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Phenolics profiles in Marquette juice and wine were not affected by grapevine plant growth regulators applications

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ABSTRACT

Red wines made from interspecific cold-hardy grape cultivars, such as Marquette, often have lower tannin concentrations compared to those made from *Vitis vinifera*, which can result in undesirable qualities like poor colour stability and oxidative aromas. To address this, various viticultural practices have been explored, including nitrogen fertilisation and plant growth regulators, to enhance phenolic content and wine quality. This study aimed to evaluate the effects of foliar phenylalanine (Phe) and prohexadione calcium (ProCa) treatments on tannin and iron-reactive phenolic (IRP) concentrations in Marquette grape juice and wine. Despite significant interaction effects between year and treatments (ProCa, Phe), the treatments themselves did not seem to have a strong impact on either IRP or tannin concentrations. This suggests that, at the current application rates, the environmental conditions play a more dominant role than the treatments.

KEYWORDS: prohexadione calcium, phenylalanine, cold-hardy hybrid grape, foliar spray, red winemaking

INTRODUCTION

Tannins and anthocyanins are phenolic compounds that are essential to maintain wine quality, because they contribute astringency and colour to the finished wine. Condensed tannins are oligomers (3 to 9 units) and polymers (≥ 10 units) of flavan-3-ols, including (–)-epicatechin (EC), (+)-catechin (C), (–)-epicatechin-3-*O*-gallate (ECG), and (–)-epigallocatechin (EGC) (Bordiga *et al.*, 2011), which are biosynthesised in skin and seed tissues (Kennedy, 2008). Those flavan-3-ols are linked through C4-C8 or C4-C6 interflavan bonds, and the number of subunits corresponds to the mean Degree of Polymerisation (mDP) of tannins. Tannins are responsible for texture and mouthfeel properties in wine, such as astringency, (Casassa & Harbertson, 2014), due to their ability to interact, form complexes, and aggregate with salivary proteins, thus decreasing lubrication and resulting in a mouth-drying or puckering sensation (Watrelot *et al.*, 2019). Anthocyanins are found in the skins, and sometimes flesh, of fruits and petals of flowers (Kennedy, 2008; Lattanzio *et al.*, 2012). Anthocyanins are present in several forms including malvidin-3-*O*-glucoside and malvidin-3,5-*O*-diglucoside. During the process of red winemaking, anthocyanins are extracted from grape skins and flesh, react with oxygen and other phenolic compounds, and are responsible for the colour intensity, stability, and hue of red wines.

Red wines made from interspecific cold-hardy (*i.e.*, non-*Vitis vinifera*) grape cultivars have lower tannin concentration than red wines made from *V. vinifera* cultivars (Springer & Sacks, 2014; Watrelot & Norton, 2020). Examples of these cultivars include Marquette, Frontenac, Crimson Pearl, and Marechal Foch, which are grown in regions with winter temperatures too cold for *V. vinifera* to survive, such as the U.S. midwest and southeastern Canada. In particular, Marquette is one of the most widely planted cold-hardy cultivars. Additionally, interspecific grape cultivars are being considered worldwide as a potential mitigation for climate-related stressors on wine grape production (Santos *et al.*, 2020). Low tannin concentration leads to low red wine quality indicators, such as high prevalence of oxidative aromas, low astringency, and poor colour stability (Watrelot & Norton, 2020). However, the final phenolics concentration in red wines depends on many factors; these include their initial content in grapes, which is influenced by viticultural practices, and their extractability during winemaking (Manns *et al.*, 2013; Springer & Sacks, 2014). Various viticultural practices have been used to manage the phenolic content in grapes and thereby impact wine quality. Two practices that have shown promise on *V. vinifera* are nitrogen fertilisation (Delgado *et al.*, 2004; Portu, *et al.*, 2015a; Portu, *et al.*, 2015b; Portu, *et al.*, 2017a; Portu, *et al.*, 2017b) and the application of plant growth regulators (LoGiudice *et al.*, 2004; Mhetre *et al.*, 2021; Portu, *et al.*, 2017a; Thomidis *et al.*, 2018; Vaquero-Fernandez *et al.*, 2009).

Nitrogen fertilisation is widely used in agriculture, because nitrogen is an essential nutrient for plant growth (Metay *et al.*, 2015). More specifically, vine nitrogen status is important for wine quality as it affects the amount of yeast assimilable nitrogen (YAN) in grapes, and adequate YAN (> 140 mg N/L) is necessary for yeasts during alcoholic fermentation to avoid the formation of reductive aromas (Bell & Henschke, 2005). Typically, nitrogen supplementation is applied to the soil, and various forms of nitrogen have been used (*e.g.*, ammonium nitrate, urea) as a fertiliser on grapevines to modify the content of phenolic compounds with mixed results (Delgado *et al.*, 2004; Hui *et al.*, 2021). For example, Delgado *et al.* (2004) reported higher anthocyanin concentrations and increased tannin degree of polymerisation after fertilisation with nitrogen and potassium; however, astringency was not affected despite larger tannins. Hui *et al.* (2021) found an increase in the content of total anthocyanins, total flavanols, and total flavonols after spray treatment with urea. Alternately, nitrogen can be applied as a foliar spray with advantages over soil application including improved usage by the vine (up to 70 %), faster incorporation into plant tissues (one to two days *vs* three to four days), and reduced fertiliser waste and environmental risk (Cheng *et al.*, 2021). Phenylalanine (Phe) is another source of nitrogen that has been used to target phenolics content, because Phe is a precursor for the biosynthesis of flavonoids via the phenylpropanoid (shikimate) pathway (Broeckling *et al.*, 2012). In one study, an increase in total anthocyanins in *V. vinifera* cv. Tempranillo grape extracts were reported after Phe was applied (1.5 kg N/ha) to the vine canopy at *véraison* and one week after *véraison* (Portu, *et al.*, 2015b). On the other hand, Phe applied at a lower rate (0.9 kg N/ha) resulted in no significant effect on phenolic content of whole grape extracts from Tempranillo vines (Portu, *et al.*, 2017b); however, the two studies were conducted under different climatic conditions, and in different soils and vintages, which may have limited the effectiveness of the lower dose. Despite conflicting results of Phe effectiveness on phenolic content in grape extracts, the same group found Phe treatment increased tannin content by 30 % in Tempranillo wines over the control wines (Portu, *et al.*, 2015a). Therefore, the use of Phe has potential to change the phenolic content in grapes and wines, which could benefit red wine quality when using cold-hardy grape cultivars initially low in tannin content.

Plant growth regulators, which are chemicals used to affect metabolic or developmental processes of plants (Rademacher, 2016; Ministry of Agriculture, 2022), have also been used to improve crop performance. Prohexadione calcium (ProCa, 3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate) is an industrially synthesised plant growth regulator with low toxicity, which has previously been shown to modify the sugar-acid balance and enhance aroma, thus improving the quality of fruit (Li *et al.*, 2024). ProCa has led to an increase in phenolic compounds concentrations in *V. vinifera* cv. Xinomavro (Thomidis *et al.*, 2018), which was attributed to a reduction in berry size due to the effects of ProCa as a late-stage gibberellin biosynthesis inhibitor

that restrains cell expansion by limiting biosynthesis of the active forms of gibberellic acid (Rademacher, 2016). In the study conducted using Xinomavro, application of ProCa (250 mg/L, 1000 L/ha) resulted in higher concentrations of anthocyanins in grape skins and total phenolics in skins and seeds (Thomidis *et al.*, 2018). Wines made from those ProCa treated vines also showed a higher tannin concentration, total phenolics, and colour intensity than control wines (Thomidis *et al.*, 2018). Other studies have reported similar increases in colour intensity, total anthocyanins, and total phenolic compounds in juice and wine following ProCa treatment on various *V. vinifera* cultivars (Avizcuri-Inac *et al.*, 2013; Lal *et al.*, 2022; LoGiudice *et al.*, 2004; Mhetre *et al.*, 2021; Reinehr *et al.*, 2021; Vaquero-Fernandez *et al.*, 2009). Although treatment with Phe and ProCa did not show an effect on phenolic compounds in the skins and seeds of interspecific cold-hardy cultivar Marquette (Gapinski *et al.*, 2024), extraction of phenolics was conducted with a non-wine matrix in a short time. For the current study, we wanted to evaluate the treatments during winemaking to understand if the changing fermentation matrix (*i.e.*, change in ethanol concentration) disrupted binding/association between phenolics and cell wall material, and thus extractability of phenolics, as previously published by Bindon *et al.* (2014). Therefore, it was hypothesised that application of ProCa on interspecific cold-hardy cultivar Marquette grapevines would lead to similar outcomes, notably increased tannin concentrations in wines. The goal of this study was to evaluate the tannin and iron-reactive phenolics contents in grape juice and wine at bottling and after ageing after the application of Phe and ProCa on Marquette grapevines in the vineyard.

MATERIALS AND METHODS

1. Chemicals

Ammonium dihydrogen phosphate, potassium metabisulfite (KMBS), sodium dodecyl sulphate (SDS), acetone, acetonitrile (HPLC grade), D-fructose, ethyl alcohol (200 proof), ferric chloride, hydrochloric acid (37.5 %), methanol (HPLC grade), and *ortho*-phosphoric acid (extra pure, 85 % solution in water) were manufactured by Fisher Scientific (Fair Lawn, NJ). Triethanolamine (TEA) was purchased from Aqua Solutions (Deer Park, TX). Sodium hydroxide (0.1 N) was purchased from Lab Chem (Zelienople, PA). (–)-Epicatechin (purity ≥ 90 %), (+)-catechin hydrate (purity ≥ 98 %), glucose, L-tartaric acid, ammonium sulphate, and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO). Malvidin-3-*O*-glucoside (M3G) and malvidin-3,5-*O*-diglucoside (M35DG) were manufactured by Extrasynthese (Genay Cedex, France). Apogee® (27.5 % (*w/w*) prohexadione calcium) was purchased from BASF (Ontario, Canada). Induce®was purchased from Helena Agri-Enterprises (Collierville, TN, USA).

