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ORIGINAL RESEARCH ARTICLE

Effect of early cane pruning on yield components, grape composition, carbohydrates storage and phenology in *Vitis vinifera* L. cv. Merlot

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ABSTRACT

Dormant cane pruning has a great impact on vineyard management both in terms of labour costs and the time required to complete this field operation in the absence of mechanisation. In this study, we investigated over three seasons the influence of five pruning dates on yield components, grape composition, phenology and carbohydrate reserves in the variety Merlot, grown in a warm climate area. Pruning was conducted soon after harvest, at the end of leaf fall, two bud dormancy stages and vine bleeding. Early pruning, carried out a few days after the grape harvest, did not significantly affect vine productivity or grape composition in the next season compared to full winter pruning. Similarly, non-structural carbohydrate concentration in trunk and roots showed no difference just before budburst, no matter the timing of pruning. Late winter pruning at vine bleeding slightly postponed budburst and flowering; it also delayed veraison in one out of two years of observation without affecting grape maturity. Our findings suggested that, in a warm-climate area where leaf fall occurs within a ten-week period after harvest, early pruning had no detrimental effects on reserve accumulation in storage organs and, therefore, in vine yield and grape composition in the next harvest, thus allowing viticulturists to operate winter pruning over an extended interval of time.

KEYWORDS: pruning, grapevine, phenology, carbohydrates, Merlot, *Vitis vinifera* L.

INTRODUCTION

Dormant pruning is an important practice in vineyard management, aimed at maintaining the set training system (Reynolds and Vanden Heuvel, 2009) and balancing the vine vigour and fruit productivity (Howell, 2001). Depending on the selected pruning technique, changes in the canopy architecture and microclimate, yield components and fruit composition can be observed (Wessner and Kurtural, 2013).

Pruning is traditionally performed during vine ecodormancy, from leaf-fall until the onset of budbreak. Waiting for the leaves to shed is thought to be particularly relevant, especially in warmer climates, where leaves are retained on the vine several weeks after harvest, allowing accumulation of carbohydrate (CHO) reserves in permanent organs (Williams, 1996). Moreover, hydrolysed proteins, CHO and nucleic acids, or minerals are reallocated into storage organs during leaf senescence (Bates *et al.*, 2002; Greven *et al.*, 2016). The effect of the pruning time on vine performance and grape quality is of particular interest in viticulture. In this regard, the incidence of late winter pruning in different environments, cultivars and training systems has been extensively studied: in cool-climate growing districts, this practice proved helpful in postponing budbreak, escaping late frost events, and increasing the yield (Howell and Wolpert, 1978; Friend and Trought, 2007; Friend *et al.*, 2011). Since the postponement of the winter pruning until after budburst was shown to shift vine phenology, in warmer areas this technique was also effective in mitigating the consequences of warming due to climate change by delaying ripening and spreading grape maturity (Frioni *et al.*, 2016; Gatti *et al.*, 2016; Palliotti *et al.*, 2017; Petrie *et al.*, 2017; Moran *et al.*, 2017; Zheng *et al.*, 2017; Moran *et al.*, 2018; Gatti *et al.*, 2018; Silvestroni *et al.*, 2018; Frioni *et al.*, 2019; Allegro *et al.*, 2020; Buesa *et al.*, 2021). By contrast, the vine response to early pruning was investigated to a much lesser extent. In a cool climate environment, Trought *et al.* (2011) showed that pruning shortly after harvest induced no significant adverse effects on either vine phenology or the yield of Sauvignon blanc grapevine. In addition, the authors indicated that carbohydrates sufficiently accumulated in the trunk by harvest, suggesting a limited role of post-harvest photosynthesis on vine development in the following seasons. In post-harvest defoliation experiments, which might be considered a proxy for early pruning, it has been demonstrated that the complete removal of leaves reduced yield in the following year from 25 % to 56 % in Sultana potted vines (Scholefield *et al.*, 1978). Similarly, in hot-climate vineyards, full defoliation in post-harvest vines decreased productivity by up to 50 % in high-yielding Semillon vines after two seasons of treatment (Holzapfel *et al.*, 2006; Smith and Holzapfel, 2009). In a cool climate region, lower production was also observed in high-yielding Sauvignon grapevines after a few consecutive years of post-harvest defoliation (Greven *et al.*, 2016). Moreover, the level of storage carbohydrates in Sultana basal canes was not affected by partial defoliation (60 %) of the vine at harvest (Scholefield *et al.*, 1978), whereas Greven *et al.* (2016) observed that complete post-harvest leaf removal caused a reduction in root starch by up to 50 %, which eventually led to lower yield and poorer vegetative growth. The timing of pruning may also affect wound susceptibility to pathogen invasion together with environmental conditions and geographical location. A higher rate of natural infection of pruning wounds by grapevine trunk disease-related fungi was observed after late (February) rather than early (November) pruning in Galicia (Martínez-Diz *et al.*, 2020) and Catalonia (Luque *et al.*, 2014), respectively. Although some artificial fungal inoculation studies indicated that the susceptibility of grapevine wounds and the period between pruning and wound inoculation were inversely related (Martínez-Diz *et al.*, 2020), contrasting results on whether pruning time could affect infections incidence have been reported, favouring the hypothesis that the climatic conditions after pruning may play a major role (van Niekerk *et al.*, 2011).

