

Research Article

Vine Performance and Phenology Postponement in Cane-Pruned Chardonnay Vines Grown in a Temperate Climate: The Effects of a Delayed Winter Pruning

Alberto Vercesi, Alessandra Garavani, Maria Giulia Parisi, Matteo Gatti, and Stefano Poni

Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, Piacenza 29122, Italy

Correspondence should be addressed to Stefano Poni; stefano.poni@unicatt.it

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In a global warming context, the advancement and compression of maturity in early ripening grape varieties suited to sparkling wine making can easily expedite harvest within the first two weeks of August (in northern Italy). Such earliness, albeit safeguarding acid retention, involves potential suboptimal grape composition and triggers logistical issues related to grape harvest and delivery. Hence, this three-year study beginning in 2020 was conducted on cane-pruned Chardonnay vines, grown in the Franciacorta district of Lombardy, to assess if a single-step delayed winter pruning was able to postpone vine phenology and ripening, without harming yield potential. Control (C) vines pruned midwinter were compared with those subjected to late winter pruning (LWP), performed when the apical shoots growing on the unpruned canes had reached the 2-3 unfolded leaf stage (T1) as well as 7-8 days later (T2). In 2022, a fourth treatment was added, consisting of a two-step procedure with finishing performed at T1 (LWP-two canes). Vegetative growth, yield components, ripening dynamics, and maturity at harvest were followed in each year of this study. Budburst delay induced by LWP treatments across seasons varied between 4 and 9 days, whereas harvest was postponed by 5-14 days. The extent of delay was especially pronounced in 2020, when the removed leaf area (LA) was also found to be the highest. The higher the removed LA, the higher the yield constrain was in the concomitant year. In 2020 and 2021, despite the harvest delay, LWP vines were able to assure full matching with desirable features in must composition, set at total soluble solids (TSS) of about 18°Brix and a titratable acidity (TA) of at least 8 g/L. In the very hot and dry phase of 2022, none of the treatments facilitated the required ripening status, while data showed that an even earlier ripening would not have allowed the harvest to reach the minimum TSS level. Notably, even in 2021 and 2022, when the removed LA was quite low, a delaying effect was prompted in the seasonal trends of all main ripening parameters. This suggested that besides the amount of LA removal, other unknown factors drive the postponement of ripening. In 2022, the behavior of added-treatment LWP-two canes was found to be quite similar to that of the C vines. Depending upon the desired yield level and local climate feature, the protocol entailing a singlestep late winter pruning, performed at any time between the T1-T2 time window used in this study, is deemed as effective in achieving a significant harvest delay while maintaining or even improving the compositional patterns recorded for midwinter pruned vines.

1. Introduction

Among the most consistent effects of global warming on viticulture across the world, two stand out: a lengthening of the vine growing period and an advancement of all phenological stages, with a general compression of the annual productive cycle [1-3]. Such effects might be associated with both good and bad consequences. For instance, a longer growing season can allow the possibility of full ripening even for medium-to-late cultivars grown in

a somewhat cool environment [4], whereas in a warmtemperate climate, the required heat summation for the desired ripening is reached with considerable anticipation as compared to that of a standard ripening calendar [5].

The latter case raises serious concerns in any viticultural district where early ripening grapevine varieties (i.e., Pinot Noir, Chardonnay etc.) are grown for sparkling and spumante vinification. In fact, according to a thermal-based model for grapevine phenology forecasts [6], a comparison made in a renowned area of the Franciacorta appellation (Erbusco site) in Italy shows that over 1981-1990, the average harvest date for Chardonnay centers around 13th September, whereas for 2011–2020, it centers around 19th August. The reason behind such a change seems quite obvious: the ripening heat summation for Chardonnay, that previous work argued as being around 1300-1400 growing degree days (GDD) [7, 8], was reached well ahead of time (typically mid-August, if not earlier). The solution to this problem seems likewise clear: harvest is to be progressively anticipated so that the overall grape composition suited to sparkling wine making can be maintained.

However, the reality is more complex. On top of the logistic related to large grape batches that must be delivered in a very short time to a necessarily open winery, other compositional issues might arise. If an anticipated ripening assures the retention of adequate acids, there might still be confusion over whether enough sugar is accumulated. Moreover, reduced daily thermal excursion and the rise in berry temperature over 40°C, leading to initial sunburn [9, 10], brown lesions, and finally undesirable secondary phenolic compounds [11], might affect overall grape quality. Eventually, an even milder berry withering, causing an artifactual increase in sugar concentration, might affect, in an overall neutral grape variety, the development of fermentation aromas which, in a typical Chardonnay sparkling or spumante wine produced in northern Italy, should not lean over excessive fruitiness, rather emphasizing apple and citrus nuances as well as fresh white flowers [12].

An alternate solution to the abovementioned problem involves adopting cultural practices that can delay the entire annual grapevine cycle by repositioning harvest to a cooler period. This fix can be especially beneficial regarding the goal of sparkling wine making. In this respect, several postponing techniques and practices have been presented and explained in recent broad review publications [3, 13–15]. Among them, the late winter pruning (LWP) protocol [16] implies that regardless of the pruning type adopted (spur or cane pruning), vines must be finally pruned when the growth of the apical nodes of unpruned canes lies typically between the "wool bud stage" and that displaying the development of 7-8 unfolded leaves on young shoots. While the execution protocol of LWP under spur and cane pruning is discussed in Poni et al. [16], the largest majority of related contributions, dealing with spurred cordons, shows the following: considering a number of factors (cultivar and climate site interaction, amount of leaf area removed during final pruning, etc.), this protocol can achieve a budburst delay of 5-55 days compared to the standard pruning performed during full dormancy, and as per a remarkable number of use cases [17–20], it can be partially followed till harvest, thus proving the postponement hypothesis mentioned above. Furthermore, in the innumerable studies conducted on red cultivars, the results surprisingly share the following observation: under LWP, the sugaring process is postponed, whereas phenolic maturity is not. The most efficient operational protocol regarding spurred cordons recommends the performance of a late winter finishing that cannot be delayed beyond the stage where 2-3 unfolded leaves are present on the apical shoots; later interventions will almost unavoidably generate significant yield losses [16].