2. Vineyard treatments

The details of the vineyard treatments were as previously published (Gapinski *et al.*, 2024). Briefly, cold-hardy interspecific (hybrid) *Vitis* spp. cv. Marquette grapevines were planted in 2011 at the Iowa State University (ISU) Horticulture Research Station in Ames, IA, USA (42° 06' N 93° 35' W, 300 m elevation, plant hardiness zone 5a, Clarion loam soil). The vines were trained on a single high-wire trellis system with north-south row orientation with 2.44 m between vines and 3.05 m between rows. Treatments were assigned to three-vine panels following a split-plot experimental design (Figure S1). The first and last panels in each row (*i.e.*, panels 1 and 12) and all of row 3 were not used to assign treatments. Twenty-four of the remaining thirty panels were selected based on homogenous pruning weights and lack of dead vines and then were randomly assigned to four treatments with six replicates.

The treatments were as follows: phenylalanine (Phe), prohexadione calcium (ProCa), both prohexadione calcium and phenylalanine (ProCa+Phe), and a water-treated control (CTL), as previously published (Gapinski *et al.*, 2024). Both Phe and ProCa were mixed with Induce® (0.6 mL/L) and ammonium sulphate (1.0 g/L) for foliar application on both sides of the vine canopy until the point of runoff using a 15 L backpack sprayer with a calibrated rate equivalent to 935 L per hectare (Solo Inc., Newport News, VA). Phe was applied at a rate of 100 mg/L at *véraison* and two weeks after the start of *véraison*. ProCa (50 mg/L) was applied every two weeks starting one week post bloom until two weeks after the start of *véraison* for a total of five treatments. ProCa was sprayed first on days when both treatments were applied. Cumulative rainfall between 1 April and harvest was 543 mm in 2022 and 472 mm in 2023. For the same time period, the average low temperature was 12 °C for both years, while the average high temperature was 25 °C in 2022 and 26 °C in 2023 (Gapinski *et al.*, 2024).

3. Preparation of juices samples

Grape clusters were sampled at five phenological time points (one week post fruit set (PFS), three weeks PFS, *véraison*, mid-ripening, and harvest) in 2022 and in 2023 (Table S1), and transported to the laboratory in a cooler with ice packs within one hour of collection. Nine clusters per treatment (one cluster per vine) were collected randomly from different parts of the vines and pooled for analysis. At harvest, each three-vine panel was harvested separately. The yield for each panel was recorded and six clusters per panel were randomly sampled for basic chemical analysis. One hundred berries were randomly sampled from the nine clusters per treatment gathered at each time point throughout development and ripening (once per treatment), and from the six clusters per panel sampled at harvest (once for each panel, three per treatment). The juice obtained from the 100-berry samples was crushed by hand then chemically analysed.

4. Winemaking

Grapes were harvested on 26 August 2022 and 31 August 2023, sprayed with a 30 mg/L sulphur dioxide (SO_2) as a KMBS solution, and transported from the ISU Horticulture Research Station to the ISU winery for processing the same day. Each panel was processed separately. However, in 2022, one panel (M-ProCa-MW) yielded only bare stems and two other panels (M-Phe-NMW and M-CTL-NMW) did not yield enough for winemaking; therefore the clusters were added to the other panels of the same condition. Twenty-one wines were made in 2022, and 24 wines were made in 2023 according to the following procedure. Crushed and destemmed berries were placed into two-gallon (7.6 L) buckets. The median must weight was 14.1 kg in 2022 and 14.7 kg in 2023. The must was inoculated with Lalvin ICV D254 yeast (Scott Laboratories, Petaluma, CA, USA) with GoFerm™ yeast rehydration nutrient (Scott Laboratories, Petaluma, CA, USA) according to supplier instructions. Each fermentation was punched down twice per day after inoculation and throughout alcoholic fermentation. After 48 hours, the musts were co-inoculated with Lalvin VP41 bacteria (Scott Laboratories, Petaluma, CA, USA). Temperature, total soluble solids, and density (portable density meter DMA 35, Anton Paar, Ashland, VA, USA) were measured after the second punch down every day to monitor fermentation progress.

The wines were pressed at the completion of fermentation into one-gallon (3.8 L) jugs with an airlock and kept at room temperature (20 °C). Once malolactic fermentation was complete and checked by HPLC-DAD/RID (Gapinski *et al.*, 2023), the wines were racked into half-gallon (1.9 L) jugs and 0.5 mg/L molecular SO_2 was added (as a 6.6 % (*w/v*) solution KMBS) according to each wine's pH. Then, after flushing with argon, the wines were cold stabilised at 4.0 °C for four weeks. A volume of 6.6 % (*w/v*) KMBS solution was added at bottling to a target of 0.8 mg/L molecular SO_2. The wines were bottled in 750 mL green bottles, flushed with argon, and closed with cork #9. Samples of wines were collected for chemical analysis at bottling, and after five months of ageing.

5. Basic chemical parameters of juice and wine

Soluble solids (digital refractometer RF153, FLIR Commercial Systems Inc., Nashua, NH, USA), pH (Orion Star™ A211 Benchtop pH Meter, Thermo Fisher Scientific, Waltham, MA, USA), and titratable acidity (TA) were measured in juices and wines at all time points throughout winemaking and after ageing. Titratable acidity was determined by titrating 5 mL of sample with 0.1 N sodium hydroxide to an endpoint of pH 8.20 and expressed in tartaric acid equivalents. Two-mL aliquots were centrifuged (5 min at 11000 × *g*) prior to colour intensity and hue measurement in a 1 mm quartz cuvette with a Genesys 150 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) using VISIONlite Wine Analysis software (ascanis OHG, Ueberlingen, Germany). The software automatically scaled the results to a 1 cm path length after scanning the sample 380 to 780 nm at 5 nm intervals. The colour intensity and hue were evaluated in analytical triplicate for all juices during development and ripening, and in biological triplicate in juice at harvest and in all wines. An Agilent Hi-Plex H (7.7 × 300 mm, 8 µm) column protected with an H-plex guard column at 60 °C (Agilent Technologies, Santa Clara, CA, USA) was used on a reversed-phase high-performance liquid chromatograph (RP-HPLC, 1260 series, Agilent Technologies, Santa Clara, CA, USA) with a diode array detector (DAD) and refractive index detector (RID) to check the levels of malic acid and lactic acid during malolactic fermentation and ethanol. The isocratic mobile phase was 5 mM sulfuric acid at a flow rate of 0.7 mL/min. A 20 µL sample previously filtered at 0.45 µm using a PTFE filter was injected for 30 min. Ethanol was detected with the RID (cell temperature of 55 °C) and the concentration was calculated using an external calibration curve (Gapinski *et al.*, 2023).

6. Total iron-reactive phenolics concentration

The total iron-reactive phenolics (IRP) concentration was measured in juices and wines following the Harbertson-Adams Assay (Harbertson *et al.*, 2002). Briefly, background absorbance at 510 nm of 75 µL of sample in 800 µL sodium dodecylsulphate/triethanolamine buffer was recorded after a 10 min incubation at room temperature. Then, 125 µL ferric chloride reagent was added, the solution was vortexed, left at room temperature to incubate for 10 min, and the final absorbance at 510 nm was recorded. A blank of the same buffer (875 µL) and ferric chloride (125 µL) solutions without sample were used to evaluate the absorbance values. A calibration curve of (+)-catechin was used to express total iron-reactive phenolics as (+)-catechin equivalents.

7. Tannin concentration

Tannin concentration was quantified in juices and wines following a previously published method using RP-HPLC-DAD (1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) (Gapinski *et al.*, 2024). The polystyrene divinylbenzene column (PLRP-S, 2.1 × 50 mm, 100 Å, 3 µm, Agilent Technologies, Santa Clara, CA, USA) was protected with a guard column of the same material (PRP-1, 3 × 8 mm, Hamilton Co., Reno, NV, USA) and held at 30 °C. A linear gradient at a flow rate of 0.30 mL/min was used as follows: time in min (% mobile phase B), 0 (14), 12.6 (34), 12.6−13.3 (34), 15.1 (70), 15.1−16.8 (70), 19.6 (14), and 19.6−28.0 (14). Mobile phases were 1.5 % (*w/w*), 85 % *ortho*-phosphoric acid (mobile phase A), and 20 % (*v/v*) mobile phase A in acetonitrile (mobile phase B). Five µL of previously filtered (0.45 µm PTFE filter) sample were injected and tannin quantification was carried out by first drawing a baseline at 0 mAU across the whole chromatogram, then integrating the peak between 16.8 to 19.8 min. An external calibration curve of (−)-epicatechin was used to express tannin concentration as (−)-epicatechin equivalents.