Labour requirements and costs for dormant pruning operations can greatly vary depending on whether pruning is manual or mechanical (Poni *et al.*, 2016). Hand dormant pruning can take up to 17 % of total vineyard management costs (Galletto and Scaggiante, 2006), while mechanical pruning saves labour by about 95 % (Intrieri and Poni, 1995; Caprara and Pezzi, 2013) and operation costs by 80 % and 90 % in Concord and Merlot grapes, respectively (Bates and Morris, 2009; Kurtural *et al.*, 2019). Nevertheless, hand pruning is still widely used, especially in premium wine production districts, as it is considered more selective and aesthetically acceptable than mechanical pruning (Poni *et al.*, 2016). Further, long-cane trellis systems, such as Guyot, can be only partially mechanised since some specific operations still require human intervention, such as the selection of the renewal cane and its positioning onto the fruiting wire (Poni *et al.*, 2016). In this context, the interval of wintertime, during which manual dormant cane pruning is normally operated, might be too short if the workforce is a limiting factor (Trought *et al.*, 2011). Furthermore, prolonged adverse weather conditions might exacerbate the abovementioned problem.

In this study, we tested the effects of operating long-cane pruning at different times, from a few days after harvest until just before budburst, on Merlot vines cultivated in a warm climate. We focused on the effect of timing on vine yield, grapes composition, non-structural carbohydrates storage in trunk and roots and phenology kinetics. The experiment aimed to evaluate whether early pruning would be an option that comes with no physiological, production or quality costs to operate winter pruning over an extended period of time.

MATERIALS AND METHODS

1. Plant material and experimental design

Field experiments were carried out across three seasons (2014–2015, 2015–2016, 2016–2017), defined as the elapsed time between two harvests, in a commercial vineyard of Merlot (clone R3) grafted onto 161.49 Couderc rootstock in Tezze di Piave (Treviso, Italy) (45°48’27.4”N

12°21'30.0"E, 40 m a.s.l.). Tomasi *et al.* (2011) placed this growing area in the warm climate maturity group based on the growing season average temperature (GST), in the Region III on the Winkler Index and in the warm index class on the Huglin Index. The vineyard was established in 2005 and vines were trained to a single Guyot. The fruiting cane was pruned to 10–12 buds and set on a supporting wire 0.8 m aboveground, with two foliage wire pairs for vertical shoot positioning. Two additional spurs with 1-2 buds were left on each vine. Vine spacing was 2.7 m × 0.88 m (inter- and intrarow, respectively) for a density of 4209 vines/ha. Five pruning treatments were applied at different phenological stages based on the modified Eichhorn and Lorenz (E-L) scale (Coombe, 1995): E-L 41 (after-harvest), E-L 47 (end of leaf fall), two E-L 1 stages (A, B) spanning across the 'winter bud' phase and an intermediate pruning time between E-L 1 and E-L 2 (bud scales opening), concomitantly with vine bleeding. Dates for every treatment per season are shown in Supplemental Table 1. Fruiting cane was wrapped on the supporting wire immediately after pruning except for the earliest treatment (E-L 41), which was positioned at the subsequent treatment date (E-L 47) after leaf fall. Within two adjacent east-west oriented rows, 20 plots of ten vines were set and randomly assigned to the five treatments resulting in four replicates per treatment. Vines at the ends of each row were not included in the experiment. The summer pruning consisted of either shoot mechanical trimmings at about 2.0 m in height and 0.5 m in depth between the vertical canopy faces and suckers removal. The vines were irrigated by a drip subsoil irrigation system. Pest management and fertilisation were carried out following local standard agronomic practices. Climatic data were retrieved from a meteorological station of the regional agency for environmental protection (ARPAV) located ~ 1.3 km apart from the experimental vineyard (45°48'42.0"N 12°20'30.8"E).