Added to the paucity of associated research, the application of LWP on cane-pruned vines is more troublesome for a number of reasons. First, a choice needs to be made between following the LWP in just one step (requiring all removal operations of the previous-year fruiting cane, as well as the selection and positioning of the new cane(s) on the support wire, to be performed all at once) or in two steps (comprising the cutting, at dormancy, of the previous-year fruiting cane and selecting two long vertical canes which, once distal growth commences during spring, will be shortened to the required length). As previously pointed out in a Pinot Noir trial [21], if the main purpose is to shift harvest to a later date, the above two-step-procedure seems too mild. In fact, the extent of the delay achieved with LWP is primarily a product of two factors: when the hand finishing is made, and how much leaf area is removed [22]. The latter factor, in turn, depends on the total cane number left on a vine after the prepruning task. If only two canes are left, the removed leaf area is inherently low and the main result is just a few days of delay at budburst, a delay which often vanishes by the time flowering occurs.

Till date, only two works on the response of Chardonnay to LWP have been published, both of which study spur pruning, with somewhat contradictory results. In a Chardonnay trial in New Zealand [23], frost is found to occur with a minimum night temperature of -1.7° C, damaging a portion of the developing buds and killing 33% of primary shoots. Conversely, in the late pruning treatments (21 and 41 days after standard winter pruning performed on 25 July), due to a considerable budburst delay, no more than 3% of the primary shoots are found to be killed, and the average yield per vine rises by about 38%. Moreover, in a trial involving a no-frost scenario [24], LWP applied one, two and three weeks after standard pruning on Chardonnay grown in Brazil (Santa Catarina Region) reinforces the argument that the later the pruning, the higher the negative impact on yield. Here, the yield per vine decreases by over 50% as a result of LWP conducted two and three weeks after traditional winter pruning.

Owing to the unavailability of any midterm trial focusing on the effect of an LWP pruning technique applied on canepruned Chardonnay grapes meant for sparkling wine making, this three-year study had the following objectives: (i) assess overall performance of vines in light of a one-step LWP carried out at two different dates and (ii) observe whether the induced phenological delay persists until harvest and unravel physiological determinants of the observed responses.

2. Materials and Methods

2.1. Plant Material and Experimental Design. This study's trial was conducted in 2020–2022, in a 0.7-hectare vineyard belonging to the Castello di Gussago la Santissima Estate, located in Gussago (45°58'N and 10°09'E, Northern Italy, elevation of 185 m a.s.l.), where cv. Chardonnay (standard material), grafted on SO4 in 2005, was planted. The spacing was 2.5 m interrow and 0.9 m intrarow for a resulting vine density of 4444 vines/ha; the vines were trained on vertical shoot positioned (VSP) cane-pruned trellis (horizontal Guyot-type) with about 10 nodes retained on the fruiting cane. No spurs were maintained for cane renewal purposes. The selected fruiting canes were tied to the horizontal support wire set 0.8 m above the ground, while three pairs of upper wires produced a canopy wall extending 1.2–1.4 m above the main wire.

The experimental plot consisted of 36 vines arranged in three complete blocks and randomly assigned to three pruning regimes. Standard winter pruning (SWP) was performed in midwinter at bud dormancy, specifically around February 20, 22, and 18 in 2020, 2021, and 2022, respectively. Late winter pruning (LWP) was usually performed in a single step at two different dates. The untouched vines were Guyot, pruned at T1 (corresponding to no more than 2-3 unfolded leaves borne on shoots sprouting from the last 3-4 apical nodes of the unpruned canes; Figure 1(a)) and T2 (corresponding to T1 + 7-8 days; Figure 1(b)). Therefore, the T1 and T2 pruning dates were 22nd April and 30th April in 2020, 7th April and 14th April in 2021, and 12th and 19th April in 2022. For the sake of comparison, in 2022, an additional LWP treatment, with canes named LWP-two, was added to the experimental layout. This treatment, hereafter named LWP-two canes, was performed at the T1 stage in a two-step mode. Vines pertaining to the LWP-two canes' treatment were prepruned in midwinter, comprising the removal of the old fruiting cane and the selection of two renewal canes which were kept as vertical and long as possible. At T1, hand finishing was performed, involving the shortening of the selected canes to the suitable length, followed by their positioning and tying on the horizontal main wire (Figure 1(c)).

12 vines per treatment (four per block) were tagged before budburst and selected for specific measurements over the three seasons of study. Moreover, local standard pest management practices were applied throughout all the trial years. The vines were mechanically shoot-trimmed once per season when the shoots outgrew the top foliage wire by about 50 cm.

2.2. Weather Data, Phenology, and Vine Growth. Seasonal weather trends relevant to this study were monitored by an automatic meteorological station located in the vineyard, which kept hourly records of maximum temperature (T_{max}), minimum temperature (T_{min}), and rainfall. The daily mean temperature (T_{avg}) was calculated, and the Winkler Index (WI) was computed as the summation of base 10°C active temperature (T) from 1st April (day of the year (DOY) 91) to 31st October (DOY 304) of each study year [25].

The assessment of budburst, flowering, and veraison occurrence was performed every year on each cane-pruned test vine. According to Baggiolini's classification [26], the attainment/crossing of stage B (defined as the stage of swollen bud) was deemed as proof of the argument that budburst had been reached. On each vine, the observed buds were those borne on the fruiting cane. Each year, the visual estimates commenced when all the buds were still dormant, and two-day assessment intervals were kept to ensure maximum accuracy of the estimates. Altogether, budburst was declared as reached when at least 50% of the observed buds of at least 50% of the tagged vines were found to have attained or crossed stage B.

The assessment of flowering (stage I) date inherently required a quantitative analysis, which was performed by a trained crew. On each test vine, two shoots were tagged, one basal and one apical on the cane, and the percentage of open flowers to total flowers was visually assessed at two-day intervals. Flowering was declared as reached when at least 50% of the observed inflorescences on each vine had crossed 50% of open flowers over total flowers number.

Further, the assessment of veraison (stage L) was performed on the same clusters that were previously assessed regarding the attainment of flowering. As color change was not very helpful in evaluating a white cultivar veraison, this assessment involved finger-touch evaluation on three berries per cluster randomly chosen from top, middle, and bottom portions of the clusters, for their classification as "hard" or "soft." Moreover, this survey was held at two-day intervals, and veraison was declared as reached when at least 50% of the observed berries on at least 50% of the tagged vines were classified as "soft."

