American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2021.20076

Research Article

Delaying Budbreak to Reduce Freeze Damage: Seasonal Vine Performance and Wine Composition in Two *Vitis vinifera* Cultivars

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Acknowledgments: This project was supported by the Pennsylvania Wine Marketing and Research Board Program, Timothy R. Crouch Program Support Endowment, and the USDA National Institute of Food and Agriculture (NIFA) Federal Appropriation under Project PEN0 4628 (Accession number 1014131). The authors would like to thank Dr. Charles Zaleski, MD, at Fero Vineyards and Winery for providing and maintaining the vineyard experimental site; Drs. Helene Hopfer and Molly Kelly for assistance with research winemaking; Dr. Rich Marini for consultation on statistical analysis; and all lab members for help with phenology and production data collection.

Manuscript submitted Dec 21, 2020, revised April 26, 2021, accepted May 7, 2021

Abstract: Spring freeze events pose a threat to vineyard productivity worldwide. We compared two methods to delay grapevine budbreak for freeze avoidance and evaluated their effects on phenology, yield components, fruit composition, and postharvest parameters, including wine chemistry, carbohydrate storage, and bud freeze tolerance. The two methods to delay budbreak were: a vegetable oil-based adjuvant (Amigo®) applied to dormant buds at 8% and 10% (v/v) and late pruning applied when apical buds reached approximately Eichhorn-Lorenz stage 7. Treatments were applied in 2018 and 2019 on two *Vitis vinifera* cultivars, Lemberger and Riesling, and compared to a control treatment (no delayed budbreak strategy applied). Amigo

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and late pruning delayed budbreak compared to control vines for both years and cultivars. The delay in budbreak varied from three to six days for Amigo 8%, five to eight days for Amigo 10%, and 10 to 11 days later for late pruning. In 2019, there was a freezing event near budbreak; compared to control vines, late-pruned Lemberger vines had lower shoot damage when measured during the growing season and higher yield at harvest. Delayed budbreak treatments did not influence wine chemistry either year or consistently affect carbohydrate storage or bud freeze tolerance the following dormant season. However, in Riesling, late pruning reduced cluster and berry weight by up to 34% and 22%, respectively, compared to control vines. Furthermore, our results indicated Amigo 10% may decrease bud survival when applied to Riesling vines. In general, late pruning more effectively delayed budbreak and mitigated freeze damage than Amigo application without negatively affecting vine health and wine composition; however, the cultivar-dependent effect of late pruning on cluster weight is a consideration prior to adoption.

Key words: carbohydrate reserve, cool climate, dormancy release, freeze stress, pruning, viticultural practice

Introduction

Spring freeze events pose a significant economic threat to cool climate wine growing regions (Evans 2000, Warmund et al. 2008). As grapevines emerge from dormancy and approach budbreak, bud metabolism increases (Gardea et al. 1994) and bud freeze tolerance decreases (Ferguson et al. 2011, 2014, Kovaleski and Londo 2019). Post-budbreak, temperatures below freezing can permanently damage young and tender shoots. If primary shoots are killed from freeze damage, secondary or tertiary buds will grow; however, these buds tend to be less fruitful,

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leading to significant reductions in yield (Friend et al. 2011). One strategy to mitigate spring freeze damage is to shift grapevine budbreak to later in the growing season, when the risk of freezing events has decreased.

Previously studied methods to delay grapevine budbreak include applying sodium alginate gels (Friend et al. 2011) or vegetable oil-based adjuvants (Dami and Beam 2004, Loseke et al. 2015, Centinari et al. 2018, Wang and Dami 2020) to vines during dormancy. These products are presumed to decrease bud respiration and therefore delay cold deacclimation, but evidence of their mode of action is not conclusive. It is reported that oil application reduces bud respiration, but this reduction is cultivar-dependent (Dami and Beam 2004), and certain oils may be phytotoxic, especially if applied above specific concentrations (Dami and Beam 2004, Centinari et al. 2018). In comparison, late pruning is an established method to delay budbreak for freeze avoidance (Howell and Wolpert 1978). In this method, pruning is not performed during the dormant season, or it is limited to the removal of several upper nodes per cane (“double pruning”). Maintaining apical buds until they break suppresses budbreak of the basal buds, which remain dormant for longer. When the risk of freeze damage subsides, the apical buds are removed (i.e., late-pruned), initiating basal budbreak. While more convenient for cordon-trained and spur-pruned vines, late or double pruning can also be adapted for cane-pruned vines by leaving the intended canes longer than needed and in an upright position; however, concerns of damaging swollen or broken buds when tying canes to the trellis and the extra labor and time needed for this operation might limit its adoption for cane-pruned vines.

Although it is a promising method to avoid freeze damage, delaying budbreak can shift other key phenological stages, including bloom and fruit set (Friend and Trought 2007).

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Delaying budbreak can also delay berry maturation (Frioni et al. 2016, Frioni et al. 2019, Petrie et al. 2017), resulting in reduced fruit soluble sugar concentration at harvest and altered wine chemical composition (Moran et al. 2018). Delaying the onset of berry ripening is desirable in warm climates, where late pruning has been used to offset rapid sugar accumulation in fruit (Frioni et al. 2016, Moran et al. 2017, Palliotti et al. 2017, Petrie et al. 2017); however, effects of delaying budbreak on fruit and wine composition may be detrimental in cool climates, where the growing season is relatively short.

It is also unknown if phenological shifts during the growing season delay vine cold acclimation and dormancy induction, including the accumulation of total non-structural carbohydrates (TNC), which equal the sum of soluble sugars and starch, and the acquisition of bud freeze tolerance. Stored soluble sugars are important for bud freeze tolerance (Jones et al. 1999), and starch fuels growth the following spring (Holzapfel et al. 2010). If delaying budbreak delays canopy development and the onset of berry ripening, vines may prioritize photosynthate allocation to vegetative growth and fruit ripening, which are stronger sinks than storage allocation (Candolfi-Vasconcelos et al. 1994). Late-pruned vines may be especially vulnerable to reductions in TNC, as they must mobilize stored TNC reserves twice: once during apical budbreak and again during basal budbreak. Therefore, identifying strategies that consistently delay budbreak, while maintaining vine health and fruit and wine quality, is paramount to helping practitioners make informed freeze protection decisions.

This two-year study compared two methods to delay grapevine budbreak for freeze damage avoidance: the application of a vegetable oil-based adjuvant (Amigo®, Loveland Products, Inc.) at 8% and 10% and late pruning. The study was performed on Lemberger and

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Riesling, two *V. vinifera* cultivars relevant to cool climate winegrowing (Ferguson et al. 2014). Our first objective was to examine if these strategies consistently delayed grapevine budbreak and to evaluate the effects of delayed budbreak on within-season (e.g., phenology, berry development, yield) and postharvest (e.g., wine chemistry, TNC accumulation, and bud freeze tolerance) parameters. If a freeze event occurred during the study period, we sought to evaluate the efficacy of our treatments in mitigating freeze damage. We tested two concentrations of Amigo (8% and 10%) based on previous work conducted at the same research site, which indicated Amigo 10% reduced bud survival (Centinari et al. 2018).

We hypothesized Amigo application and late pruning would delay grapevine budbreak and reduce spring freeze damage, but also shift key phenological stages (e.g., bloom and veraison) compared to control vines. Based on previous work conducted in cool climate regions (Dami and Beam 2004, Loseke et al. 2015, Wang and Dami 2020), we did not expect a shift in phenology to affect fruit composition at harvest and wine chemistry, unless the delay in budbreak was extensive (> 10-14 days). However, we hypothesized vines might prioritize fruit ripening at the expense of total TNC storage, which in turn could negatively affect bud freeze tolerance or growth the following spring.