2. Yield parameters and must composition at harvest

At harvest, in 2015 and 2016, grapes from three vines per replicate were hand-picked the same day within the same year, and yield and cluster number per vine were noted. The average cluster weight was calculated. One hundred berries were randomly sampled from ten representative clusters per replicate for fruit composition analyses; a further set of 20 berries per replicate was collected, immediately frozen and stored at –80 °C for polyphenols evaluation. Mean berry weight and berries per cluster were calculated. The 100 berries were hand-crushed and the resulting juice was used to measure total soluble solids (TSS, °Brix), pH and titratable acidity (TA, g/L) according to Alessandrini *et al.* (2016). Yeast assimilable nitrogen (YAN) concentration in the must also was assessed according to Nicolini *et al.* (2004a). Skin anthocyanins and flavonols were extracted and analysed according to Downey and Rochfort (2008): extracts were run on a 1220 Infinity LC system (Agilent Technologies, Santa Clara, CA, USA) coupled to a Diode Array Detector (DAD) Gilson 170 (Agilent Technologies, Santa Clara, CA, USA) and separated in a ProteCol® C18 G120A column (150 mm × 4.6 mm, 3 µm) (SGE, Ringwood, Australia) protected by a C18 guard column (SGE, Ringwood, Australia). Anthocyanins and flavonols were quantified against calibration curves made with oenin chloride (malvidin 3-O-glucoside chloride) and quercetin-3-O-glucoside (Extrasynthese, Genay, France), respectively. Anthocyanins were detected at 520 nm and flavonols at 353 nm.

3. Non-structural carbohydrate storage in permanent organs

Soon after the latest treatment (E-L 1/2), vine root and trunk samples were collected to determine the carbohydrates reserve status on two vines per replicate in 2015, 2016 and 2017. Two-four mm diameter roots were taken at 20–30 cm soil depth. After bark removal, specimens from the central portion of the trunk were sampled through a cork borer (5 mm diameter) and then wounds were sealed with a protective paste (Zapi, Padova, Italy). Samplings were repeated on different vines each year. The concentration of soluble sugars and starch in both organs was determined using a colorimetric method (Loewus, 1952). Absorbance readings at 620 nm were performed using a UV Mini-1240 spectrophotometer (Shimadzu, Kyoto, Japan).

4. Phenology

Key phenological stages (budburst, flowering, veraison) were registered in 2016 and 2017. Budburst was evaluated using the modified E-L scale (Coombe, 1995); fruiting cane buds from two tagged vines per replicate were scored and E-L numbers for all nodes were averaged to describe the fruiting cane median value at each observation date. Flowering and veraison were determined as the percentage of open flowers and coloured berries, respectively. At each observation date, the median value of the replicate was assessed by monitoring vines on both sides of the row.

5. Statistical analysis

A two-way ANOVA was performed on yield parameters, fruit composition and non-structural carbohydrates stored in roots and trunk using the JMP® software (JMP 7.0, SAS Institute Inc., NC, USA). Treatment and year were considered fixed factors. One-way ANOVA was performed to compare the phenological trends of the five treatments at each observation date within a given season; significant differences among means were determined using the Tukey HSD test ($p < 0.05$).

The effect of timing of winter pruning on the kinetics of budburst, flowering and veraison were also assessed using a survival analysis technique (Rich *et al.*, 2010) performed with the Statistica software (v 7.1, StatSoft Inc., Tulsa, OK, USA). The Kaplan–Meier method was used to estimate the survival curve for the veraison in response to pruning treatments, following indications by Herrera and Castellarin (2016). Similarly, both in budburst and flowering, the survival functions were defined as the probability for buds and flower bottoms to remain closed. Significant differences among treatments were tested using the log-rank test ($p < 0.05$).