Shoot fertility was assessed in mid-May of each year by counting the number of inflorescences per shoot and per vine. The leaves inserted at nodes 3, 6, 9, and 12 of a distal and proximal shoot of each tagged vine were collected at harvest, together with two representative leaves of a lateral shoot developing below the trimming cut. The area of each leaf was measured with a LI-3000A leaf area meter (LI-COR Biosciences, Lincoln, N.E., U.S.A.). Immediately after leaf fall, the number of nodes per cane and per lateral shoot was counted. Then, the final leaf area was estimated by considering the main and lateral shoots per vine on the basis of node counts and leaf-blade areas. Thereafter, the total vine leaf area was calculated as a sum of the two above components.

2.3. Ripening Course and Harvest Parameters. In the study area, the desirable maturity thresholds for high-quality Chardonnay sparkling wines were set as the following: TSS of about 18°Brix and TA not less than 8 g/L. To track the ripening course, a sample of 25 berries was collected from each vine, at almost weekly intervals, from preveraison (TSS ~ 5°Brix) until their best match with the desired ripening thresholds. In addition, post-C-harvest grape samples were taken and analyzed to further assess ripening progress in response to the delayed pruning treatments. When the target ripening thresholds were possibly met, all the tagged



FIGURE 1: Images of shoot development reached in 2022 before performing LWP-T1 (a), LWP-T2 (b), and LWP-two canes (c).

vines were harvested, the yield per vine was measured with a portable field scale, the number of clusters per vine was recorded, and the average cluster mass was also calculated.

A pool of 100 berries per vine was used to determine fruit composition at harvest. The total soluble solids (TSS) concentration of the pool was measured using a temperature-compensating RX 5000 refractometer (Atago, Bellevue, WA, USA), and its must pH was assessed with a digital PHM82 pH meter (Radiometer Analytical, Villeurbanne, France). The TA was measured via titration with 0.1 N NaOH to an end-point of pH 8.2 and expressed as the g/L of tartaric acid equivalents. To assess the tartaric and malic acid concentrations in all samples taken seasonally and at harvest, an aliquot of the must was diluted four times and then filtered through a $0.22 \,\mu m$ polypropylene syringe for highperformance liquid chromatography (HPLC) analysis before being transferred to autosampler vials. Notably, all solvents were of an HPLC grade. In addition, water of Milli-Q quality, acetonitrile, and methanol were obtained from VWR chemicals. L-(+)-tartaric acid and L-(-)-malic acid standards were purchased from Sigma-Aldrich. The chromatographic method was developed using an Agilent 1260 Infinity Quaternary LC (Agilent Technology) consisting of a G1311B/C quaternary pump with an inline degassing unit, a G1329B autosampler, a G1330B thermostat, a G1316B thermostated column compartment, and a G4212B diode array detector (DAD) fitted with a 10 mm path, along with a $1 \mu L$ Max-Light cartridge flow cell. This instrument was controlled using the Agilent ChemStation software, version A.01.05.

Moreover, for the analysis of organic acids, this study used an Allure Organic Acid column ($300 \times 4.6 \text{ mm}$, $5 \mu \text{m}$; Restek). Separation was performed in isocratic conditions using water, with pH adjusted to 2.5 using ortho-phosphoric acid, at a flow rate of 0.8 mL/min. The column temperature was maintained at 30 ± 0.1 °C, and 15μ L of the sample was injected. Thereafter, the elution was monitored at 200–700 nm and detected by UV-Vis absorption with the DAD at 210 nm. Indeed, the organic acids were identified using authentic standards, and quantification was based on peak areas and performed by external calibration using robust standards.

2.4. Statistical Analysis. This study's data were analyzed using a two-way analysis of variance (ANOVA) test conducted with the SigmaStat 3.5 software package (Systat Software, San Jose, C.A., U.S.A.). The means were separated using the Student–Newman–Keuls test. In case of significant interactions between treatment type and year, the results were shown separately for the two experimental factors in specific figures as vertically grouped bars with standard error (SE) per treatment combination.

Repeated measures of the same parameters (berry weight, TSS, pH, TA, tartaric acid, and malic acid) taken at different dates along the study seasons were analyzed with the Repeated Measures ANOVA routine embedded in the XLSTAT software package (Addinsoft, New York, N.Y., U.S.A.). Furthermore, the least squares (LS) mean method (at P < 0.05) was used for multiple comparisons within dates.

3. Results

3.1. Weather, Phenology, Vegetative Growth, and Yield Components. The daily means over the 1 April–31 October period for minimum (T_{min}) , mean (T_{mean}) , and maximum (T_{max}) temperatures and for precipitation in each trial season are reported in Figure S1. For each year, the cumulated seasonal GDD, introduced by [25] and commonly referred to as the Winkler Index (WI), are shown in Figure S2.

The climatic features of the three study seasons were distinctly different: 2020 could be easily defined as a warm and wet season with WI = 2251° C and a total precipitation (1 Apr-31 Oct) of 785 mm; 2021 was warm and semidry with WI = 2286° C and a total precipitation (1 Apr-31 Oct) of 420 mm; finally, 2022 was a hot and dry year, with WI peaking at 2613°C and a total precipitation (1 Apr-31 Oct) of only 268 mm. In addition, 2021 was also marked by an especially cool and wet spring season with GDD = 346° C throughout April and May (as compared to GDDs of 461° C and 481° C reached at the same time in 2020 and 2022, respectively). Over the same time window, total precipitation in 2021 was 160 mm (against 127 mm and 50 mm precipitation recorded in 2020 and 2022, respectively).

The amount of the removed leaf area at T1 and T2 in the LWP treatments considerably varied across seasons and generated a significant treatment × year ($T \times Y$) interaction (Table 1 and Figure 2(a)). The least amount of LA/vine was removed in 2021 (383 cm² and 815 cm² at T1 and T2, respectively), while the highest amount was removed in 2020 (4914 cm² and 8863 cm² at T1 and T2, respectively), with 2022 registering intermediate values in this regard. All final leaf area components (total, main, and lateral) were less responsive to the imposed treatments, although the total amount of LA/vine also showed a significant $T \times Y$ interaction (Figure 2(b)). This, however, was not a crossover interaction, as in the successive years, LWP treatments constantly showed higher values than C vines.

General delays in all the surveyed phenological stages as well as in the harvest date were found every year, albeit with large differences in magnitude (Table 2). Regardless of the amount of LA removed each year, both LWP-T1 and LWP-T2 induced a budburst delay of 4 to 9 days (Table 2). Interestingly, in each season, and with a decidedly greater extent in 2020, this delay became much more pronounced at the flowering stage (interval of variation ranged from 6 to 28 days) and was either maintained or variably reduced at the time of harvest (Table 2).

