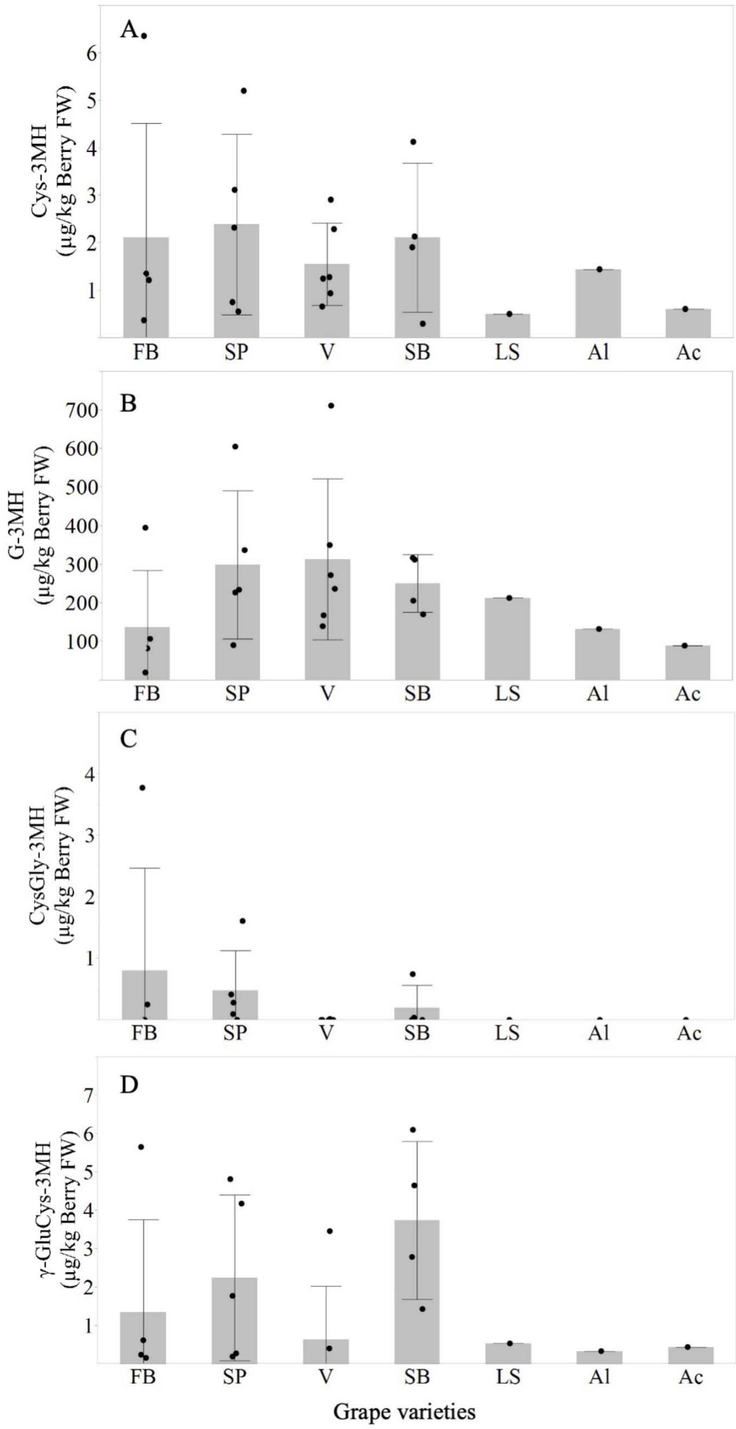

FIGURE 1. Concentration (µg/kg berry FW) of cysteine-3MH (A, Cys-3MH), glutathione-3MH (B, G-3MH), cysteinyl-glycine-3MH (C, CysGly-3MH) and γ-glutamyl-cysteine-3MH (D, γ -GluCys-3MH) in the hybrid *Vitis* varieties Frontenac blanc (FB; n = 5), Saint-Pepin (SP; n = 5), Vidal (V; n = 6), Seyval blanc (SB; n = 4), Louise Swenson (LS; n = 1), Adalmiina (Al; n = 1) and L'Acadie blanc (Ac; n = 1) harvested in the province of Quebec, Canada, during the 2016 season. The bar represents the mean concentration and the error bar the standard deviation.

4. Statistical analyses

Analysis of variance (One-way ANOVA) of the varieties screening results was carried out using the JPM software (version 11.0.0; SAS Institute Inc., Cary, NC, USA). The Standard Least Squares personality within the Fit Model platform was used, and pairwise comparisons of least squares means using Tukey HSD (α = 0.05) were performed to find significant differences between varieties. Principal component analysis (PCA) was performed with the JMP software to explore the relationship between the different variables. Varieties with only one sample were excluded from these analyses.

Analyses of variance (ANOVA) of results from the urea and sulfur supplementation experiment were conducted with the JMP software. The main effect of factors was determined, as well as their interactions. Squares personality within the Fit Model platform was used, and pairwise comparisons of least squares means using Student's t tests or Tukey HSD ($\alpha = 0.05$) were performed to find any significant differences between varieties or treatments.

RESULTS AND DISCUSSION

1. Thiol precursors in the berries

Seven white interspecific hybrid varieties grown in the cold climate of Quebec (Canada) were analysed for the first objective of this study. The varieties studied included selections from different breeders (e.g., French hybrids, such as Seyval blanc and Vidal and American hybrids, such as Saint-Pepin and Frontenac blanc) that have different genetic backgrounds and are locally known to produce wines with very different aroma profiles.

