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ORIGINAL RESEARCH ARTICLE

Delaying grapevine budbreak to prevent spring freeze damage impacts Lemberger wine flavour compounds under variable weather conditions

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ABSTRACT

Delaying grapevine budbreak through viticultural management practices is a promising method to prevent spring freeze damage for small vineyards. However, in cool-climate regions, delaying budbreak can potentially delay fruit development and maturation, negatively impacting wine quality. In this three-year study, 2017–2019, we evaluated the impacts of delaying budbreak on wine volatile and nonvolatile composition for *Vitis vinifera* c.v. Lemberger at a cool-climate site, and we related treatment impacts on wine composition to consumer perception. We also assessed if treatment impacts were similar across vintages, or if seasonal weather conditions were more important for wine composition than delaying budbreak. We evaluated four treatments each year: a control (C) (no delayed budbreak strategy applied), a vegetable oil-based adjuvant (Amigo®) applied at either 8 % or 10 % (v/v) concentration during dormancy (A8, A10), and late pruning conducted shortly after budbreak (1–4 leaves unfolded) of apical buds (LP). Delays in budbreak between treatments and C vines ranged from 5 days (A8, 2017) to 23 days (LP, 2017) across the 3 years. Furthermore, berry colour change, one of the parameters used to assess *véraison*, started later in LP vines than in C vines each year. Within each vintage, we found that delayed budbreak treatments, especially LP, had lower concentrations of several volatile compounds than C wines. Furthermore, LP wines tended to have higher monomeric anthocyanins relative to C wines, ranging from 18 % (2019) to 36 % higher (2018). Results from consumer discrimination testing broadly reflected differences in wine chemical composition: consumers perceived LP wines as different from C or A8, depending on the year, and all treatments were different in 2019. However, we found that vintage impacted wine composition more than the delayed budbreak treatments. Of the 49 volatile and nonvolatile compounds detected every year, about half showed significant vintage effects, while only three were consistently different by treatment. Together, our results suggest that delaying grapevine budbreak at a cool-climate site can impact wine chemical composition and perception, especially if phenological delays are still present around *véraison*; however, seasonal weather conditions remain a more important driver of wine chemical composition than relatively small changes in phenological development.

KEYWORDS: freeze avoidance, phenology, climate change, wine composition, sensory perception

INTRODUCTION

Spring freeze events are a global challenge to grape production (Ault *et al*., 2013; Gu *et al*., 2008; Vitasse and Rebetez, 2018). As grape buds emerge from dormancy in spring, temperatures close to or below freezing can damage green tissues (Johnson and Howell, 1981), decreasing yield potential and compromising fruit quality by harvest (Friend *et al*., 2011; Persico *et al*., 2021). Bud freeze damage may become more frequent if global warming accelerates budbreak without a concurrent decrease in spring freeze events (Ault *et al*., 2013; Leolini *et al*., 2018). One promising strategy to prevent freeze damage is to delay budbreak, for example by applying chemical products during dormancy (Centinari *et al*., 2018; Wang and Dami, 2020) or by postponing winter pruning until after budbreak (i.e., "late-pruning") (Howell and Wolpert, 1978). While delaying budbreak is effective at mitigating freeze damage (Friend *et al*., 2011; Persico *et al*., 2021), it can also delay the onset of fruit development (e.g., bloom, fruit-set) (Friend and Trought, 2007) and maturation (Moran *et al*., 2017; Morgani *et al*., 2023; Petrie *et al*., 2017), leading to lower sugar accumulation and higher acidity in grapes at harvest (Frioni *et al*., 2016). Delaying budbreak to postpone the onset of fruit development and maturation is desired in warm climates, where high temperatures can compromise fruit and wine quality (Sadras *et al*., 2013; Sweetman *et al*., 2014). However, in cool climates where low temperatures constrain the length of the growing season, delaying budbreak may hinder the accumulation of berry primary and secondary metabolites necessary for optimum wine quality.

Berry metabolites, which develop and accumulate during the growing season, impart wine flavour properties related to sensory perception and quality (Charters and Pettigrew, 2007; Ferreira, 2010; Hopfer *et al*., 2015). Abiotic factors, primarily temperature, dictate the progression of vine phenology and berry development stages (Parker *et al*., 2011; van Leeuwen *et al*., 2004) and consequently influence the accumulation of berry metabolites (Coombe, 1973; Kalua and Boss, 2009; Kennedy *et al*., 2001). It is possible that delaying budbreak to prevent spring freeze damage changes the berry metabolite profile by shortening the time between the onset of berry development and harvest. For example, delaying budbreak may affect terpenes, which begin to accumulate pre-*véraison* and are involved in berry aroma (Kalua and Boss, 2009; Matarese *et al*., 2014; Yang *et al*., 2011), and anthocyanins, responsible for pigmentation, which accumulate starting at *véraison* (Boss *et al*., 1996).

In previous work conducted in warm climates, delaying fruit maturation into cooler late-summer temperatures slowed berry sugar accumulation and organic acid degradation (Frioni *et al*., 2016), increased wine anthocyanin and tannin concentration (Moran *et al*., 2018, 2021), and improved wine sensory characteristics (Moran *et al*., 2018). However, there is limited data on how potential shifts in fruit development and maturation in cool-climate regions may affect wine sensory properties and perception. Our research group previously investigated the effects of applying a chemical spray product and late-pruning on budbreak, freeze damage, yield parameters, and basic wine chemistry of Lemberger (*Vitis vinifera*) vines over two years at a cool-climate site (2018 and 2019; Persico *et al*., 2021). We found that delayed budbreak treatments delayed 50 % budbreak between 5 and 10 days and did not affect basic fruit and wine chemistry at harvest; however, late-pruned vines had less berry colour change than control vines when assessed on the same day during *véraison* each year (Suppl. Table 1). These visual colour differences suggested a potential delay in berry compound accumulation related to ripening that went uncharacterised with basic fruit and wine chemistry measurements. Furthermore, in the pilot year of our prior study, 2017 (unpublished data), late pruning was applied at a later phenological stage, and budbreak was delayed up to 23 days, which was over twice as much as the longest delay in 2018 and 2019. This 23-day delay in budbreak led to an even more extensive delay in the onset of *véraison* and primary metabolite concentrations for late-pruned vines in 2017 than in the subsequent two years (Suppl. Table 1).

In this study, we evaluated the impact of the same techniques to delay budbreak reported in Persico *et al.* (2021) on volatile and nonvolatile compounds in finished Lemberger wines over three years, from 2017-2019, and we related the impacts on wine composition to consumer perception. We assessed how delaying budbreak and the onset of fruit ripening impact wine chemical composition and whether outcomes of delaying budbreak are consistent over years, or if they mainly depend on factors such as seasonal weather. We hypothesised that, among vintages, seasonal weather metrics would more strongly impact overall wine composition than delaying key phenological stages (i.e., budbreak and *véraison*), regardless of the extent of the delay. Within each vintage, we hypothesised that wines made from delayed budbreak treatments would have lower concentrations of compounds that synthesise pre-*véraison* (e.g., terpenes) and those associated with berry ripening (e.g., anthocyanins), especially if there was still a delay at *véraison*. We expected that if delayed budbreak affected wine composition, consumers could perceive sensory differences between treatments.

