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Research Article

Post-Spring Frost Canopy Recovery, Vine Balance, and Fruit Composition in cv. Barbera Grapevines

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Background and Aims. In viticulture, one of the effects of warming trends is the advance of budburst date and the consequent increased risk of spring frost-related damage. In 2021, severe frost events affected a large fraction of European viticulture. In a cv. Barbera vineyard, located in NW Italy, primary bud shoots (PBS), secondary bud shoots (SBS), and suckers (SK) were tagged after the occurrence of freezing temperatures in spring. The goal of the study was to clarify if SBS could partially restore yield loss and analyze their contribution to fruit composition. *Methods and Results*. The number of developing SBS and SK correlated with the number of killed PBS. While PBS bore 1.44 inflorescences per shoot, SBS had much lower fertility (0.4), with SK at intermediate levels (0.85). The vine yield was 40% of the previous season, with SBS bunches contributing just 17% of the total. SBS produced smaller and looser bunches, as compared with PBS (−28% mass and −27% compactness). At harvest, no difference was found in grape total soluble solids (TSS) among different shoot types. However, the TSS average was notably higher than that observed in the previous season (27.8°Brix vs. 23.3°Brix in 2020). Interestingly, while in PBS and SK, a direct correlation (linear and quadratic, respectively) existed between the leaf area to yield ratio (LA/Y) and grape TSS or total anthocyanins, this did not occur for SBS. *Conclusions*. In the case of spring frost damage, the number of PBS avoiding fatal injuries will drive agronomic results at harvest since SBS contribution to total yield is modest due to low shoot fruitfulness. The frost-induced increase in vine LA/Y leads to a dramatic rise in grape TSS and phenolics. *Significance of the Study*. When spring frosts kill a significant number of primary shoots, an altered grape composition at harvest should be expected due to changes in vine balance. Therefore, the vineyard management should be adjusted accordingly early in the season. Further studies are needed to test specific post-frost canopy management strategies ensuring yield, optimal fruit composition, and cane renewal.

1. Introduction

Spring frost is one of the most dramatic events that might occur in a vineyard, as freezing temperatures ≤ −1.5°C are known to cause severe injuries to swelling buds and developing shoots [1–3]. The release from endodormancy encompasses a quick rehydration of buds and young green tissues moving from a water concentration of approximately 40% to > 80% [2]. Consequently, tissue tolerance to low temperatures decreases, since cytoplasmic freezing temperature depends on the concentration of solutes, which get diluted by progressive rehydration [2–8]. In such context, the occurrence of "false springs" due to warming trends accelerates and advances budburst and early shoot development, causing a likely increase in grapevine susceptibility to spring frost [9–12]. Many wine regions in France, Germany, and Italy have experienced drastic yield losses between 2016 and 2021 due to spring frost occurrence [11, 13].

Frost damage can vary from total desiccation of the shoot to partial injury, preserving the viability of some shoot parts [4, 14]. A further complication of the damaging scenario is that, within a single vine and depending on pruning type, damage can vary for buds/shoots at different positions along a cane or for even adjacent spurs on the same cordon [4, 14–16].

Vitis vinifera L. has compound buds, and, indeed, secondary buds are inhibited by primary buds, therefore maintaining a lower tissue relative water content and, in turn, a higher tolerance to low temperatures. If frost kills the primary shoot, the inhibition of secondary meristems ceases allowing them to sprout and develop [17, 18].

Depending on the total number of primary shoots killed by frost, growers need to decide quickly whether any recovery interventions are required and how they are implemented. Likewise, as a function of the observed damage, a given operation might still attempt to preserve or stimulate some of the current year cropping or, in case of a very late frost damaging the shoots having already a considerable length, the primary objective might be to promote the formation of adequate fruiting wood for the forthcoming winter pruning and next season crop.

The state of knowledge about grapevine canopy structure and yield performances after spring frosts is limited [3, 7, 8]. Few works describe yield and fruit composition after spring freezing temperatures, yet different shoot types were not separately studied and the contribution of primary and secondary shoots remains largely undefined [4, 19–22]. Upon a late frost event that killed 33% of primary shoots in cv. Chardonnay, Friend et al. [23] reported that secondary shoots development was proportional to the number of killed primaries and that secondaries were significantly less fruitful. Conversely, Montague et al. [24], in Cabernet Sauvignon and Grenache, artificially removed primary shoots at about 15 cm length and promoted the growth of secondary shoots, whose yield was about 50% lower than primary ones. No further information is available in the literature for *Vitis vinifera*.

In the *Vitis* hybrid cv. Marquette, when spring freezing temperatures killed over 80% of primary shoots, secondaries restored a full canopy with a yield that was about 60% of a standard year [15]. In this variety, primary shoot fruitfulness and secondary shoot fruitfulness was comparable. This is also hinted by Sanchez and Dokoozlian [25], who found a largely varying number of inflorescence primordia in secondary buds of Chardonnay, Cabernet Sauvignon, Flame Seedless, and Thomson Seedless. Notably, spring freezing temperatures could also affect secondary bud shoots and inflorescences formation, and in-field fruitfulness of shoots from secondary buds can be lower than the potential fruitfulness of the secondary buds, assessed at dormancy. However, the abovementioned results pave the way for the hypothesis that fruitfulness of secondary bud shoots might be a relevant aspect in yield recovery after a spring frost event.