8. Anthocyanin content

Anthocyanin content was determined in wine at bottling and after ageing using RP-HPLC-DAD (1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) with a LiChrospher column (100-5 RP18 250 mm × 4.0 mm, 5 µm, Agilent Technologies) at 40 °C. A previously published mobile phase gradient at a flow rate of 0.5 mL/min was used (Ritchey & Waterhouse, 1999). Mobile phases were A: 50 mM ammonium dihydrogen phosphate adjusted to pH 2.5 with *ortho*-phosphoric acid, B: 20 % A with 80 % acetonitrile, and C: 0.2 M *ortho*-phosphoric acid adjusted to pH 1.5 with sodium hydroxide. Twenty µL of previously filtered (0.45 µm PTFE filter) sample were injected and anthocyanins were monitored at 520 nm with M3G and M35DG as external calibration standards for mono- and di-glucoside, respectively.

9. Statistical analysis

Data collected on juices were analysed using a three-way analysis of variance (ANOVA) for an additive effects linear regression model with random intercepts, for which the *lme* function from the *nlme* package (Pinheiro & Bates, 2023) in R statistical software (RStudio version 4.4.0) was used. Treatments were assigned using a split-plot design including row as a blocking factor, half rows assigned to mowed (MW) or not mowed (NMW) as the whole plot, and individual panels assigned to treatments as the subplots (Figure S1). MW and NMW panels for the same treatment were averaged for analysis. Values for time points one week PFS to mid-ripening are means of MW and NMW analytical triplicates ± standard deviation, and values at harvest are means of MW and NMW biological triplicates ± standard deviation. Mowing treatment was included in the model as a physical application in the vineyard but was not part of the study. The effect heredity principle (Hamada & Wu, 1992) was employed to aid interpretation of significant effects. See supplementary data for the R code (Code S1).

Data collected on wines were analysed with JMP Pro 17.0 software (SAS Institute Inc., Cary, SC, USA) using two-way (ethanol) and three-way (pH, titratable acidity, hue, colour intensity, iron-reactive phenolics, tannin, and anthocyanins) analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$ for the effects of year, time point, and treatment.

RESULTS AND DISCUSSION

1. Basic chemical parameters of juices

During berry development and ripening, the soluble solids and pH decreased from one week PFS to three weeks PFS and then increased to a maximum at harvest in both years, as expected (Table 1). Year had a significant effect on soluble solids and pH at each time point except harvest (Table 2). Additionally, the effects of ProCa, Year2023:ProCa, Year2023:Phe, and Year2023:ProCa:Phe on soluble solids were significant at three weeks PFS while Year2023:ProCa and Year2023:ProCa:Phe were also significant at one week PFS and *véraison*, respectively. The pH of juice was affected by Year2023:ProCa:Phe at *véraison*, and Year2023:ProCa and Year2023:Phe at mid-ripening, which indicates that year had more of an effect on juice pH than treatment with ProCa or Phe. Titratable acidity (TA) increased from one week PFS to *véraison*, when it reached a maximum for all four treatments in both years, then decreased to a minimum at harvest (Table 1). Statistical analysis showed TA was affected by year at every time point throughout development and ripening with lower TA in 2023 than in 2022, which could be related to warmer pre-*véraison* temperatures in 2023 lessening organic acid content (Blank *et al.*, 2019). The effect of Year2023:ProCa on TA was significant at three weeks PFS, and the effect of Year2023:Phe was significant at *véraison* and mid-ripening. The three-way interaction between year, ProCa, and Phe was significant at three weeks PFS and *véraison* (Table 2). The effect of year on hue was significant at mid-ripening but on colour intensity at mid-ripening and harvest. Hue and colour intensity of juice were minimally affected by treatment. However, the effect of Phe treatment on hue was significant at harvest with higher hue when Phe was applied, which indicates a higher degree of yellowness than redness. This is contrary to the hypothesis that adding Phe would increase the biosynthesis rate of flavonoids, and potentially leading to higher anthocyanin content, thus deeper-coloured (*i.e.*, redder) juice.

Overall, application of Phe and ProCa exhibited limited effect on basic chemical properties of juices including hue and colour intensity as previously reported on Tempranillo juices after application of Phe on vines (Portu, *et al.*, 2015a; Portu, *et al.*, 2015b; Portu, *et al.*, 2017b) and on Beauty Seedless juices after application of ProCa on vines (Mhetre *et al.*, 2021). However, the TA of Beauty Seedless juices was higher when ProCa was applied at 200 mg/L but lower when applied at 400 mg/L, compared to the control. Additionally, a study conducted with cultivars Grenache and Tempranillo reported no change in soluble solids, pH, or TA after application of ProCa at pre-bloom (Avizcuri-Inac *et al.*, 2013), while another study across two years, also with Tempranillo, observed higher juice TA concentrations after ProCa application than control (Vaquero-Fernandez *et al.*, 2009). Another study applied ProCa to Cabernet-Sauvignon and Cabernet franc observed increased colour intensity, which the authors attributed to a reduction in berry weight (LoGiudice *et al.*, 2004). Therefore, ProCa seems to have variable effect on basic chemical properties, especially TA, and may be dependent on dose and cultivar or the climate and soil in which it is applied.

2. Iron-reactive phenolics and tannin concentrations in juices

The concentration of iron-reactive phenolics (IRP) in juice was highest at one week PFS (Figure 1), which was affected by year with higher IRP concentration in 2023 than in 2022 (Table 3). This could also be caused by the differences in temperature and precipitation, since warmer temperatures

TABLE 1. Basic chemical parameters and colour parameters in juice at each phenological time point during berry development and ripening.

Year	Treatment	Time point	Soluble solids (°Brix)	pH	TA (g/L)	Hue	Colour intensity (AU)
2022	CTL	1 week PFS	4.5 ± 0.1	2.66 ± 0.03	23.4 ± 0.1		
		3 weeks PFS	3.9 ± 0.1	2.49 ± 0.02	33.9 ± 0.6		
		Véraison	7.8 ± 0.1	2.59 ± 0.02	36.5 ± 0.5		
		Mid-ripening	18.5 ± 0.3	2.84 ± 0.03	22.1 ± 0.5	0.7 ± 0.1	4.1 ± 0.5
		Harvest	22.1 ± 1.3	3.00 ± 0.08	13.4 ± 0.8	1.0 ± 0.3	4.9 ± 0.9
	Phe	1 week PFS	4.4 ± 0.0	2.64 ± 0.02	23.3 ± 0.7		
		3 weeks PFS	3.6 ± 0.1	2.50 ± 0.07	33.3 ± 0.5		
		Véraison	7.1 ± 0.7	2.56 ± 0.04	36.2 ± 0.4		
		Mid-ripening	18.7 ± 0.3	2.88 ± 0.03	21.2 ± 0.6	0.7 ± 0.2	4.1 ± 0.9
		Harvest	21.5 ± 1.3	2.98 ± 0.08	14.5 ± 2.1	1.0 ± 0.3	5.2 ± 1.7
	ProCa	1 week PFS	4.5 ± 0.1	2.67 ± 0.01	23.1 ± 0.6		
		3 weeks PFS	3.5 ± 0.0	2.51 ± 0.04	31.7 ± 0.4		
		Véraison	7.8 ± 0.9	2.55 ± 0.02	34.8 ± 3.5		
		Mid-ripening	18.4 ± 0.0	2.79 ± 0.06	21.7 ± 0.7	0.8 ± 0.2	3.7 ± 0.3
		Harvest	22.8 ± 1.3	2.99 ± 0.11	13.1 ± 1.5	0.8 ± 0.1	4.9 ± 0.9
	ProCa+Phe	1 week PFS	4.6 ± 0.3	2.66 ± 0.02	23.5 ± 0.9		
		3 weeks PFS	3.5 ± 0.0	2.48 ± 0.02	32.8 ± 0.5		
		Véraison	7.0 ± 0.0	2.59 ± 0.01	37.5 ± 0.4		
		Mid-ripening	18.3 ± 0.3	2.84 ± 0.04	21.8 ± 0.2	0.7 ± 0.1	4.5 ± 0.4
		Harvest	22.6 ± 0.9	3.03 ± 0.08	13.2 ± 0.7	1.0 ± 0.1	5.4 ± 1.6
2023	CTL	1 week PFS	4.4 ± 0.4	2.72 ± 0.05	16.4 ± 1.1		
		3 weeks PFS	3.4 ± 0.0	2.59 ± 0.01	33.9 ± 0.5		
		Véraison	8.3 ± 1.0	2.62 ± 0.02	37.7 ± 0.6		
		Mid-ripening	15.7 ± 0.4	2.81 ± 0.05	19.8 ± 1.2	1.0 ± 0.3	2.4 ± 0.4
		Harvest	22.4 ± 1.1	3.00 ± 0.04	11.9 ± 0.2	0.8 ± 0.2	2.3 ± 0.7
	Phe	1 week PFS	4.2 ± 0.1	2.71 ± 0.01	19.1 ± 2.3		
		3 weeks PFS	3.4 ± 0.0	2.60 ± 0.02	34.4 ± 0.4		
		Véraison	8.8 ± 0.4	2.65 ± 0.01	37.6 ± 1.5		
		Mid-ripening	15.5 ± 0.4	2.79 ± 0.02	21.9 ± 0.7	1.0 ± 0.1	2.3 ± 0.3
		Harvest	22.1 ± 1.6	3.00 ± 0.05	12.3 ± 0.4	1.0 ± 0.4	2.3 ± 0.7
	ProCa	1 week PFS	4.2 ± 0.1	2.73 ± 0.04	17.4 ± 2.1		
		3 weeks PFS	3.4 ± 0.0	2.59 ± 0.04	34.1 ± 0.6		
		Véraison	9.0 ± 0.5	2.62 ± 0.03	38.0 ± 1.7		
		Mid-ripening	15.5 ± 0.1	2.79 ± 0.00	18.5 ± 5.1	1.0 ± 0.1	2.1 ± 0.2
		Harvest	22.7 ± 1.3	2.98 ± 0.07	12.5 ± 0.7	1.0 ± 0.2	2.7 ± 0.7
	ProCa+Phe	1 week PFS	4.0 ± 0.3	2.70 ± 0.02	18.6 ± 3.8		
		3 weeks PFS	3.4 ± 0.0	2.60 ± 0.03	34.5 ± 0.4		
		Véraison	7.9 ± 0.6	2.64 ± 0.04	36.7 ± 0.8		
		Mid-ripening	15.1 ± 0.2	2.79 ± 0.03	21.0 ± 2.1	1.2 ± 0.1	2.2 ± 0.3
		Harvest	21.3 ± 1.0	3.01 ± 0.04	12.7 ± 1.1	1.3 ± 0.3	2.7 ± 0.5

PFS: post fruit set, TA: titratable acidity expressed as tartaric acid equivalents, AU: Absorbance unit. Time point (GDD: 2022, 2023): 1 week PFS (582, 503), 3 weeks PFS (751, 731), *véraison* (1000, 903), mid-ripening (1191, 1157), harvest (1389, 1456).

