THE IMPACT OF DELAYED GRAPEVINE BUDBREAK ON LEMBERGER WINE SENSORY COMPOUNDS UNDER VARIABLE WEATHER CONDITIONS

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Introduction

Spring freeze events threaten grape production globally [1–3]. As grape buds emerge from dormancy in spring, freezing temperatures have the potential to damage green tissues [4,5], decreasing yield potential and compromising fruit quality by harvest [2,6,7]. Bud freeze damage may become more frequent if global warming accelerates budbreak without a concurrent decrease in spring freeze events [3,8]. One promising strategy to prevent grapevine freeze damage is to delay budbreak, for example by applying chemical products during dormancy [7,9,10] or by postponing winter pruning until after budbreak [11]. Although these methods are effective at preventing freeze damage, in cool climates with short fruit ripening periods, delaying budbreak has the potential to delay the development of sugars, phenolics, and volatile compounds in fruit, negatively impacting the flavor, aroma, and mouthfeel of finished wines. In this study, we evaluated the impact of two techniques to delay grapevine budbreak on volatile and nonvolatile compounds of Lemberger wines (*Vitis vinifera*), and we related the impacts on wine composition to consumer perception, over three vintages at a cool-climate site.

This study builds on our past work, where we investigated the effects of applying a chemical spray product and delayed winter pruning on Lemberger budbreak, freeze damage, yield parameters, and basic wine chemistry in 2018 and 2019 [7]. While we found no differences in basic fruit and wine chemistry by harvest, vines with later budbreak tended to show a delay in berry color change around veraison, assessed in mid-August each year, suggesting a delayed onset of phenolic compounds that went uncharacterized. Furthermore, in the pilot year of that study (2017, unpublished data), budbreak was delayed up to 23 days, over twice as much as the highest delay in 2018 and 2019, which led to an even more extensive delay in the onset of veraison. Due to distinct variations in key phenological stages and seasonal weather conditions over the three years of the study, we expanded our research to understand the extent to which delaying budbreak affects volatile and nonvolatile compounds in wines from 2017-2019.

Here, we aimed to determine how delaying budbreak and the onset of fruit ripening impacts wine chemical composition and whether impacts of delaying budbreak are consistent among years, or if they mainly depend on factors such as seasonal weather. We also evaluated if consumers could detect differences between wines made from vines that experienced a different degree of delayed budbreak and onset of veraison. We hypothesized that, among vintages, seasonal weather metrics would more strongly impact overall wine composition than delaying key phenological stages (i.e., budbreak and veraison), regardless of the extent of delay. However, within each vintage, we hypothesized that if there was still a delay in fruit phenological development at veraison, delayed budbreak treatments would lead to lower concentrations of nonvolatile (e.g., tannins and anthocyanins) and volatile (e.g., terpenoids and ethyl esters) compounds in finished wines, which consumers would be able to detect.

Research Objectives

The objectives of this study were to: 1) Determine if treatments that delay grapevine budbreak, and consequently the onset of berry ripening, impact chemical composition and sensory perception of finished Lemberger wines over three years, and 2) Explore if treatment impacts on wine chemical composition are consistent across years, or if seasonal weather parameters more strongly affect wine chemical composition than delaying key phenological stages. Broadly, results from this study will inform grape growers and winemakers of how delaying budbreak to various extents may impact wine quality and consumer perception in a cool-climate region. This study also seeks to contextualize the relative impact of delaying budbreak and onset of fruit ripening on wine flavor and aroma profiles compared to the impacts of overall seasonal weather.