The aims of this paper were as follows: (i) to investigate the consequences of a spring frost which occurred in 2021 in north-western Italy on vines cv. Barbera, a widely cultivated genotype, featuring high average bud fruitfulness and early budburst; (ii) to clarify if shoot types other than the killed primary ones could restore yield and ensure adequate fruit composition.

2. Materials and Methods

2.1. Site Description. This paper describes an observational study carried out in 2020 and 2021 in a nonirrigated vineyard of cv. Barbera (*Vitis vinifera* L.), clone AT84, grafted on 420A, planted in 2003 in Bacedasco Basso, Vernasca (PC), "Azienda Vitivinicola Villa Rosa" (44°50′ N, 9°54′ E; 183 m a.s.l), Italy. The rows were NW-SE oriented with a 2.5 m × 1.2 m vine spacing for a resulting density of 3,333 vines/ha. Vines were trained to a vertically shoot-positioned, bilateral double cane-pruned Guyot trellis, with 12 buds on the canes and 4 nodes on the 2 renewal spurs. Canes were tied to the first wire at 0.80 m from the ground, with three pairs of surmounted catching wires forming a canopy wall extending approximately 1.2 m above the first wire.

2.2. Weather Data, Shoot Type Identification, and Experimental Layout. The core of this work involved the following: (a) the comparison of (i) primary bud shoots (PBS), (ii) secondary bud shoots (SBS), and (iii) suckers (SK), in 2021, after spring frost occurrence; (b) the evaluation of 2021 vine performances, as compared with those of the previous year (2020), when spring frost did not occur, and data for the same experimental vines were collected for different purposes (unpublished material).

Daily rainfall and maximum (T_{max}), mean (T_{mean}), and minimum (T_{min}) temperatures were recorded from 1 January to 31 December 2020 and 2021 from a weather station located within the vineyard, about 20 m from experimental vines. Growing degree days (GDD) were calculated according to Winkler et al. [26].

Four adjacent rows were selected in March 2020 and assigned to four different blocks. Within each block, three vines were tagged (3 vines per 4 blocks = 12 vines). In 2020, budburst (BBCH 09), identified according to Lorenz et al. [27], occurred on 13 April (DOY 103). According to standard practice, only primary shoots were retained, removing all secondary shoots and suckers that progressively developed. In 2021, the budburst occurred earlier (DOY 90) due to the higher temperatures recorded in March, specifically between DOYs 84 and 94. On 7–9 April 2021 (DOYs 97–99), air temperatures dropped below 0°C for three consecutive nights (lowest temperature = −3°C recorded on DOY 99) and damaged ~75% of developing primary shoots, which were at the stage of first leaf unfolded, as a median value (BBCH 11). On 15–17 April 2021, the budburst of secondary buds occurred. On 22 April 2021 (DOY 112), on the abovementioned 12 vines, killed and survived primary shoots were counted and the survived ones were tagged, while secondary bud shoots were identified based on the angle of projection on the cane, according to Mullins et al. [28]. In more detail, for each node, if the alive shoot had an angle of ~45° with the cane, in the absence of other necrotic shoot residues, it was classified as a survived primary bud shoot; conversely, if a necrotic shoot was present alongside an alive shoot exhibiting a ~90° angle of projection on the cane, this was classified as a secondary bud shoot (Figure 1). Lastly, any sucker developing from the trunk head, retained to ensure cane renewal, was tagged (27 May 2021, DOY 147). In 2021, shoots were not thinned, and data were collected separately among the three shoot types (PBS, SBS, and SK). Three shoots per type were selected and tagged on each vine