MATERIALS AND METHODS

1. Experimental design

The grapevines used in this experiment were *Vitis vinifera* cv. Lemberger scion grafted onto 101-14 Mgt rootstock. The vines were located at a commercial vineyard in Lewisburg, Pennsylvania, USA (40°57′N; 76°53′W) and planted in 2010. A weather station at the vineyard collected hourly air temperature, solar radiation, and rainfall data. Daily growing degree days (GDD, base 10 °C) were calculated as GDD = [maximum daily temperature + minimum daily temperature)/2] – 10), and total daily cumulative solar exposure (CSE) was calculated by summing hourly solar radiation averages (MJ/m^2) as in Harner *et al*. (2019).

Cumulative GDD, CSE, and rainfall (mm) were calculated monthly, for the whole growing season (1 May to harvest), and for the fruit ripening period (1 Aug to harvest) each year. Weather data and phenology measurements (discussed below) were used to calculate GDD, CSE, and rainfall between key phenological stages (e.g., budbreak to harvest) for each treatment.

The experimental design was a randomised complete block design with four treatments and six replications per treatment. The treatments were: (1) control (no delayed budbreak strategy applied; "C"); (2) Amigo® (Loveland Products, Inc), a vegetable-oil based adjuvant, applied at 8 % (v/v) concentration during dormancy ("A8"); (3) Amigo® applied at 10 % (v/v) concentration during dormancy ("A10"); and (4) late pruning (LP) applied shortly after budbreak. In 2017, LP was applied when the apical buds averaged at approximately stage 10, or "four leaves unfolded" on the Eichhorn-Lorenz (E-L) scale (Coombe, 1995). In 2018 and 2019 LP was applied when the three most-apical buds averaged at stage 7, or "first leaf separated." We applied two concentrations of Amigo® to test potential bud mortality from a higher concentration application (A10) based on results from previous work (Centinari *et al.*, 2018). More information about the experimental design and delayed budbreak treatments can be found in Persico *et al*. (2021).

Twice per week phenology measurements, using the E-L scale, started approximately one week before C vines reached 50 % budbreak (28 April 2017, 9 May 2018, and 6 May 2019) and were recorded until C vines reached at least full bloom (16 June 2017, 16 June 2018, and 18 June 2019). Detailed phenological data for the 2018 and 2019 seasons are reported by Persico *et al.* (2021). In mid-August, around *véraison*, the percentage change in berry colour was visually assessed for each cluster on the same vines selected for phenology measurements. Berry samples (100 berries per experimental unit) were collected twice before harvest to evaluate juice chemistry (total soluble solids, titratable acidity, and pH) using protocols outlined in Persico *et al.* (2021); one sampling occurred the same day as the berry colour change assessment (mid-August) and a second about three weeks later (early September). Each year, all treatments were harvested on the same date, based on the fruit maturity of C vines. The dates of harvest were 10 Oct 2017, 4 Oct 2018, and 3 Oct 2019, within a day of commercial harvest each year. The fruit was unlikely to continue maturing beyond the dates that we harvested due to canopy senescence by October at our experimental site.

2. Winemaking and wine analysis

On the day of harvest, fruit (approx. 90–100 kg per treatment) was transported to Pennsylvania State University, University Park, PA for winemaking each year. For each treatment, fruit from two adjacent field replicates was combined into one winemaking replicate (e.g., fruit from field replicates one and two combined into winemaking replicate one), resulting in three fermentation replicates per treatment. We combined adjacent field replicates because our winemaking equipment was not designed to process the volume of fruit yielded from one field replicate. For all three years, wines were made according to procedures outlined in Persico *et al.* (2021), and finished wines were stored for up to six months in coolers (~7 °C) before sensory evaluation and wine composition analysis. Several days before sensory discrimination testing, each wine was tested for obvious faults (e.g., spoilage, oxidation, etc.), and one replicate was chosen to move forward for sensory testing from C, A8, and LP. A10 was not tested due to the expected similarity with A8. A triangle test (ASTM 2011), statistically powered to also conclude sensory similarity at beta = 10 % and Pd = 20 % (Castura and Franczak, 2017; Sinkinson, 2017), was then conducted in the Sensory Evaluation Center (SEC) at Penn State. Consumers (n = ~100 each year), the majority of whom reported drinking red wine between one and three times per week, performed three triangle tests: C versus A8, C versus LP, and A8 versus LP. Wines were presented in ISO wine-tasting glasses in randomised presentation order and identified only by three-digit codes. Participants were asked to smell, taste, and expectorate the sample before selecting which wine they believed to be the odd sample out of the three presented. Water was provided for participants to rinse between individual samples and flights of wines, and data were collected using Compusense® Cloud software (Academic Consortium, Guelph, ONT, Canada). Procedures were deemed exempt by the institutional review board at Penn State (STUDY08551).

To quantify volatile compounds, wine samples were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) in analytical triplicate and reported as d8-naphthalene internal standard equivalents (ug/L) (Keller *et al*., 2021). The following 15 nonvolatile compounds related to wine quality were quantified using High Performance Liquid Chromatography (HPLC) at ETS Laboratories (St. Helena, CA) and reported in mg/L: quercetin and quercetin glycosides (flavonols), catechin and epicatechin (flavan-3-ols), *p*-coumaric acid, caftaric and caffeic acid (hydrocinnamic acids), resveratrol (stilbene), gallic acid (hydroxybenzoic acid), total tannins, malvidin glucoside, delphinidin, and peonidin (monomeric anthocyanins), total polymeric anthocyanins, and total anthocyanins (monomeric plus polymeric anthocyanins).

3. Data analysis

All data analysis was performed in the R computing environment v4.1.3 (Team, 2021). To identify differences in wine compounds among treatments and years, volatile and nonvolatile compounds were first subjected to multivariate analysis of variance (MANOVA), including "treatment," "vintage," and their interaction effect used as fixed independent effects. A large sample size (over 10 blocks) is suggested to detect differences at the 5 % level under field conditions (Marini, 1999); therefore, we elected to report values as different if $p < 0.1$. Following MANOVA, significantly different compounds were subjected to analysis of variance (ANOVA) with either "treatment" or "vintage" as the fixed independent effect and fermentation replication as a random effect using "lme4" (Bates *et al*., 2015), and

pairwise comparisons were evaluated using Tukey's Honest Significance (HSD). Pairwise comparison *p*-values are reported in the text. In 2018, samples from only two wine replicates per treatment were submitted for nonvolatile analysis due to low wine volume after sensory analysis. In 2019, one C fermentation replicate was a clear outlier after volatile analysis and was removed from data analysis; therefore, standard errors are reported for nonvolatile compounds in 2018 and volatile compounds in 2019 in the ANOVA tables. To compare treatment impacts across years, while accounting for the seasonal differences in wine profiles, the relative change of each treatment from the control was calculated for compounds of interest (e.g., anthocyanins). Relative change (%) for anthocyanins was calculated for each year by subtracting the control value from the treatment value, dividing it by the control value, and then multiplying it by 100.