Total soluble solids, pH, monosaccharide and organic acid concentrations of the juices from the sampled varieties are shown in the Supplementary Material (Table S1). No significant differences were found between the varieties for those parameters, except for Frontenac blanc, which showed a higher citric and malic acid content than the other varieties. Frontenac blanc is known to be a highly acidic variety compared to other white hybrids grown in Eastern Canada (Slegers *et al*., 2017).

A total of eight thiol precursors were investigated in the sampled varieties. Among those, only the four precursors of 3MH (Cys-3MH, G-3MH, CysGly-3MH and γ-GluCys-3MH) were detected in all varieties and, for a given precursor, no significant differences were found from one variety to another (Figure 1; means and standard deviations are available in Table S2 of Supplementary Material). The absence of 4MMP precursors from all samples is likely attributable to the varieties' genotypes. Indeed, 4MMP precursors have been found in only two out of 38 recently analysed hybrid varieties (Nicolini *et al*., 2020), suggesting that the genes responsible for the biosynthesis of these compounds have a lower occurrence in hybrid *Vitis* varieties than in *Vitis vinifera*.

The concentrations of the 3MH precursors showed large variations in the biological replicates of Frontenac blanc, Vidal, Seyval blanc and Saint-Pepin. Such results were expected due to the completely randomised plan selected for the sampling in order to carry out a broad survey of 3MH precursors in hybrid varieties. However, the high variability also suggests that the accumulation of these compounds is likely highly affected by bioclimatic conditions, cultural practices and/or berry ripeness; for instance, the concentration of G-3MH, which was the main thiol precursor found in the studied varieties, varied from 21 to 395 µg/kg berry FW in Frontenac blanc, from 91 to 605 µg/kg berry FW in Saint-Pepin and from 141 to 711 µg/kg berry FW in Vidal (Figure 1B). The other 3MH precursors (Cys-3MH, CysGly-3MH and γ-GluCys-3MH) were found in quantities equal to or lower than 7 µg/kg berry FW and, except for CysGly-3MH, were detected in all varieties. However, additional analyses would be necessary to validate the presence/absence or the variability of 3MH precursors concentration in L'Acadie blanc, Aldamiina and Louise Swenson berries, as only one replicate could be analysed for those varieties (Figure 1A, 1C, and 1D).

Vidal and Saint-Pepin showed the highest concentration of G-3MH, with 50 % of the replicates showing a concentration higher than or close to the overall average value of 240 µg/kg berry FW (Figure 1B). During winemaking, the conversion rate of 3MH precursors to 3MH volatile thiol is very low and ranges from 0.1 to 12 % (Robinson *et al.*, 2014). This process is affected by oenological conditions, such as must turbidity, temperature and yeast strain used for winemaking. However, it ultimately depends on yeast assimilable nitrogen (Subileau *et al.*, 2008), with ammonium ions and amino acids as the main sources in grape must. Since the olfactory perception threshold of 3MH is 60 ng/L in wine, the concentration of G-3MH found in the studied varieties could significantly impact the resulting wine aroma. Furthermore, in *Vitis vinifera* varieties, freezing whole grape berries has been shown to increase the concentration of G-3MH in berries (Chen *et al*., 2019; Capone *et al*., 2011). Such a phenomenon has not yet been demonstrated in interspecific hybrids; therefore, an analysis of thiol precursors in fresh berries would be necessary to fully account for the thiol potential of these varieties in table wine production. However, most of the studied varieties, especially Vidal, are used for ice wine production in Quebec. Our results suggest that thiols contribute to the aroma of these speciality wines, and that their concentration can increase given the selection of proper yeast strain and fermentation conditions (Santiago and Gardner, 2015).

2. Amino acids in the berries

Thiol precursors contain an amino acid or a peptide moiety. Despite not being fully understood, the accumulation of thiol precursors has been suggested to relate to amino acid and glutathione biosynthesis in berries (Chen *et al*., 2019). Glutathione (L-γ-glutamyl-L-cysteinyl-glycine and GSH) is a tripeptide that results from the condensation of glutamic acid, cysteine and glycine: Glu and Cys are first condensed to form γ-glutamylcysteine through the action of γ-glutamylcysteine synthetase, and then Gly is added via GSH synthetase activity to form GSH (Kritzinger *et al*., 2012). In berries, GSH may combine with hexenal via the activity of GSH S-transferases (VvGST3 and VvGST4), resulting in the production of the thiol precursor G-3MH (Kobayashi *et al*., 2010). Other thiol precursors (CysGly-3MH and Cys-3MH) derive from G-3MH through the subsequent action of γ-glutamyl transferase (CysGly-3MH), and carboxypeptidase (Cys-3MH) (Dubourdieu and Tominaga, 2009).

In the present study, 21 amino acids and the GSH content of seven hybrid varieties were quantified and their potential relationship with 3MH precursors was evaluated. The total amino acid contents (including GSH) of the varieties analysed were similar (Table 1), but large ranges were observed for biological replicates; for instance, the total amino acid + GSH content of Frontenac blanc ranged from 196 to 2167 mg/kg berry FW. Such variation is likely attributable to the different climatic conditions, soil composition (e.g., organic matter content and pH) and/or vine management practices (including fertilisation) that occurred in the commercial vineyards where the berries were sampled (Ortega-Heras *et al*., 2014). However, large variations in nitrogen compound accumulation (e.g., primary amino nitrogen and yeast assimilable nitrogen) are frequent in cold-hardy and disease-resistant grape varieties, such as interspecific hybrids (Pedneault and Provost, 2016).