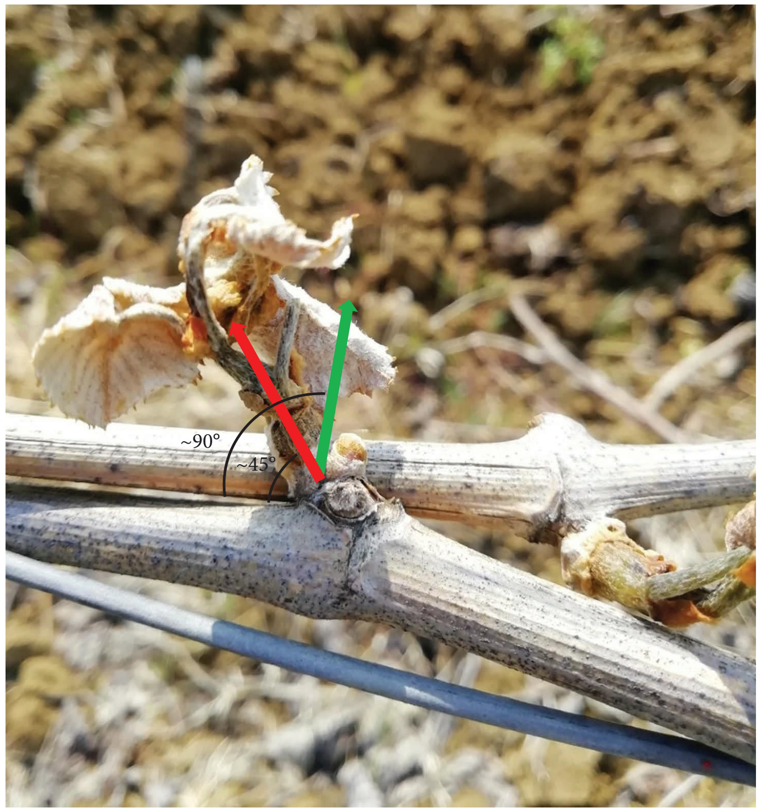

Figure 1: In 2021, shoots from primary (PBS) and secondary (SBS) buds were identified based on the angle formed with the two-year-old wood direction [15]. The figure shows PBS killed by frost and growing with an angle of ~45° to the fruiting cane (red arrow) and a secondary bud pushing with an angle of ~90° (green arrow). The photo was taken on 22 April 2021 (DOY 112).

as subreplicates. Then, data considered on a per-vine basis were calculated as the mean or the total of the three shoot types. In 2020, only PBS were present, and data were included to provide a reference against which to evaluate total vine agronomic performances of 2021.

2.3. Vegetative Growth, Shoot Fruitfulness, and Physiological Performances. On each vine, the number of shoots and inflorescences was counted at flowering (BBCH 61, [27]). However, in 2020, only PBS were present, and in 2021, shoots and inflorescences were counted separately for the three shoot types. Shoot fruitfulness was then calculated as the number of inflorescences per shoot. The total leaf area per vine was estimated as described by Gatti et al. [29], considering the proportion of different shoot types. At harvest, on three representative shoots per vine (one per type in 2021), main and lateral leaves were sampled and brought to the lab, where the leaf number and leaf area/shoot were measured. Then, the mean leaf area for the main and lateral leaves of PBS, SBS, and SK was calculated. After leaf fall, the number of nodes was counted on a per-vine basis and according to shoot type. The vine leaf area was estimated by multiplying the number of nodes and the respective mean leaf area. Gas exchange parameters (leaf assimilation rate A, transpiration rate E, and stomatal conductance g_s) were measured on 22 July 2020 (DOY 203) and on 6 August 2021 (DOY 218), corresponding to the mid-veraison phenological stage (BBCH 85), between 12 : 00 and 13 : 00, using a Lci T Pro (ADC Bioscientific Ltd., Hoddesdon, Herts., UK). Measurements were conducted on one main leaf per shoot type (one per PBS in 2020, one per shoot type in 2021) inserted at nodes 4–7 on the stem in PBS (2020) and in PBS, SBS, and SK (2021) under saturating light conditions (PAR >1,400 μmol·m^{-2}·s^{-1}) and ambient relative humidity, with the adjustment of airflow to 350 mL·min^{-1}. The leaf cuvette chamber had a 6.25 cm^2 window. Instantaneous leaf water use efficiency (WUE) was calculated as the A/E ratio.

2.4. Yield, Bunch Morphology, Vine Balance, and Fruit Composition. Harvest in both seasons was performed concurrently for all the experimental vines when, based on destructive samplings from untagged vines, the two following thresholds were achieved: (i) grape total soluble solids (TSS) concentration ~23°Brix; (ii) titratable acidity ~10 g/L. This occurred on 2 September 2020 (DOY 245) and 15 September 2021 (DOY 258). At harvest, on each vine, bunches from different shoot types were counted and weighed. On each vine, a sample of three representative bunches per PBS in 2020 and per all shoot types in 2021 was collected, stored in a cooler, and transported to the lab for bunch morphology and grape composition analysis. Bunch and rachis mass and length were determined, and bunch compactness was expressed as the ratio of bunch mass to rachis length. The number of berries per bunch was counted, and the average berry mass was then calculated. Sixty randomly selected berries per sample were frozen and stored for the determination of total anthocyanins and phenolics, whereas the remaining grapes were crushed to obtain a must. Must TSS concentration was measured with a digital refractometer SMART-1 (Atago, Bellevue, WA, USA), while pH analysis was performed with a pH meter (pH 60 VioLab Giorgio Bormac, Carpi, MO, Italy). Titratable acidity (TA), expressed as g/L of tartaric acid equivalents, was determined by titration with 0.1 N NaOH to a pH 8.2 endpoint and expressed as g/L of tartaric acid equivalents, using an AT 1000 Series Potentiometric Titrator (Hach Company, Loveland, CO, USA). Malic and tartaric acid concentrations were quantified via HPLC (Agilent Technologies, Santa Clara, CA, USA) into auto-sampler vials through a Synergy 4u Hydro-RP80 A column (Phenomenex Inc., Torrance, CA, USA), 250 × 4.6 mm, after juice dilution and 0.22 μm polypropylene syringe filtration. The buffer solution utilised for separation was a 0.2 M KH_2PO_4 adjusted to 2.4 pH with orthophosphoric acid. The 15 μL sample ran through the column maintained at 30°C ± 0.1°C. The run was monitored at 200–700 nm with a diode array detector (DAD) at 210 nm UV. Calibration curves were built with authentic standards, and organic acids concentration was quantified, determining areas of peaks corresponding to malic and tartaric acid.