Principal component analysis (PCA) was used to calculate and visualise the relationships among seasonal metrics (e.g., weather parameters, days between budbreak and harvest), wine compounds, and treatments across years using the package "factoextra" (Kassambara and Mundt, 2020), and a PCA biplot was graphed based on a correlation matrix. Wine compounds that were both present in wine samples each year and had a significant "vintage" effect following MANOVA were included in the PCA biplot. Pearson's correlation coefficients and corresponding *p*-values between weather parameters and wine compounds significant by "vintage" were calculated using functions from the package "Hmisc" (Harrell, 2020).

RESULTS

1. Weather Conditions

Each growing season had distinct weather characteristics (Table 1). Seasonal (1 May–harvest) GDD was lowest in 2017 (1596), while 2018 and 2019 had similar GDD (1766 and 1783, respectively); however, GDD during the ripening period tended to be higher in 2018 compared to the other two years. Seasonal rainfall was highest in 2018 (851 mm), and about half of this rainfall occurred between 1 August and harvest (401 mm). In comparison, seasonal rainfall was 600 in 2017 and 623 mm in 2019, and about 20 % of seasonal rainfall occurred between 1 August and harvest (125 mm and 118 mm, in 2017 and 2019, respectively). Solar exposure was highest during the growing season in 2019 (2620 MJ/m^2), followed by 2018 (2289 MJ/m^2), and then 2017 (2080 MJ/m^2). In summary, 2017 had the lowest heat accumulation, 2018 was warm but with above-average rainfall, particularly in August and September, and 2019 had the highest overall solar radiation than the other two seasons.

2. Treatment impacts on budbreak and berry parameters

In all three years, both A8 and A10 vines reached a 50 % budbreak approximately one week later than C vines, whereas LP vines reached budbreak 23 days later than C vines in 2017 and 10 days later in 2018 and 2019 (Persico *et al.*, 2021; 2017 data not shown). To note, the greater delay in budbreak for LP vines in 2017 compared to 2018 and 2019 is likely attributed, at least in part, to the later phenological stage LP was applied in 2017. Delays in budbreak tended to agree with differences in berry colour and juice chemistry assessed prior to harvest: in mid-August each year, LP vines had a lower percentage of berries that changed colour than C vines, while only in 2018, A8 vines had a lower percentage of berries that changed colour than C vines (Supplementary Table 1). Additionally, in 2017, LP vines had lower TSS, lower pH, and higher TA in mid-August compared to C, A8, and A10 vines, and this trend persisted for TSS and TA until at least three weeks later (Suppl. Table 1; Suppl. Table 2). In 2018, LP wines again had lower pH and higher TA than all other treatments in September, but treatment differences were not significant in mid-August (Supplementary Tables 1 and 2).

TABLE 1. Cumulative seasonal weather parameters at the experimental site for 2017, 2018, and 2019. Reported are cumulative monthly growing degree days (GDD), cumulative monthly rainfall, and cumulative monthly solar exposure (CSE), in addition to cumulative metrics for the growing season (May to Harvest) and estimated ripening period (August to harvest), at the experimental site.

	GDD (base 10 °C)			Rainfall (mm)			CSE (MJ/m^2)		
Month	2017	2018	2019	2017	2018	2019	2017	2018	2019
May	170	270	246	178	124	164	451	480	493
June	318	335	319	49	91	129	265	419	555
July	411	408	463	248	236	82	498	586	598
August	334	428	382	64	161	54	456	480	539
September	278	298	282	29	221	50	332	292	402
October*	87	26	32	32	19	14	77	32	33
May–harvest**	1596	1766	1783	600	851	623	2080	2289	2620
August–harvest	697	753	696	125	401	118	866	804	974

*Data for the month of October are from 1 October to harvest, which occurred on 10 Oct 2017, 3 Oct 2018, and 4 Oct 2019.
**C vines reached 50 % budbreak on 28 April 2017, 9 May 2018, and 6 May 2019.

3. Wine chemical composition and sensory triangle test data for each vintage

In total, 70 wine volatile compounds were detected in at least one year and belonged to the following classes: acetate esters (8), alcohols (15), aldehydes (1), ethyl esters (17), ketones (4), methyl esters (3), organic acids (6), terpenoids (12) and "other esters" (4). Thirty-five of these volatile compounds were in every wine each year (data not shown). For nonvolatile compounds, the 15 targeted compounds were detected in every wine each year, except for quercetin, which was not in any sample and not included in the analysis. Overall, delayed budbreak treatments impacted wine volatile and nonvolatile compounds to varying degrees each vintage (Figure 1; Table 2). Differences in wine composition tended to be greater and more consistent among years between wines made from C and LP vines, in general agreement with larger differences in budbreak dates and berry parameters (i.e., berry colour change and juice chemistry) between these two treatments.

The largest differences among wines were in 2017, the year with the greatest differences in budbreak dates and berry parameters between LP vines and the other treatments (Suppl. Table 1; Suppl. Table 2). In 2017, all volatile and nonvolatile compounds that were significantly different between treatments (n = 12) differed between LP wines and at least one other treatment (all comparisons, $p < 0.1$) (Figure 1). For volatile compounds, LP wines had lower concentrations of several aromatic compounds than C wines (i.e., "total ethyl esters", 2-Nonanone, and β-myrcene; all comparisons, $p < 0.1$). In addition, A8 wines tended to have lower concentrations of several ethyl esters than C wines ($p = 0.090$), and both A8 and A10 had a lower concentration of 2-Nonanone than C (both, $p < 0.05$). For nonvolatile compounds, anthocyanins were higher in LP wines than C and A8 wines (both, $p < 0.1$) in 2017, while both LP and A8 wines had lower tannin concentrations than C wines (both, $p < 0.05$). Differences in total anthocyanins were driven by monomeric anthocyanins, especially delphinidin, which was significantly higher in LP wines (11.3 mg/L) than in C, A8, and A10 wines (C: 6.2 mg/L; A8: 4.7 mg/L; A10: 8.7 mg/L, respectively; all comparisons, $p < 0.05$). Malvidin, the most abundant monomeric anthocyanin in our wines, was slightly higher in LP (229 mg/L) and A10 (229 mg/L) wines than in C (211 mg/L) and A8 (216 mg/L) although not significantly different between treatments ($p = 0.350$) (data not shown). For basic wine chemistry, LP wines had slightly lower pH than C wines (3.90 vs 3.80, $p = 0.027$) and 1.0 % lower alcohol than C, A8, A10 (12.7 % vs 11.7 %, all comparisons $p < 0.05$) in 2017 (data not shown). Overall, sensory discrimination results reflected treatment differences in wine chemical data in 2017: consumers perceived no sensory difference between C and A8 wines (sensory similarity at beta = 0.10), while both C and A8 wines were perceived as significantly different from LP wines ($p < 0.05$).