RESULTS

1. Climate characterisation of the experimental site

Total rainfall throughout the pruning period (from 16 September to 31 March) was higher in the 2014–2015 season than in the 2015–2016 and 2016–2017 seasons (563.2 mm, 471.8 mm and 491.0 mm, respectively), distributed over 91, 97 and 73 rain days (Figure S1). Historical data (1993–2014) over the same interval showed mean total rainfall values of 625.3 mm and ~ 91 rainy days (data not shown). Rainfall was concentrated between November and December (290.6 mm, ~ 52 % of the total rainfall) along the pruning period in 2014–2015, in February–March (304.6 mm, ~ 65 %) in 2015–2016, and more evenly distributed from September to the end of November in 2016–2017 (357.2 mm, ~ 73 %) (Figure S1). The lowest air temperature within the pruning period was observed between the end of December and January in all seasons, whilst the 2014–2015 season was characterised by milder mean daily temperatures (9.2 °C) than 2015–2016 (8.1 °C) and 2016–2017 (7.7 °C) over the entire span of treatments

TABLE 1. Yield components at harvest from Merlot vines subjected to five winter pruning treatments.

	Yield/vine (Kg)	Cluster weight (g)	Clusters/ vine	Berry weight (g)	Berries/ cluster
Treatment					
E-L 41	5.21	260.07	19.96	1.88	140.32
E-L 47	5.31	259.22	20.50	1.96	133.43
E-L 1 (A)	4.85	237.03	20.58	1.92	125.72
E-L 1 (B)	5.31	258.39	20.67	1.92	136.03
E-L 1/2	4.95	239.10	20.79	1.92	126.13
p-value*	0.7805	0.2419	0.9796	0.6462	0.1928
Year					
2015	5.10	256.78	19.77	2.15	119.36
2016	5.15	244.74	21.23	1.69	145.30
p-value	0.8507	0.1736	0.1076	< 0.0001	< 0.0001
Treatment x Year interaction					
p-value	0.7178	0.8575	0.8329	0.6690	0.6848

*Data were analysed through two-way ANOVA.

TABLE 2. Must composition at harvest from Merlot vines subjected to five winter pruning treatments. (SS = Soluble Solids, TA = Titratable Acidity, expressed as g/L tartaric acid equivalent, YAN = Yeast Assimilable Nitrogen).

	SS (Brix°)	pH	TA (g/L)	YAN (mg/L)
Treatment				
E-L 41	20.24	3.40	5.72	121.53
E-L 47	20.26	3.40	5.63	129.77
E-L 1 (A)	20.24	3.42	5.82	137.03
E-L 1 (B)	20.40	3.40	5.76	123.37
E-L 1/2	20.36	3.41	5.83	123.64
p-value*	0.8131	0.8490	0.7087	0.4798
Year				
2015	20.42	3.52	5.09	114.05
2016	20.19	3.30	6.41	140.09
p-value	0.0428	< 0.0001	< 0.0001	0.0002
Treatment x Year interaction				
p-value	0.5572	0.9446	0.8480	0.7977

*Data were analysed through two-way ANOVA.

application. Heat accumulation, expressed as growing degree days (GDD, >10 °C) from 1 April to 30 September, was higher in 2015 than in 2016 and 2017 (1884, 1784 and 1774 GDD, respectively), with the latter two more similar to the historical mean (1737 GDD, 1993–2014) (Figure S2). The highest monthly maximum air temperature was recorded in July in 2015 and 2016 (32.4 and 30.5 °C, respectively) and in August in 2017 (31.4 °C).