Methods and Materials

Experimental design

The grapevines used in this experiment were 10-year-old *Vitis vinifera* cv. Lemberger scion grafted onto 101-14 Mgt rootstock in a commercial vineyard in Lewisburg, PA. A weather station at the vineyard collected hourly air temperature, solar radiation, and rainfall data, which was used to calculate cumulative growing degree days (GDD, base 10 °C), the sum of hourly solar radiation averages (cumulative solar exposure, CSE, MJ/m^2), and total rainfall (mm) each growing season. The experimental design was a randomized 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 in 2018 and 2019 when the three most-apical buds averaged at stage 7, or “first leaf separated,” on the Eichhorn-Lorenz (E-L) scale [12]. In 2017, late pruning was applied when the apical buds averaged at approximately stage 10, or "three leaves unfolded". We chose to apply two concentrations of Amigo to test potential bud mortality from a higher concentration product (A10). More information about the experimental design and delayed budbreak treatments can be found in Persico et al. (2021).

Weekly phenology measurements started approximately one week before C vines reached budbreak and were taken until at least full bloom. In mid-August, around veraison, we recorded the berry color change percentage for each cluster on the same vines selected for phenology measurements, and fruit chemistry data was taken on the same day. Detailed phenological data for the 2018 and 2019 seasons are reported in Persico et al. (2021). To provide background information to the wine results reported below, it is important to note that in all three years, both A8 and A10 vines reached 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. Each year, all treatments were harvested on the same date (mid-October).

Winemaking and wine analysis

The day of harvest, fruit (approx. 400 kg/year) was transported to Penn State and wines were made using methods outlined in Persico et al. (2021). Wines were fermented in biological triplicate for each treatment, and bottled wines were stored for up to six months in coolers (~7 °C) prior to sensory evaluation. 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 similarity with A8. A triangle discrimination test (ASTM 2011) was then conducted in the Sensory Evaluation Center (SEC) at Penn State, where regular wine consumers (n = ~100 each year) performed three triangle tests: C versus A8, C versus LP, and A8 versus LP. Wines were presented in ISO wine tasting glasses in randomized presentation order and identified only by 3-digit codes. Participants were asked to smell, taste, and expectorate the sample prior to selecting which wine they believed to be the odd sample out of the three presented. Water was permitted to rinse in 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-napthalene internal standard equivalents (ug/L) [13].The following non-volatile compounds related to wine quality were quantified using High Performance Liquid Chromatography (HPLC) via a third-party service (ETS Laboratories) and reported in mg/L: tannins (total, epicatechin and catechin), anthocyanins (total, monomeric, polymeric, delphinidin, peonidin, and malvidin), quercetin, quercetin glycoside, hydroxycinnamic acids (caffeic, *p*-coumaric), caftaric acid, gallic acid, and resveratrol.

Data analysis

To identify differences in wine compounds among treatments and years, volatile and nonvolatile compounds were first subjected to multivariate analysis of variance, including “treatment,” “vintage,” and their interaction effect used as fixed independent effects. Following, significantly different compounds at $p < 0.1$ were subjected to analysis of variance with either “treatment” or “vintage” as the fixed independent effect and fermentation replication as a random effect, and pairwise comparisons were performed using Tukey’s Honest Significance and reported if $p < 0.1$. Principal Component Analysis was used to calculate and visualize the correlations among weather metrics, wine compounds, and delayed budbreak treatments.

Results

Delayed budbreak treatments impacted wine chemical compounds to varying degrees each vintage. Overall, differences 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 between these two treatments (see methods section). Wine chemical results also tended to agree with differences in berry color assessed in mid-August: every year, LP vines had lower berry color change percentage than C vines, while only in 2018, A8 vines had lower berry color change percentage than C vines.

The strongest differences among wines were in 2017, which was the year with the largest difference in budbreak dates between LP vines and the other treatments. All volatile and nonvolatile compounds that were significantly different among treatments (n=12) differed between LP wines and at least one other treatment (all, $p < 0.1$), and LP wines had lower concentrations of volatile aroma compounds than C wines (e.g., “total ethyl esters”; Figure 1). Sensory discrimination results supported treatment differences in wine chemical data: 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 ($p < 0.05$). Notably, anthocyanins were higher in LP wines than C and A8 wines (both, $p < 0.1$), while C wines had a higher tannin concentration than LP and A8 wines (both, $p < 0.05$) (Figure 1). We are currently exploring potential factors driving higher anthocyanins in LP wines than other treatments.