Compared to 2017, there were fewer differences in volatile and nonvolatile compounds among treatments in the following two vintages (Table 2), when budbreak delays ranged between 5 (A8 and A10, 2018) and 10 days (LP, 2018 and 2019). In 2018, wines from all delayed budbreak treatments had lower concentrations of the volatile compounds hexyl acetate and 1-hexanol than C wines, while only LP had a lower concentration of the volatile compound phenylethyl alcohol than C wines (all $p < 0.05$). For the nonvolatile compounds, delphinidin tended to be higher in LP wines than the other treatments, while gallic acid did not show a clear treatment trend. Sensory discrimination test results indicated that, in 2018, consumers deemed C and A8 wines as similar to each other once again (beta = 0.10), although only A8 differed significantly from LP wines ($p < 0.05$).

In 2019, there were the fewest differences in compounds between treatments and inconsistent treatment effects overall (Table 2). Ethyl 2-hexenoate tended to be lower in LP wines than in C wines, and LP wines tended to have had higher 3-Methylbutyl acetate than A8 and A10 wines. In addition, A8 wines had higher gallic acid concentration than all other treatments (all comparisons, $p < 0.1$). Despite few compounds that were significantly different between treatments, and no differences in other wine chemistry measurements (Persico *et al.,* 2021), in 2019, consumers detected differences between each treatment (all comparisons, $p < 0.05$).

TABLE 2. Significant differences in volatile and non-volatile compounds among treatments in 2018 and 2019

	2018					2019		
Treatment	Phenethyl Alcohol (µg/L)	Hexyl Acetate (µg/L)	1-Hexanol (µg/L)	Delphinidin (mg/L)	Gallic Acid (mg/L)	3-Methylbutyl Acetate (µg/L)	Ethyl 2-Hexenoate (µg/L)	Gallic Acid (mg/L)
C	1882 a*	37.7 a	632 a	2.0 +/- 0.45	17.5 +/- 0.88	830 +/- 79	35.9 +/- 2.4	17.0 b
A8	1518 ab	30.3 b	560 b	2.5 +/- 0.50	19.0 +/- 0.88	614 +/- 59	29.5 +/-1.8	19.3 a
A10	1669 ab	24.1 c	514 b	2.0 +/- 0.50	21.3 +/- 0.78	791 +/- 59	30.4 +/- 1.8	16.0 b
LP	1241 b	25.3 bc	528 b	4.0 +/- 0.50	18.5 +/- 0.88	919 +/– 59	26.1 +/- 1.8	16.0 b
p-value	0.002	0.002	0.023	NA**	NA	NA	NA	0.010

*Different letters within each column indicate significant differences among treatments at $p < 0.1$ following Tukey's HSD. Treatments included a control (C), two concentrations (8 % and 10 %) of dormant oil (Amigo®) application (A8, A10), and late pruning (LP) applied at EL 7 in 2018.

**Nonvolatile compound analysis was only performed on two fermentation replicates in 2018, so standard errors are reported for those data. One control fermentation replicate was removed from volatile analysis in 2019, so standard errors are reported for those data.

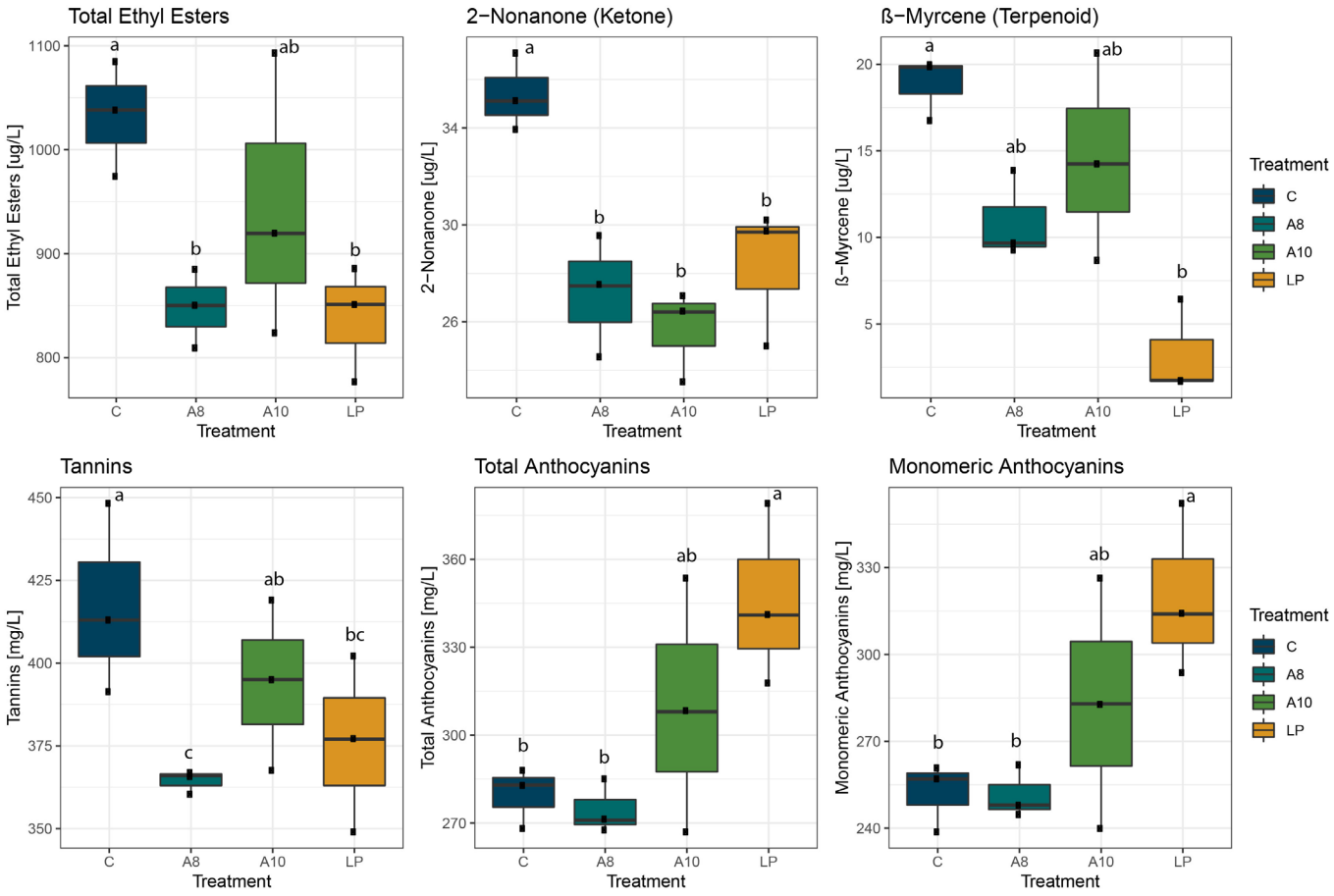


FIGURE 1. Significant differences in volatile and non-volatile compounds among treatments in 2017