2. Yield parameters and must composition at harvest

Statistical analysis showed no significant ($p < 0.05$) impact of pruning treatments on plant productivity either in 2015 or 2016 (Table 1). The same held true for the number of clusters per vine and cluster weight ($p = 0.2419$ and 0.9796, respectively). Berry weight and the number of berries per cluster were not affected by treatments, whereas a seasonal effect ($p < 0.0001$) was observed on these components with approximately 21 % lighter berries in 2016 than in 2015, concomitantly with ~ 22 % fewer berries per cluster in 2015 (Table 1). In both 2015 and 2016, pruning treatments had no significant ($p < 0.05$) effect on must soluble solids, pH, TA and YAN at harvest (Table 2). The warmer 2015 vintage (Figure S2) slightly ($p = 0.0428$) influenced the soluble solids accumulation (+1.12 %) compared to 2016, while a stronger effect was observed for pH (3.52 vs. 3.30, $p < 0.0001$), and TA (–25.9 %); average YAN concentration was approximately 26 mg/L lower in 2015. Total anthocyanin and flavonol concentrations and contents were unaffected by pruning treatments (Table 3). Similarly, the proportions of the skin tri-substituted and methoxylated 3-O-monoglucoside anthocyanins were not influenced by any treatment ($p = 0.3434$ and 0.8090, respectively), as well as that of acylated anthocyanins ($p = 0.9369$), resulting in mean values of ~ 77 %, ~ 88 % and ~ 39 %, respectively (Table S2). Conversely, the year effect was evident in the concentration (+41 % in 2015) and content (+25 % in 2015) of both total anthocyanins and flavonols; similar trends were observed for the tri-substituted 3-O-glucoside (+9.8 %), methoxylated (+5.4 %) and acylated anthocyanins (+8.2 %) (Table S2).

3. Effect of pruning on non-structural carbohydrates in permanent organs

The concentration of non-structural carbohydrates in roots and trunk was assessed over three seasons to evaluate the effect of different pruning dates on grapevine reserves. Starch concentration was not affected by treatments in the trunk ($p = 0.7915$, 149 mg/g), nor in roots ($p = 0.3155$, 252 mg/g) (Table 4). Similarly, no variation was observed for soluble sugars in the trunk, while post-harvest pruning (E-L 41) versus the earlier winter bud phase treatment (E-L 1A) showed a slightly higher concentration ($p = 0.0344$) in roots. Carbohydrate concentration was significantly affected by seasonal trends both in the trunk and roots (Table 4).

4. Impact of early pruning on vine phenology

Budburst, flowering and veraison of Merlot grapevines were scored throughout the vegetative phase in 2016 and 2017. No significant differences were observed in budburst (corresponding to E-L 4) progression between anticipated (E-L 41) and traditional winter pruning (E-L 47, E-L 1A, E-L 1B) treatments (Figure 1 A, D). Although the latest pruning (E-L 1/2) was not applied beyond vine bleeding in order not to damage the buds through the cane wrapping, it significantly postponed both bud swelling and budburst, which was achieved four and about three days later in 2016 and 2017, respectively (Figure 1 A, D); from 227 DAH (2016, 2017), one-way ANOVA did not detect differences

TABLE 3. Anthocyanins and flavonols concentration and content at harvest from Merlot vines subjected to five winter pruning treatments. (FW = Fresh Weight).

	Total anthocyanins (mg/g skin FW)	Total anthocyanins/ berry (mg)	Total flavonols (mg/g skin FW)	Total flavonols/ berry (mg)
Treatment				
E-L 41	5.64	1.11	0.50	0.10
E-L 47	5.87	1.03	0.66	0.12
E-L 1 (A)	4.84	0.94	0.43	0.08
E-L 1 (B)	5.80	1.10	0.56	0.11
E-L 1/2	5.87	1.08	0.62	0.11
p-value*	0.0702	0.1891	0.0843	0.2769
Year				
2015	7.03	1.20	0.70	0.12
2016	4.17	0.90	0.41	0.09
p-value	< 0.0001	< 0.0001	< 0.0001	0.0089
Treatment x Year interaction				
p-value	0.0944	0.8315	0.6205	0.9663

*Data were analysed through two-way ANOVA.

TABLE 4. Stored carbohydrates concentration in trunk and roots from Merlot vines subjected to five winter pruning treatments. (DW = Dry Weight).