Moreover, the yield per vine and its main components (i.e., shoot fruitfulness, cluster number, and cluster weight) showed a significant $T \times Y$ interaction (Table 3), whose partitioning is displayed in Figure 3. Upon its first year of application, LWP, regardless of the timing of execution, drastically reduced yield and its associated components. These effects were considerably relieved over the second year of LWP application (2021), when most within-treatment differences were rendered insignificant except for smaller clusters in LWP-T2 (vs. C) vines (Figure 3(c)). On the other hand, LWP behavior recorded in 2022 was somewhat intermediate compared with those of the two previous seasons, although the added treatment (LWP-two canes) allowed a consistent recovery in any yield component, in contrast to the fates of the more severe LWP-T1 and LWP-T2 (Figure 3). Vine balance, given as final leaf area-to-fruit ratio, essentially reflected scant among-treatment variation in LA and indicated large differences in yield. Therefore, the LWPs had a surplus of source as compared to C, whose leaf area-toyield ratio was set at $1.15 \text{ m}^2/\text{kg}$ (Table 3).

When the amount of leaf area removed at the time of winter pruning was correlated with the fraction of yield change versus C vines for data pooled over the study years, a close ($R^2 = 0.85$) negative exponential relationship was fitted to the data, indicating a high sensitivity of yield loss in the current year compared to the amount of removed LA at the time of pruning (Figure 4). According to the calculated exponential model ($y = 75.74 * (1 - \exp (-0.0006 * x, P < 0.0031)$), a 50% yield loss occurred when about 2000 cm² of the leaf area was removed.

3.2. Ripening Course and Harvest Parameters. The seasonal data sampling performed each year to evaluate berry mass and the main ripening variables are shown in Figure 5 (berry mass, TSS and pH) and 6 (TA, tartaric acid and malic acid).

In 2020, LWP strongly slowed down berry growth. On the first sampling date (20 July), the berries were already deemed smaller in LWPs as compared to C vines, and this difference amplified over time (Figure 5(a)). When C vines were harvested (12 August), their berries were 26% and 28% smaller in LWP-T1 and LWP-T2, respectively. Post C vines' harvest, the berries under LWP treatments continued to recover, and although the berry sizes of T1 and T2 were quite comparable, at their respective harvest dates, the LWP treatments resulted in a final berry size that was similar to or even higher than the values recorded for C vines. The response of berry size to the timing of winter pruning was definitely less pronounced in 2021 (Figure 5(b)), as differences among treatments were rather occasional and inconsistent until 13 August (the harvest date of C vines). At the harvest of the LWP treatments (8 days later), their berry sizes were quite comparable (1.22 g in LWP-T1 vs. 1.19 g in LWP-T2). In 2022, when an additional LWP was added (LWP-two canes), no differences in berry size were recorded among treatments over the first two sampling dates; conversely, LWP-T1 and LWP-T2 had smaller berries than those of the C and LWP-two canes over the two following dates (Figure 5(c)).

The accumulation of TSS in berries in 2020 was greatly delayed for LWP from the very beginning (20 July) until the harvest of the C vines (12 August), when LWP-T1 and LWP-T2 were found to have 5.2°Brix and 6.8°Brix less TSS than C, respectively (Figure 5(d)). Over the remainder of that season, the LWPs quickly recovered and their final TSS were found to have exceeded 18°Brix by their respective harvest dates. In 2021, the effects on the berry sugaring were milder yet quite interesting (Figure 5(e)). Initially, no effects on TSS were seen over the sampling dates of 6th July and 12th July. Thereafter, sugar accumulation in LWPs started to slow down consistently, and at C harvest (13th August), LWP-T1 and LWP-T2 had 2.1°Brix and 1.4°Brix less TSS, respectively, compared to C vines (Figure 5(e)). Then, in an eight-day span, the same treatments surged to show TSS values of 18.9°Brix and 19.6°Brix, respectively. In 2022, the dynamics of berry sugar accumulation in the different treatments decidedly mirrored those of 2021, with a progressive buildup of a sugar-delaying effect in LWPs, as compared to the C

TABLE 1: Effects of different winter pruning treatments on vegetative growth given as the removed leaf area (LA) and final leaf area components recorded over three years (2020–2022) on mature Chardonnay grapevines. C = control vines; LWP = late winter pruning. In 2022 only, an additional LWP treatment was imposed as LWP-two canes.

	Removed LA at winter pruning (cm ²)	Total main LA/vine (m ²)	Total lateral LA/vine (m ²)	Total LA/vine (m ²)
Treatment				
С	0	1.78b	0.20	1.98b
LWP-T1	1929.90b	1.99ab	0.20	2.19ab
LWP-T2	4280.66a	2.16a	0.24	2.40a
F-prob	* *	*	ns	* *
Year				
2020	6888.55a	1.92	0.30a	2.22
2021	599.25c	2.01	0.22b	2.23
2022	1828.03b	2.01	0.12c	2.13
F-prob	* *	ns	**	ns
$T \times Y$	* *	ns	ns	*

*and ** denote significant differences between treatments at P < 0.05 and 0.01 according to within column mean separation performed with SNK test for treatment levels and with *t*-test for year levels. ns = not significant.



FIGURE 2: Partitioning of the significant year × treatment interactions recorded for the removed leaf area/vine (a) and total leaf area/vine (b). Color codes for pruning treatments are blue (C), orange LWP-T1 (LWP-T1), grey (LWP-T2), and yellow (LWP-two canes, in 2022). Data are means for each year × treatment combination (n = 12) and vertical bars are standard errors (SE). In (a) value of LWP-two canes is at the limit of readability.

and LWP-two canes that behaved quite similarly (Figure 5(f)).

Further, the must pH was severely delayed in 2020 by the LWP treatments, and when the C vines were harvested on 12^{th} August at a pH of 2.98, the pH values measured in LWP-T1 and LWP-T2 were 2.76 and 2.61, respectively (Figure 5(g)). Over the remaining dates, the LWP treatments completed their pH recovery, with harvest pH values of 3.11 and 3.08 for T1 and T2, respectively. In 2021, a milder delay in must pH was recorded in LWPs, which started to become significant by July 27 but, however, was maintained until C harvest (Figure 5(h)). Even in the 2022 season, the tendency of a delayed must pH was maintained in LWP-T1 and LWP-T2 as compared to C and LWP-two canes (P < 0.05 in three dates out of four). However, a five-day delay in the harvests of LWP-T1 and LWP-T2 led their pH values to be noted as higher than 3.3 (Figure 5(i)).