Overall, there were few differences in volatile and non-volatiles compounds among treatments in the following two vintages, when budbreak delays ranged overall between 6 and 10 days. Yet, C and LP wines were still the most different. In 2018, C wines had higher concentrations of hexanol and phenylethyl alcohol than LP wines and higher concentrations of hexyl acetate than A8, A10, and LP wines (all $p < 0.05$). Sensory discrimination test results indicated that consumers deemed C and A8 wines as similar to each other once again (beta = 0.10), although only A8 differed significantly from LP wines ($p < 0.05$).

Two-thousand-nineteen had the fewest differences in compounds among treatments and inconsistent treatment effects. Ethyl 2-hexenoate was higher in C vines than LP wines ($p = 0.037$), LP wine had higher 3-Methylbutyl acetate than A8 wines ($p = 0.033$), and A8 wines had higher gallic acid concentration than all other treatments (all comparisons, $p < 0.1$). Despite few compounds that were significantly different among treatments, and no differences in other wine chemistry measurements (e.g., alcohol percentage), consumers were able to detect differences between treatments. We are currently investigating the detection thresholds and characteristics of the compounds significantly different among treatments in 2019 (e.g., 3-Methybutyl acetate), which may explain perceived sensory differences.

Although we found differences in wine chemical concentrations among treatments each year, vintage more strongly characterized wine chemical profile than treatments (Figure 2, see weather metrics in caption). “Vintage” effect was significant for 25 out of the 49 volatile and nonvolatile compounds quantified each year, while “treatment” effect was significant for 7 compounds only. It is likely that seasonal weather metrics affected compounds to a greater degree than potential changes in ripening time observed in our study; for example, GDD, which was higher in 2018 and 2019 than 2017, was highly correlated to the grape-derived compounds α-Myrcene and D-Limonene (r = 0.88 and 0.95, respectively) but negatively correlated to terpinen-4-ol (r = - 0.96). Given strong seasonal differences, we are analyzing the relative change between each treatment and C wines for each compound and vintage.

Conclusions

Seasonal weather conditions affected wine chemical composition more than delayed budbreak treatments. However, within each vintage, delaying the onset of veraison impacted wine chemical composition and related sensory perception, especially for LP in 2017. Across years, we did not find a consistent treatment effect on chemical composition, even in instances where treatments induced a similar delay in budbreak between years. We are currently exploring how the delayed budbreak treatments, especially late pruning, shifted the onset of veraison and wine chemical composition compared to the control treatment each year.