	Starch (mg/g DW)		Soluble sugars (mg/g DW)	
	Trunk	Roots	Trunk	Roots
Treatment				
E-L 41	147.40	240.84	87.00	43.53
E-L 47	150.26	258.92	74.14	35.18
E-L 1 (A)	151.29	259.18	85.11	31.20
E-L 1 (B)	146.90	257.96	86.09	34.52
E-L 1/2	149.22	243.12	85.39	34.85
p-value*	0.7915	0.3155	0.0548	0.0344
Year				
2015	146.19	258.09	66.51	40.88
2016	145.56	228.14	108.73	34.26
2017	155.29	263.69	75.40	27.42
p-value	0.0047	0.0004	0.0001	< 0.0001
Treatment x Year interaction				
p-value	0.4535	0.1343	0.7145	0.6220

*Data were analysed through two-way ANOVA.

in shoot development among treatments. Similarly, the flowering progression was generally not affected by early nor dormant pruning treatments (Figure 1 B, E), while it occurred (50 % caps off) approximately two (2016) and three (2017) days later in response to more delayed pruning (E-L 1/2). Veraison in 2016 was virtually unaffected by any pruning treatments (Figure 1 C). In 2017, although berry colour assessment was not extended till full veraison, post-harvest and dormancy winter pruning treatments did not show any difference at veraison (50 % of coloured berry), which was instead estimated to be reached about two days later in vines pruned at E-L1/2 (Figure 1 F). The survival analysis tested on phenology data sharply confirmed that anticipated pruning did not significantly modify budburst, flowering and veraison onset compared to full winter pruning, whereas late winter pruning slightly retarded phenology (Figure S3, Table S3).