[a]Following MANOVA, compounds that were significantly different between treatments ($p < 0.1$) were subjected to ANOVA. Different letters above each box indicate treatment differences at $p < 0.1$ after Tukey's HSD. Treatments in 2017 included a control (C), two concentrations (8 % and 10 %) of oil (Amigo®) application during dormancy (A8, A10), and late pruning (LP) applied at EL 10. For each compound, box plots show a distribution of values for the three wine replicates per treatment. Each replicate is represented by a point. The line in the middle of the box represents the median value of the three fermentation replicates per treatment, and the lower and upper limits of each box represent the first and third quartiles, respectively. The category "Total Ethyl Esters" includes Ethyl Decanoate, Ethyl Dodecanoate, Ethyl 2-Methylbutanoate, and Pentadecanoic acid, 3-methylbutyl ester.

4. Wine composition across vintages

Although wine composition differed among treatments each year, vintage more strongly characterised wine chemical profile than treatments. Following MANOVA, the "vintage" effect was significant for 25 of 49 compounds detected each year, compared to the "treatment" effect, which was significant for only three compounds: monomeric anthocyanins, delphinidin, and hexyl acetate (data not shown). Monomeric anthocyanins, due primarily to delphinidin, were higher in LP wines relative to C wines by 27 % in 2017, 36 % in 2018, and 18 % in 2019 (Figure 2), but differences in monomeric anthocyanins and delphinidin between C and LP wines were only statistically significant in 2017. In comparison, hexyl acetate did not show a consistent trend between treatments each year but tended to be lower in A8 than in LP wines ($p = 0.052$). Only two compounds, gallic acid and caffeic acid, had a significant "treatment" by "vintage" interaction effect ($p = 0.003$ and $p = 0.002$, respectively) and were not included in the PCA intended to compare "vintage" effects.

It is likely that seasonal differences, particularly weather parameters, affected compounds to a greater degree than potential changes in ripening time due to our applied treatments (Figure 3). The correlations among weather parameters, days from budbreak to harvest, and specific wine compounds are visually apparent in Figure 3, where PC 1 explained 43.7 % and PC 2 explained 30.6 % of the total variation. Pearson's correlation coefficients reflected strong relationships between weather parameters and specific compounds (Supplementary Figure 1); for example, growing degree days between budbreak and harvest, which was higher in 2018 and 2019 than in 2017, was positively correlated to β-myrcene and D-Limonene (r = 0.88 and r = 0.95, respectively, both $p < 0.001$) but negatively correlated to terpinen-4-ol and tannins (r = -0.89 and r = -0.96, respectively, both $p < 0.001$). Cumulative solar exposure between budbreak and harvest, which was highest in 2019, was positively correlated to catechin, quercetin glycoside, ethyl-3-methyl butanoate, and ethyl octanoate (r = 0.88, r = 0.95, r = 0.96, and r = 0.88, respectively, all $p < 0.001$)

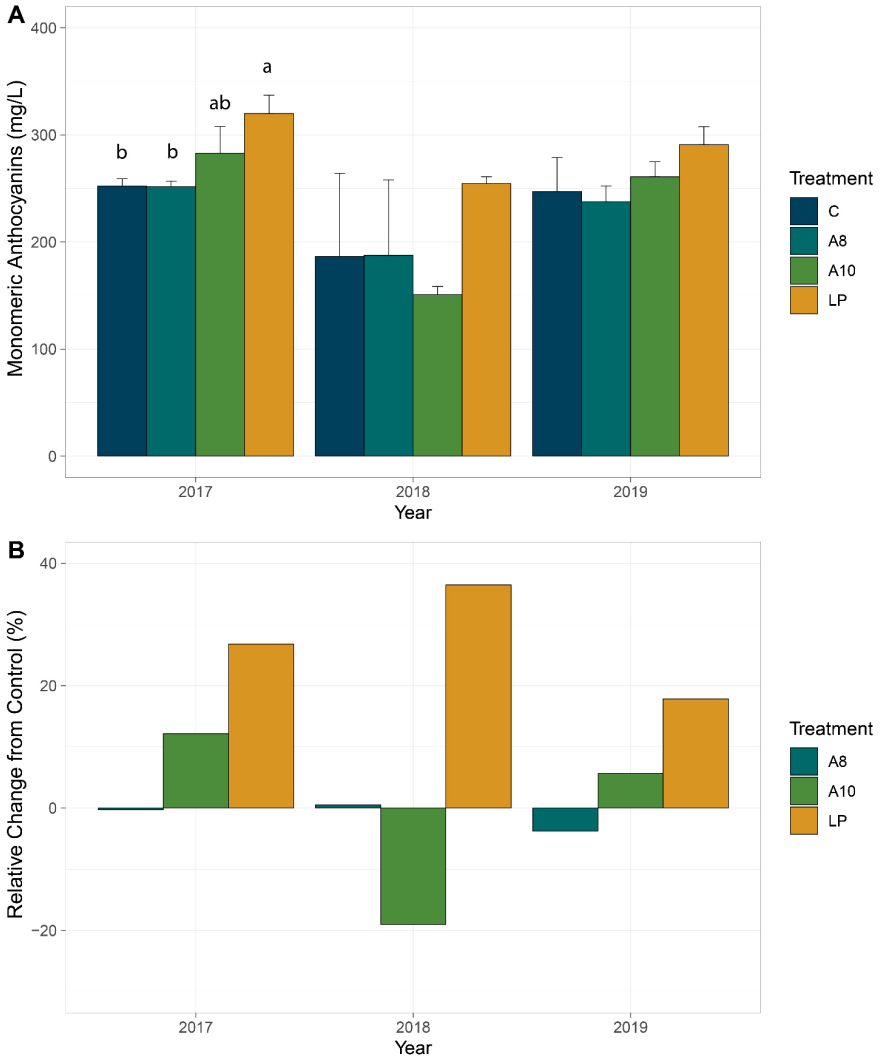


FIGURE 2. The concentration of monomeric anthocyanins and their relative change from the control for each treatment across three years

[a]A) Average concentrations of monomeric anthocyanin for all treatments and years (2017, 2018, and 2019). Treatments included a control (C), two concentrations (8 % and 10 %) of dormant oil (Amigo®) application (A8, A10), and late pruning (LP) applied at EL 10 in 2017 and EL 7 in 2018 and 2019. Different letters indicate treatment differences at $p < 0.1$ following ANOVA and Tukey's HSD, and B) Relative change of monomeric anthocyanins from the control for each treatment and each year.

and negatively correlated to resveratrol, linalool, methyl octanoate, and ethyl hexanoate (r = -0.97, r = -0.88, r = -87, and r = -0.94, respectively, all $p < 0.001$). Rainfall between budbreak and harvest, which was highest in 2018, especially between August and harvest, was positively correlated to caftaric acid (r = 0.90, $p < 0.001$) and negatively correlated to monomeric anthocyanins and epicatechin (r = -0.85 and r = -0.87, respectively, both $p < 0.001$).