Concerning grape acidity, the TA did not differ among treatments on the first sampling date (20th July) in 2020 (Figure 6(a)). Thereafter, a wide gap was progressively noticed between TA measured in C vines versus LWPs. In fact, C vines were harvested on 12th August at a TA of 11.9 g/L compared to TAs of 24.3 g/L and 27.9 g/L that were still retained between LWP-T1 and LWP-T2, respectively, on the same date. Over the remaining dates, the TAs in LWPs decreased quite rapidly, although at their respective harvest dates, they still preserved 8.7 g/L (LWP-T1, 1st September) and 8.6 g/L (LWP-T2, 4th September) of TA. A somewhat similar trend was observed in 2021, albeit milder in its extent (Figure 6(b)): at C harvest (13th August), the TA of SWP vines was 10.0 g/L, whereas LWP-T1 and LWP-T2 still held 12.3 g/L and 12.0 g/L, respectively. When the latter were harvested (seven days later), the TA values had reached up to 8.64 for LWP-T1 and 8.82 for LWP-T2. In 2022, again, the

est dates recorded each year in the three winter pruning systems. cional LWP treatment was imposed as LWP-two canes.	2022
wering, and veraison and actual winter pruning and harve lay (in days) as compared to C. In 2022 only, an additi	2021
ABLE 2: Estimated dates (DD/MO) of occurrence for budburst, flo abels and numbers between brackets indicate either DOY or de	2020

		2020			2021			20	22	
	U	LWP-T1	LWP-T2	C	LWP-T1	LWP-T2	C	LWP-T1	LWP-T2	LWP-two canes ¹
Date of winter pruning	20/02 (DOY 51)	22/04 (DOY 113)	30/04 (DOY 121)	22/02 (DOY 53)	07/04 (DOY 97)	14/04 (DOY 104)	18/02 (DOY 49)	12/04 (DOY 102)	19/04 (DOY 109)	12/04 (DOY 102)
Bud burst	25/03 (DOY 85)	30/03 (5)	01/04 (7)	29/03 (DOY 88)	03/04 (5)	05/04 (7)	21/03 (DOY 80)	26/03 (5)	30/03 (9)	25/03 (4)
Flowering	25/05 (DOY 146)	16/06 (22)	22/06 (28)	01/06 (DOY 152)	15/06 (14)	15/06 (14)	24/05 (144)	01/06 (8)	01/06(8)	30/05 (6)
Veraison	24/07 (DOY 206)	14/08 (21)	18/08 (25)	23/07 (DOY 204)	30/07 (7)	30/07 (7)	13/07 (DOY 194)	19/07 (6)	19/07 (6)	17/07 (4)
Harvest	12/08 (DOY 2249)	01/09 (20)	04/09 (23)	13/08 (DOY 225)	21/08 (8)	21/08 (8)	05/08 (DOY 217)	10/08 (5)	10/08 (5)	05/08 (0)
¹ Added only in 2022. I	Hand finishing perf	ormed at T1.								

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TABLE 3: Effects of different timings of winter pruning on yield components and supply-demand function given as leaf area-to-fruit ratio recorded over three years (2020–2022) on mature Chardonnay grapevines. C = control vines; LWP = late winter pruning. In 2022 only, an additional LWP treatment was imposed as LWP-two canes.

	Shoots/ vine	Inflorescences/ vine	Inflorescences/ shoot	Clusters/ vine	Yield/vine (kg)	Cluster weight (g)	Berry weight (g)	Leaf area-to-fruit ratio (m ² /kg)
Treatment	ţ							
С	12b	17a	1.2a	16a	2.03a	127a	1.31	1.15b
LWP-T1	14a	11b	0.8b	12b	1.12b	93b	1.27	3.47ab
LWP-T2	14a	11b	0.7b	10b	0.79b	79b	1.23	7.50a
F-prob	* *	**	**	* *	* *	* *	ns	*
Year								
2020	13	8c	0.6c	9b	0.97	108a	1.45a	7.61
2021	14	17a	1.2a	15a	1.56a	104a	1.23b	2.59
2022	13	12b	0.9b	14a	1.23b	88b	1.12c	3.59
F-prob	ns	* *	* *	* *	* *	*	* *	ns
$T \times Y$	ns	* *	* *	* *	* *	* *	ns	ns

* and ** denote significant differences between treatments at P < 0.05 and 0.01 according to within column mean separation performed with the SNK test for treatment levels and with *t*-test for year levels. ns = not significant.



FIGURE 3: Partitioning of the significant year × treatment interactions recorded for yield components defined as inflorescences/shoot (a), clusters/vine (b), cluster weight (c), and for total yield/vine (d). Color codes for pruning treatments are blue (C), orange LWP-T1 (LWP-T1), grey (LWP-T2), and yellow (LWP-two canes, in 2022). Data are means for each year × treatment combination (n = 12), and vertical bars are standard errors (SE).



FIGURE 4: Relationship between the leaf area removed at winter pruning (*x*) and percentage of yield/vine decrease as compared to C vines. Dots represent treatment mean data pooled over years. A negative exponential model was fitted to the data yielding the following equation: $y = 75.74 * (1 - \exp(-0.0006 * x)), P < 0.0031, R^2 = 0.85).$



FIGURE 5: Ripening curves for berry weight (a–c), total soluble solids (d–f), and must pH (g–i) in 2020 (top), 2021 (middle), and 2022 (bottom). The repeated measure ANOVA performed on each annual data set has resulted in a significant treatment × date interaction at P < 0.05. Within sampling dates, the asterisk indicates that multiple pair comparisons performed by Tukey's test were significant at P < 0.05.

same scenario was replicated: on 1st August (i.e., four days before harvest of C vines and LWP-two canes, both setting around 8.5 g/L), LWP-T1 and LWP-T2 still had 11.1 and 15.8 g/L of TA, respectively. However, a week later (i.e., two days before harvest), in these treatments, TA had already dropped to 7.0 g/L (LWP-T1) and 6.6 g/L (LWP-T2) (Figure 6(c)).