Materials and Methods

Vineyard site and experimental design. This experiment was performed in 2018 and 2019 on two *V. vinifera* cultivars: 10-year-old Lemberger (red-fruited) and Riesling (white-fruited) vines at a commercial vineyard in Lewisburg, Pennsylvania, USA (40°57′N; 76°53′W; USDA hardiness zone 6b). The vineyard soil was classified as Washington silt-loam

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(https://websoilsurvey.sc.egov.usda.gov/). Vines from each cultivar were grafted on 101-14Mgt rootstock and spaced 1.5 m apart; vineyard rows were north-south oriented and spaced 2.1 m apart. Lemberger and Riesling vines were trained to a low-wire bilateral cordon at 0.80 m height with vertical shoot positioning. Shoot density was adjusted to 15 shoots per vine if they exceeded this number. All vines received standard cultural and disease control practices for *V. vinifera* in the eastern USA (Wolf 2008).

The experimental design was a randomized complete block design with four treatments and six replications per treatment, and there were approximately eight contiguous vines in each experimental unit. Four consecutive rows of each cultivar were selected, and different rows were chosen in 2018 and 2019 to prevent additive treatment effects. The treatments were: 1) control (no delayed budbreak strategy applied); 2) Amigo® (Loveland Products, Inc.) applied at 8% (v/v) concentration during dormancy, 3) Amigo® (Loveland Products, Inc.) applied at 10% (v/v) concentration during dormancy; and 4) late pruning applied in both years when the three-most-apical buds averaged stage 7, or “first leaf separated from shoot tip,” on the Eichhorn-Lorenz (E-L) scale system (Coombe 1995), except for Riesling buds which averaged stage E-L 9, “two to three leaves separated,” in 2018. Amigo is composed of 9.2% oil (soybean-based) and 0.7% emulsifier, and the product was diluted with water to reach the desired 8% and 10% concentrations before application.

Vines assigned to the control and Amigo 8% and 10% treatments were spur-pruned to two basal buds on 28 Feb 2018 and 13 Mar 2019, while vines assigned to late pruning were trimmed to the top catch wire on the same day (approx. 2 m high). Amigo 8% and 10% were applied with a backpack sprayer until runoff was observed on both sides of the canopy on 28 Feb

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2018 and 18 Mar 2019; oil was applied before any significant heat accumulation occurred (< 10 growing degree days, GDD, base 10 °C). Late-pruning treatments were applied 7 May 2018 and 1 May 2019 for Lemberger and 11 May 2018 and 6 May 2019 for Riesling.

Weather parameters. Air temperature and rainfall data were measured throughout the study with a weather station (MK-III, RainWise, Trenton, ME) at the site. Growing degree days (base 10 °C) were calculated as GDD = [maximum daily temperature + minimum daily temperature)/2] – 10. To record the incidence of below freezing events, wireless temperature dataloggers (iButton Fob, Model DS1921G-F5#, accuracy ± 0.5 °C; Embedded Data Systems, Lawrenceburg, KY) with radiation shields were placed in each experimental unit on the first trellis wire, at the approximate height of the vine cordon. Air temperature was recorded every 20 minutes by the dataloggers from mid-March through April to capture a potential spring freeze event.

Grapevine phenology and freeze damage. Four vines and either the north or south facing cordon were randomly selected in each experimental unit for phenological assessment. On each cordon, phenological growth stage was determined for each node using a modified E-L scale. Phenology measurements were conducted on the same buds approximately twice per week, starting about one week before control vines reached budbreak and ending shortly after control vines reached fruit-set (E-L 27) in 2018 and full bloom (E-L 23) in 2019. Buds were deemed at budbreak when they were at E-L 5, “rosette of leaf tips visible.” For each date that phenology was recorded, the percentage of buds at or beyond budbreak was calculated for each experimental unit, and to estimate the date of 50% budbreak, the percentage of budbreak was interpolated between the last pre-budbreak and first post-budbreak dates. Therefore, the estimate

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assumes phenology was linear between the period before and post-budbreak, and it is an approximation. A veraison assessment was conducted in Lemberger by visually determining the percentage of berries per cluster that changed color on the same vines selected for phenology on 15 Aug 2018 and 15 Aug 2019. The veraison assessment was not conducted on the Riesling vines.

A freezing event occurred on 29 Apr 2019, when phenological stage of the Lemberger control averaged between E-L 3 and 4, “woolly buds” and “green leaf tips visible,” respectively. Green tissue damage was visually assessed in Lemberger on the same vines selected for phenology about five weeks later (6 June 2019) when signs of freeze damage (i.e., leaf discoloration and necrosis) were clearly visible. Presence of green tissue damage on each shoot was evaluated either “yes” or “no.” Freeze damage was not visible on Riesling and therefore not assessed.

Berry chemistry analysis. In each cultivar, 100 to 200 berries were randomly sampled per experimental unit three times during fruit ripening, beginning near veraison and ending at harvest. Berry samples were placed on ice for transport from the vineyard and frozen at -20 °C until chemical analysis. To determine total soluble sugars (TSS), pH, and titratable acidity (TA) for each sample, frozen berries were first counted and weighed, then placed in a water bath at 60 °C to thaw. The thawed berries were then pressed for juice and strained through cheesecloth to remove solids as described in Homich et al. 2017. TSS was measured using a handheld refractometer (Master, Atago, Bellevue, WA); pH was measured using a pH meter (Orion Star A111, Themo Fisher Scientific, Waltham, MA), and TA was measured using an automatic titrator (G20, Mettler Toledo, Columbus, OH), in which 10 mL of juice sample was diluted with 30 mL deionized water and titrated to a pH of 8.2 using 0.10 N sodium hydroxide.

Yield components and vegetative growth. Vines were hand harvested on the same day, or just prior to, commercial harvest: 3 Oct 2018 and 4 Oct 2019 for Lemberger; 26 Sept 2018 and 30 Sept 2019 for Riesling. Clusters of all the vines in each experimental unit, except the first and last vines, were counted and weighed using a hanging scale accurate to 0.02 kg (ES-55 Electro, Samson Brecknell, Fairmont, MN), from which average yield and cluster number per vine were calculated. Vines were spur-pruned to 5 or 6, 2-bud spurs, per meter of cordon on 13 Mar 2019 and 7 Apr 2020, and pruning weights of all experimental vines were collected on the same day with a hanging scale accurate to 0.02 kg (ES-55 Electro, Samson Brecknell). Crop load was calculated and expressed as Ravaz index (yield/pruning weight).

Winemaking and wine chemical analysis. On the day of harvest, approximately 375 kg Lemberger (each year) and 160 kg Riesling (2019 only) fruit was transported to The Pennsylvania State University Department of Food Science and stored overnight at 3 °C. Lemberger wines were made in 2018 and 2019, but Riesling only in 2019, due to a high level of fruit rot present across all treatments in 2018. In both years, Lemberger fruit was separated by treatment and divided into three winemaking replicates by combining grapes of two consecutive blocks (i.e., blocks 1&2; 3&4; 5&6). Riesling grapes were pooled by treatment and fermented in duplicate due to an uneven volume of fruit among field blocks.