TABLE 2. Three-way analysis of variance *p*-values (α = 0.05) for the effects of year, treatment, and their interactions on basic chemical parameters in juice at each phenological time point.

		p-value(sign of regression coefficient)						
Parameter	**Time point**	**Year**	**ProCa**	**Phe**	**ProCa+Phe**	**Year2023:ProCa**	**Year2023:Phe**	**Year2023:ProCa:Phe**
Soluble solids	1 week PFS	< 0.0001(–)	n.s.	n.s.	n.s.	0.0433(–)	n.s.	n.s.
	3 weeks PFS	< 0.0001(–)	0.0377(–)	n.s.	n.s.	< 0.0001(+)	< 0.0001(+)	< 0.0001(–)
	Véraison	< 0.0001(+)	n.s.	n.s.	n.s.	n.s.	n.s.	0.0313(–)
	Mid-ripening	< 0.0001(–)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Harvest	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
pH	1 week PFS	< 0.0001(+)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	3 weeks PFS	< 0.0001(+)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Véraison	< 0.0001(+)	n.s.	n.s.	n.s.	n.s.	n.s.	0.0026(–)
	Mid-ripening	< 0.0001(–)	n.s.	n.s.	n.s.	0.0154(+)	0.0005(–)	n.s.
	Harvest	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Titratable acidity	1 week PFS	< 0.0001(–)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	3 weeks PFS	< 0.0001(+)	n.s.	n.s.	n.s.	< 0.0001(+)	n.s.	0.0006(–)
	Véraison	0.0133(+)	n.s.	n.s.	n.s.	n.s.	0.0461(+)	0.0294(–)
	Mid-ripening	0.0244(–)	n.s.	n.s.	n.s.	n.s.	0.0236(+)	n.s.
	Harvest	0.0014(–)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hue	Mid-ripening	< 0.0001(+)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Harvest	n.s.	n.s.	0.0185(+)	n.s.	n.s.	n.s.	n.s.
Colour intensity	Mid-ripening	< 0.0001(–)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Harvest	< 0.0001(–)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

PFS: post fruit set, n.s.: not significant. Time point (GDD: 2022, 2023): 1 week PFS (582, 503), 3 weeks PFS (751, 731), *véraison* (1000, 903), mid-ripening (1191, 1157), harvest (1389, 1456).

and less rainfall occurred in 2023, similar to the findings of Blank *et al.* (2019). From one week PFS, IRP concentration decreased through harvest, and IRP concentration was lower in 2023 for each time point. Although the interaction effects of Year2023:ProCa, Year2023:Phe, and Year2023:ProCa:Phe were significant at various time points, individual ProCa and Phe treatments did not have any significant effects on juice IRP concentrations at any time point (Table 3), which means year had more of an effect than treatment at the current application rates.

Similar to juice IRP concentration, juice tannin concentration was highest at one week PFS (Figure 2). At three weeks PFS, the tannin concentration decreased by 10- to 20-fold compared to one week PFS. At *véraison* and later, the juice tannin concentration was below the limit of quantification (< 35 mg/L) for all the treatments (Figure 2). Year had a significant negative effect at every time point, except *véraison* (Table 3), with lower juice tannin concentration in 2023 than in 2022. Also similar to juice IRP concentration, the effects of Year2023:ProCa and Year2023:Phe were significant at various time points; however, individual treatments showed no effect on juice tannin concentration (Table 3).

The low doses of Phe and ProCa used in this study may explain why limited effects on IRP and tannin concentrations were observed in juices. However, short contact time between juice, skins, and seeds during juice sample preparation may also be a factor, as other studies reported limited changes to total polyphenol index, total anthocyanins, and total flavanols in juice from Tempranillo and Grenache cultivars at harvest after application of Phe (Portu, *et al.*, 2015b; Portu, *et al.*, 2017a). Contrary to the present study, ProCa application on vines has been shown to increase total phenols and anthocyanins concentrations in Cabernet-Sauvignon and Cabernet franc

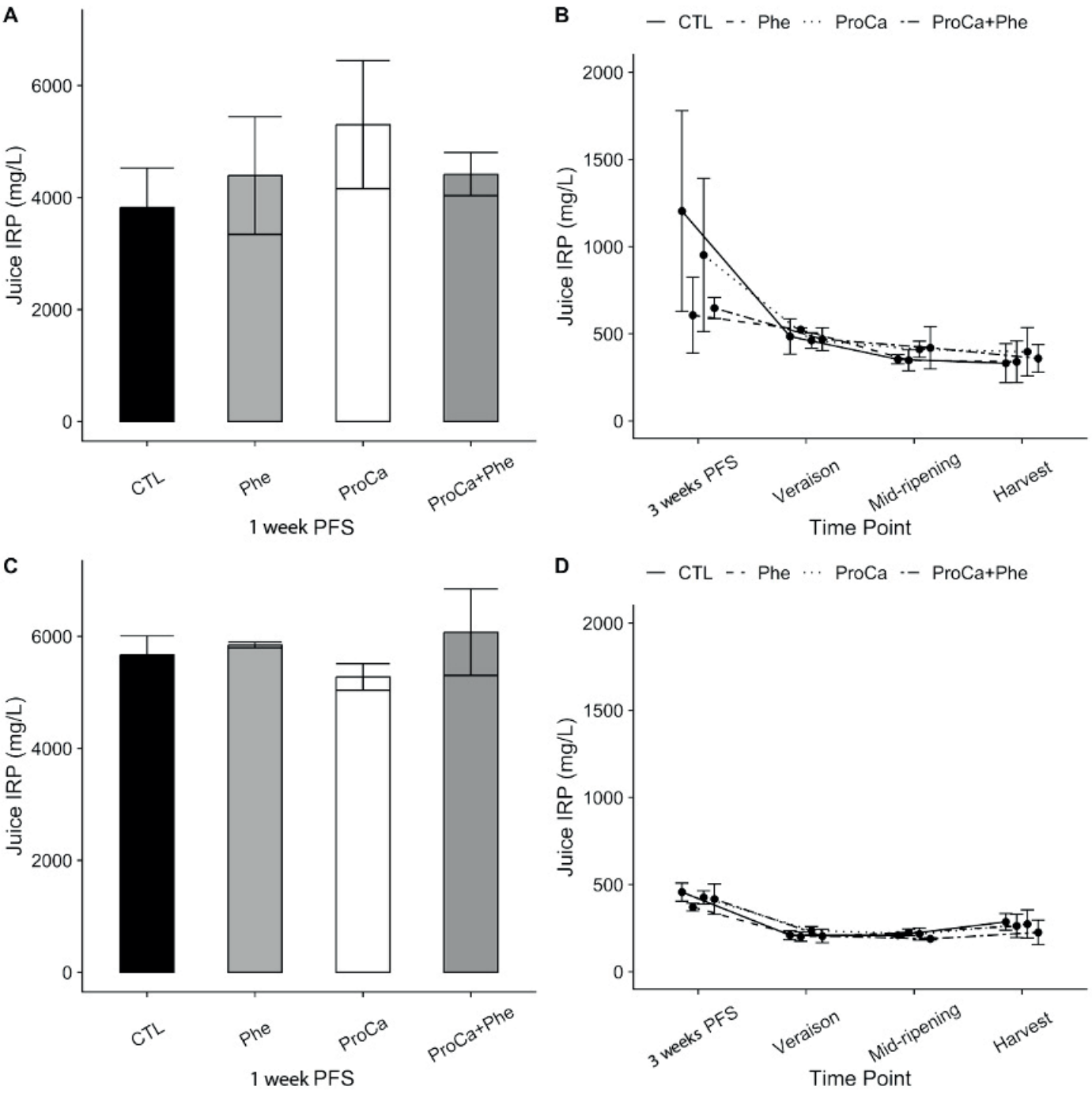


FIGURE 1. Iron-reactive phenolics (IRP) concentration, expressed as (+)-catechin equivalents, in juice from four treatments across five time points in 2022 (A, B) and 2023 (C, D). PFS: post fruit set. Time point (GDD: 2022, 2023): one week PFS (582, 503), three weeks PFS (751, 731), *véraison* (1000, 903), mid-ripening (1191, 1157), harvest (1389, 1456).