DISCUSSION

1. Treatment impacts each vintage

Our results supported the hypothesis that delaying grapevine budbreak can shift the onset of berry compound accumulation and influence wine sensory properties. For volatile compounds, we found that delayed budbreak treatments had the greatest impact in 2017 and 2018. For example, the extensive phenological delay of LP vines in 2017 might explain why β-myrcene was significantly lower in LP wines compared to the control. β-myrcene, attributed to "orange" and "balsam" aromas in red wine (Slegers *et al*., 2015), is a monoterpene previously found to accumulate between *véraison* and harvest (Yue *et al*., 2020). A shorter period for berry maturation for LP vines might have caused a reduction in β-myrcene relative to C wines, as all treatments were harvested on the same day. Likewise, in 2017, wines made from delayed budbreak treatments, except A10, tended to have lower concentrations of several compounds associated with floral and fruit aromas than C wines (e.g., "Total ethyl esters" and 2-Nonanone) (Hu *et al*., 2018; Wang *et al*., 2017). Concentrations of ethyl esters and 2-Nonanone in finished wines depend on grape-derived precursors and their concentrations (Boss *et al*., 2015; Dennis *et al*., 2012; Wang *et al*., 2017).

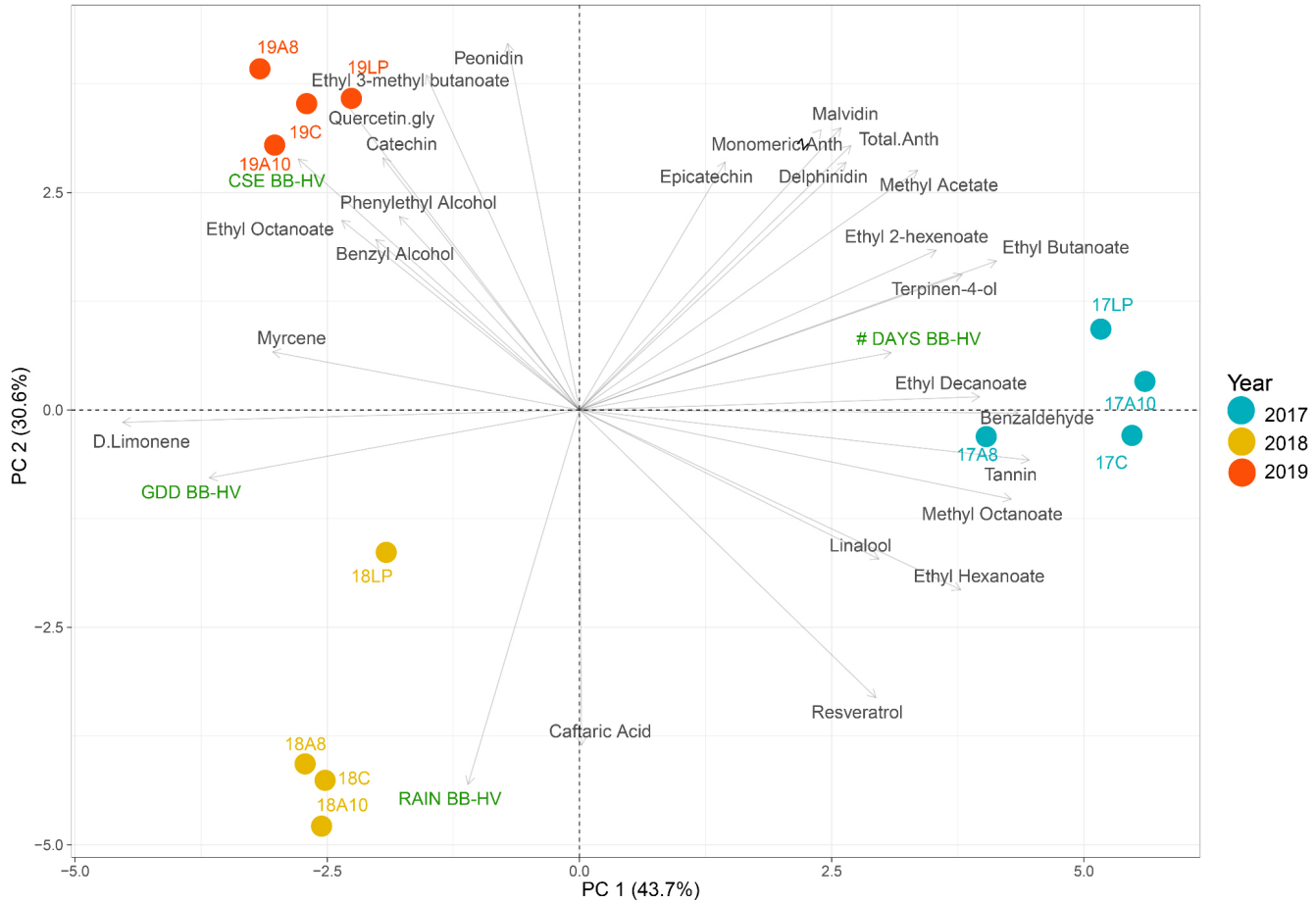


FIGURE 3. Principal component analysis (PCA) biplot of wine compounds for all years

[a]Wine compounds were included in the PCA biplot if they had a significant "vintage" effect and an insignificant "vintage × treatment" effect following MANOVA at $p < 0.1$. Treatments included a control (C), two concentrations (8 % and 10 %) of dormant oil (Amigo®) application (A8, A10), and late pruning (LP) applied at EL 10 in 2017 and EL 7 in 2018 and 2019. Individual points represent a year and treatment combination (e.g., 18LP is 2018 late pruning), and greater proximity of points indicates higher similarity. The number of days between budbreak and harvest, and rain, GDD, and CSE variables between budbreak and harvest are denoted as BB-HV in the plot.

Treatments to delay budbreak may have delayed the accumulation of these grape-derived compounds during the growing season. We cannot exclude that fruit from delayed budbreak treatments would continue to ripen if harvested at a later date than C vines; however, low temperatures in fall at our experimental site suggested that fruit was unlikely to continue maturation. For example, cumulative GDD between harvest and 31 December was only 59 in 2017, 133 in 2018, and 66 in 2019 (data not shown).