Total anthocyanins and phenolics were determined after Iland [30]. Berries were homogenized at 24,000 rpm with an Ultra-Turrax T25 (Rose Scientific Ltd., Edmonton, Canada) homogenizer for 5 min; afterwards, 2 g of the homogenate was put into a centrifuge tube added with 10 mL of aqueous ethanol extraction solution (50%, pH 5) and kept for 1 h mixing every 10 min. After the extraction period, the solution was centrifuged at 3,500 rpm, and after 5 min, 0.5 mL supernatant was added to 10 mL 1M HCl. After three hours, absorbance was read at 520 nm for total anthocyanins and 280 nm for total phenolics, on a JascoV-530 spectrophotometer (Jasco Analytical Instruments, Easton, MD, USA). Total anthocyanins and phenolics concentration was expressed as mg per g of fresh weight.

2.5. Statistical Analysis. Data obtained in 2021 from different types of shoots were subjected to a one-way analysis of variance (ANOVA), and means were separated by the SNK test ($p < 0.05$). Data on a vine basis were then calculated as a sum or a weighted average, according to the type of parameter (details provided in the tables), and compared with 2020 data, separating means by Student's t-test ($p < 0.05$). Statistical analysis was performed with SPSS 12 (IBM, Armonk, NY, USA). Graphs and correlations were built with Sigma Plot 12 (Systat Software Inc., San Jose, CA, USA).

3. Results and Discussion

3.1. Weather Course and Freezing Temperatures. In 2020, a total of 2,062 GDDs were recorded from 1 April to 31 October. The heat summation corresponded to the region average, and the total yearly rainfall was 847 mm. No freezing temperatures were recorded after the budburst in 2020 (Figure 2). In 2021, higher temperatures recorded in March (23.4°C T_{max} on DOY 90) brought forward budburst by 13 days, as compared with the previous year. Afterwards, on DOYs 97, 98, and 99, T_{min} dropped below 0°C for three consecutive nights (down to −3°C on DOY 99), killing part of the developing primary shoots (Figure 2). GDDs recorded in 2021 from 1 April to 31 October were 2,022, and the total yearly rainfall was 688 mm.

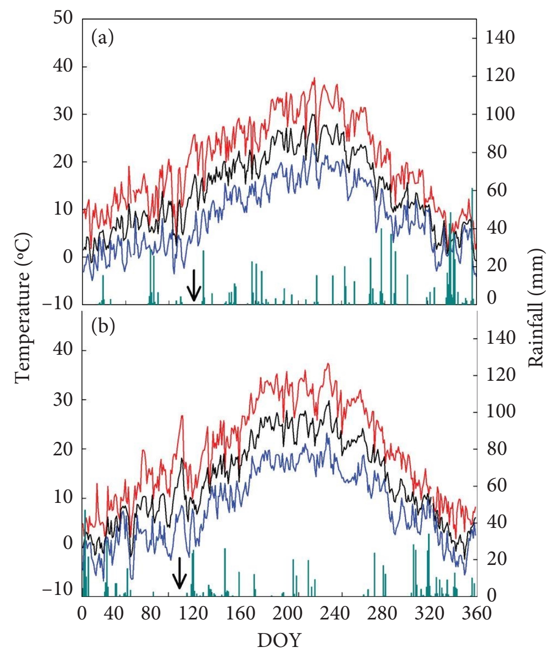

Figure 2: Seasonal evolution from 1 Jan to 31 Dec of maximum (T_{max}, —), mean (T_{mean}, —), and minimum (T_{min}, —) temperatures and rainfall (|) at the site of the experiment in 2020 (a) and 2021 (b). Arrows indicate budburst (BBCH 09). DOY, day of year.

3.2. Frost Damages, Vegetative Growth, Shoot Fruitfulness, and Leaf Gas Exchange. In 2021, the spring frost killed 75% of the developing PBS. Of 17 shoots per vine, at the end of the season, 4 survived PBS, 6 SBS, and 7 SK per vine were counted, whereas, in 2020, the canopy consisted of 16 PBS (Table 1). The reason why some of the PBS avoided fatal injuries could be related to the variability in the phenological stage at the time of freezing temperatures. Cane-pruning promotes a certain heterogeneity of node development at budburst related to the node position along the cane and due to acrotony favoring distal positions rather than basal ones or other growth-inhibiting factors. In this framework, it is likely that buds surviving freezing temperatures were those more inhibited at that time and that different pruning strategies could change the number of killed PBS per vine for the same minimum temperatures [14]. The number of killed PBS was directly correlated with the sum of SBS and SK subsequently developed (Figure 3). This is in line with the study by Friend et al. [23], even if they removed all the developing suckers and the correlation was built between PBS and SBS only. In addition, in our conditions, contribution of SBS to the total vine leaf area (13% of the total vine

Table 1: Canopy growth components, yield components, and vine balance, according to different shoot types, developed in response to a spring frost event occurred in April 2021 in a Barbera vineyard.