Lemberger fruit was crushed/destemmed the day after harvest using a stainless-steel crusher and destemmer. The must from each winemaking replicate was then poured into open-top, low density polyethylene fermentation bins (Nalgene, Thermo Fisher Scientific, Waltham,

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MA), yielding between 12 and 30 L must per replicate. Riesling fruit was crushed and destemmed within 48 hours after harvest and immediately pressed in a vertical stainless-steel hydraulic basket press, yielding between 11 and 22 L per treatment. Pressed Riesling juice was evenly divided into two glass carboys per treatment. For both cultivars, juice chemistry (TA, pH, TSS) and yeast assimilable nitrogen (YAN) were measured on a 50 mL sample the day of crushing. YAN was determined by adding Ammonia Nitrogen (AN) and Primary Amino Acid Nitrogen (PAAN) values, which were determined separately using enzymatic test kits (Kit 4A120 and 4A110, respectively, Vintessential Laboratories, New South Wales, Australia). Before primary fermentation, each replicate was adjusted to 50 ppm SO_2 using potassium metabisulfite (KMBS), and Lemberger 2018 must and Riesling 2019 juice were adjusted to 21 Brix using granulated sucrose. Following, Lemberger must and Riesling juice were inoculated with 0.25 g/L *Saccharomyces cerevisiae* strain ICV-GRE (Lallemand, Milwaukee, WI) and rehydrated with 0.3 g/L GoFerm (Scott Laboratories, Petaluma, CA) for primary fermentation. YAN of each replicate was adjusted to 0.25 g/L using Fermaid K (Lallemand) nutrient at one-third sugar depletion. Fermentations were considered complete when residual sugar concentration reached <0.1%, confirmed by Clinitest tablets (Bayer, Hanover, NJ), followed by enzymatic quantification of glucose and fructose concentrations using a test kit (Kit 4A140, Vintessential Laboratories).

During primary fermentation, Lemberger pomace caps were punched down and temperatures measured three times daily, while TSS was measured once per day using a hydrometer. At dryness, each replicate was pressed into glass carboys using a vertical stainless-steel hydraulic basket press, and a 250-mL wine sample was taken from each fermentation

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replicate then stored at -20 °C. Lemberger wines were then inoculated with *Oenococcus oeni* Alpha MBR (Lalleland) for malolactic fermentation, which was monitored using paper chromatography. Malolactic fermentation was confirmed complete using an enzymatic assay for L-malic concentration (Kit 4A165, Vintessential Laboratories).

Both Lemberger and Riesling wines were adjusted to 0.8 mg/L molecular free SO_2 based on pH prior to bottling, which occurred on 4 Feb 2019 (Lemberger 2018 vintage) and 18 and 19 Dec 2019 for Riesling and Lemberger 2019 vintage, respectively. For each cultivar, 250-mL wine samples were collected at bottling and frozen at -20 °C. All wine samples were tested for alcohol, residual sugar, pH, TA, malic acid, lactic acid, and volatile acidity, using near-infrared technology (WineScan, Model 8388621, FOSS, Denmark), and free and total SO_2, using flow injection analysis (FIAstar Analyzer 5000, FOSS), at the Cornell Craft Beverage Analytical Laboratory (New York State Agricultural Experiment Station, Geneva, NY). Lemberger wine samples were analyzed for color intensity and hue according to Zoecklein et al. 1995.

Total non-structural carbohydrates. Concentrations of TNC (starch and soluble sugars) were measured in canes, trunks, and roots during vine acclimation. Two vines per experimental unit were sampled on 12 Nov 2018 and 11 Nov 2019. Two internode sections were cut from the base of two canes per vine. Trunk tissue was collected by drilling three holes using a 3.57 mm drill bit into the bottom, middle, and uppermost portion of the trunk and removing the collected trunk material from the drill bit flute. Lignified roots between 1 mm and 4 mm were collected from shallow soil layers (0-20 cm) at both sides of the vine. All cane, trunk, and root samples were immediately placed on dry ice for transport and stored at -80 °C until processing. Root samples

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were washed with de-ionized water to remove debris and any fine, absorptive root (<1 mm) was trimmed before storage.

Tissues were lyophilized for one week at a temperature of -50 °C and pressure of 0.100 mbar (FreeZone 12 Liter Freeze Dryer, Labconco, Kansas City, MO) before TNC extraction. Once dried, cane and root samples were ground using a mill with 1.0 mm mesh sieve (Wiley Model 4 Mill, Thomas Scientific, Swedesboro, NJ), while trunk samples were hand-ground using a mortar and pestle. Total non-structural carbohydrates concentration was determined using the protocol outlined in Comas et al. 2005, with a spectrophotometer at 520 nm (UV1600, VWR International); TNC concentrations are expressed in glucose equivalents.

Bud freeze tolerance. Bud freeze tolerance was determined during vine acclimation (November), midwinter (maximum freeze tolerance; January or February), and deacclimation (March or April) using differential thermal analysis (DTA) as described in Mills et al. 2006. This method detects the low-temperature exotherm (LTE) created by the freezing of intracellular water, which is lethal to the cell (Burke et al. 1976). Four healthy canes per experimental unit were sampled on the following dates for Lemberger: 6 Nov 2018 and 11 Nov 2019, 28 Jan 2019 and 20 Jan 2020, and 9 Apr 2019 and 25 Mar 2020; in Riesling, canes were sampled on: 8 Nov 2018 and 11 Nov 2019, 7 Feb 2019 and 20 Jan 2020, and 9 Apr 2019 and 25 Mar 2020. Bud freeze tolerance was measured on the same day samples were collected, except for Riesling acclimation sampling in 2019, midwinter sampling dates in both years, and deacclimation sampling in 2020; in these instances, half the samples of each block were measured on the same day they were collected and half the following day with canes stored in a 2 °C walk-in cooler overnight, or at ambient temperatures (10 °C) for Riesling midwinter sampling in 2019.

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To measure bud freeze tolerance, nodes 3 and 4 on each cane were excised with a razorblade and placed in thermoelectric modules on trays (Melcor Corporation, Lawrenceville, NJ). Four buds were placed in each module and two modules were used for each experimental unit in each DTA run. The trays were placed in a temperature-controlled freezer chamber (Tenney, Thermal Product Solutions, New Columbia, PA) and temperature slowly lowered to -40 °C, as described in Mills et al. 2006. Bud freeze tolerance for each experimental unit was estimated as the median low-temperature exotherm (LT_{50}); or, the temperature at which 50% of sampled buds died (Mills et al. 2006).

Bud survival and fruitfulness. Bud survival was assessed on the same cordons used for the phenology assessment on 29 May 2018 for both cultivars and on 3 and 4 June 2019 for Lemberger and Riesling, respectively, prior to shoot thinning; bud survival was calculated as percentage of total buds that opened. Carry-over treatment effects were evaluated the year after the 2018 and 2019 treatment applications on 3 June 2019 and 6 June 2020, respectively. The number of live buds, number of shoots, and number of clusters per shoot were measured on each cordon of the vines used for phenological measurements, and the percentage of live buds, and clusters per shoot (bud fruitfulness) were calculated.

Statistical Analysis. All data analysis was performed using SAS statistical software (SAS Institute, Inc.) All viticulture data were subjected to analysis of variance (ANOVA) using the MIXED procedure with block included as a random effect. All viticulture data were analyzed separately by year because we wanted to assess vine response to a spring freeze event, which occurred in one out of the two years of the study (2019). Tukey's honest significant difference test was used to identify significant treatment differences at the 0.05 alpha level. Data collected

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under field conditions can be quite variable, and a large sample size (over 10 blocks) might be needed to detect differences at the 5% level (Marini 1999); therefore, we elected to report exact pairwise comparison *p*-values in text to assist the reader in data interpretation.

Lemberger wine chemical data was analyzed similarly to viticulture data, as wine was fermented in biological triplicate. For Riesling wine chemistry, biological replicates could not be maintained, and wine was fermented in duplicate; therefore, differences among treatments were not statistically analyzed and standard errors are reported for Riesling wine data.

Results

Weather conditions. Cumulative GDD between 50% budbreak and harvest were similar between the two growing seasons: 1706 GDD (2018) and 1689 GDD (2019) for Lemberger control vines and 1635 GDD (2018) and 1612 GDD (2019) for Riesling control vines. GDD accumulated from budbreak to harvest for Lemberger treatments were 39 GDD (Amigo 8%, 2018) to 74 GDD (late pruning, 2018) lower than the control; for Riesling, the range varied from 13 (Amigo 8%, 2018) to 76 (late pruning, 2018) fewer GDD than control vines (data not shown). Rainfall from May through September was almost double in 2018 (836 mm) compared to 2019 (480 mm). The summer of 2019 (July to September) was relatively dry with only 187 mm of total rainfall, while 2018 was much wetter with 617 mm of rainfall. There was a freezing event on 29 Apr 2019; temperatures decreased below 0 °C starting at 0200 hr and did not increase above 0 °C until 0600 hr. The minimum temperature reached during this period was -2.0 °C at 0500 hr. About 40% of the control buds were at or beyond budbreak (E-L 5) at the time of the freezing event (Figure 1D).