Furthermore, seasonal tartaric acid concentration was also affected by the timing of winter pruning (Figures 6(d)–6(f)). In 2020, there was a significant delay in the decrease of tartaric acid concentration in LWPs starting from the first sampling date (20 July), which was maintained until harvest of C vines, with the latter settling at 8.1 g/L and LWPs showing about 2 g/L more of tartaric acid concentration (Figure 6(d)). Over the remainder of the season, no differences were observed between LWP-T1 and LWP-T2, which at their respective harvest dates displayed concomitant values of 7.6 g/L and 7.1 g/L, respectively. An overall mild effect on tartaric acid concentration was seen in 2021 (Figure 6(e)), whereas in 2022, this concentration was noted as higher in LWPS on the sampling dates of 20th July and 1st August (Figure 6(f)).

In 2020, the effects of the timing of winter pruning on the seasonal trend of malic acid concentration in berries were very strong. Malate was found to be much higher in LWPs since the first sampling date (20 July), and this gap widened over time; when C vines were harvested (12 August) at a malic acid concentration of 6.4 g/L, LWP-T1 and LWP-T2 had 18.7 g/L and 20.5 g/L of malic acid, respectively (Figure 6(g)). When LWP-T1 and LWP-T2 were harvested on 1st and 4th September, respectively, their final malic acid concentrations were still, respectively, 4.6 g/L and 4.0 g/L. Malic acid was also responsive to treatments in 2021, albeit with a different seasonal evolution (Figure 6(h)). On the initial sampling date (6 July), C vines had a considerably higher malate concentration that delayed winter-pruned vines. Thereafter, for two consecutive dates, malate concentration did not differ among treatments, and thereafter, LWPs retained higher malic acid concentration until the harvest of C vines. Plus, a good pool of malic acid (3.2-3.4 g/ L) was retained from LWPs at their harvest dates (Figure 6(h)). During the considerably hot 2022 season, from 20th July and until the preharvest of C vines, the LWPs preserved a higher malic acid pool (Figure 6(i)); however, when LWPs were harvested (five days later than C vines), their malate concentration was found to be quite low as well (~1.5-2.0 g/L).

The total picture of grape composition at the respective harvest dates is provided in Table 4. Unsurprisingly, significant $T \times Y$ interactions were found to occur among TSS, pH, TA, and malic acid (Figure 7). Despite harvest delays that, over seasons, varied from 5 to 23 days for the LWP-T1 and LWP-T2 treatments, their TSS at harvest were always higher as compared to the TSS observed for C vines' on the C harvest dates. Although the absolute values of TA and malic acid concentration at harvest reflected seasonality, not surprisingly, the minimum concentrations across treatments were reached in the hot 2022 season, it was quite remarkable that, in 2020 and 2021, the final TAs of LWP-T1 and LWP-T2 were still higher than the minimum required threshold of 8 g/L. Finally, pH varied quite erratically. However, when the harvest delay was high (i.e., in 2020), the final pH of LWP musts was lower than that of the C vines, whereas the opposite happened in 2021 and, to some extent, in 2022 as well.

4. Discussion

Chardonnay is well known as being an early-ripening cultivar, requiring about 1300-1400 GDD to reach a ripening stage, a range still suitable for sparkling vinification [27, 28]. A recent study [29] on projected changes in grape composition by 2050 and 2070, associated with two different emission scenarios (RCP4.5 and RCP8.5) and referring to the warm region of La Mancha, Spain, predicted the following for Chardonnay: further phenology advancement by 4-10 days, and TA and malic acid losses up to -0.56 g/L and -0.43 g/L, respectively, for the 2070-RCP8.5 combination. However, it is usually difficult to compare the crossregional behavior of Chardonnay ripening, as, for instance, the desired final grape composition might also vary across locales. In our study, once we defined a TSS threshold around 18°Brix and a minimum TA of about 8 g/L, combining the required heat (1300-1400 GDD) with the time of the season when these thresholds were reached yielded the following, potentially optimal, ripening windows: 31st July-5th August (DOY 212-217) in 2020; 30th July-5th August (DOY 211-217) in 2021, and 19th July -23rd July (DOY 200-204) in 2022. Therefore, in 2020 and 2021, the actual harvest dates of C vines were delayed by about a week compared to the maximum value of the optimal date range, whereas in 2022, such a delay was extended by 13 days (DOY 217 vs. DOY 204). These simple comparisons lead to two interesting outcomes: (i) in 2020 and 2021, despite local WI being largely in excess compared to the Chardonnay heat requirements, a fairly early harvest of C vines allowed for the fulfilment of the TA requirement, albeit the final sugar level was found to lie below the set threshold (Figure 7(a)); in fact, retarding the harvest by a few more days (3-4 days) could have generated the ideal balance; (ii) in 2022, when the WI summation was exceptionally high (2613°C), an even earlier C harvest date (5 August) did not allow the preservation of the required minimum TA level (Figure 7(c)). Based on the ripening curves reported in Figure 5, harvest should have been hastened by another week, which, however, would have also resulted in undesirably low TSS in the berries (Figure 5(f)). The message from these results is that, depending on specific seasonal climate trends, a simple "early harvest" solution can still be valid and allow a compromise with the desirable compositional characteristics of sparkling-targeted grapes. However, the validity of such a solution weakens in extrahot seasons likely associated with drought events, when extreme harvest anticipation for the sake of TA preservation clashes against the minimum sugar level and, presumably, a good baseline for flavor evolution. If the latter is associated with climate model predictions unanimously forecasting a trend of further harvest earliness and a steeper drop in acid concentrations [5, 29], then the



FIGURE 6: Ripening curves for titratable acidity (a-c), tartaric acid (d-f), and malic acid concentrations (g-i) in 2020 (top), 2021 (middle), and 2022 (bottom). The repeated measure ANOVA performed on each annual data set has resulted in a significant treatment × date interaction at P < 0.05. Within sampling dates, the asterisk indicates that multiple pair comparisons performed by Tukey's test were significant at P < 0.05.

issue of shifting ripening to a later date confirms its importance.