juices (LoGiudice *et al.*, 2004), in Beauty Seedless juices (Mhetre *et al.*, 2021), and in Grenache and Tempranillo juices (Avizcuri-Inac *et al.*, 2013), with no impact on total tannin concentrations. These results could indicate that higher doses than five applications of 50 mg/L per year of ProCa are needed to improve extraction of phenolic compounds in Marquette juices. Additionally, research on apples and pears (Halbwirth *et al.*, 2002; Roemmelt *et al.*, 2003), hops (*Humulus lupulus,* Kavalier *et al.,* 2014), and tomato (Ghiasi & Razavi, 2013) have demonstrated changes in activities of enzymes involved in phenolics biosynthesis. Specifically, Halbwirth *et al.* (2002) reported ProCa blocking formation of common flavonoids by inhibiting flavanone-3-hydroxylase (FHT), which means that production of leucocyanidins, and thus flavan-3-ols, is decreased, despite enhanced dihydroflavonol reductase (DFR) activity. Roemmelt *et al.* (2003) found a similar inhibition of FHT leading to higher hydroxycinnamic acids and lower falvan-3-ols and flavonols. Complementarily, when ProCa was applied to hops, researchers found an increase in metabolic

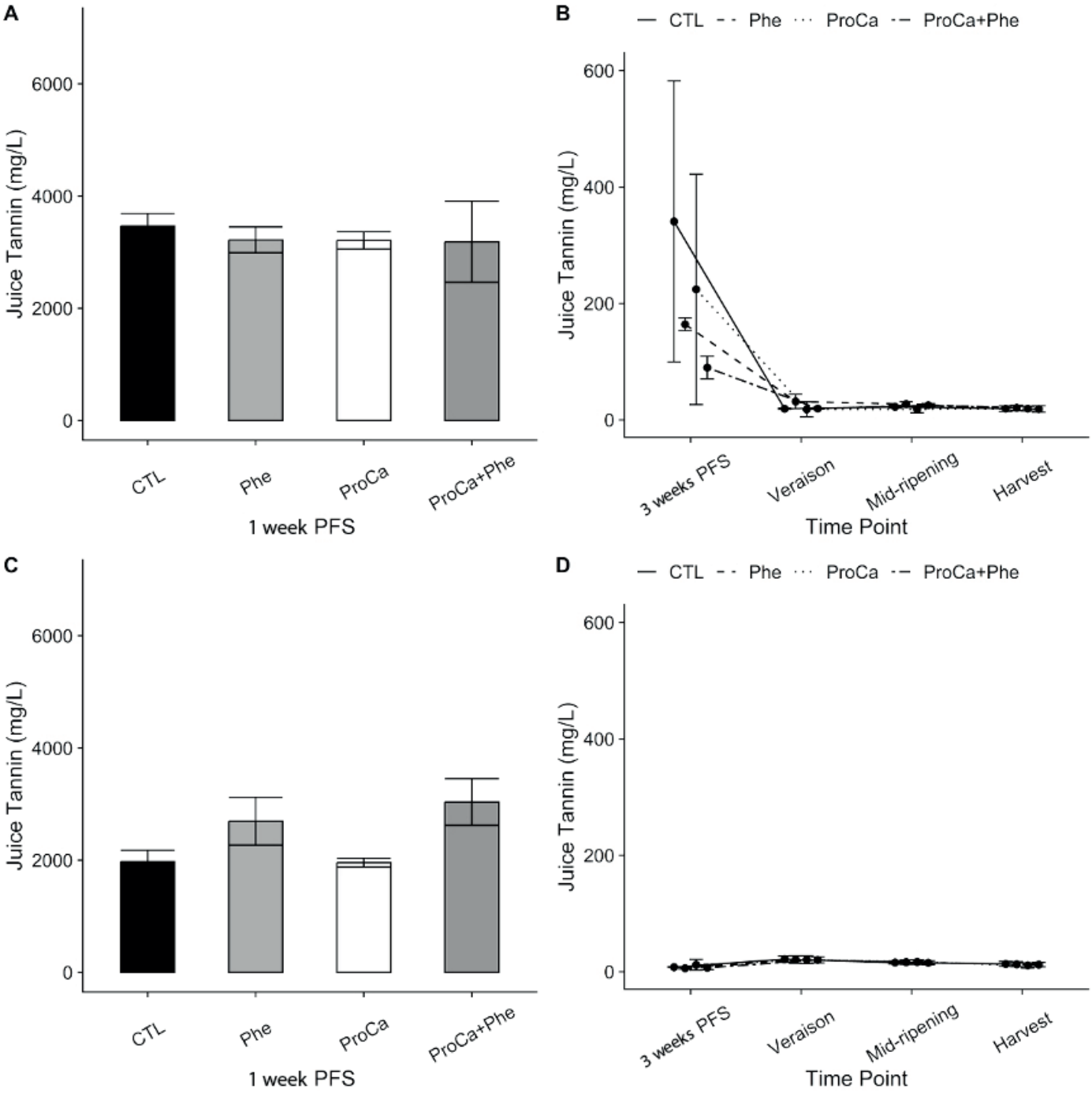


FIGURE 2. Tannin concentration, expressed as (–)-epicatechin equivalents, in juice from four treatments across five time points in 2022 (A, B) and 2023 (C, D). PFS: post fruit set. Time point (GDD: 2022, 2023): one week PFS (582, 503), three weeks PFS (751, 731), *véraison* (1000, 903), mid-ripening (1191, 1157), harvest (1389, 1456).

precursors upstream from flavanon-3-hydroxylase (F3H) and decreases in flavonoid products (catechin, epicatechin, and quercetin) downstream from F3H due to F3H inhibition by ProCa. However, those differences were only present 7 and 15 days after treatment, and concentrations of the same metabolites returned to that of the control 22 days following treatment (Kavalier *et al.*, 2014). Additionally, Ghiasi and Razavi (2013) reported increased phenylalanine ammonia-lyase (PAL) activity but no difference in total phenols content in tomato treated post-harvest with ProCa.

3. Basic chemical parameters of wines

As expected, pH in wines increased from harvest to bottling, and pH did not change after ageing (Table 4). The effect of year was significant with higher pH at bottling in 2022 (3.34 to 3.47) than in 2023 (3.23 to 3.29). A decrease in TA was observed between harvest and bottling, and TA was significantly affected by time point, year*time point, and year*time point*treatment. This indicates the winemaking process (*e.g.*, cold stabilisation) changed TA more than treatment with ProCa or Phe, at least at the current doses.

TABLE 3. Three-way analysis of variance *p*-values (α = 0.05) for the effects of year, treatment, and their interactions on iron-reactive phenolics (IRP) and tannin concentrations in juice at each phenological time point.

		p- value(sign of regression coefficient)						
Parameter	**Time point**	**Year**	**ProCa**	**Phe**	**ProCa+Phe**	**Year2023:ProCa**	**Year2023:Phe**	**Year2023:ProCa:Phe**
IRP	1 week PFS	< 0.0001(+)	n.s.	n.s.	n.s.	0.0160(–)	n.s.	0.0034(+)
	3 weeks PFS	< 0.0001(–)	n.s.	n.s.	n.s.	n.s.	0.0017(+)	n.s.
	Véraison	< 0.0001(–)	n.s.	n.s.	n.s.	0.0139(+)	0.0448(–)	n.s.
	Mid-ripening	< 0.0001(–)	n.s.	n.s.	n.s.	0.0116(–)	n.s.	n.s.
	Harvest	0.0009(–)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Tannin	1 week PFS	< 0.0001(–)	n.s.	n.s.	n.s.	0.0002(+)	< 0.0001(+)	n.s.
	3 weeks PFS	< 0.0001(–)	n.s.	n.s.	n.s.	0.0433(+)	0.0025(+)	n.s.
	Véraison	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Mid-ripening	< 0.0001(–)	n.s.	n.s.	n.s.	n.s.	0.0074(–)	n.s.
	Harvest	< 0.0001(–)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

PFS: post fruit set, n.s.: not significant.

TABLE 4. Basic chemical and colour parameters in wine at bottling and after ageing, and three-way analysis of variance *p*-values (α = 0.05) for the effects of year, treatment, and their interactions.