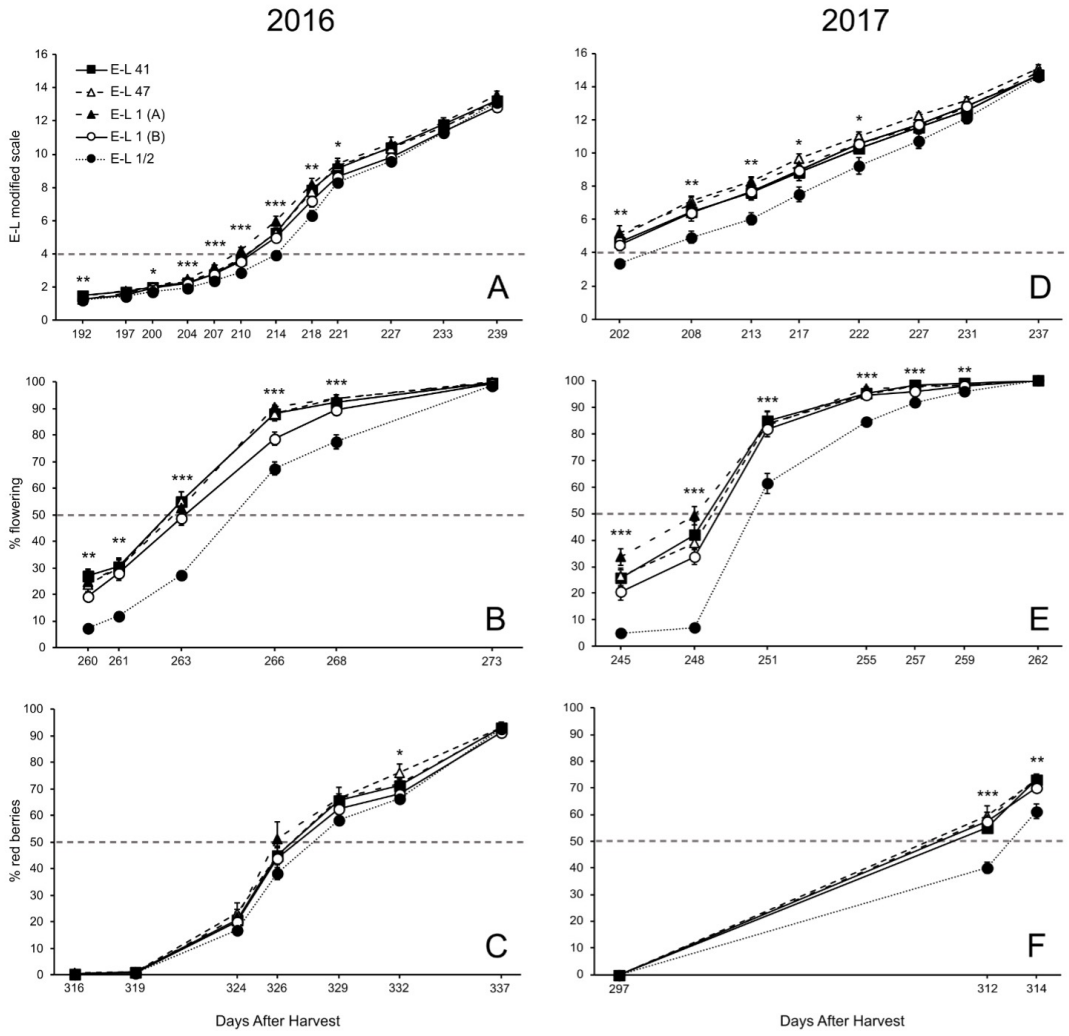
DISCUSSION

The main goal of the study was to evaluate the effect of early pruning on different vine parameters. This was compared with full winter pruning, which in normal practice is operated over an extended period during eco-dormancy, and with late winter pruning at the sap bleeding stage. This late winter pruning, before budburst, was used to avoid the risk of damaging the swelling buds or emerging shoots and, thus, a potentially time-consuming and expensive manual operation due to the extreme care that would be needed for the fruiting cane wrapping.

In spring, the vegetative resumption in growth relies on the non-structural CHO reserves, especially those stored within the roots, until photosynthesis becomes the primary source of new carbon skeletons. Since then, and throughout the vegetative season, the plant replenishes these reserves until leaf fall. It is known that an excessive crop load may reduce the accumulation of vine reserves by harvest (Greven *et al.*, 2015). Nevertheless, in our experiment, the vine yield seems not to be a limiting factor, as, in both years of observation, the yield consistently ranged around five kg per plant, which can be considered a low to moderate production in the selected area.

The photosynthesis efficiency sharply decreases from late summer to autumn; however, it seems to play an important role in replenishing storage reserves, as a complete post-harvest defoliation could lead to lower yield in the subsequent season (Scholenfield *et al.*, 1978). Our study showed that anticipated pruning did not influence Merlot shoot fruitfulness nor cluster and berry size, resulting in similar yield among treatments. This would likely indicate that in such a warm climate area, where the interval between harvest and leaf fall may last ten weeks, Merlot vines can accumulate sufficient reserves by harvest. Thus, the subsequent photosynthetic activity and reallocation from the leaves apparently do not play a significant role in the replenishment of storage organs necessary for the development of floral primordia. After post-harvest pruning, the foliage of the selected fruiting cane may reasonably represent less than ten per cent of the pre-pruned canopy, favouring the hypothesis of a limited contribution imparted by the post-harvest interval. The analysis of non-structural carbohydrates also supported this evidence as trunk starch and soluble sugars concentrations before budbreak were not influenced by pruning timing over three seasons, consistent with what was observed in New Zealand

FIGURE 1. Progression of phenological stages in response to five pruning treatments in 2016 (A, B, C) and 2017 (D, E, F).



Grey dotted lines indicate budburst (E–L 4) (A, D), flowering (50 % caps off) (B, E) and veraison (50 % coloured berries) (C, F). Each point is the mean value of four replicates at a given observation date. Bars represent the standard error (n = 4). *, **, *** indicate significant difference among treatments at $p < 0.05$, 0.01 and 0.001, respectively, based on the one-way ANOVA. Means comparison according to the Tukey HSD test ($p < 0.05$) at each observation date is reported in Supplementary file 1. No asterisks represent no significance.

on Sauvignon blanc (Trought *et al.*, 2011). A similar trend was also observed in the roots. Although we did not assess the concentration of non-structural CHOs in the shoots, it is unlikely that differences among treatments could greatly impact vegetative and reproductive cycles.

Comparing early and late pruning, Trought *et al.* (2011) observed significant differences in the level of TSS and titratable acidity from Sauvignon blanc grapes, though not coupled in the same season. No effects of early pruning on the berry composition traits were assessed in the present work; the warmest 2015 season led either to a slight increase in berry sugars and a more evident acidity decrease compared to 2016. The YAN is an important component of must since it can influence the fermentation kinetics and the biosynthesis of several compounds (Carrau *et al.*, 2008); YAN concentration in grapes varies according to diverse factors such as the genetics of cultivars and rootstocks, the nutritional status of the plant, the environmental conditions and the vineyard management. The median YAN concentration measured in the present study was consistent with that observed in Italy for Merlot grapes (Nicolini *et al.*, 2004b); the vintage effect on YAN accumulation was evident and much likely due to climate differences between years. In 2015, clusters had, on average, fewer and heavier berries than in 2016, which might be due to weight compensation in response to poorer fruit-set during a rainy flowering period in 2015. However, the concentration of anthocyanins and flavonols in the peel were higher in 2015 and the same was for their berry content, suggesting a higher biosynthesis of these molecules in the berry skin. The biosynthesis of anthocyanins and flavonols