In our study, except for the LWP-two canes' treatment, which was added in 2022 for internal comparison with the other treatments, the LWP-T1 and LWP-T2 were always effective in delaying budburst, flowering, veraison, and harvest dates, albeit with different magnitudes depending on the given season. However, a distinction needs to be made between a strategy seeking simply a budburst delay for improved prevention of frost damages and the goal of significantly retarding the whole annual productive cycle [16]. With the aim of frost prevention, the interval in budburst delay obtained in our study (four-nine days across treatments and seasons) is interesting, yet not spectacular. Indeed, the interval is shorter than the delay range (9-23 days) that was induced in a similar study carried out for three years on cane-pruned Pinot Noir vines [21], where the LWP was executed when about three unfolded leaves

were present at node 10 of the unpruned canes. Besides the influence exerted by varietal sensitivity and the vines' interaction with spring weather trends, the images reported in Figure 1 are quite helpful in delineating the additional reasons behind an overall mild effect of budburst postponement. As discussed in Poni et al. [16], extent of budburst delay achievable through a LWP technique is primarily a function of the acrotony of buds and shoots development along the cane, which is supposed to be maximum when the still-unfinished canes are as vertical and long as possible [30]. Of course, the inhibition of the median and basal nodes is also dependent upon the timing of cane shortening. Figure 1, referring to LWP-T1 and LWP-T2 in 2022, accounts for two possible factors driving the partial spoiling of the desired budburst delay: (i) at T1 and T2, some subtending median and basal nodes had likely already crossed the B stage, therefore anticipating the target of 50% and (ii) it became apparent that some canes are not vertical, and it is

	Total	ни	Titratable acidity (ø/L)	Tartaric acid (ø/L)	Malic acid (ø/L)	Citric acid (ø/L)	Tartate/malate ratio
	soluble solids ([°] Brix)	L	(7,9) (minion acommitte	(T 19) minn arimitat	(T)9) min aimit	(1, 19) man anno	omnt omnitti lonn int
Treatment							
C	17.0b	3.16b	9.54a	7.33	3.62a	0.14	4.03
LWP-T1	18.3a	3.19ab	8.23b	6.78	2.81b	0.13	4.52
LWP-T2	18.9a	3.21a	8.58b	7.69	3.96a	0.11	3.76
F-prob	*	* *	* *	Su	*	ns	SU
Year							
2020	16.25c	3.06c	10.86a	7.29	5.87a	0.10b	1.35b
2021	18.36b	3.21b	9.15b	6.99	3.43b	0.14a	2.27b
2022	19.63a	3.30a	6.40c	7.52	1.10c	0.13a	8.73a
F-prob	¥	* *	* *	su	* *	*	* *
$T \times Y$	¥	*	¥	su	*	ns	su

TABLE 4: Effects of winter pruning treatments on grape composition at harvest recorded over three years (2020–2022) on mature Chardonnay grapevines. C = control vines; LWP = late winter pruning. In 2022 only, an additional LWP treatment was imposed as LWP-two canes.



FIGURE 7: Partitioning of the significant year × treatment interactions recorded for must parameters defined as total soluble solids (a), pH (b), titratable acidity (c), and malic acid (d). Color codes for pruning treatments are blue (C), orange LWP-T1 (LWP-T1), grey (LWP-T2), and yellow (LWP-two canes, in 2022). Data are means for each year × treatment combination (n = 12), and vertical bars are standard errors (SE).

known that any deviation from verticality (e.g., inclined or almost horizontal canes) favors the sprouting of more basal nodes [31]. In more general terms, it appears that an LWP applied on prepruned spurred cordons is more effective in inducing stronger budburst delays, as reported elsewhere [17, 19, 32–34].

While a general expectation is that an initial phenology delay is progressively eroded throughout a season until it might be fully offset [35], this did not happen in our study as the delay recorded at budburst actually amplified to various extents by the time of flowering, with the greatest concomitant magnitude observed in 2020, and, thereafter, was either maintained or slightly eroded (Table 2). Further, as per data pooled over seasons and treatments, the harvest delay went from a minimum of five days (LWPs in 2022) to a maximum of 23 days (LWP-T2 in 2020). Thus, it is evident that, especially in 2020, in the time window between budburst and flowering, other factors played a role in the harvest delay. It is especially remarkable that in 2020, in transitioning from budbreak to flowering, LWP-T1 and LWP-T2, respectively, took up 77 and 82 days compared to the 61 days taken by C vines for the same transition, and as per GDD, 623°C and 692°C were observed for the LWPs

compared to only 422°C for C. This observation corresponds to temperature shifts of 6.9°C/day in C vines versus those of 8.1°C/day and 8.4°C/day in LWP-T1 and LWP-T2, respectively. The rationale behind this observation is that despite a higher amount of daily heat load [36] being available for the LWPs within the budburst flowering period, a large delay still occurred. The main explanation behind this apparently puzzling behavior is the considerable amount of the leaf area removed at T1 and T2. Still, the LA fraction could be considered as still acting as a sink [36–38], its loss representing a waste of resources. Moreover, the LWPs were forced to restart with basically new leaf formations which, besides entailing high respiration costs [39, 40], would make it necessary to again tap into storage pools. Overall, this serves as a source limitation condition that might explain slow or inhibited vine reactivity to an energy potential (i.e., GDD/day), a quality which was actually found to be higher in the LWPs. Moreover, the delay of flowering expanded less in 2021 and 2022 when compared to that estimated at budburst; overall, this accords with the fact that in both seasons, a much lesser leaf area was removed especially at T1 (Figure 2(a)). Here, a possible point of criticism is as follows: if the criteria for the timing of LWP were established, why did the amount of removed LA largely differ across the study years? Besides the limitations that, for T1, are associated with visual assessment, it is relevant that in 2020, the time period elapsing between T1 and T2 was characterized by unseasonably high temperatures with T_{max} reaching 26°C between 7th and 14th April. In contrast, in 2021, the same period was definitely cold, with a recorded T_{\min} of -1.1° C on DOY 98. In both the abovementioned years, the number of days between T1 and T2 was very similar (eight and seven, respectively), and the heat availability significantly affected leaf formation until T2, therefore increasing between-year variability. In terms of vegetative growth, it is notable that none of the LWP treatments impaired canopy growth, as the final leaf area was always higher, sometimes significantly higher, than the LA values estimated on the C vines (Figure 2(b)). While this finding accords with the data reported by the Pinot Noir trial [21], the full recovery capacity for LA development shown by the vines subjected to LWP (and associated with their source excess), in turn related to the induced yield limitation, supports the hypothesis of no negative carryover effects of the LWP technique over nextyear vine performance.