Arg, Ala, Gln and Pro were the main amino acids found in all analysed varieties, accounting for 61 (Louise Swenson) to 82 % (Adalmiina) of the analysed amino acids (Table 1). Thirteen of the 22 amino acids showed significantly different concentrations from one variety to another, with Frontenac blanc generally showing the highest concentration for most amino acids. Arg was the main amino acid in L'Acadie blanc (42 %), Frontenac blanc (33 %) and Louise Swenson (26 %),

TABLE 1. Amino acid and glutathione content (mg/kg berry; fresh weight) in berry juices from seven white interspecific hybrid grape varieties (Frontenac blanc, *n = 5*; Saint-Pepin, *n = 5*; Seyval blanc, *n = 4*; Vidal, *n = 6*, Adalmiina, *n = 1*; L'Acadie blanc, *n = 1*; and Louise Swenson, *n = 1*) which were harvested in the province of Quebec, Canada, during the 2016 season.

AA[2]	Frontenac blanc	Seyval blanc	Saint-Pepin	Vidal	Adalmiina	L'Acadie blanc	Louise Swenson	p-value
Ala	185 ± 102	177 ± 105	235 ± 143	66.5 ± 31	147	43.4	116	0.0727
Arg	474 ± 273 b[1]	90.1 ± 109 a	175 ± 137 ab	130 ± 111 a	257	258	221	0.0114
Asn	13.2 ± 6.1	11.9 ± 9.9	10.5 ± 6.7	4.88 ± 2.14	6.62	8.92	5.45	0.1823
Asp	32.0 ± 13	15.1 ± 9.8	20.0 ± 10.4	16.9 ± 3.7	26.0	22.2	48.9	0.0601
Cys	0.26 ± 0.11 b	0.19 ± 0.08 ab	0.17 ± 0.05 ab	0.12 ± 0.02 a	0.12	0.14	0.24	0.0415
GABA	53.5 ± 19	23.6 ± 11	34.6 ± 14.5	27.5 ± 6.6	34.4	30.0	47.5	0.0144
Gln	263 ± 167	221 ± 239	228 ± 171	73.2 ± 61	359	103	125	0.2468
Glu	53.5 ± 29 b	20.1 ± 14 a	19.0 ± 11.3 a	17.0 ± 5.4 a	40.6	21.3	59.1	0.0089
Gly	4.09 ± 2.1 b	2.58 ± 1.9 ab	2.61 ± 1.2 ab	1.24 ± 0.51 a	1.24	1.72	1	0.0451
His	20.2 ± 10.5	6.08 ± 4.6	12.8 ± 6.1	13.9 ± 7.0	8.91	6.76	17.3	0.0833
Ile	11.1 ± 5.0 b	5.73 ± 4.3 ab	2.52 ± 0.80 a	11.1 ± 5.5 b	1.36	1.57	7.1	0.0141
Leu	28.0 ± 14 b	8.04 ± 6.2 a	8.47 ± 4.9 a	12.8 ± 3.6 a	6.94	3.22	16.8	0.0041
Lys	5.07 ± 2.2 b	1.32 ± 0.38 a	1.31 ± 0.61 a	1.41 ± 0.76 a	1.62	1.87	2.62	0.0003
Met	5.18 ± 3.0 b	1.21 ± 0.83 a	1.29 ± 0.94 a	2.55 ± 1.36 ab	0.95	0.38	2.09	0.0109
Phe	16.4 ± 8.3 ab	6.28 ± 3.52 a	8.88 ± 4.2 a	19.3 ± 4.8 b	2.89	2.93	4.99	0.0061
Pro	135 ± 76	166 ± 200	42.8 ± 24.9	214 ± 169	135	57.0	50.8	0.2462
Ser	48.5 ± 27	25.9 ± 23.2	34.8 ± 21	17.6 ± 7.4	20.8	18.3	26.3	0.1201
Thr	43.1 ± 25	23.3 ± 19	21.8 ± 13	17.5 ± 8.1	17.0	15.3	29.8	0.1152
Trp	8.21 ± 2.9 b	2.56 ± 0.55 a	3.83 ± 1.09 a	4.78 ± 1.95 a	6.77	5.15	6.09	0.0023
Tyr	6.78 ± 4.1 b	1.17 ± 0.49 a	3.64 ± 2.89 ab	4.85 ± 1.18 ab	3.61	0.56	11.6	0.0344
Val	23.7 ± 11.2	11.6 ± 8.1	11.3 ± 5.67	12.9 ± 3.6	7.98	4.04	16.0	0.0556
GSH	7.81 ± 5.2 a	18.92 ± 11.4 b	5.44 ± 3.44 a	16.6 ± 8.8 ab	7.37	7.24	29.3	0.0299
TOTAL	1438 ± 767	839.45 ± 769.53	883 ± 564	686 ± 346	1093	612	845	

[1] Data represent mean values ± standard deviations of 4 to 5 biological samples, except for the Adalmiina, L'Acadie blanc and Louis Swenson varieties, for which n = 1 biological sample; samples were analysed in duplicate. For a given variable, values followed by different lower-case letters within a row are significantly different from each other according to Tukey's HSD test at $p \leq 0.05$.
[2] Abbreviations: Amino acid, AA; L-alanine, Ala; L-arginine, Arg; L-asparagine, Asn; L- aspartic acid, Asp; L-cysteine, Cys; γ-aminobutyric acid, GABA; L-glutamic acid, Gln; L-glutamine, Glu; L-glycine, Gly; glutathione, GSH; L-histidine, His; L-isoleucine, Ile; L-leucine, Leu; L-lysine, Lys; L-methionine, Met; L-phenylalanine, Phe; L-proline, Pro; L-serine, Ser; L-threonine, Thr; L-tryptophan, Trp; L-tyrosine, Tyr; L-valine, Val.

whereas Gln was the main amino acid in Adalmiina (33 %) and Seyval blanc (26 %). Ala was predominant in Saint-Pepin (27 %). Other amino acids, as well as GSH, were found in lower concentrations. In contrast with the other varieties studied, the Vidal amino acid profile showed a significant proportion of Pro (31 %). This is noteworthy because, as a secondary amino acid, Pro cannot be assimilated by yeast. Furthermore, Vidal generally accumulates a lower concentration of primary amino nitrogen than other hybrid varieties (Pedneault and Provost, 2016; Slegers *et al*., 2017). Thus, sometimes, in Vidal juice it is necessary for the supplementation of primary amino nitrogen to ferment correctly.