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Grapevine budbreak and phenology. Amigo and late pruning treatments delayed budbreak compared to the control in both cultivars and years (Figure 1 and 2). In Lemberger, control vines reached 50% budbreak around 9 May 2018 and 6 May 2019, while those treated with Amigo 8% and 10% reached 50% budbreak six and seven days later in 2018 ($p = 0.012$ and $p = 0.005$, respectively) and five days later in 2019 ($p = 0.030$) (Table 1). Late-pruned Lemberger vines reached 50% budbreak 10 days later than the control in both years ($p < 0.001$). In general, there were not significant differences in phenological growth stage between Lemberger vines treated with Amigo 8% or 10%; therefore, results of the two Amigo treatments will be discussed together.

Control vines had a higher percentage of buds at or beyond budbreak than Amigo 8% and Amigo 10% through 15 May 2018 ($p = 0.028$ and $p = 0.019$, respectively) and approximately 15 May 2019 ($p = 0.034$ and $p = 0.072$, respectively) (Figure 1C and 1D). The delay in rate of budbreak was more pronounced for late pruning than Amigo treatments; control vines had a significantly higher percentage of buds at or beyond budbreak compared to late-pruned vines through 21 May 2018 ($p = 0.003$) and 23 May 2019 ($p = 0.016$) (Figure 1C and 1D).

The delay in phenological development of late-pruned Lemberger vines was visible at veraison (Table 2), while differences between Amigo and control vines disappeared by bloom (Figure 1A and 1B). Specifically, the Amigo treatments were still behind in phenological development when the control was at E-L 19 “beginning of flowering” in 2018 (control vs. Amigo 8%, $p = 0.008$; control vs. Amigo 10%, $p = 0.024$) and E-L 11 “four leaves separated” in 2019 (control vs. Amigo 8%, $p = 0.006$; control vs. Amigo 10%, $p = 0.009$). The phenological delay of the late pruning treatment was more pronounced than Amigo treatments; late-pruned

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vines were still significantly different than control vines when the control was at fruit set (E-L 27) in 2018 ($p = 0.010$) and past full bloom (E-L 23) in 2019 ($p = 0.006$). The veraison visual assessment indicated that, in mid-August, a higher percentage of berries had changed color in the control compared to late-pruned clusters in both years, while differences between Amigo and control treatments were not significant or consistent between years (Table 2).

In Riesling, vines treated with Amigo 8% and 10% reached 50% budbreak three and six days later than control vines in 2018 ($p = 0.249$ and $p = 0.004$, respectively) and six and eight days later in 2019 ($p = 0.050$ and $p = 0.013$, respectively) (Table 1). Late-pruned Riesling vines reached 50% budbreak 11 (2018) and 10 (2019) days after the control vines (2018 and 2019; $p < 0.001$) (Table 1). In 2018, Riesling control vines had a greater percentage of buds at or beyond budbreak than Amigo 10% through 21 May ($p = 0.023$), but there were no differences between the control and Amigo 8% treatments (Figure 2C). In comparison, in 2019, control vines had a higher percentage of buds at or beyond budbreak through 14 May for Amigo 8% ($p = 0.007$) and 20 May for Amigo 10% ($p = 0.025$) (Figure 2D). Late-pruned Riesling vines had the lowest percentage of buds at or beyond budbreak, remaining lower than the control through 21 May 2018 ($p < 0.001$) and 4 June 2019 ($p = 0.008$); except for 30 May 2019, when the percentage of budbreak did not differ among treatments (Figure 2C and 2D). Late pruning and Amigo 10% delayed phenological development compared to control vines in Riesling, but there were not consistent differences between Amigo 8% and control vines (Figure 2A and 2B). Phenological growth stage of late-pruned vines remained behind the control through the last day of measurements in both years, while Amigo 10% in 2019 only. In 2019, Amigo 10% and late

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pruning vines were at E-L 20, “0% caps off,” while control vines were already past full bloom, E-L 23 (p = 0.017 and p = 0.037, respectively) on the last day of measurements (Figure 2B).

Freeze damage. On 29 Apr 2019, the day of the spring freeze event, 40% of Lemberger control buds were at budbreak (E-L 5), while only 15%, 11%, and 16% of Amigo 8%, Amigo 10%, and late pruning buds, respectively, were at budbreak (c vs. each delayed budbreak treatment, $p < 0.001$) (Figure 1D). In Riesling, less than 10% of the buds were at budbreak, regardless of treatment; the average growth stage was around E-L 3 “woolly bud,” thus green tissue was not yet visible (Figure 2B). The post-freeze damage assessment indicated Lemberger control vines had significantly higher percentage of shoots with visible green tissue damage than late-pruned vines (58% vs. and 31% of total shoots, respectively; p = 0.004). The control also tended to have a higher percentage of shoots with visible damage (58%) than Amigo 8% (42%, p = 0.098) and Amigo 10% (41%, p = 0.067) (data not shown).

Yield components and pruning weight. Treatment effects on yield components at harvest varied between cultivars (Table 3). In Lemberger, Amigo and late pruning treatments did not affect yield components in 2018. In 2019, the year with a spring freeze, late-pruned vines had 61% higher yield than control vines (p = 0.038); although there were no significant differences in yield components, the number of clusters per vine tended to be higher in the late-pruned vines compared to control vines (p = 0.077). In contrast, late pruning negatively affected Riesling average cluster and berry weight in both years, while production parameters of Amigo and control vines did not differ (Table 3). In 2018, late-pruned Riesling vines had lower yield compared to control vines (p = 0.027), likely attributed to lower berry weight (p = 0.023) and fewer berries per cluster (p = 0.074).

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Amigo and late pruning treatments did not affect vine vegetative growth, assessed through pruning weights, in either cultivar or year (Table 3). Overall, crop load indices were low for both cultivars and years (data not shown). In 2018, Ravaz indices did not differ across treatments and ranged from 1.32 (control) to 2.40 (late pruning) for Lemberger and from 2.47 (Amigo 8%) to 3.15 (control) for Riesling. In 2019, Lemberger Ravaz indices ranged from 1.75 (control) to 2.68 (late pruning; control vs late pruning, $p = 0.063$). Ravaz indices for Riesling 2019 were very low, ranging from 0.67 (late Pruning) to 1.11 (Amigo 10%), but crop load was not affected by the treatments.

Juice and wine chemistry. In both cultivars, juice chemistry (TSS, pH, and TA) during ripening did not consistently or significantly vary across treatments (harvest data shown in Table 2). The only significant difference among treatments at harvest was higher juice TA of late-pruned Riesling vines than Amigo 10% in 2018 ($p = 0.015$) and lower pH of Amigo 8% and late-pruned vines compared to Amigo 10% in 2019 ($p = 0.041$ and $p = 0.004$, respectively) (Table 2). Wine chemistry confirmed harvest juice chemistry results; there were no significant differences among treatments for any parameter analyzed (pH, TA, alcohol, color intensity and hue) for Lemberger wines (Table 4).

Total non-structural carbohydrates. The delayed budbreak treatments did not decrease TNC concentration in canes, trunks, or roots in November (Table 5). In 2019, canes of Lemberger control vines had significantly lower concentration of TNC than all the other treatments (Amigo 8%, $p = 0.018$; Amigo 10%, $p < 0.001$; late pruning, $p = 0.004$).