	2021—based on shoot types				Vine total		
	PBS	SBS	SK	*Sig*	2020$	2021$	*Sig*
Shoots per vine (n)	4 b¶	6 a	7 a	***	16	17	Ns
Leaf area (m^2/vine)	1.51 a	0.52 b	1.93 a	***	4.69	3.96	Ns
Yield (kg/vine)	0.98 b	0.51 c	1.56 a	***	6.67	2.65	***
Bunches per vine (n)	6 a	4 b	7 a	***	29	17	***
LA/Y (m^2/kg)	1.60	1.18	1.39	ns	0.70	1.45	***

PBS = primary bud shoots; SBS = secondary bud shoots; SK = suckers. ***Significant difference per $p < 0.001$. ns = no significant difference. $In 2020, canopies were composed by PBS only. 2021 data were calculated according to the contribution of the three different shoot types (sum). Means separated by Student's *t*-test. ¶Different letters within rows indicate significant difference per $p < 0.05$ (Student–Newman–Keuls test, $n = 12$).

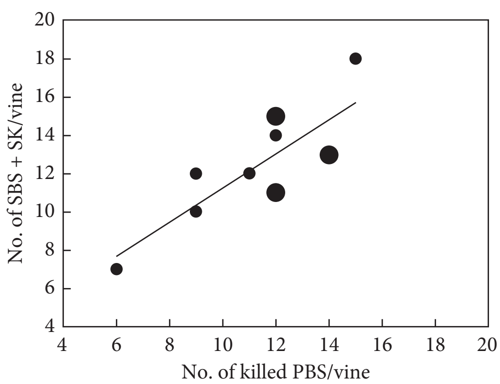

Figure 3: Correlation between the number of shoots from primary buds (PBS) killed by freezing temperatures in 2021 and the sum of new shoots growing from secondary buds (SBS) and suckers (SK) ($y = 0.89x + 2.33$; $R^2 = 0.63$, $p < 0.05$) in cv. Barbera grapevines ($n = 12$). Larger circles are shown when two dots coincide.

leaf area) was significantly lower than the contribution of PBS and SK (49% and 38% of the total vine leaf area, respectively) (Table 1). Overall, data suggest that vines can recover from frost damage by developing new shoots from the whole range of available bud types and that when suckers have to be retained for the need of cane renewal, a weaker secondary shoot development is likely to occur (Table 1). This can be a relevant aspect also in view of the renewal of canes in the subsequent winter. The low leaf area per shoot of SBS (0.09 m^2/shoot) (Table 2) suggests that SBS are inadequate to ensure the training system renewal and that PBS and SK are the only shoot types producing adequate wood for the purpose.

Vine fruitfulness was dramatically affected by the loss of PBS due to spring frost. SBS and SK had a significantly lower fruitfulness than PBS (0.40 and 0.84 inflorescences per shoot, respectively, compared with 1.44 found in PBS), resulting in an average fruitfulness of 0.85 inflorescences/shoot, much lower than the 1.76 inflorescences/shoot recorded in 2020 (Table 2). This agrees with the observations of Friend et al. [23], who found an average of 0.5 inflorescences per SBS in cv. Chardonnay. Conversely, Frioni et al. [15] reported that in the interspecific hybrid cv. Marquette, PBS and SBS exhibited a similar shoot fruitfulness. Our data confirm that in frost-affected *Vitis vinifera* vines, SBS fruitfulness is significantly lower than PBS fruitfulness.

SBS exhibited higher leaf A than SK (+34%) and lower leaf E than PBS (−20%), resulting in the highest leaf WUE (+13% than PBS and +22% than SK) (Table 2). Gatti et al. [31] demonstrated that canopies growing with a delay of up to 31 days, as compared with regularly growing control vines, showed higher canopy efficiency in terms of (i) shorter time needed to reach maximum net canopy photo-assimilation, (ii) higher maximum photosynthetic rates, and (iii) better canopy physiological performances from veraison to the end of the season. SBS develop only after spring freezing temperatures kill a part or the total of PBS. In our conditions, the budburst of SBS occurred about 15 days later than PBS. Therefore, also considering the lower SBS final leaf area, it can be assumed that SBS had a delayed growth and phenological pattern, as compared with PBS [15,23]. In this framework, the higher WUE of SBS can be linked to the different age of the SBS basal leaves vs. PBS and SK at the time of measurement.