The presence of an amino moiety in thiol precursors suggests that the biosynthesis of those precursors may relate to the biosynthesis of certain amino acids, such as Cys and GSH. In the current study, the potential relationships between the accumulation of thiol precursors (Cys-3MH and G-3MH) and the amino profile of berries were investigated using correlation analysis. The results show moderate ($0.56 \leq r \leq 0.68$, $P \leq 0.01$) to strong ($r \geq 0.70$, $P \leq 0.001$) positive correlations between variables (Table 2). The strongest correlations occurred between amino acids, rather than between amino acids and thiol precursors. Despite the high concentrations of both GSH and thiol precursors found in certain varieties, such as Seyval blanc and Vidal (19 mg/L and 17 mg/L for GSH respectively), no significant correlation was found between those variables.

In contrast with Chen *et al*. (2019), no significant correlation was found between thiol precursors and the amino acids Glu, Cys and Gly, which, as constituents of GSH, may be involved in the metabolism of G-3MH and Cys-3MH. Conversely, a significant correlation was found between G-3MH and Pro ($r = 0.57$, $P \leq 0.01$). As Pro is biosynthesised from Glu, and Glu is necessary for GSH biosynthesis, Pro is likely a competitor of G-3MH from a metabolic point of view; for this reason, this correlation was unexpected. Furthermore, in contrast with our results, Chen *et al*. (2019) found a negative relationship between G-3MH content of Sauvignon blanc berries and their content in Pro and Glu, among other amino acids. Such differences between the results suggest that interspecific hybrid *Vitis* varieties accumulate thiols differently than *V. vinifera*.

Interest in the amino acid content of grape juice with regards to thiols is also driven by the relationship between the presence of specific amino acids and the release of free thiols during winemaking. The amino acid content of berry juice and the relative content of amino acids to each other's have been found to impact the consumption of thiol precursors by yeast and the consequent release of free thiol in wine (Alegre *et al*., 2017). For instance, previous studies on *Vitis vinifera* sp. Sauvignon blanc juice have shown moderate relationships ($r = 0.33$ to 0.43) between the concentration of amino acids Glu, GABA and Gln and the release of free 3MH in wine (Pinu *et al*., 2014; Chen *et al*., 2019). In model solutions, a high proportion of Asp, Thr, Phe and Ser were related to a higher release of 3MH during fermentation, whereas His, Pro and Met were associated with a lower release of free thiols (Alegre *et al*., 2017).

The potential of releasing free thiol from the studied hybrid varieties was evaluated using principal component analysis (PCA) and the relative proportion (as a percentage) of amino compounds (amino acids and GSH). The result primarily showed the compositional differences between the Seyval blanc and Saint-Pepin varieties (Quadrat I), Frontenac blanc (Quadrat II) and Vidal (Quadrats III), as can mainly be seen in PC 1 (39.5 % of variance explained; Figure 2). The variables graph shows that amino acids that readily release thiols (e.g., Asp, Thr and Ser) are mostly located in Quadrat II of the PCA, while amino acids associated with a lower rate of thiol release (e.g., Pro, His and Met) are in Quadrat III. Quadrat II is associated with Frontenac blanc, suggesting that thiol release could be facilitated in this variety. In contrast, amino acids associated with lower thiol release rates are mostly found in Vidal (Quadrat III), which also showed a higher range of G-3MH. Based on results from Chen *et al*. (2019), a high Pro content may negatively impact the release of thiols during fermentation. However, it is unclear whether the positive impact of thiol-promoting amino acids would bypass the negative impact of Pro, His and Met during the fermentation for this variety. On the other hand, some varieties containing high levels of thiol precursors (e.g., Saint-Pepin and Seyval blanc; Quadrat I) showed low amounts of amino acids associated with low-thiol conversion rates (Quadrat III). These results suggest that adjusting the amino profile by adding thiol-promoting amino acids can contribute to higher conversion rates in certain varieties, including Vidal, Saint-Pepin and Seyval blanc. However, the impact of the amino profile on the rate of conversion of thiol precursors into free thiols during winemaking using interspecific hybrid varieties remains to be studied

3. Impact of foliar urea and sulfur spraying on thiol precursors and vine physiology

Previous studies (Buica *et al*., 2016; Dufourcq *et al*., 2010; Dufourcq *et al*., 2009) showed that nitrogen and sulfur supplementation increased thiol precursor concentration in *V. vinifera* berries. However, in a recent trial on the interspecific hybrid Floreal, which is a new disease-resistant variety released in France in 2018, nitrogen sprays significantly increased the concentration of G-3MH in early harvested berries, but its content quickly decreased as berries ripened (Dournes *et al*., 2020).

The effect of foliar urea and sulfur supplementation on vine physiology, berry ripening and thiol precursors was investigated on the Vidal variety grown on two sites. Most physiological (e.g., yield, average cluster weight, berry FW and SECV) and basic berry chemistry variables (e.g., pH and TA) differed significantly between the two experimental sites. However, the impact of the treatments (urea and sulfur) on each site and their interactions were limited or absent (Table 3); for instance, sulfur supplementation significantly impacted juice pH and TA, but the interaction of urea and sulfur supplementation on pH was significant and site dependent.