Bud freeze tolerance. Overall, treatments did not consistently affect bud freeze tolerance throughout dormancy in either cultivar (Table 6). In 2018 only, LT_{50} of Amigo and late-pruned

Lemberger vines was between 2.1 (Amigo 8%) and 2.6 °C (late pruning) higher than that of the control in November (Amigo 8%, $p = 0.024$; Amigo 10%, $p = 0.008$; late pruning, $p = 0.005$). These differences were not significant by January 2019.

Bud survival and fruitfulness. In both years, Riesling vines treated with Amigo 10% had lower bud survival than control vines. In 2018, Riesling vines treated with Amigo 10% had 82.4% bud survival, whereas control vines had 91.8% bud survival ($p = 0.042$); in 2019, bud survival was 59.2% for Amigo 10% and 73.6% for control vines ($p = 0.045$) (data not shown). Lemberger bud survival did not differ among treatments in either year and ranged from 82.4 % (control) to 90.2% (late pruning) in 2018, and from 74.8% (Amigo 8%) to 83.8% (control) in 2019 (data not shown). There were no carry-over effects the year after treatment application. The percentage of live buds and bud fruitfulness did not differ between vines assigned to control and delayed budbreak treatments the previous year for either cultivar (data not shown).

Discussion

In general, our results indicated Amigo and late pruning consistently delayed grapevine budbreak. The delay in budbreak was most pronounced and consistent between years in late-pruned vines than those treated with Amigo 8% or 10%. Consequently, late pruning was most effective in mitigating grapevine freeze damage. Our results on the effects of Amigo and late pruning on delaying budbreak support previous research conducted on *V. vinifera* cultivars. For instance, Amigo 10% applied during dormancy to Riesling and Lemberger grown at the same site of our study delayed budbreak between six and 11 days (Centinari et al. 2018). Rapeseed oil applied to Grüner Veltliner and Zweigelt delayed budbreak between five and 12 days,

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respectively (Herrera et al. 2018). These ranges, with few exceptions (see for example, Riesling Amigo 8% in 2018) are similar to what we reported for the Amigo treatments. Several factors can influence the efficacy of vegetable oil-based adjuvants in delaying budbreak, including cultivars (i.e., *Vitis* hybrids vs *V. vinifera*), time of application during dormancy, and weather conditions prior to budbreak (Loseke et al. 2015, Centinari et al. 2018, Wang and Dami 2020). Cultivars with relatively late budbreak may benefit from later application of Amigo during dormancy (Dami and Beam 2004), and an early application might increase chance of oil weathering by rainfall. More research is required to clarify the effect of weather conditions (i.e., heat accumulation and rainfall) after Amigo application on year-to-year variation in budbreak delay.

In comparison to applications of vegetable oil-based adjuvants, the extent of delay induced by late pruning is mostly attributed to the phenological stage of the apical buds at the time of removal (Frioni et al. 2016, Gatti et al. 2016). Late pruning applied at a similar phenological stage of our study (between two-to-three leaves unfolded) delayed budbreak from 17 days (Gatti et al. 2016) in Sangiovese vines, to four weeks or more (Frioni et al. 2019, Moran et al. 2017) in Pinot Noir and Shiraz vines, respectively, compared to standard pruning. Delays in budbreak of late-pruned vines were less pronounced in our study, but still consistent between years and cultivars. Among several factors, including chilling requirement and genotype (Londo and Johnson 2014), different temperatures around the time of budbreak may explain different results across studies. Although our research was conducted in a cool climate, it is possible that at our site, temperatures around the time of budbreak were higher than in northern Italy and Australia (Gatti et al. 2016, Moran et al. 2017), leading to a shorter delay in budbreak between

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control and late-pruned vines; for example, in our study, budbreak of Lemberger and Riesling control vines occurred approximately three to four weeks later than in Gatti et al. 2016.

Delayed budbreak in the Amigo and late pruning treatments resulted in the delay of subsequent key phenological stages. In Lemberger, differences between the late pruning and control treatments were still visible at veraison, while differences in phenology between the Amigo treatments and control tended to converge by bloom. In Riesling, differences between the control and late pruning treatments were observed through bloom, and Amigo 10% tended to be more effective in delaying phenology than 8%. These delays in phenology did not correspond to treatment differences in berry ripening. In agreement with our hypothesis, there were no effects of Amigo or late pruning on fruit and wine chemistry compared to control vines. Our results support previous studies on Amigo where the extent of delayed budbreak was similar to what we observed (i.e., 11 days or less), and there were no effects on juice or wine chemistry in either *V. vinifera* (Centinari et al. 2018) or hybrid cultivars (Dami and Beam 2004). Previous research on the effects of late pruning on juice chemistry reported lower TSS accumulation and higher TA in berries during ripening (Frioni et al. 2016, Frioni et al. 2019); however, these differences were observed when pruning was performed at a later phenological stage (Frioni et al. 2016), or when a more extensive delay in budbreak was reported (Frioni et al. 2019) than in our study. Similarly, differences in wine chemistry between late pruning and control treatments were reported when delays in budbreak were more extensive (i.e., over two weeks) than we measured under our study conditions (Moran et al. 2018).

We hypothesized preferential allocation of photosynthates to vegetative and reproductive growth might reduce TNC storage in perennial tissues, especially if the delay in budbreak was

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extensive. However, there was no evidence of reduced TNC concentration for the tissues analyzed or related negative, carry-over effects, such as lower bud freeze tolerance, in response to delayed budbreak. It is possible leaves of Amigo and late-pruned vines had increased net photosynthetic efficiency (Gatti et al. 2016), which may have supplied sufficient photosynthates for both fruit ripening and storage by fall. Alternatively, the delays in phenology were too modest to cause a photosynthate ‘deficit’ strong enough to affect fruit development or TNC storage; although late pruning may have altered source-sink relationships earlier in the season, reducing fruit set in Riesling, as discussed later.

The efficacy of late pruning to mitigate freeze damage, without negatively affecting within-season and postharvest parameters, is relevant to regions where spring freeze events are a recurrent issue. In 2019, Lemberger late-pruned vines had 61% greater yield than control vines, reflecting differences in shoot freeze damage between the two treatments. Freezing temperatures may have killed a higher percentage of young shoots in the control, leaving less-fruitful secondary buds to develop (Friend et al. 2011); or, freezing temperatures might have damaged inflorescences in buds and young primary shoots (Centinari et al. 2016). Freeze damage did not affect fruit or wine chemistry but may have reduced TNC accumulation in canes of control vines at the end of the season (November) vis-à-vis damage to vegetative growth and a potential reduction in carbon assimilation. Although the Amigo and late-pruned vines appeared to be at a similar phenological stage on the day of the freeze event, the ability for late pruning to reduce freeze damage (i.e., prevent vegetative tissue damage and crop loss) was more pronounced in late pruning than Amigo treatments. It is plausible that slight differences in bud development were not detectable at such early phenological stages; this is supported by the fact that Amigo-

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treated vines achieved budbreak earlier than late-pruned vines. It is worth noting that while Amigo treatments did not mitigate crop loss, they reduced vegetative damage in Lemberger compared to control vines to some degree.