Year	**Time point**	**Treatment**	**pH**	**TA (g/L)**	**Ethanol (vol %)**	**Hue**	**Colour intensity (Au)**
2022	Bottling	CTL	3.40 ± 0.13	8.3 ± 1.5	11.4 ± 0.4	0.6 ± 0.1	10.0 ± 2.3
		Phe	3.47 ± 0.15	8.6 ± 0.6	11.1 ± 0.3	0.6 ± 0.2	8.7 ± 2.1
		ProCa	3.34 ± 0.09	9.2 ± 0.2	11.4 ± 1.0	0.5 ± 0.1	10.9 ± 0.6
		ProCa+Phe	3.47 ± 0.08	9.3 ± 1.1	11.2 ± 0.4	0.6 ± 0.1	8.5 ± 1.0
	Ageing	CTL	3.45 ± 0.13	8.3 ± 1.1	nd	0.7 ± 0.1	9.7 ± 1.7
		Phe	3.42 ± 0.09	8.6 ± 0.7	nd	0.7 ± 0.2	8.6 ± 1.5
		ProCa	3.38 ± 0.09	9.0 ± 0.2	nd	0.6 ± 0.1	10.3 ± 0.8
		ProCa+Phe	3.41 ± 0.09	8.2 ± 0.2	nd	0.7 ± 0.1	8.7 ± 0.9
2023	Bottling	CTL	3.23 ± 0.07	9.0 ± 0.2	12.6 ± 0.9	0.5 ± 0.1	12.6 ± 2.1
		Phe	3.25 ± 0.12	9.0 ± 0.6	12.5 ± 0.9	0.5 ± 0.1	12.3 ± 3.3
		ProCa	3.29 ± 0.10	8.7 ± 0.6	12.4 ± 1.1	0.5 ± 0.1	10.9 ± 4.3
		ProCa+Phe	3.29 ± 0.08	8.7 ± 0.5	11.9 ± 0.5	0.5 ± 0.1	9.9 ± 2.3
	Ageing	CTL	3.23 ± 0.06	9.7 ± 0.4	nd	0.6 ± 0.0	11.9 ± 2.2
		Phe	3.27 ± 0.14	9.7 ± 0.7	nd	0.6 ± 0.1	11.2 ± 3.0
		ProCa	3.30 ± 0.10	9.3 ± 0.5	nd	0.6 ± 0.1	10.5 ± 3.3
		ProCa+Phe	3.32 ± 0.09	9.7 ± 1.1	nd	0.6 ± 0.1	9.4 ± 1.9
Year			< 0.0001	n.s.	< 0.0001	–	–
Time point			< 0.0001	< 0.0001		< 0.0001	< 0.0001
Treatment			n.s.	n.s.	n.s.	–	–
Year*time point			< 0.0001	< 0.0001		0.0105	< 0.0001
Year*treatment			0.0393	n.s.	n.s.	–	–
Time point*treatment			n.s.	n.s.		n.s.	n.s
Year*time point*treatment			n.s.	0.0369		n.s.	n.s

TA: titratable acidity expressed as tartaric acid equivalents, AU: Absorbance unit, n.s.: not significant, nd: not determined, –: missing data resulted in lost degrees of freedom and no viable test.

TABLE 5. Three-way analysis of variance *p*-values (α = 0.05) for the effects of year, time point, treatment, and their interactions on iron-reactive phenolics (IRP) and tannin concentrations in wine.

Parameter	Year	Time point	Treatment	Year*time point	Year*treatment	Time point*treatment	Year*time point*treatment
IRP	< 0.0001	< 0.0001	0.0005	< 0.0001	0.0186	n.s.	n.s.
Tannin	< 0.0001	< 0.0001	0.0358	< 0.0001	0.006	n.s.	n.s.

n.s.: not significant.

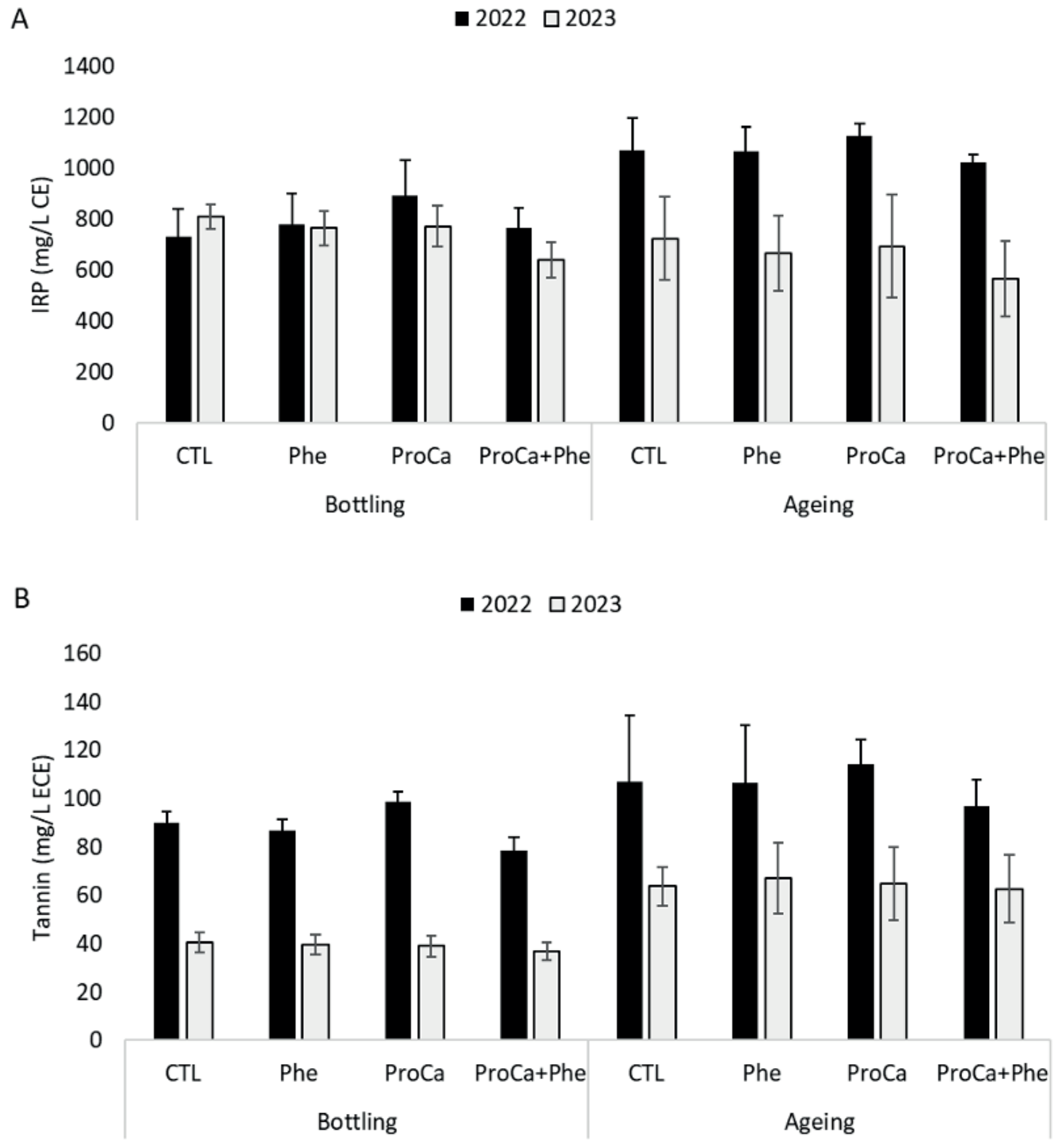


FIGURE 3. Iron-reactive phenolics (IRP) (A) and tannin (B) concentrations, expressed as (–)-epicatechin and (+)-catechin equivalents, respectively, in wine from four treatments at bottling and after ageing in 2022 and 2023.

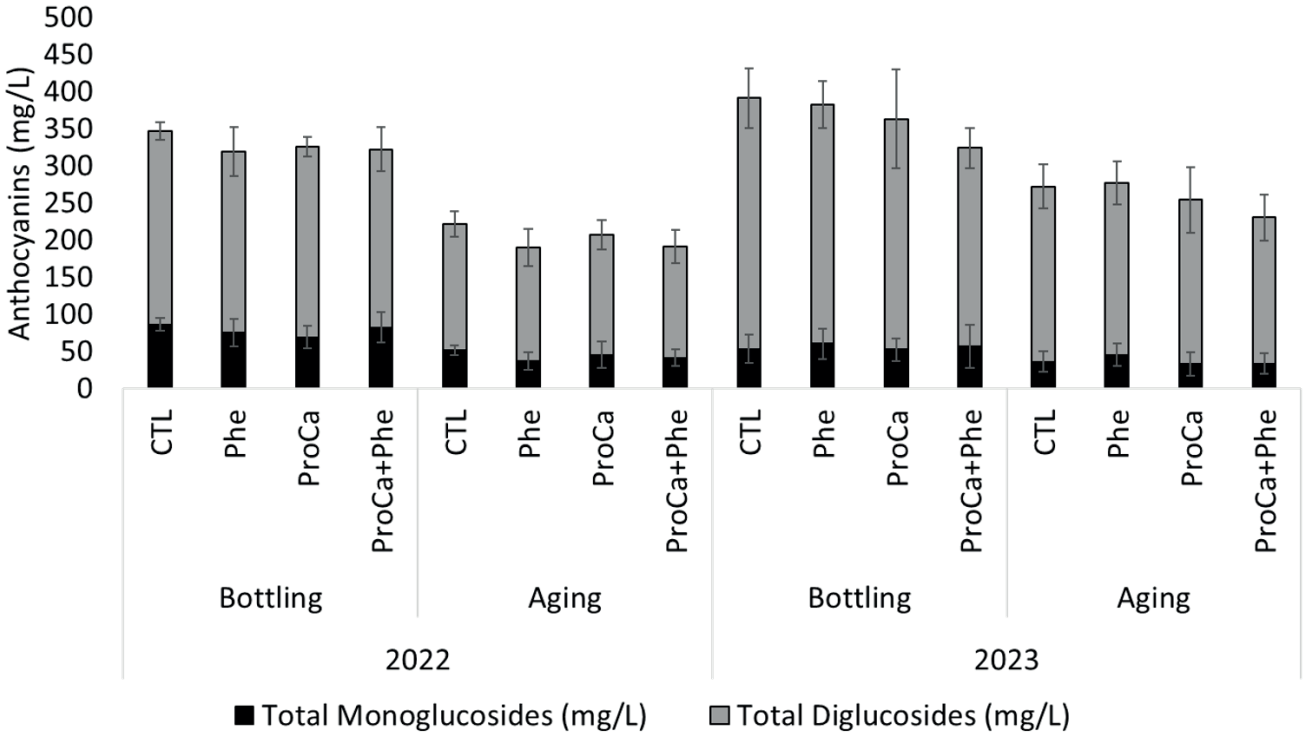


FIGURE 4. Anthocyanins in wine at bottling and ageing in 2022 and 2023. Anthocyanins monoglucosides expressed as malvidin-3-*O*-glucoside equivalents. Anthocyanin diglucosides expressed as malvidin-3,5-*O*-diglucoside equivalents.