TABLE 2. Correlation matrix of thiols precursors (Cys-3MH; G-3MH) and amino compounds (amino acids, glutathione) content in berry juices from seven white interspecific hybrid grape varieties (Frontenac blanc, *n* = *5*; Saint-Pepin, *n* = *5*; Seyval blanc, *n* = *4*; Vidal, *n* = *6*, Adalmiina, *n* = *1*; L'Acadie blanc, *n* = *1*; and Louise Swenson, *n* = *1*) harvested in the province of Quebec, Canada, during the 2016 season.

Compounds[1]	Cys-3MH	Glut-3MH	Ala	Arg	Asn	Asp	Cys	GABA	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	GSH
Cys-3MH	1[2]																							
Glut-3MH	0.52	1																						
Ala	0.37	0.37	1																					
Arg	0.32	0.10	*0.62*	1																				
Asn	0.15	0.16	**0.86**	**0.68**	1																			
Asp	0.38	0.23	**0.71**	**0.92**	**0.74**	1																		
Cys	0.10	-0.09	*0.69*	**0.82**	**0.78**	**0.78**	1																	
GABA	0.14	-0.04	*0.60*	**0.87**	*0.66*	**0.81**	**0.80**	1																
Gln	0.21	0.16	**0.89**	**0.70**	**0.96**	**0.75**	**0.76**	*0.64*	1															
Glu	0.29	0.03	*0.60*	**0.97**	*0.70*	**0.90**	**0.89**	**0.85**	**0.72**	1														
Gly	0.00	-0.03	*0.65*	**0.68**	**0.87**	*0.67*	**0.76**	**0.75**	**0.75**	**0.71**	1													
His	0.33	0.39	0.54	**0.90**	0.54	**0.85**	*0.64*	**0.76**	*0.58*	**0.83**	0.53	1												
Ile	0.07	0.11	0.05	*0.58*	0.28	0.51	0.39	0.46	0.28	*0.59*	0.29	**0.70**	1											
Leu	0.29	0.04	0.44	**0.94**	0.56	**0.89**	**0.76**	**0.82**	*0.58*	**0.96**	0.59	**0.88**	**0.74**	1										
Lys	0.13	-0.12	0.36	**0.88**	*0.57*	**0.76**	**0.76**	**0.84**	0.49	**0.89**	**0.78**	**0.73**	0.55	**0.87**	1									
Met	0.24	0.00	0.33	**0.86**	0.44	**0.79**	*0.67*	**0.72**	0.48	**0.88**	0.51	**0.84**	**0.80**	**0.96**	**0.82**	1.00								
Phe	0.14	0.31	0.13	*0.62*	0.21	*0.59*	0.32	0.53	0.26	*0.58*	0.20	**0.82**	**0.86**	**0.73**	0.51	**0.77**	1							
Pro	0.16	*0.57*	0.12	0.28	0.32	0.34	0.17	0.16	0.24	0.29	0.21	0.44	0.56	0.34	0.21	0.26	0.50	1						
Ser	0.38	0.25	**0.85**	**0.88**	**0.90**	**0.92**	**0.82**	**0.82**	**0.90**	**0.87**	**0.82**	**0.79**	0.41	**0.79**	**0.74**	**0.68**	0.45	0.31	1					
Thr	0.26	0.15	**0.72**	**0.92**	**0.87**	**0.91**	**0.88**	**0.86**	**0.85**	**0.94**	**0.82**	**0.82**	*0.59*	**0.88**	**0.83**	**0.79**	*0.56*	0.41	**0.95**	1				
Trp	0.05	-0.18	0.27	**0.81**	0.35	*0.67*	*0.67*	**0.76**	0.40	**0.81**	0.48	**0.77**	*0.66*	**0.85**	**0.80**	**0.88**	**0.69**	0.01	*0.57*	**0.68**	1			
Tyr	0.14	0.11	0.31	**0.76**	0.27	**0.69**	0.52	*0.60*	0.38	**0.74**	0.31	**0.81**	*0.57*	**0.79**	*0.60*	**0.80**	**0.76**	0.16	0.53	*0.59*	**0.79**	1		
Val	0.30	0.15	*0.59*	**0.93**	**0.72**	**0.92**	**0.81**	**0.84**	**0.72**	**0.95**	**0.70**	**0.88**	**0.73**	**0.97**	**0.84**	**0.92**	**0.71**	0.43	**0.89**	**0.95**	**0.77**	**0.73**	1	
GSH	-0.16	0.09	-0.02	-0.15	0.1	-0.01	0.24	-0.13	0.1	0.02	-0.07	-0.11	0.23	0.01	-0.2	0.02	0.19	0.39	-0.05	0.05	-0.1	0	0.09	1

[1] Amino compounds: L-alanine, Ala; L-arginine, Arg; L-asparagine, Asn; L- aspartic acid, Asp; L-cysteine, Cys; γ-aminobutyric acid, GABA; L-glutamic acid, Gln; L-glutamine, Glu; L-glycine, Gly; L-histidine, His; L-isoleucine, Ile; L-leucine, Leu; L-lysine, Lys; L-methionine, Met; L-phenylalanine, Phe; L-proline, Pro; L-serine, Ser; L-threonine, Thr; L-tryptophan, Trp; L-tyrosine, Tyr; L-valine, Val; glutathione, GSH. Thiol precursors: cysteine-3MH, Cys-3MH; glutathione-3MH, G-3MH.

[2] Values in bold are significant at P ≤ 0.001 and values in italics at P ≤ 0.01.