3.3. Yield Components and Vine Balance. In 2021, the spring frost reduced vine yield by 60% as compared with the previous year (Table 1), and this was due to the decrease in all yield components (bunches/vine, bunch, and berry mass). A direct linear correlation was fitted between the number of PBS per vine and total vine yield (Figure 4(a)), meaning that the final yield after spring frost depends on the number of PBS killed by freezing temperatures. Conversely, no significant correlation was found between the number of SBS or SK per vine and total vine yield (Supplementary Figure 1(a)). Interestingly, spring freezing temperatures seemed to affect PBS bunch morphology. In 2021, PBS had lower bunch size, rachis length, and berry mass than that of the previous season. Although seasonal effects cannot be excluded, this seems to confirm that PBS avoiding total desiccation may be subjected to partial injuries to developing florets and other reproductive structures. Our data are in agreement with the findings of Friend et al. [23] in cv. Chardonnay and reveal that even in varieties having high PBS fruitfulness, the contribution of SBS to the final yield is low and not comparable to that exhibited, for instance, by interspecific hybrids such as cv. Marquette [15]. Then, the relatively good SBS yield reported in Cabernet Sauvignon and Grenache by Montague et al. [24] was likely to be linked to the absence of competition for SBS, since all PBS and other shoots were artificially removed in their experiment. Notably, in our

Table 2: Shoot fruitfulness, leaf area and gas exchange, bunch characteristics, and grape composition, according to different shoot types, developed in response to a spring frost event that occurred in April 2021 in a Barbera vineyard.

	2021—based on shoot types				Vine average		
	PBS	SBS	SK	*Sig*	2020[$]	2021[$]	*Sig*
Shoot fruitfulness (inflorescences/shoot)	1.4 a[¶]	0.4 c	0.8 b	***	1.8	0.8	***
Leaf area (m^2/shoot)	0.38 a	0.09 b	0.28 a	***	0.29	0.23	Ns
Leaf A ($\mu mol\ m^{-2} \cdot s^{-1}$)	12.15 a	11.26 a	7.43 b	***	9.87	10.04	Ns
Leaf E ($mmol\ m^{-2} \cdot s^{-1}$)	3.83 a	3.06 b	2.55 b	***	4.46	3.07	***
Leaf g_s ($mol\ m^{-2} \cdot s^{-1}$)	0.257 a	0.237 b	0.223 c	***	0.138	0.238	***
Leaf WUE ($\mu mol\ m^{-2} \cdot s^{-1}/mmol\ m^{-2} \cdot s^{-1}$)	3.24 b	3.66 a	2.93 c	***	2.20	3.25	***
Bunch mass (g)	178 a	121 b	208 a	***	228	166	***
Rachis length (cm)	13.63 a	11.00 b	10.04 c	***	16.75	11.39	***
Bunch compactness (g/cm)	13.06 b	11.00 c	20.71 a	***	13.6	14.57	ns
Berry mass (g)	1.43 b	1.31 b	1.74 a	***	2.34	1.54	***
TSS (°Brix)	28.4	26.9	28.1	ns	23.3	27.8	***
TA (g/L)	10.05	9.72	10.61	ns	9.37	10.12	ns
TSS/TA	2.82	2.77	2.65	ns	2.49	2.75	***
pH	3.20	3.19	3.13	ns	3.05	3.17	ns
Malate (g/L)	3.83 a	3.53 ab	3.37 b	*	2.82	3.58	**
Tartrate (g/L)	10.50 a	10.79 a	9.58 b	**	10.89	10.34	ns
Total anthocyanins (mg/g)	1.67	1.75	1.66	ns	1.02	1.72	***
Total phenolics (mg/g)	2.86	3.00	2.87	ns	1.91	2.92	***

PBS = primary bud shoots; SBS = secondary bud shoots; SK = suckers. The symbols *, **, and *** denote significant difference per $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ns = not significant difference. [$]In 2020, canopies were composed by PBS only. 2021 data were calculated according to the contribution of the three different shoot types (weighed average). Means separated by Student's *t*-test. [¶]Different letters within rows indicate significant difference per $p < 0.05$ (Student–Newman–Keuls test, $n = 12$).