Throughout our study, there were no negative effects of Amigo application on yield components in Lemberger or Riesling. Late pruning did not have any detrimental effect in Lemberger, but it negatively affected several yield components in Riesling (i.e., reduced cluster and berry weight). Previous studies have reported contrasting effects of late pruning on crop yield. Depending on location, cultivar, and time of application, late winter pruning decreased (Frioni et al. 2016, Gatti et al. 2016, Petrie et al. 2017), increased (Friend and Trought 2007, Moran et al. 2017, Petrie et al. 2017), or did not affect crop yield (Frioni et al. 2016, Moran et al. 2017). In our study, late-pruned Riesling vines had lower berry weight than the control in both years, which could be attributed to a delay or shorter "Stage 1" of berry ripening, when the relative increase in berry fresh weight rapidly increases (Staudt et al. 1986). Fewer berries per cluster in response to late pruning has been reported in previous literature (Frioni et al. 2016, Gatti et al. 2016), but the mechanism behind this reduction is not clear. Late-pruned vines tended to have fewer berries per cluster compared to the control in the first season ($p = 0.074$) of our study. Possible explanations for fewer berries per cluster in late-pruned vines include compromised flower development in basal buds or decreased fruit set in response to late pruning. Growth of apical buds in late-pruned vines might have limited carbohydrates availability to basal buds, reducing their floral development (Mason et al. 2014); or, a carbohydrate deficit induced by late pruning may have prompted vines to prioritize vegetative growth over fruit set, as reported when pre-bloom leaf removal is implemented (Frioni et al. 2018). Future studies could

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explore effects of late pruning on flower development, fertilization, and berry growth, which, at least under our experimental conditions, varied between cultivars and vintages.

Crop load values in our study for both cultivars and years were below the range (4 to 10) suggested for optimal vine balance (Kliewer and Dokoozlian 2005). Vines were overly vegetative likely due to high-vigor potential of the site and possible winter cold damage, specifically for Riesling in 2019, when crop load was low regardless of treatment. Although not directly quantified, the overall low yield and number of clusters per vine in Riesling may be associated with winter cold damage prior to the second study year. The lowest winter temperature was -19 °C on 31 Jan 2019; based on LT_{10} (data not shown) and LT_{50} data of the 2018 experimental vines collected three days earlier (Lemberger) and a week later (Riesling), we did not expect extensive bud damage in either cultivar. However, the vines used in 2019 were in different rows than the vines used in 2018, and the 2019 vines might have experienced winter cold damage, resulting in a lower-than-expected overall bud survival. Percent of bud survival for Riesling control vines was, indeed, 91.8 % in 2018, but 73.6% in 2019. However, neither Amigo application nor late pruning affected the inherent vigor of Riesling or Lemberger vines, and these treatments had no relevant or consistent effect on the crop load.

The application of Amigo 10% consistently reduced bud survival in Riesling compared to control vines, supporting previous work (Centinari et al. 2018). In that study, oil phytotoxicity together with below-average winter temperatures were suggested as potential causes for reduced bud survival of Amigo-treated vines. In our study, it is possible the higher concentration of Amigo (10%) induced excessive CO_2 or endogenous hormone accumulation in buds, eventually causing the reduced bud survival when measured later in the season. Our results indicate the

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effect of Amigo 10% on bud survival was cultivar-specific; moreover, reduced bud survival was not significant enough to negatively affect other parameters (e.g., yield or pruning weight) in Riesling vines treated with Amigo 10%.

Conclusion

Amigo and late pruning treatments tended to delay budbreak in both years and cultivars in our study, and late pruning provided the most consistent and extensive delay in budbreak and subsequent phenological stages. Furthermore, Lemberger late-pruned vines sustained significantly lower freeze damage and had higher yield than control vines in the year with a freeze event. There were several drawbacks of Amigo application and late pruning on Riesling vines suggesting cultivar-dependent responses should be tested and expected. There were no negative effects of Amigo application on within-season or postharvest parameters in Lemberger; however, the extent of delay in phenological growth can be less predictable in Amigo-treated vines and, in our study, it was not large enough to cause a significant reduction in freeze damage. Testing Amigo on a small number of vines is suggested; this could also limit the cost of application (material and labor) which could be prohibitive for some growers. Late pruning applied relatively soon post-apical budbreak (i.e., E-L 7) may be a more reliable method to delay budbreak for cordon-trained vines. Because it is a labor-intensive practice, late pruning is best suited for small, freeze-prone areas of the vineyard.

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Table 1 Dates of 50% budbreak and harvest for Lemberger and Riesling grapevines at the experimental vineyard in 2018 and 2019.

	2018		2019	
Treatment[a]	Date of 50% budbreak	Date of harvest	Date of 50% budbreak	Date of harvest
Lemberger				
Control	9 May a[b]	3 October	6 May a	4 October
Amigo 8%	15 May b		11 May b	
Amigo 10%	16 May b		11 May b	
Late pruning	19 May b		16 May c	
p-value	<0.001		<0.001	
Riesling				
Control	12 May a	26 September	13 May a	30 September
Amigo 8%	15 May a		19 May b	
Amigo 10%	18 May b		21 May b	
Late pruning	23 May c		23 May b	
p-value	<0.001		0.004	

[a] Treatments were a control (no delayed budbreak strategy applied), Amigo® applied during dormancy at 8% or 10% (v/v), and late pruning applied when the three-most-apical buds averaged stage 7 on the Eichhorn-Lorenz (E-L) scale, except for Riesling buds in 2018 which averaged E-L 9.

[b] Treatment means within columns followed by different letters are significantly different based on Tukey's honest significant difference test at 95% confidence.

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Table 2 Effect of Amigo and late pruning treatments on Lemberger percent veraison in mid-August and Lemberger and Riesling juice chemistry at harvest.

Treatment[a]	Veraison[b] (%)		TSS (Brix)		pH		TA (g/L)	
	2018	2019	2018	2019	2018	2019	2018	2019
Lemberger								
Control	80 a[c]	75 a	18.2	23.5	3.59	3.56	7.79	6.62
Amigo 8%	51 b	74 a	18.2	22.3	3.57	3.50	7.88	6.44
Amigo 10%	58 ab	74 a	17.0	22.5	3.60	3.54	7.38	6.66
Late pruning	37 b	53 b	18.4	23.5	3.54	3.54	8.40	6.38
p-value	<0.001	0.009	0.420	0.192	0.148	0.516	0.340	0.361
Riesling								
Control	N/A[d]	N/A	15.5	18.6	3.42	3.46 ab	8.71 ab	7.49
Amigo 8%	N/A	N/A	15.5	18.9	3.39	3.43 b	8.89 ab	7.74
Amigo 10%	N/A	N/A	14.8	18.5	3.37	3.54 a	8.39 b	7.69
Late pruning	N/A	N/A	15.3	17.9	3.34	3.36 b	9.39 a	8.40
p-value			0.726	0.496	0.063	0.006	0.022	0.176

[a] Treatments were a control (no delayed budbreak strategy applied), Amigo® applied during dormancy at 8% or 10% (v/v), and late pruning applied when the three-most-apical buds averaged stage 7 on the Eichhorn-Lorenz (E-L) scale, except for Riesling buds in 2018 which averaged E-L 9.

[b] Percentage of berries per cluster that changed color measured on 15 Aug 2018 and 15 Aug 2019.

[c] Treatment means within columns followed by different letters are significantly different based on Tukey's honest significant difference test at 95% confidence.

[d] Riesling berry color-change was not measured.

Table 3 Effect of Amigo and late pruning treatments on Riesling and Lemberger yield components and pruning weight in 2018 and 2019.

	Yield (kg/vine)		Clusters/vine (n)		Cluster weight (g)		Berry weight (g)		Berries/cluster (n)		Pruning wt (kg/vine)	
Treatment[a]	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Lemberger												
Control	2.60	2.03 b[b]	19 b	15	139	139	1.82	1.83	76	77	1.47	1.63
Amigo 8%	2.86	3.02 ab	22 ab	19	129	157	1.80	1.86	71	84	1.24	1.44
Amigo 10%	2.96	2.72 ab	24 a	17	125	154	1.77	1.72	71	90	1.18	1.44
Late pruning	3.24	3.27 a	25 a	21	130	151	1.86	1.72	70	87	1.23	1.44
p-value	0.150	0.045	0.011	0.094	0.679	0.404	0.551	0.379	0.550	0.344	0.103	0.231
Riesling												
Control	3.52 a	1.12	33	13	107 a	79 a	1.97 a	2.06 a	55	39 ab	1.15	1.42
Amigo 8%	3.05 ab	1.27	35	15	86 ab	87 a	1.94 a	1.88 ab	44	46 ab	1.24	1.33
Amigo 10%	2.96 ab	1.51	33	16	88 ab	95 a	1.95 a	1.86 ab	45	53 a	1.00	1.38
Late pruning	2.28 b	0.75	31	12	73 b	52 b	1.76 b	1.60 b	43	33 b	0.92	1.11
p-value	0.027	0.171	0.542	0.645	0.012	0.003	0.018	0.010	0.081	0.023	0.309	0.161

[a] Treatments were a control (no delayed budbreak strategy applied), Amigo® applied during dormancy at 8% or 10% (v/v), and late pruning applied when the three-most-apical buds averaged stage 7 on the Eichhorn-Lorenz (E-L) scale, except for Riesling buds in 2018 which averaged E-L 9.