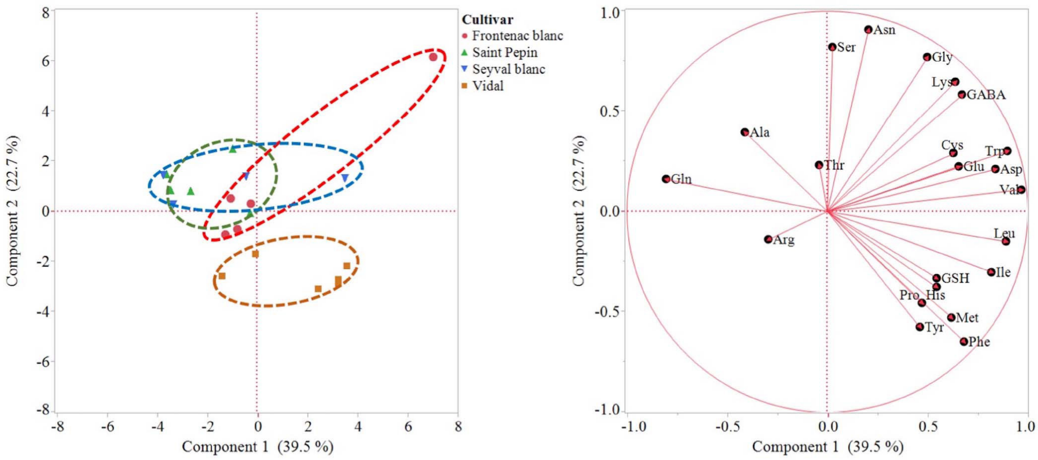

FIGURE 2. Principal component analysis showing (A) the distribution of 20 samples from 4 white interspecific hybrid varieties (Frontenac blanc, n = 5, purple diamond; Saint-Pepin, n = 5, blue triangle; Seyval blanc, n = 4, brown square; and Vidal, n = 6, green circle) from the province of Quebec, Canada, during the 2016 season, and (B) the loadings plot based on concentrations of amino acids and glutathione (refer to Table 1 for abbreviations).

TABLE 3. P-values for physiological parameters (yield per plant (kg/plant), number of clusters per plant, average cluster weight (g), average berry weight (g), exposed leaf area (SECV, m2) and ratio of exposed leaf area to fruit yield (SECV/PR; m2 leaf/kg fruit)), basic juice composition (total soluble solids (TSS, Brix), pH, titratable acidity (TA, g/L tartaric ac. eq.) and thiol precursors content (Cys-3MH and G-3MH, mg/kg berry FW) of Vidal grapevines supplemented with urea (0,10 and 20 kg/ha) and sulfur (0 and 5 kg/ha) (two treatments) on two sites (Dunham and Saint-Rémi), with treatments repeated on three blocks (1, 2, and 3).

Source of variation	Physiological parameters						Basic juice composition			Thiol precursors	
	Yield	# Cluster per plant	Cluster weight	Berry weight	SECV	SECV/PR	TSS	pH	TA	Cys-3MH	G-3MH
Site	**0.0099**[1]	0.8095	**< 0.0001**	**0.0021**	**< 0.0001**	0.8572	0.2680	**0.0260**	**0.0110**	**0.0001**	0.3639
Urea	0.6669	0.9463	0.4342	0.8280	0.3379	0.5212	0.6630	0.2420	0.3990	0.5435	0.3448
Sulfur	0.8989	0.6811	0.8222	0.3706	0.1652	0.9071	0.1060	**0.0480**	**0.0250**	0.5306	0.1025
Site x Urea	0.3077	0.3142	0.6282	0.5749	0.1603	0.4394	0.7210	0.4830	0.1150	0.7769	0.1327
Site x Sulfur	0.2072	0.1995	0.9296	0.8593	0.1302	0.1028	0.8340	0.1780	0.3040	0.0597	0.5564
Urea x Sulfur	0.8272	0.9173	0.1828	0.6086	0.9642	0.3175	0.6060	0.4440	0.5480	0.2597	0.1392
Site x Urea x Sulfur	0.3146	0.5113	0.0793	0.9330	0.4231	0.2972	0.8550	**0.0040**	0.6560	0.7534	0.2674
Block	0.6956	0.7680	0.9386	n/a[2]	**0.0034**	0.7674	n/a	n/a	n/a	n/a	n/a
Site x Block	**0.0032**	**0.0013**	0.4670	n/a	0.3949	0.1725	n/a	n/a	n/a	n/a	n/a
Urea x Block	0.6065	0.4625	0.5511	n/a	0.2458	0.1947	n/a	n/a	n/a	n/a	n/a
Site x Urea x Block	0.6197	0.2085	0.4839	n/a	0.4433	0.5985	n/a	n/a	n/a	n/a	n/a
Sulfur x Block	0.5712	0.7260	0.6196	n/a	**0.0012**	0.2335	n/a	n/a	n/a	n/a	n/a
Site x Sulfur x Block	0.8296	0.8980	0.6182	n/a	0.3425	0.5467	n/a	n/a	n/a	n/a	n/a
Urea x Sulfur x Block	0.3636	0.5685	0.1638	n/a	**0.0206**	0.7577	n/a	n/a	n/a	n/a	n/a
Site x Urea x Sulfur x Block	0.3708	0.7475	0.0589	n/a	0.2957	0.3437	n/a	n/a	n/a	n/a	n/a

[1] p-values ≤ 0.05 are in bold. [2] Block effect could not be measured on berry weight, juice composition and thiol precursor content.