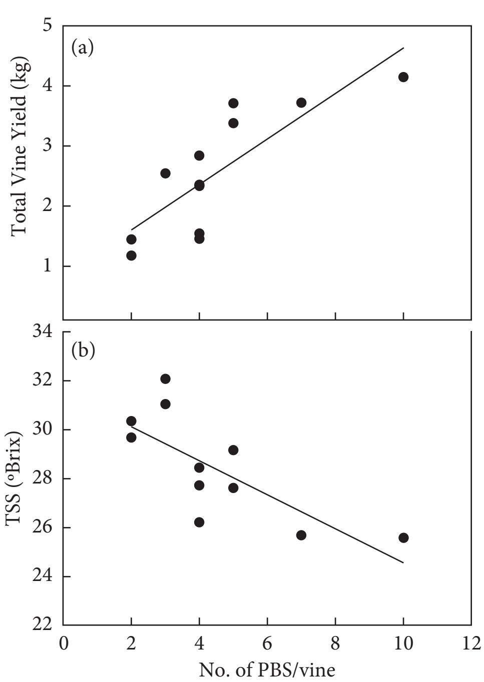

Figure 4: (a) Correlation fitted between the number of primary bud shoots (PBS) per vine (●) and total vine yield ($y = 0.38x + 0.85$, $R^2 = 0.66$, $p < 0.05$, $n = 12$). (b) Correlation fitted between the number of PBS per vine and PBS grape total soluble solids (TSS) concentration ($y = -0.69x + 31.49$, $R^2 = 0.55$, $p < 0.05$, $n = 12$).

conditions, SBS produced smaller (−28%) and looser bunches (−27%), compared with PBS and SK (Table 2, Figure 5). Overall, our data suggest that in cane-pruned vines, postfrost canopy management could be based on survived PBS and developing SK. These were indeed the only shoot types ensuring both some fruits and training system renewal, in our conditions.

Interestingly, the LA/Y ratio changed significantly between the two seasons, passing from 0.70 m^2/kg in the absence of frost damage to 1.45 m^2/kg in 2021. Therefore, in the absence of spring frosts, the vineyard was settling slightly below the adequate threshold of vine balance proposed by Kliewer and Dokoozlian [32] (corresponding to 0.8–1.2 m^2/kg), while following spring frost events, LA/Y values were well above the maximum threshold proposed. This was something that might be expected in the presence of a considerable reduction in yield with less than proportional changes in the total vine leaf area.

3.4. Grape Composition at Harvest. At harvest, no differences were found in grape TSS, pH, and TA between the different types of shoots in 2021 (Table 2). On the other hand, fruit composition was considerably different from the previous season (2020), when TSS was about 23°Brix (Table 2). In 2021, after the spring frost, the average TSS concentration rose to 27.8°Brix, far above the optimal threshold for any type of red wine. Early harvest to lower the TSS concentration was not an option, as the TA would have been >11 g/L. Similar variations as compared with the previous season were found in total anthocyanins and phenolics concentration (Table 2). The correlation depicted in Figure 4(b) shows that such an increase in average TSS can be linked to the decrease in the number of PBS composing the canopy after spring frost damage. While an inverse linear correlation was fitted between the number of PBS and TSS (Figure 4(b)), no relationship existed between the number of SBS or SK

(a) (b) (c)

Figure 5: Bunches of *Vitis vinifera* cv. Barbera bore on shoots from primary buds (a), latent buds (suckers) (b), and secondary buds (c). Pictures were taken at the end of the harvest season in 2021.

and TSS of grapes on those shoot types (Supplementary Figure 1(b)).

While no difference between different shoot types was found in terms of TA, SBS had lower malate (−0.46 g/L) and tartrate than PBS (−0.92 g/L). TSS/TA was significantly different between the two seasons. In this regard, the smaller berry size in 2021 hints that some of the berries could have undergone moderate dehydration at the time of harvest, leading to a further concentration of solutes, promoting the rise of TSS, and slowing down the decrease of acidity. This made it even more difficult to achieve an optimal TSS/TA ratio and the best organic acids composition during ripening.

Such a dramatic increase in grape TSS after spring frost has never been reported in the literature before. In Michigan, after spring freezing temperatures of 2012, grape TSS concentration at harvest was lower than in the subsequent season, when no frost occurred [15]. In addition, in that case, the high fruitfulness of cv. Marquette SBS avoided significant yield losses and LA/Y changes. Thus, the reasons for the increase in TSS observed in our work could be linked to the drastic changes induced by vine balance. In support of this hypothesis, Centinari et al. [19] reported that in Pennsylvania, in 2015, freezing temperatures caused a significant crop loss in cvs. Noirette, Lemberger, Riesling, and Traminette, with a drastic reduction in the Ravaz Index and an increase in TSS concentration by 1.4 to 3.0°Brix, as compared with the previous season. However, Pennsylvania is defined as a cool climate for viticulture, and under such environmental conditions, the increase in sugars could be something desirable [19, 33]. In our experiment, LA/Y increased by 107%. In addition, Table 2 shows that, in 2021, single leaves were more efficient than in the previous season, in terms of leaf photosynthetic rates and water use efficiency. Therefore, higher LA/Y and higher physiological efficiency in the hot climate where we operated were likely factors leading to the excessive grape sugars load found regardless of shoot type (+4.5°Brix as an average).