[b] Treatment means within columns followed by different letters are significantly different based on Tukey's honest significant difference test at 95% confidence.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2021.20076
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Table 4 Effect of Amigo and late pruning treatments on Lemberger and Riesling on wine composition at bottling, vintage 2018 and 2019.

Treatment[a]	pH		TA (g/L)		Alcohol[b] (%)		Color Intensity (Au)		Color Hue (Au/Au)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Lemberger										
Control	3.76	3.86	5.23 a[c]	5.53	10.9	12.6	2.46	2.23	1.21	1.03
Amigo 8%	3.79	3.78	4.90 b	5.60	10.7	12.5	2.18	2.24	1.18	0.93
Amigo 10%	3.75	3.78	4.90 b	5.67	10.7	12.5	2.40	2.06	1.10	0.98
Late pruning	3.72	3.76	5.20 a	5.50	10.8	12.7	2.27	2.16	1.24	0.90
p-value	0.396	0.427	0.011	0.902	0.939	0.763	0.529	0.756	0.381	0.759
Riesling										
Control	N/A[d]	3.21 ±0.01[e]	N/A	7.55 ± 0.15	N/A	11.8 ± 0.1	N/A	N/A[f]	N/A	N/A
Amigo 8%	N/A	3.20 ± 0.01	N/A	7.75 ± 0.05	N/A	12.1 ± 0.1	N/A	N/A	N/A	N/A
Amigo 10%	N/A	3.17 ± 0.05	N/A	7.65 ± 0.05	N/A	11.8 ± 0.1	N/A	N/A	N/A	N/A
Late pruning	N/A	3.17 ± 0.05	N/A	7.75 ± 0.05	N/A	11.1 ± 0.1	N/A	N/A	N/A	N/A

[a] Treatments were a control (no delayed budbreak strategy applied), Amigo® applied during dormancy at 8% or 10% (v/v), and late pruning applied when the three-most-apical buds averaged stage 7 on the Eichhorn-Lorenz (E-L) scale, except for Riesling buds in 2018 which averaged E-L 9.

[b] Lemberger must was chaptalized to 21 Brix with granulated sucrose prior to inoculation in 2018. Riesling juice was chaptalized to 21 Brix with granulated sucrose prior to inoculation in 2019. Lemberger must was not chaptalized in 2019.

[c] Lemberger wines were fermented in biological triplicate; means within columns followed by different letters are significantly different based on Tukey's honest significant difference test at 95% confidence.

[d] Riesling wine was not made in 2018.

[e] Riesling wine was fermented in duplicate in 2019; standard error of treatment means are reported.

[f] Color intensity and hue were not measured for Riesling (white wine).

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Table 5 Effect of Amigo and late pruning treatments on total non-structural carbohydrates (TNC) concentration in Lemberger and Riesling cane, trunk, and root tissues during vine acclimation in November 2018 and 2019. Concentrations are reported in milligrams of glucose equivalents per gram of dry tissue weight.

Treatment[a]	Cane (mg/g)		Trunk (mg/g)		Root (mg/g)	
	2018	2019	2018	2019	2018	2019
Lemberger						
Control	78.5	55.7 b[b]	57.8	62.4	81.3	121.0
Amigo 8%	79.7	63.1 a	56.5	55.7	90.1	123.6
Amigo 10%	85.0	67.6 a	54.5	57.8	88.3	115.3
Late pruning	81.6	64.7 a	54.9	63.1	92.8	109.8
p-value	0.411	< 0.001	0.583	0.098	0.160	0.753
Riesling						
Control	73.0	61.8	59.6	46.9	N/A[c]	N/A
Amigo 8%	78.4	59.9	62.5	43.3	N/A	N/A
Amigo 10%	75.5	63.9	61.8	45.8	N/A	N/A
Late pruning	75.1	60.6	66.8	46.1	N/A	N/A
p-value	0.307	0.841	0.383	0.547		

[a] Treatments were a control (no delayed budbreak strategy applied), Amigo® applied during dormancy at 8% or 10% (v/v), and late pruning applied when the three-most-apical buds averaged stage 7 on the Eichhorn-Lorenz (E-L) scale, except for Riesling buds in 2018 which averaged E-L 9.

[b] Treatment means within columns followed by different letters are significantly different based on Tukey's honest significant difference test at 95% confidence.

[c] Vine undergrowth prohibited accurate root sampling of Riesling.

Table 6 Effect of Amigo and late pruning treatments on bud median low-temperature exotherm (LT$_{50}$; °C) for Riesling and Lemberger vines at acclimation (November), maximum freeze tolerance (January or February) and deacclimation (March or April) during 2018-2019 and 2019-2020 dormant seasons.

Treatment[a]	**November 2018**	**November 2019**	**January 2019**	**January 2020**	**April 2019**	**March 2020**
Lemberger						
Control	-17.10 b[a]	-17.43	-22.33	22.28	-11.02	-13.82
Amigo 8%	-15.00 a	-17.61	-20.35	-22.60	-10.45	-13.77
Amigo 10%	-14.67 a	-17.79	-22.53	-22.53	-12.03	-14.08
Late pruning	-14.48 a	-17.78	-22.90	-22.91	-11.25	-13.99
p-value	<0.003	0.751	0.078	0.825	0.427	0.936
	November 2018	**November 2019**	**February 2019**	**January 2020**	**April 2019**	**March 2020**
Riesling						
Control	-15.12	-19.35	-22.95	-22.85	-10.42	-15.92
Amigo 8%	-15.14	-19.02	-23.99	-22.95	-10.85	-16.11
Amigo 10%	-14.11	-18.96	-22.30	-23.18	-9.83	-16.34
Late pruning	-14.63	-19.03	-22.78	-22.80	-10.60	-15.85
p-value	0.191	0.951	0.142	0.908	0.326	0.764

[a] Treatments were a control (no delayed budbreak strategy applied), Amigo® applied during dormancy at 8% or 10% (v/v), and late pruning applied when the three-most-apical buds averaged except for Riesling buds in 2018 which averaged E-L 9.

[b] Treatment means within columns followed by different letters are significantly different based on Tukey's honest significant difference test at 95% confidence.