TABLE 6. Three-way analysis of variance *p*-values (α = 0.05) for the effects of year, time point, treatment, and their interactions on anthocyanin concentration in wine at bottling and after ageing.

Parameter	Year	Time point	Treatment	Year*time point	Year*treatment	Time point*treatment	Year*time point*treatment
Total monoglucosides	< 0.0001	< 0.0001	n.s.	0.0266	n.s.	n.s	n.s.
Total diglucosides	< 0.0001	< 0.0001	0.0023	n.s.	n.s.	n.s.	n.s.
Total anthocyanins	< 0.0001	< 0.0001	0.0193	n.s.	n.s.	n.s.	n.s.

n.s.: not significant.

Ethanol concentration was significantly higher in 2023; however, as mentioned previously, soluble solids were not affected by year at harvest. It is likely that the difference in ethanol concentration was due to a one-day-longer fermentation in 2023 than 2022. Hue and colour intensity were affected by time point and year*time point, with higher hue and lower colour intensity after ageing than at bottling (Table 4). However, treatment had no effect, similar to previous reports considering Phe applications (Portu, *et al.*, 2015a).

4. Iron-reactive phenolics, tannins, and anthocyanins concentrations in wines

Year had a significant effect on wine IRP and tannin concentrations (Table 5), which were higher in 2022 than in 2023 (Figure 3). The effect of time point was also significant with the highest IRP and tannin concentrations after ageing. The wines made from ProCa treated vines had the highest IRP and tannin concentrations, while the ProCa+Phe wines had the lowest concentrations across both years. Interestingly, the IRP concentration – averaged over year and time point – in the CTL wines was not different from either the Phe or ProCa treatments, but it was significantly higher than the ProCa+Phe treatment. Additionally, ProCa wines (72 mg/L) had significantly higher tannin concentration than ProCa+Phe wines (63 mg/L), but no treatment (including Phe, 68 mg/L) was significantly different from the CTL (67 mg/L).

Total anthocyanins were higher at bottling (347 mg/L) than after ageing (230 mg/L), with overall higher concentration in 2023 (312 mg/L) than 2022 (265 mg/L, Figure 4). Coupled with the changes observed for hue and colour intensity, the decrease in anthocyanins with ageing indicates formation of polymeric pigments over time (Picariello *et al.*, 2017). Interestingly, the effect of treatment on total anthocyanins was significant (Table 6) with the highest concentration present in the CTL wines (308 mg/L), followed by Phe,

ProCa, and ProCa+Phe (292, 287, and 267 mg/L, respectively, Figure 4). However, only the CTL and ProCa+Phe treatments were significantly different from each other. It is possible that there is an antagonistic effect between ProCa and Phe when both compounds are applied, similar to what has been previously proposed between Phe and methyl jasmonate (Portu, *et al.*, 2017a). However, questions remain about exactly what that interaction may be. It is possible that the effects ProCa has on the phenylpropanoid pathway enzymes inhibited the ability for the added Phe to be converted to phenylpropanoid end products.

Previous work has shown limited or negative influence of foliar-applied nitrogen on wine phenolics concentrations when vine nitrogen status is within normal ranges and YAN is above threshold values (> 140 mg N/L), as they were in this study (Gapinski *et al.*, 2024). For example, Cheng *et al.* (2021) concluded that foliar nitrogen supplementation is only beneficial in nitrogen-deficient vineyards, while Gutiérrez-Gamboa *et al.* (2017) found that flavonoid concentration in Cabernet-Sauvignon wines decreased, because YAN was sufficient. The short half-life of ProCa in plants (a few weeks) may have limited effectiveness at influencing phenolics concentration in grape berries by harvest, and thus the finished wines, since the final treatment of ProCa occurred about one month before harvest (Kavalier *et al.*, 2014). Further, ProCa is a gibberellin inhibitor, which limits cell expansion, and may have affected cell wall porosity by thickening cell walls, thus limiting phenolics extraction. A previous study by Bindon *et al.* (2014) described changes in tannin content in grape skin extracts attributed to the degradation of the middle lamella (thus increasing surface area for tannin binding) as berries matured. Although the current dose of ProCa may not have been high enough to show an effect, the cell wall material in cold-hardy, hybrid grape cultivars may already be thicker than *V. vinifera* cultivars, resulting in lower tannin extractability, and the changes observed here could be due to different weather patterns between years leading to changes in cell wall material composition. Alternately or in combination, disruptions in enzyme activities for the normal phenylpropanoid pathway ending in flavan-3-ols by ProCa (as described above for juice) may have led to the formation of 3-deoxyflavans instead (Kavalier *et al.*, 2014; Roemmelt *et al.*, 2003).

CONCLUSION

Year and time point (both phenological and winemaking) affected basic chemical parameters of juices and wines more than treatments with Phe and/or ProCa. The same was observed for IRP and tannin concentrations. Treatment effectiveness may have been limited by low application rate (dose) or by inhibitory effects of ProCa on phenylpropanoid pathway enzymes. Although ProCa was hypothesised to act on the biosynthesis pathway, no effects were observed; therefore, interspecific cultivars such as Marquette may have some other way of regulating the pathway due to a genome that includes *V. riparia* heritage that differs from *V. vinifera*. More work is needed to better understand the effect of ProCa on those enzymes in interspecific cold-hardy grape cultivars, which would lead to insights on how to best use ProCa to modify phenolics content in those cultivars and the resulting wines. Future work will also include the use of abscisic acid as this plant hormone plays a significant role in plant stress response, which could impact biosynthesis of stress-mitigating phenolic compounds.

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REFERENCES

Avizcuri-Inac, J. M., Gonzalo-Diago, A., Sanz-Asensio, J., Martínez-Soria, M. T., López-Alonso, M., Dizy-Soto, M., Echávarri-Granado, J. F., Vaquero-Fernández, L., & Fernández-Zurbano, P. (2013). Effect of cluster thinning and prohexadione calcium applications on phenolic composition and sensory properties of red wines. *Journal of Agricultural and Food Chemistry*, *61*(5), 1124–1137. https://doi.org/10.1021/jf304257r

Bell, S. J., & Henschke, P. A. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. In *Australian Journal of Grape and Wine Research* (Vol. 11, Issue 3, pp. 242–295). Australian Society of Viticulture and Oenology. https://doi.org/10.1111/j.1755-0238.2005.tb00028.x

Bindon, K. A., Madani, S. H., Pendleton, P., Smith, P. A., & Kennedy, J. A. (2014). Factors affecting skin tannin extractability in ripening grapes. *Journal of Agricultural and Food Chemistry*, *62*(5), 1130–1141. https://doi.org/https://doi.org/10.1021/jf4050606

Blank, M., Hofmann, M., & Stoll, M. (2019). Seasonal differences in *Vitis vinifera* L. cv. Pinot noir fruit and wine quality in relation to climate. *Oeno One*, *53*(2), 189–203. https://doi.org/10.20870/oeno-one.2019.53.2.2427

Bordiga, M., Travaglia, F., Locatelli, M., Coïsson, J. D., & Arlorio, M. (2011). Characterisation of polymeric skin and seed proanthocyanidins during ripening in six *Vitis vinifera* L. cv. *Food Chemistry*, *127*(1), 180–187. https://doi.org/10.1016/j.foodchem.2010.12.141

Broeckling, C. D., Li, K.-G., & Xie, D.-Y. (2012). Comparative metabolomics of transgenic tobacco plants (*Nicotiana tabacum* var. Xanthi) reveals differential effects of engineered complete and incomplete flavonoid pathways on the metabolome. In *Transgenic Plants - Advances and Limitations* (pp. 379–396). InTech. https://doi.org/10.5772/32872

Casassa, L. F., & Harbertson, J. F. (2014). Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. *Annu. Rev. Food Sci. Technol.*, *5*, 83–109. https://doi.org/10.1146/annurev-food-030713-092438

Cheng, X., Liang, Y., Zhang, A., Wang, P., He, S., Zhang, K., Wang, J., Fang, Y., & Sun, X. (2021). Using foliar nitrogen application during veraison to improve the flavor components of grape and wine. *Journal of the Science of Food and Agriculture*, *101*(4), 1288–1300. https://doi.org/10.1002/jsfa.10782

Delgado, R., Martín, P., Del Álamo, M., & González, M. R. (2004). Changes in the phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilisation rates. *Journal of the Science of Food and Agriculture*, *84*(7), 623–630. https://doi.org/https://doi.org/10.1002/jsfa.1685

Gapinski, A. D., Horton, A. C., & Watrelot, A. A. (2023). Effect of whole cluster fermentation on phenolics in cold-hardy hybrid wines. *Food and Bioprocess Technology*. https://doi.org/10.1007/s11947-023-03010-7

Gapinski, A. D., Slack, S. M., & Watrelot, A. A. (2024). Foliar applications of phenylalanine and prohexadione calcium for managing tannin content in cold-hardy hybrid grape cultivars. *Oeno One*. https://doi.org/10.20870/oeno-one.2024.58.3.8166

Ghiasi, N., & Razavi, F. (2013). Impact of postharvest prohexadione calcium treatment on PAL activity in tomato fruit in response to chilling stress. *Iranian Journal of Plant Physiology*, *4*(1), 865–871.