The differences between the sites show that, on average, Saint-Rémi exhibited a higher yield (2.64 vs 2.05 kg/plant), cluster weight (165 vs 132 g/cluster), berry weight (2.17 vs 2.01 g/berry) and exposed leaf area (SECV; 4.79 vs 4.44 m^2 leaf) compared to Dunham (Table 4). Meanwhile, the juice from the Dunham berries had a higher pH and a lower titratable acidity than those from Saint-Rémi (2.53 vs 2.49 pH units and 18.9 vs 21.2 g/L tartaric acid eq. respectively; Table 5). Furthermore, the treatment combinations that received 5 kg/ha of sulfur had higher titratable acidity than those without sulfur (data not shown). The urea, sulfur and site treatments significantly interacted, affecting pH: the urea and sulfur supplementation had no impact on juice pH on the Dunham site, whereas the urea and urea + sulfur treatments

TABLE 4. Physiological parameters (yield per plant (kg/plant), number of clusters per plant, average cluster weight (g), average berry weight (g), exposed leaf area (SECV, m^2) and ratio of exposed leaf area to fruit yield (SECV/PR; m^2 leaf/kg fruit)) of Vidal grapevines grown on the Dunham and Saint-Rémi experimental sites during the 2017 season (Site effect).

Site	Yield per plant (kg)	Cluster per plant (#)	Cluster weight (g)	SECV (m2)	SECV/PR (m2 leaf/kg fruit)	Berry weight (g)
Dunham	2.05 ± 0.90 a[1,2]	15.7 ± 6.4a	132 ± 33a	4.44 ± 0.39a	4.17 ± 2.1a	2.01 ± 0.13a
Saint-Rémi	2.64 ± 1.13b	16.3 ± 7.0a	165 ± 32b	4.79 ± 0.37b	4.11 ± 2.1a	2.17 ± 0.13b

[1] Data represent mean values ± standard deviations of 54 biological replicates. [2] For a given variable, values followed by different letters are significantly different from each other according to Tukey's HSD test at $p \leq 0.05$.

TABLE 5. Total soluble solids (Brix), pH, titratable acidity (g/L tartaric ac. eq.), and thiol precursors (cysteine-3MH, glutathione-3MH, in mg/kg berry FW) content of juice of Vidal berries harvested from experimental sites (Dunham, Saint-Rémi) located in Quebec, Canada, and treated with foliar urea (0, 10, 20 kg/ha) and sulfur (0, 5 kg/ha) supplements during the 2017 season.

Site	Urea (kg/ha)	Sulfur (kg/ha)	TSS (°Brix)	pH	TA (g/L tartaric ac. eq.)	Cys-3MH (µg/kg berry FW)	G-3MH (µg/kg berry FW)
Dunham	0	0	18.8 ± 0.1[1]	2.52 ± 0.07 A	16.8 ± 1.6	1.40 ± 0.55	95.7 ± 44
	0	5	19.7 ± 1.1	2.57 ± 0.06 A	19.6 ± 3.7	2.09 ± 0.30	139 ± 26
	10	0	19.3 ± 0.8	2.57 ± 0.10 A	17.8 ± 0.7	1.46 ± 0.33	121 ± 15
	10	5	19.3 ± 2.0	2.50 ± 0.05 A	21.3 ± 3.9	2.02 ± 0.36	145 ± 37
	20	0	18.0 ± 2.2	2.49 ± 0.03 A	17.6 ± 0.7	1.98 ± 0.95	154 ± 80
	20	5	19.7 ± 0.6	2.54 ± 0.02 A	20.1 ± 4.0	1.86 ± 0.39	188 ± 51
	Average		19.1 ± 1.3 a[3]			1.79 ± 0.52 a	140 ± 49 a
Saint-Rémi	0	0	20.0 ± 1.4	2.50 ± 0.04 A[2]	21.6 ± 4.3	0.98 ± 0.17	86.3 ± 39
	0	5	20.3 ± 1.1	2.55 ± 0.10 A	24.7 ± 1.4	0.93 ± 0.21	145 ± 41
	10	0	19.0 ± 1.2	2.37 ± 0.04 B	20.2 ± 0.7	1.13 ± 0.71	158 ± 51
	10	5	19.6 ± 1.7	2.55 ± 0.05 A	21.5 ± 3.0	1.08 ± 0.22	104 ± 40
	20	0	19.0 ± 2.0	2.50 ± 0.05 A	20.2 ± 0.5	1.30 ± 0.38	82.5 ± 9.2
	20	5	20.1 ± 1.0	2.46 ± 0.04 AB	19.1 ± 0.5	0.95 ± 0.22	131 ± 67
	Average		19.7 ± 1.3 a			1.06 ± 0.30 b	117 ± 46 a

[1] Data represent mean values ± standard deviations of 3 biological replicate (blocks). [2] For a given variable, data followed by different capital letters are significantly different from each other according to Tukey's HSD test at $p \leq 0.05$ (Urea x sulfur x Site effect). [3] For variables with only a site effect, data (in grey; TSS, Cys-3MH and G-3MH) represent the mean values ± standard deviations of 18 biological replicates; each value followed by different lower-case letters are significantly different from each other according to Tukey's HSD test at $p \leq 0.05$ (Site effect).

were related to significantly lower pH values in Saint-Rémi (Table 5), even though the data shows no clear trend.

Significant block effects were found for certain variables and treatment combinations, including a Site x Block (yield, number of clusters per plant) effect, a Sulfur x Block (SECV) effect and a Urea x Sulfur x Block (SECV) effect. This suggests that the blocks had some heterogeneity in terms of physiology (e.g., productivity and vigour). For example, the analysis of the block effect showed that that each respective Block 3 of the Dunham and Saint-Rémi sites differed from Blocks 1 and 2 in terms of yield and number of clusters per plant (see Table S3 of Supplementry Material for data). Thus, the yield and the number of clusters per plant were lower in Block 3 of the Dunham site than in Block 3 of the Saint-Rémi site (1.62 vs. 3.18 kg/plant and 12.9 and 19.8 clusters per plant respectively; data not shown). The interactions between treatments and blocks did not result in any clear trends in terms of SECV, again suggesting that these effects are attributable to variations in growing conditions.