Before discussing the effects of LWP on the dynamics of ripening, a point needs to be made about the impact of the technique on the current and relative yields. As explained in physiological terms in Gatti et al. [22], there is usually a negative relationship between the total leaf area removed at LWP and the yield/vine recorded in the same year. An exponential model fitted to our data (Figure 4) confirms this relationship, indicating that in order to avoid major yield loss (e.g. higher than 30% compared to C vines) less than 1000 cm^2 leaf area per vine should be removed. The (caused) source limitation is likewise responsible for this relationship, and it is indeed robust, considering that when the removed LA was lower than 500 cm^2 (2021 data), the final yield per vine did not statistically differ among treatments (Figure 3(d)). However, it is interesting to investigate which vield components are more or less sensitive to source limitation. Indeed, while berry weight was not affected at all in this study (Table 3), it is enlightening that the potential fruitfulness, given as inflorescences/cluster, was drastically reduced by LWPs already in the first year of application (Figure 3(a)). While it is unlikely that such large differences in node fertility could have been set prior to treatment imposition, the most likely hypothesis here is that the severe source limitation caused by late pruning either led to the conversion of young inflorescences into tendrils, and for those inflorescences which were maintained, their flower number was limited [41-43]. In 2021, with minimal leaf removal, no major consequences were seen regarding node fruitfulness and clusters per vine (Figures 3(a) and 3(b)). This behavior also suggests as unlikely a carryover effect of amount of the removed LA on next-year bud induction and differentiation.

One of the main goals of our study was to ascertain if, under the specific trial conditions and the oenological target, a significant temporal delay in vine harvesting could be achieved while maintaining the desirable quality thresholds. Over the study seasons, the chronological harvest delay went

from five to 23 days and, if an evaluation was made on the basis of the main effects reported in Table 4, it would be evident that while C vines maintained TA ≥ 8 g/L, their sugar concentration at harvest did not reach the set threshold, whereas both those parameters were achieved by LWPs. Then, if the same evaluation was made by analyzing $T \times Y$ interactions, it would be evident that none of the treatments were able to maintain the required TA in the exceptionally hot and dry 2022. However, when assessment of ripening delay effects is left to different picking dates which, hopefully, should center the set ripening parameters, variability due to different ripening pace post C harvest is almost unavoidable. In our study, this effect was quite apparent in 2020, when chronological ripening dates for LWP-T1 and LWP-T2 were 1st and 4th September. However, graphical extrapolation on the ripening curves of dates on which LWP-T1 and LWP-T2 reached TSS and TA levels registered at harvest in C vines would lead to the more conservative and reliable delay of maximum 14 days (DOY 224 vs. DOY 239).

On a general basis, it can be stated that induced harvest delay is primarily a function of LA removed at pruning. However, this study's data imply that the mechanism is perhaps more complex. In fact, taking into consideration the ripening curves of 2021 and 2022, two years when the amount of removed LA went from very low to moderate (Figure 2(a)), for any of the considered variables, there was a significant delay in LWPs compared to C vines on most of the sampling dates (Figures 5(b), 5(e), 5(h), 6(b), 6(e) and 6(h)). This suggests that, indeed for mostly unknown reasons, any one-step delayed winter pruning made even at budburst commencement has the potential to delay phenology and, ultimately, harvest. This is good news indeed, as under such circumstances, the treatment combination which will successfully compromise between acceptable yield decrease and significant cycle postponement can be more easily identified.

Besides the potential negative impact on yield, another possible objection to the use of this delaying technique is that, by the time the winter pruning is performed, there are no hints to forecast about an especially early harvest fed by an eventually hot and dry season. Then, if a standard year occurs, motivation to recur to the delayed winter pruning seems quite weak. However, according to the climate survey method described in Cola et al. [6], harvest dates in the specific area have shown a 24-day advancement over the last four decades and it can be currently stated that a "standard" season places harvest around mid-August. Moreover, it should not be forgotten that all main phenological stages are delayed by this practice. The area is quite prone to frost damage due to either anticipated budburst or due to the fact that mostly early varieties are grown. Having even a few days of budburst delay (5 to 9 days in our study over years) under LWP can be quite significant.

According to expectations and in agreement with previous research [21], the LWP-two canes' treatment added in 2022 showed a behavior that, especially in terms of ripening dynamics and final harvest, closely resembled that of the C vines. This likely happened because when only two long canes were left for hand finishing, the amount of LA removal inherently turned out to be quite low and growth compensation by the retained nodes likely became expedited.

5. Conclusions

This three-year study conducted on Chardonnay targeted for sparkling wine making, in an environment (Franciacorta district, Italy) where heat availability is nowadays largely exceeding the cultivar requirements, shows that, in at least two seasons, a good compromise between suitable TSS and the minimum required acidity can be reached by anticipating the harvest date. When the weather course becomes extreme in terms of available heat and meteorological drought, further harvest anticipation does not remain a viable option. A delayed one-step winter pruning, applied on untouched vines first when apical shoots have formed 2-3 unfolded leaves and then 7-10 days later, induces a consistent delay in all main phenological stages compared to the standard winter pruning. Interestingly, the delay interval is wider (5–14 days) for harvest than for budburst (4–9 days), suggesting that delaying effects progressively build up along seasons, with high chances of persistence until harvest.

The concluding remark for anyone willing to adopt this delaying technique is simple: apply it as a one-stage operation in a time span that does not risk the removal of more than 1000 cm^2 of the leaf area. Trespassing such threshold will result in increasingly limited yield, which might then compromise economic sustainability of the technique. Conversely, if the main purpose is to postpone harvest, the two-step mode of the LWP technique proves to be quite ineffective.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Figure S1: cumulated growing degree days (GDD, base temperature 10°C) calculated from 1st April to 31st October in 2020, 2021, and 2022. From left to right, symbols on the upper right corner indicate harvest dates in 2020 (squares), 2021 (triangles), and 2022 (diamonds) for C and LWP treatments. In 2022, the LWP-two-canes treatment had same harvest date. Figure S2: seasonal climate trends (1 April–31 October) for daily mean air temperature (T_{mean} , °C), minimum air temperature (T_{min} , °C), maximum air temperature

 $(T_{\text{max}}, ^{\circ}\text{C})$, and daily rainfall recorded by a weather station located nearby the vineyard (less than 200 (m) in 2020, 2021, and 2022. Arrows indicate timing of late winter pruning (LWP). From left to right, stars on the upper right corner indicate harvest dates for C and LWP treatments. In 2022, the LWP-two-canes treatment had same harvest date as C. (*Supplementary Materials*)

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