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Portu, J., Moreno-Simunovic, Y., & Martínez-Gil, A. M. (2017). Foliar nitrogen application in Cabernet Sauvignon vines: Effects on wine flavonoid and amino acid content. *Food Research International*, *96*, 46–53. https://doi.org/10.1016/j.foodres.2017.03.025

Halbwirth, H., Kampan, W., Stich, K., Fischer, T. C., Meisel, B., Forkmann, G., & Rademacher, W. (2002). Biochemical and molecular biological investigations with respect to induction of Fire Blight resistance in apple and pear by transiently altering the flavanoid metabolism with specific enzyme inhibitors. *Acta Horticulturae*, *590*, 485–492. https://doi.org/10.17660/ActaHortic.2002.590.75

Hamada, M., & Wu, C. J. (1992). Analysis of designed experiments with complex aliasing. Journal of quality technology, 24(3), 130-137. https://doi.org/10.1080/00224065.1992.11979383

Harbertson, J., Kennedy, J., & Adams, D. (2002). Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot Noir berries during ripening. *American Journal of Enology and Viticulture*, *53*(1), 54–59. https://doi.org/10.5344/ajev.2002.53.1.54

Hui, Y., Wang, J., Jiang, T., Ma, T., & Wang, R. (2021). Effect of nitrogen regulation on berry quality and flavonoids during veraison stage. *Food Science & Nutrition*, *9*(10), 5448–5456. https://doi.org/10.1002/FSN3.2503

Kavalier, A. R., Ma, C., Figueroa, M., Kincaid, D., Matthews, P. D., & Kennelly, E. J. (2014). Targeted analysis of polyphenol metabolism during development of hop (Humulus lupulus L.) cones following treatment with prohexadione-calcium. *Food Chemistry*, *145*, 254–263. https://doi.org/10.1016/j.foodchem.2013.08.023

Kennedy, J. A. (2008). Grape and wine phenolics: Observations and recent findings. *Ciencia e Investigación Agraria*, *35*(2). https://doi.org/10.4067/S0718-16202008000200001

Lal, M., Sood, Y., Singh, H., Kumar, A., Wani, A. W., & Kumar, S. (2022). Influence of prohexadione-calcium on temperate fruit crops – A Review. *Ecology, Environment and Conservation*, *28*, 164–172. https://doi.org/10.53550/eec.2022.v28i07s.028

Lattanzio, V., Cardinali, A., & Linsalata, V. (2012). Plant Phenolics: A biochemical and physiological perspective. In V. Cheynier, P. Sarni-Manchado, & S. Quideau (Eds.), *Recent Advances in Polyphenol Research* (First, Vol. 3, pp. 1–40). John Wiley & Sons. https://doi.org/10.1002/9781118299753.ch1

Li, D., Yang, J., Dai, Z., Chen, Y., Shao, Z., Wang, C., Jin, X., Wang, Y., & Feng, L. (2024). Prohexadione-calcium improves grape quality by regulating endogenous hormones, sugar and acid metabolism and related enzyme activities in grape berries. *BMC Plant Biology*, *24*(1). https://doi.org/10.1186/s12870-024-04803-4

LoGiudice, D., Wolf, T. K., & Zoecklein, B. W. (2004). Effects of prohexadione-calcium on grape yield components and fruit and wine composition. *American Journal of Enology and Viticulture*, *55*(1). https://doi.org/10.5344/ajev.2004.55.1.73

Manns, D. C., Coquard Lenerz, C. T. M., & Mansfield, A. K. (2013). Impact of processing parameters on the phenolic profile of wines produced from hybrid red grapes Maréchal Foch, Corot noir, and Marquette. *Journal of Food Science*, *78*(5). https://doi.org/10.1111/1750-3841.12108

Metay, A., Magnier, J., Guilpart, N., & Christophe, A. (2015). Nitrogen supply controls vegetative growth, biomass and nitrogen allocation for grapevine (cv. Shiraz) grown in pots. *Functional Plant Biology*, *42*(1), 105–114. https://doi.org/10.1071/FP14062

Mhetre, V. B., Patel, V. B., Singh, S. K., Verma, M. K., Mishra, G. P., Dahuja, A., & Kumar, C. (2021). Effect of new generation bio-regulators on anthocyanins and berry quality of grape cv.Beauty Seedless. *Indian Journal of Agricultural Sciences*, *91*(6), 920–923. https://doi.org/10.56093/ijas.v91i6.114300

Ministry of Agriculture, F. and R. A. (2022). *Plant Growth Regulators*.

Picariello, L., Gambuti, A., Picariello, B., & Moio, L. (2017). Evolution of pigments, tannins and acetaldehyde during forced oxidation of red wine: Effect of tannins addition. *LWT - Food Science and Technology*, *77*, 370–375. https://doi.org/10.1016/J.LWT.2016.11.064

Pinheiro, J., & Bates, D. (2023). _nlme: Linear and Nonlinear Mixed Effects Models_. In *R package version 3.1-162*. https://CRAN.R-project.org/package=nlme

Portu, J., González-Arenzana, L., Hermosín-Gutiérrez, I., Santamaría, P., & Garde-Cerdán, T. (2015a). Phenylalanine and urea foliar applications to grapevine: Effect on wine phenolic content. *Food Chemistry*, *180*, 55–63. https://doi.org/10.1016/j.foodchem.2015.02.008

Portu, J., López-Alfaro, I., Gómez-Alonso, S., López, R., & Garde-Cerdán, T. (2015b). Changes on grape phenolic composition induced by grapevine foliar applications of phenylalanine and urea. *Food Chemistry*, *180*, 171–180. https://doi.org/10.1016/j.foodchem.2015.02.042

Portu, J., López, R., Santamaría, P., & Garde-Cerdán, T. (2017a). Elicitation with methyl jasmonate supported by precursor feeding with phenylalanine: Effect on Garnacha grape phenolic content. *Food Chemistry*, *237*, 416–422. https://doi.org/10.1016/j.foodchem.2017.05.126

Portu, J., Santamaría, P., López, R., & Garde-Cerdán, T. (2017b). Phenolic composition of Tempranillo grapes following foliar applications of phenylalanine and urea: A two-year study. *Scientia Horticulturae*, *219*, 191–199. https://doi.org/10.1016/j.scienta.2017.03.014

Rademacher, W. (2016). Chemical regulators of gibberellin status and their application in plant production. In *Annual Plant Reviews: The Gibberellins* (Vol. 49, pp. 359–404). John Wiley and Sons, Ltd. https://doi.org/10.1002/9781119210436.ch12

Reinehr, J., Bogo, A., Rufato, L., Casa, R. T., Silva, F. N., Kretzchmar, A. A., Furini, G., & Nerbass, F. R. (2021). Prohexadione-calcium on downy mildew and botrytis bunch rot epidemiology, and yield and technological and phenolic parameters of the merlot vine cultivar in the highland region of southern Brazil. *Ciencia Rural*, *51*(7). https://doi.org/10.1590/0103-8478cr20200480

Ritchey, J. G., & Waterhouse, A. L. (1999). A standard red wine: Monomeric phenolic analysis of commercial Cabernet Sauvignon wines. *American Journal of Enology and Viticulture*, *50*(1), 91–100. https://doi.org/https://doi.org/10.5344/ajev.1999.50.1.91

Roemmelt, S., Zimmermann, N., Rademacher, W., & Treutter, D. (2003). Formation of novel flavonoids in apple (Malus × domestica) treated with the 2-oxoglutarate-dependent dioxygenase inhibitor prohexadione-Ca. *Phytochemistry*, *64*(3), 709–716. https://doi.org/10.1016/S0031-9422(03)00389-3

Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L. T., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S., Kartschall, T., Menz, C., Molitor, D., Junk, J., Beyer, M., & Schultz, H. R. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. In *Applied Sciences (Switzerland)* (Vol. 10, Issue 9). MDPI AG. https://doi.org/10.3390/app10093092

Springer, L. F., & Sacks, G. L. (2014). Protein-precipitable tannin in wines from *Vitis vinifera* and interspecific hybrid grapes (*Vitis* ssp.): Differences in concentration, extractability, and cell wall binding. *Journal of Agricultural and Food Chemistry*, *62*, 7515–7523. https://doi.org/10.1021/jf5023274

Thomidis, T., Zioziou, E., Koundouras, S., Navrozidis, I., & Nikolaou, N. (2018). Effect of prohexadione-Ca on leaf chlorophyll content, gas exchange, berry size and composition, wine quality and disease susceptibility in *Vitis vinifera* L. cv Xinomavro. *Scientia Horticulturae*, *238*, 369–374. https://doi.org/10.1016/J.SCIENTA.2018.05.008

Vaquero-Fernandez, L., Fernandez-Zurbano, P., Sanz-Asensio, J., Lopez-Alonso, M., & Martinez-Soria, M. T. (2009). Treatment of grapevines with prohexadione calcium as a growth regulator. The influence on production, winemaking and sensory characteristics of wines. In *Int. Sci. Vigne Vin* (Vol. 43). https://doi.org/10.20870/oeno-one.2009.43.3.788

Watrelot, A. A., & Norton, E. L. (2020). Chemistry and reactivity of tannins in *Vitis* spp.: A Review. *Molecules*, *25*(9), 2110. https://doi.org/10.3390/molecules25092110

Watrelot, A. A., Heymann, H., & Waterhouse, A. L. (2019). Red wine dryness perception related to physicochemistry. https://doi.org/10.1021/acs.jafc.9b01480