As in 2017, delayed budbreak treatments reduced the concentration of several volatile compounds related to sensory properties in 2018. The volatile berry compound 1-hexanol ("hexan-1-ol"), an alcohol that begins accumulation shortly post-fruit set and increases between *véraison* and harvest (Kalua and Boss, 2009; Previtali *et al.*, 2021), was significantly lower in wines made from all delayed budbreak treatments compared to C wines. Delayed budbreak treatments also had significantly lower hexyl acetate, an acetate ester derived in part from 1-hexanol (Dennis *et al.*, 2012) and attributed to "red-berry" aromas in wine (Forde *et al.*, 2011). As with 1-hexanol and hexyl acetate, a shorter period from *véraison* to harvest in LP vines may have led to a lower phenylethyl alcohol concentration in LP wines compared to C wines. Phenylethyl alcohol, which can develop late in berry maturation (Kalua and Boss, 2009), has been associated with "rose" aroma in prior work (Fang and Qian, 2005).

Our results that delaying budbreak, particularly by late pruning, can impact berry development and wine composition support prior work conducted in warm regions of Argentina (Morgani *et al.*, 2023), Spain (Buesa *et al.*, 2021; Zheng *et al.*, 2017), Italy (Frioni *et al.*, 2016; Frioni *et al.*, 2019; Gatti *et al.*, 2018), and Australia (Moran *et al.*, 2018; Moran *et al.*, 2021). However, the implications of altering wine flavour compounds are different for studies conducted in warmer regions than our site. For example, in studies conducted in the Barossa Valley of Australia, LP tended to improve wine sensory attributes, and this improvement was attributed to cooler temperatures during fruit ripening for LP vines compared to C vines (Moran *et al.*, 2018; Moran *et al.*, 2021). In contrast, delaying budbreak at our cool-climate site tended to decrease several wine volatile compound concentrations relative to C wines, consistent with our expectations. In 2019, when growing season conditions were relatively sunny and warm compared to the prior

two years, only two volatile compounds were significantly different between treatments. In past work, sun exposure and temperature have been positively associated with certain volatile compounds (e.g., 1-hexanol) in finished wines (Feng *et al*., 2015; Lu *et al*., 2022). Therefore, relatively high CSE and GDD may have diminished the negative effects of delaying budbreak on volatile compounds in 2019 compared to 2017 and 2018 in our study.

Delayed budbreak treatments impacted nonvolatile compound concentrations each year, although not in the ways we expected. For example, tannins, which influence wine mouthfeel and colour stability (Hornedo-Ortega *et al*., 2021; McRae *et al*., 2013), were lower in A8 and LP wines compared to C wines in 2017. Tannins are synthesised pre-*véraison* in seeds and skins and tend to decrease between *véraison* and harvest (Downey *et al*., 2003; Kennedy *et al*., 2001). Therefore, we expected that wines made from delayed budbreak treatments would have higher tannin concentrations than C wines if there was less time between *véraison* and harvest. Although not measured directly, we can speculate that our result for tannins is attributed to lower heat accumulation between fruit-set and *véraison* for delayed budbreak vines compared to C vines. In past work, higher heat accumulation, particularly between fruit set and *véraison*, was linked to higher concentrations of tannins in seeds and skins (del Rio and Kennedy, 2006). In our study, we did not measure phenology between fruit-set and *véraison*; however, in 2017, phenological development was delayed for all the delayed budbreak treatments at least until bloom compared to C vines, resulting in lower total GDD between bloom and harvest (C: 1286; A8: 1260; A10: 1260; LP: 1220; data not shown). Regardless of treatment, tannin concentrations, which ranged from 350-450 mg/L, were consistent with ranges previously reported for relatively low-tannin *V. vinifera* cultivars (e.g., Pinot Noir and Syrah) (Harbertson *et al*., 2008) and unlikely influenced sensory perception among treatments, discussed below.

In contrast to our hypothesis, anthocyanin concentration tended to be highest in LP wines across the years, though this difference was only statistically significant in 2017 (LP > C and A8). Although differences in yield parameters (e.g., berry size) and leaf area-to-fruit ratio could explain differences in berry colour (Dokoozlian and Kliewer, 2005), there were no consistent differences in yield components (e.g., yield/vine, cluster weight, berry weight) among treatments between years (Persico *et al.*, 2021; 2017 data not shown). We did not measure leaf area but pruning weight did not differ among treatments and all canopies looked fully and equally developed by mid-August by our visual assessment. Similarly, past work conducted in warmer climates reported that late pruning at similar stages to our study increased berry and wine anthocyanin concentrations (Frioni *et al*., 2016; Moran *et al*., 2018, 2021). In past work, higher berry anthocyanin accumulation due to late pruning was attributed to lower temperatures within two weeks post-*véraison* compared to control vines (Moran *et al*., 2021). Our site did not often reach temperatures that can inhibit anthocyanin synthesis (> 30 °C) (Kliewer, 1970; Mori *et al*., 2007); however, it is possible that the delayed onset of *véraison* in LP vines into cooler late-summer temperatures enhanced anthocyanin accumulation in LP wines relative to C wines, as has been reported in warmer regions.

Alternatively, higher anthocyanins in LP wines relative to C wines in our study could be attributed to vine shifts in carbon balance. In prior work, late-pruned vines had higher net photosynthetic efficiency compared to standard-pruned vines (Gatti *et al*., 2016), and carbon fixation has been positively correlated to anthocyanin and sugar accumulation in berries during ripening (Bobeica *et al*., 2015). We did not measure photosynthesis. However, it is possible that delaying shoot growth when temperatures were warmer led to a higher capacity for photosynthesis for LP vines, prompting higher anthocyanin accumulation in berries at the onset of ripening. Higher net-photosynthesis for LP vines compared to C, A8, and A10 vines may explain how LP vines reached TSS concentrations similar to the other treatments at harvest, even in 2017. The relationship between delayed budbreak, vine carbon balance, and primary metabolite accumulation in a cool climate is an avenue for future study.

We expected that treatment differences in primary metabolites during ripening would reflect differences in finished wine composition; however, this was not always true. For example, in 2017, juice TSS, pH, and TA values indicated that LP berries differed from all other treatments before harvest (Suppl. Tables 1 and 2). Treatment differences in wine anthocyanins followed a similar trend (i.e., LP was different from C and A8). However, concentrations of several compounds (ethyl esters, 2-Nonanone, and tannins) were similar between LP and A8 wines and different from C wines. Our results suggest that even though A8 and A10 vines achieved similar phenological development to C vines by *véraison*, A8, and to a lesser extent A10, still impacted wine composition. As mentioned earlier, delayed budbreak treatments remained delayed in phenology until at least bloom in 2017, resulting in lower GDD (and CSE) between bloom and *véraison* compared to control vines. Therefore, it is possible that A8 and A10 were still capable of impacting wine compounds that synthesised pre-*véraison* (e.g., tannins and ethyl ester precursors). Alternatively, these trends could be explained by limitations in pre-harvest berry sampling. It is possible that collecting only 100 berries per experimental unit did not capture slight differences in ripeness between C and Amigo®-treated vines. Instead, the full number of grapes at harvest, used to make the wines, might have better-captured differences in ripeness.