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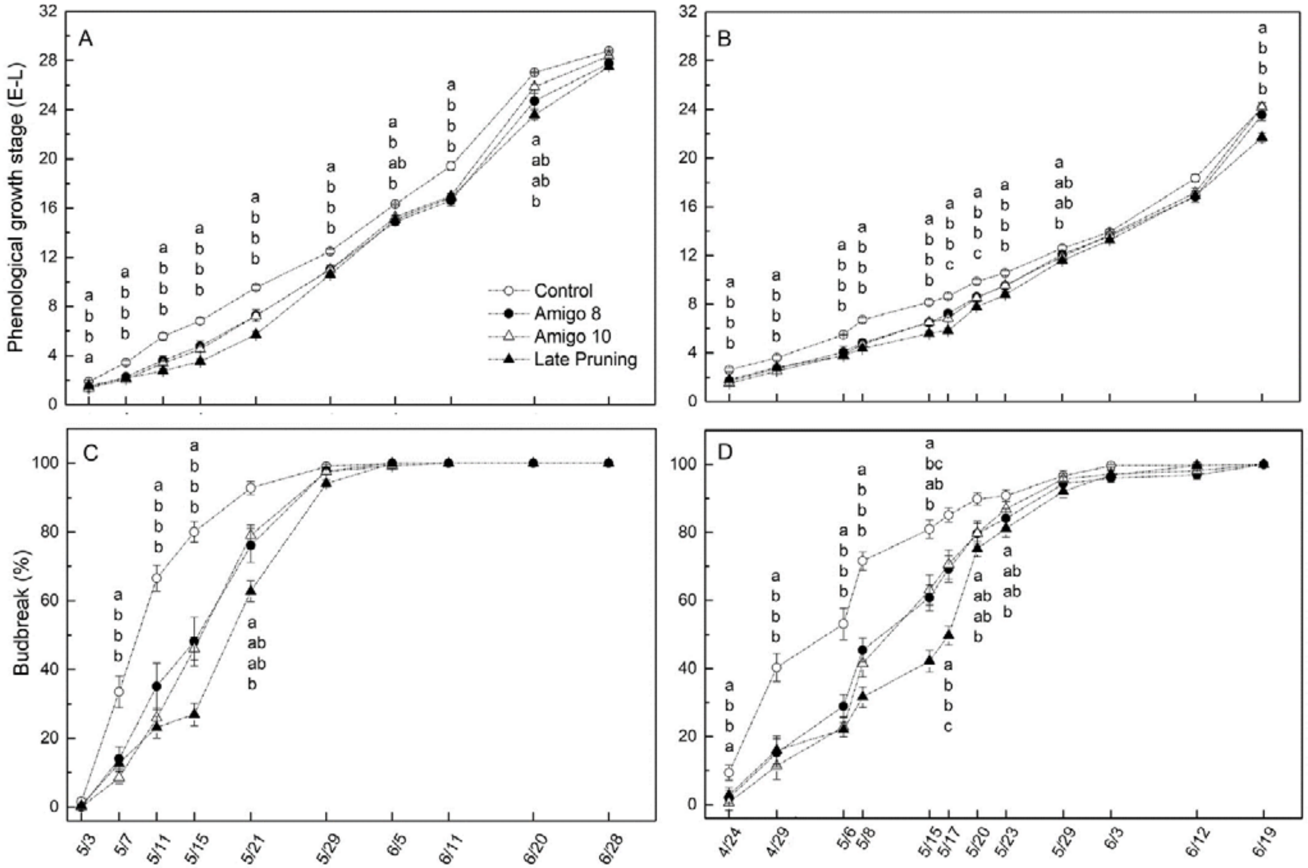

Figure 1 Average phenological growth stage of Lemberger vines in 2018 (A) and 2019 (B) and percentage of buds that reached budbreak (E-L 5) in 2018 (C) and 2019 (D) at each sampling date. Treatments were a control (no delayed budbreak strategy applied), Amigo® applied during dormancy at 8% or 10% (v/v), and late pruning applied when the three-most-apical buds averaged stage 7 on the Eichhorn-Lorenz (E-L) scale. For each date, different letters indicate differences between treatments based on Tukey's honest significant difference test at 95% confidence. Letters of significance are ordered from top-down at each date as follows: 1) Control, 2) Amigo 8%, 3) Amigo 10%, and 4) Late pruning.

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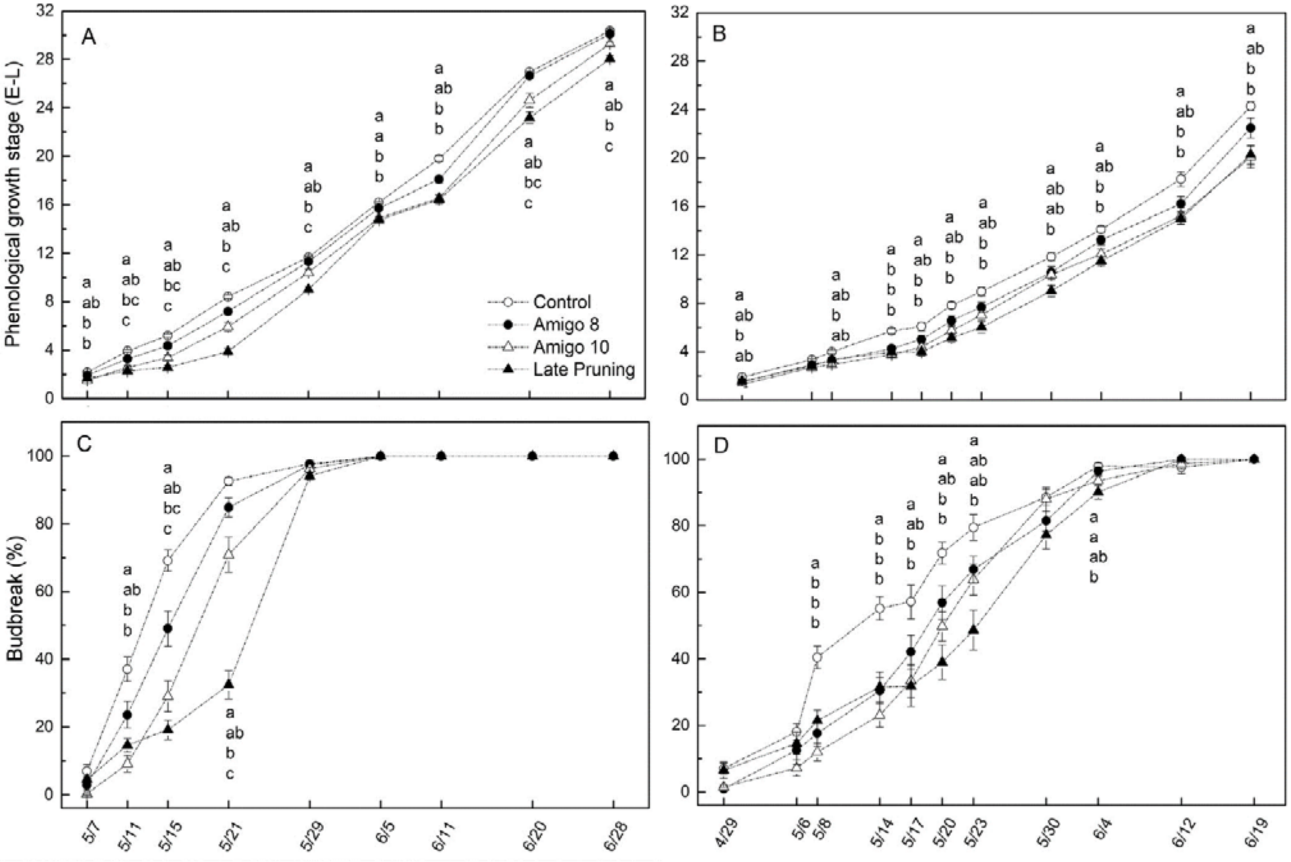

Figure 2 Average phenological growth stage of Riesling vines in 2018 (A) and 2019 (B) and percentage of buds that reached budbreak (E-L 5) in 2018 (C) and 2019 (D) at each sampling date. Treatments were a control (no delayed budbreak strategy applied), Amigo® applied during dormancy at 8% or 10% (v/v), and late pruning applied when the three-most-apical buds averaged stage 7 on the Eichhorn-Lorenz (E-L) scale, except for Riesling buds in 2018, which averaged E-L 9. For each date, different letters indicated differences between treatments based on Tukey's honest significant difference test at 95% confidence. Letters of significance are ordered from top-down at each date as follows: 1) Control, 2) Amigo 8%, 3) Amigo 10%, and 4) Late pruning.