4. Thiol precursors in grape berry juices

As observed with the screening of white hybrid varieties, Cys-3MH and G-3MH were the only thiol precursors detected in the juice of Vidal berries from both sites, with G-3MH largely dominant over Cys-3MH for all treatments combinations (Table 5). Similar to most physiological measurements and basic juice parameters, urea and sulfur supplementation, alone or combined, did not affect the content of G-3MH and Cys-3MH of Vidal berries on either site (Table 3). Considering that the berries were harvested at full maturity, our results agree with the findings of Dournes *et al.* (2020) in their study on the hybrid variety Floreal, in which the positive effect of nitrogen supplementation on G-3MH concentration observed in early ripened berries decreased as berries reached full maturity (Dournes *et al*., 2020).

In contrast, a significant site effect was found for Cys-3MH, which was more concentrated in berries from Dunham (1.79 µg/kg berry FW) when compared to Saint-Rémi (1.06 µg/kg berry FW; Table 5). Thiol precursor concentrations were highly variable among treatments, possibly due to some heterogeneity in experimental blocks (significant interactions were detected with some of the physiological variables), which suggests that differences between treatments may have been underestimated. Indeed, the urea and sulfur supplementation on the Dunham site showed a trend of a higher accumulation of thiol precursors in berries treated with urea and sulfur compared to the control; in contrast, there is no such trend on the Saint-Rémi site. Discrepancies between sites following nitrogen application at the soil level had been observed in Bordeaux and Sancerre, on Sauvignon blanc grapevine: nitrogen fertilisation increased G-3MH concentration of grape must on one site, but not on the other in the same year, and in the second year, nitrogen had no impact on thiol precursors accumulation on both sites (Helwi *et al.*, 2016). Authors suggested that supplementation may, in some instances, increase vine vigour, which seems to impact thiol accumulation in berries negatively. In agreement with this study, thiol precursor accumulation seems highly variable in interspecific hybrid varieties, not only from one site to another, but from one vintage to another. For instance, it is noteworthy that the Vidal sample collected on the Saint-Rémi site in 2016 for the screening of interspecific *Vitis* varieties (first part of the present study) showed a much higher concentration of G-3MH than samples collected during the 2017 urea and sulfur assay (351 µg/kg berry FW in 2016, data not shown, vs 86 to 158 µg/kg berry FW in the field trial conducted in 2017). Nitrogen accumulation is highly variable from one hybrid variety, site and vintage to another (Pedneault and Provost, 2016; Slegers *et al*., 2017). The accumulation of thiol precursors has been found to be related to nitrogen accumulation in *Vitis vinifera* (Choné *et al.*, 2006; Lasa *et al.*, 2012). Thus, nitrogen variations could relate to large variations in thiol precursors content in hybrid varieties. Abiotic and biotic factors affecting nitrogen accumulation and, by extension, the accumulation of thiol precursors are still little understood in hybrid varieties. Thus, further research is needed to better understand the relationship between nitrogen and thiol precursor metabolism in hybrid varieties in order to optimise further field practices that would enhance the thiol precursor content of berries.

CONCLUSIONS

Thiol precursors, amino acids and glutathione were characterised for the first time in 23 grape samples from seven white hybrid varieties (Adalmiina, Frontenac blanc, L'Acadie blanc, Louise Swenson, Saint Pepin, Seyval blanc and Vidal) grown in the province of Quebec, Canada. Precursors of 3-mercaptohexan-1-ol (3MH) were the only thiol precursors identified in the hybrid varieties analysed, glutathione-3MH being the main abundant thiol precursor. The varieties Vidal, Seyval blanc and Saint-Pepin showed the highest concentrations of glutathione-3MH. Correlation analyses on amino acids, glutathione and thiol precursors (glutathione-3MH and cysteine-3MH) in grape berry juices showed little correlation between amino acids and thiol precursors in berries, except for a positive correlation between glutathione-3MH and proline; this contrasts with previous findings for *V. vinifera*, suggesting that thiol/amino acid metabolisms differ in interspecific hybrids when compared to *V. vinifera*.

Based on literature data, principal component analysis was used to evaluate the potential of amino acid profile to promote thiol precursors conversion during winemaking. The results showed that certain varieties containing high levels of thiols precursors (e.g., Saint-Pepin and Seyval blanc) have low amounts of amino acids typically associated with low-thiol conversion rates (e.g., proline, histidine and methionine), whereas others such as Vidal have high levels of proline. Although amino nutrition is usually considered as a whole in winemaking (e.g., yeast assimilable nitrogen), these results suggest that supplementing juice with specific amino acids could be a strategy to increase thiol conversion in interspecific hybrid varieties.

In the second part of the study, the effect of urea and sulfur foliar supply on vine physiology, berry maturity and thiol precursor production was investigated on Vidal. To our knowledge, this was the first essay to be conducted on Vidal grapes grown in Quebec. No significant differences were found between treatments (combinations of urea and sulfur) on yield components and thiol precursor concentrations in grape berry juice. However, despite berries being at a similar ripening stage, cysteine-3MH accumulation was significantly different between each experimental site, suggesting that other factors, such as cultural practices, soil and climate, also affect thiol precursors concentration in berries.

These findings open avenues for further investigations on the biogenesis of thiol precursors in berries and on the improvement of wine quality in interspecific hybrid wine production.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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