2. Wine sensory perception each vintage

We expected that delayed budbreak treatments would impact wine composition and that consumers would detect these differences through a triangle discrimination test. Broadly, consumer perception reflected wine composition when differences in wine chemistry were pronounced. For example, in 2017, LP wines were perceived as different from C and A8 wines. At the same time, LP wines had the lowest per

cent alcohol of all treatments, which could have influenced the release of other odorants (Villamor *et al*., 2013), and had the highest anthocyanins concentration, which may have impacted wine colour. Furthermore, consumers in 2017 may have detected lower concentrations of several volatile aroma compounds (e.g., β-myrcene and ethyl esters) in LP wines than in C wines, as discussed earlier, leading them to discriminate between C and LP wines. Differences in composition between C and A8 wines in 2017 (e.g., tannins and ethyl esters) might not have been distinctive enough to influence sensory discrimination between these two treatments. For tannins, it is possible that concentrations in C wines (400–450 mg/L) were not high enough (e.g., > 800 mg/L) to impact astringency compared to A8 and LP wines in 2017, consistent with prior work comparing tannin concentration and perceived astringency (Landon *et al*., 2008).

Similarly, in 2018, LP wines tended to have lower volatile compounds (e.g., phenylethyl alcohol, hexyl acetate, and 1-hexanol) than C wines, but this was not enough to influence consumer perception; instead, consumers only detected differences between LP and A8 wines in 2018 despite no clear differences in wine composition between these treatments. Wine perception depends on several factors, including the "wine aroma buffer" (i.e., compounds that suppress additive sensory compounds), sensory thresholds, and compound interactions (Ferreira, 2010). Although treatment differences in consumer perception broadly aligned with our expectations in 2017 and 2018 (i.e., LP wines were most different), we can only speculate on the connection between wine chemical compounds and resulting sensory perception.

Interestingly, all wines were perceived as different in 2019 despite a few differences in wine chemical composition. This result could be due to a single, impactful compound. Generally, aroma attributes such as "floral" are attributed to several compound groups acting together (Loscos *et al*., 2007); however, certain compounds can break the aroma buffer on their own. In our study, 3-Methylbutyl Acetate ("isoamyl acetate"), which is attributed to a "banana smell" was highest in LP wines in 2019 and has been found capable of breaking the aroma buffer of neutral wines (Escudero *et al*., 2004). In quantities between 200–1400 mg/L, as in our study, isoamyl acetate can be detected and differentiated from wines that do not contain this compound (Ferreira, 2010). Although there is no field-related explanation for higher isoamyl acetate in LP wines than the other treatments, this compound may explain consumer discrimination between LP and C and A8 wines. Furthermore, slight differences between C and A8 in isoamyl acetate could explain sensory discrimination between these two treatments, considering a few other differences in wine composition we found in 2019.

3. Wine composition across vintages

Weather factors are well-established drivers of vine phenological development (Parker *et al*., 2011) and berry metabolism (Kliewer and Lider, 1968; Lu *et al*., 2022; Sweetman *et al*., 2014). Therefore, we expected that wine composition would vary among vintages. We also expected that if weather characteristics were distinct in each vintage, treatment impacts on wine composition would not be consistent across vintages. In agreement with our hypothesis, our "vintage" effect was significant for a greater number of compounds than any treatment in any year. Furthermore, seasonal factors (i.e., weather and growing season length) were highly correlated to certain compounds (e.g., CSE and quercetin glycoside) and corresponded to different wine profiles for each vintage. Even treatments that had a similar delay in budbreak across years (i.e., A8 and A10 in all three years, LP in 2018 and 2019) did not have a consistent effect on the same compound or compound class in each vintage. The only exception was anthocyanins, which tended to be higher in LP wines than C wines regardless of vintage, as previously discussed.

Our wine chemical results each vintage were best explained by two factors combined: the later onset of berry development induced by delaying budbreak and weather conditions each year. For example, in 2019, the delays in budbreak ranged from 6 to 10 days and ripening conditions were conducive to primary and secondary metabolite development (i.e., relatively high CSE and GDD). As a result, there were fewer differences in finished wine chemical composition in 2019 than in the two previous vintages. In 2018, budbreak delays were similar to 2019 but weather conditions were less favourable for fruit ripening (e.g., high seasonal rainfall); consequently, delays in key phenological stages (i.e., budbreak and potentially *véraison*) impacted more wine compounds in 2018 than in 2019. There was the highest delay in budbreak in 2017 (LP, 23 days), which was likely in part due to the later phenological stage at which LP was applied. In addition, 2017 had the lowest seasonal GDD and CSE of all three years. Subsequently, 2017 was the year with the most differences in wine chemical composition between treatments, particularly between C and LP. Similar to our study, Moran *et al.* (2018) found that the impact of late pruning on wine composition and sensory traits differed between the two years of their study, likely due to variations in weather and season length. Our results, along with past work, suggest treatment impacts on wine chemical composition depend on both the magnitude of delay in the onset of the annual growing season and seasonal weather conditions.

CONCLUSION

In our study, LP vines reached *véraison* later than C vines each year and, in agreement with our hypothesis, LP wines had a lower concentration of several wine volatile compounds than C wines. Although LP did not consistently impact the same wine compounds each year, LP wines were always perceived as different from C vines; however, determining how differences in wine composition affected wine preference was beyond the scope of our work. For Amigo®-treated vines, delays in berry parameters were not often present at *véraison*, and effects on wine chemistry were, therefore, more subtle. In some years, A8 and A10 wines had lower volatile and nonvolatile concentrations compared to C wines, but differences were less frequent than between LP and C wines.

Overall, and consistent with our expectations, we found that vintage impacted a higher number of wine compounds than any delayed budbreak treatment across years, and this was likely due to distinct weather in each vintage.

Late pruning is a more traditional and commonly used approach to delay grapevine budbreak than oil application. Previously, our research group reported that a 10-day-delay in budbreak from LP more effectively reduced spring freeze damage, including vegetative tissue damage and crop loss, than a 5-day-delay in budbreak due to A8 and A10 application (Persico *et al*., 2021). In this study, we reported some differences in wine composition and sensory perception between C and LP when budbreak was delayed by 10 days. However, for practitioners, the benefits of a 10-day delay in budbreak to prevent freeze damage may outweigh the impacts on wine composition and sensory perception that we found. In comparison, the application of late-pruning at EL 10 in 2017 resulted in the greatest delay in budbreak (23 days) and the highest impact on wine composition and perception among years. We cannot exclude that a 23-day-delay in budbreak would have had fewer impacts on wine composition and perception in a year warmer and sunnier than 2017; however, the potential consequences of applying LP at EL 10 are unlikely worth the risk to wine composition, and we would not recommend the application of LP beyond EL 7 in our cool-climate.

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