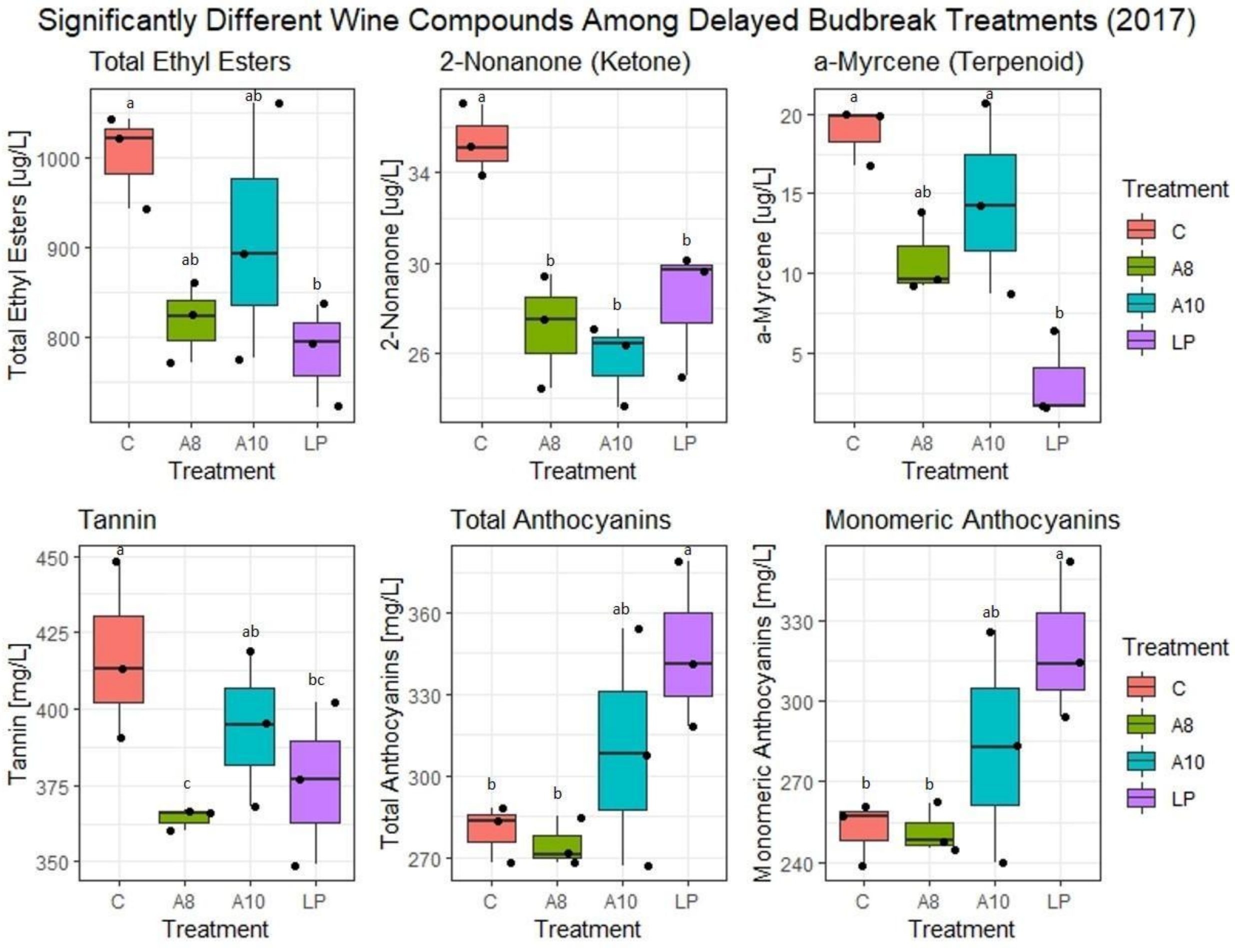

Figure 1. Significantly different wine compounds among treatments in 2017, identified by multivariate analysis of variance (MANOVA) at $p < 0.1$. Following MANOVA, significant compounds were subjected to analysis of variance and Tukey's honest significant difference pairwise comparisons. Different letters indicate treatment differences at $p < 0.1$. The category "Total Ethyl Esters" includes Ethyl Decanoate, Ethyl Dodecanoate, Ethyl-2-methylbutanoate, and Pentadecanoic acid, 3-methylbutyl ester.