is influenced by several stimuli, including temperature, abiotic and biotic stress and light exposure (Castellarin *et al.*, 2012). Thus, a combination of sub-optimal conditions in 2016 might have led to a lower amount of polyphenols. The anthocyanin profile is genetically determined in each red-grape variety; nevertheless, external factors can alter the proportion of anthocyanins. Early pruning had no effects on the Merlot anthocyanin profile and the anthocyanin composition was consistent with former studies (Mattivi *et al.*, 2006; Yan *et al.*, 2020). On the other hand, the warmer temperature registered in 2015 likely favoured a higher proportion of 3',4',5'-substituted, methoxylated and acylated anthocyanins, accordingly to previous experiments (Mori *et al.*, 2007; Tarara *et al.*, 2008; De Rosas *et al.*, 2017; Yan *et al.*, 2020). Although we did not observe significant effects of early pruning on vine performance and berry composition, it must be noted that the present study was focused on a limited number of years and possible carry-over effects from reiterated early pruning on the Merlot cultivar cannot be excluded.

The phenology was not affected by early pruning (applied from 4 to 14 DAH), which showed the same trends as observed by pruning after leaf fall (E-L 47) and during the winter bud phase (E-L 1A, E-L 1B), similar to what was obtained by Trought *et al.* (2011) in Sauvignon blanc. Conversely, the latest pruning slightly shifted onwards both budburst and flowering stages regardless of vintage. It is well-known that apical dominance in grapevine shoot suppresses the growth of basal nodes; thus, the abovementioned delay was possibly due to the unpruned apical buds that, by the time of the sap bleeding, had already started to exert their repressive effect on the experimental 10-12 basal nodes. However, budburst postponement was quite small and could hardly be effective in protecting the vine from late frost. Nevertheless, a longer interval was obtained in Pinot Noir by pruning the unpruned canes when the shoots on node ten had three unfolded leaves (Gatti *et al.*, 2019). Despite the delay, at harvest, all treatments showed no differences in berry composition, indicating that vines could fill the gap throughout the season; interestingly, in 2016, no phenology differences were observed among treatments by veraison. In this regard, it has been demonstrated that late winter pruned vines show higher canopy efficiency and cumulated carbon than standard pruning, likely in response to more favourable climate conditions (Gatti *et al.*, 2016).

Although the effect of pruning timing on vine susceptibility to wood diseases was beyond the aim of the present study, we did not observe symptomatic plants within the experiment length. A longer evaluation would be required as the time for visible symptoms might be not compatible with our experiment.

Our results may also prove helpful for viticulturists that intend to perform on-vine withering. This technique, also known as Double Reasoned Maturation (DRM), allows for grapes to wilt in the field via the total or partial cutting of fruit-bearing canes at harvest (Corso *et al.*, 2013). By interrupting the exchanges between perennial organs and the canopy, DRM induces dehydration of grapes and, consequently, the concentration of sugars, organic acids and polyphenols in the berry. Since we observed that such interruption did not decrease Merlot vine performances within a short to mid-term interval in a warm climate location,the DRM could be safely applied at least in the same time range.

CONCLUSION

To the best of our knowledge, this is the first study that has focused on the effect of early cane pruning on the performance of a red grape variety cultivated in a warm-climate region. Long-cane training systems, e.g., the Guyot, are quite labour demanding, and sometimes its requirement and availability cannot be matched. In addition, climate change may be responsible for extreme and prolonged adverse conditions, which could still shorten the favourable interval for pruning. Anticipating the winter pruning soon after the harvest is a valuable solution to extend the time for performing this operation while not resulting in detrimental effects on Merlot vine yield, berry composition, storage reserves and phenology trends. A longer observation period would be required to assess possible carry-over consequences of early pruning in Merlot; nevertheless, the present study clearly showed that anticipated pruning could be adopted either in unfavourable vintages or by rotating vineyard plots.

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