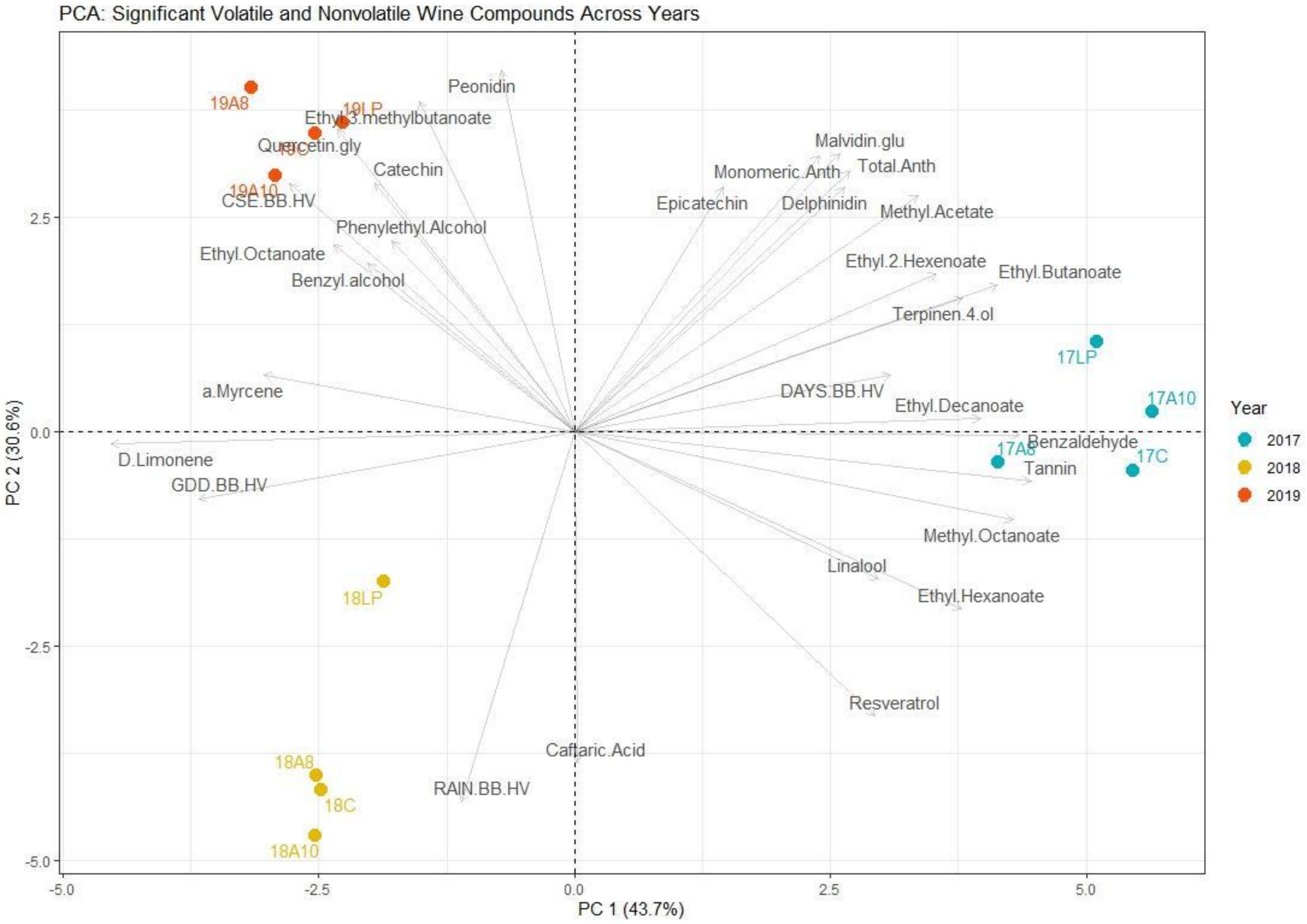

Figure 2. Principal Component Analysis (PCA) biplot of significantly different compounds across years. Wine compounds were included in the PCA biplot if they had a significant "vintage" effect and an insignificant "vintage x treatment" effect following multivariate analysis of variance (MANOVA) at $p < 0.1$. Individual points represent a year and treatment combination (e.g., 18LP is 2018 Late Pruning), and proximity of points indicate greater similarity. Rain, GDD and CSE variables between budbreak and harvest, denoted as BBHV in the plot, are reported in the figure; 2019 had the highest cumulative solar exposure (CSE.BB.HV, MJ/m^2; 17C = 2124; 18C = 2152; 19C = 2568), 2018 had the highest rainfall (RAIN.BB.HV, mm; 17C = 600, 18C = 824, 19C = 464), and 2017 had the lowest growing degree days (GDD.BB.HV, 17C = 1621; 18C = 1706; 19C = 1690).

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