In the interspecific hybrid cv. Marquette, SBS exhibited a delayed pattern of TSS and anthocyanins accumulation, as well as of TA decrease [15], as compared with PBS. Similarly, in Cabernet Sauvignon and Grenache, SBS had lower sugars than PBS [24]. In our conditions, no difference in average TSS at harvest was found between shoot types. However, Figure 6 shows that a direct correlation between TSS or total anthocyanins and LA/Y existed in PBS and SK (according to a linear and a quadratic model, respectively), whereas in SBS, the TSS and anthocyanin concentration did not directly respond to vine balance (Supplementary Figure 2). In fact, at LA/Y > 1.2 m^2/kg, some vines showed very high TSS concentration on PBS and SK (between 26 and 33°Brix) and an SBS TSS concentration lower than 26°Brix. The same can be said for total anthocyanins. These outcomes can be linked to the low average leaf area of SBS, as compared with PBS and SK. In fact, ripening bunches can be fed by shoots other than the ones where they originated, but if the proximal photosynthetic leaf area (i.e., leaf area on the same shoot of the inflorescence) is low, sugars and other metabolite accumulation rates can be reduced [34]. Moreover, since SBS bunches had a smaller size and an average number of berries, as compared with PBS and SK, it is possible that their sink strength was lower [35]. Furthermore, our data cannot totally exclude an eventual postponement of SBS fruit development and ripening, as observed in other works [15,24]. However, if this was the case, in our work, any ripening delay was offset at harvest. Probably, the large availability of total photosynthetic area per unit of the crop (Table 1) was the reason for such an occurrence. Finally, our experiment shows that, after spring frost injuries, yield (and consequently LA/Y) and TSS are directly correlated to the number

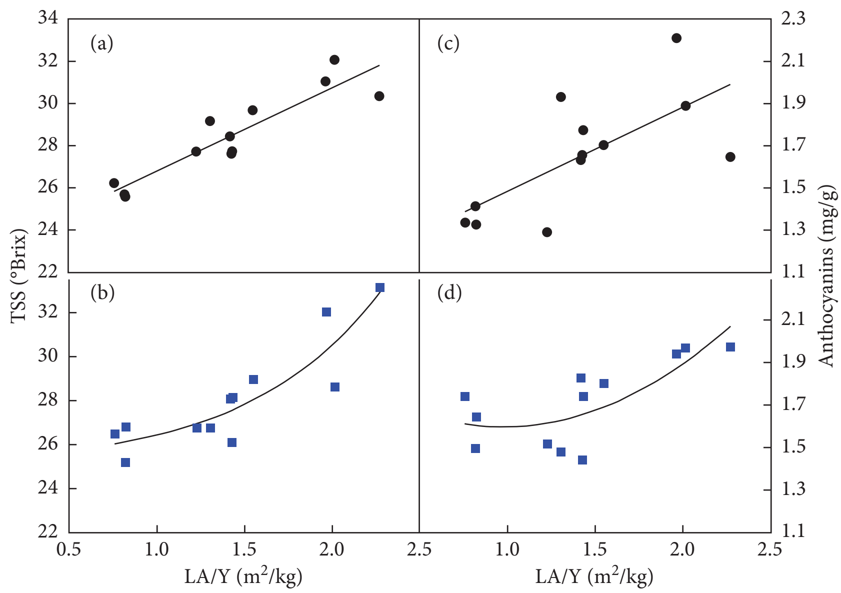

Figure 6: Correlations fitted between vine leaf area to yield ratio (LA/Y) and grape total soluble solids (TSS) in primary bud shoots (PBS, ●) ($y = 3.95x + 22.83$; $R^2 = 0.84$, $p < 0.05$) (a) and suckers (SK, ■) ($y = 2.75x^2 - 3.89x + 27.5$; $R^2 = 0.79$, $p < 0.05$) (b) of cv. Barbera grapevines affected by a severe spring frost event in April 2021 ($n = 12$). Correlations fitted between vine LA/Y and grape total anthocyanins in PBS ($y = 0.40x + 1.38$; $R^2 = 0.48$, $p < 0.05$) (c) and SK ($y = 0.28x^2 - 0.55x + 1.87$; $R^2 = 0.58$, $p < 0.05$) (d) ($n = 12$).

of survived PBS (Figure 4). This confirms that even in cultivars with high bud fruitfulness like Barbera, the magnitude of spring frost-related damages is related to the amount of PBS that avoid fatal injuries. If the number of killed PBS is limited, vine yield is preserved, LA/Y is not affected, and grape sugar concentration settles around varietal standards. Conversely, if most of the PBS are killed, SBS and SK cannot restore vine yield, then LA/Y increases to excessive values and grapes sugar concentration, and TSS/TA balance is consequently affected independently by the shoot type.

4. Conclusions

Post-budburst freezing temperatures significantly reduce yield, even in cultivars displaying high PBS fruitfulness, since SBS and SK cannot replace yield lost from the killed PBS due to their low fruitfulness. Grapes ripening is significantly altered by the increased LA/Y, with a dramatic increase in TSS that occurs well before adequate TA thresholds are achieved. In a such situation, no ripening delay can be found at harvest in SBS. While for some specific cultivars and conditions, this may represent an acceptable compromise, in such a frost damage scenario, a grower could also be encouraged to forego the current season crop and focus on training system renewal. Data support the hypothesis that this decision can also be taken early in the season, according to the number of surviving PBS. After spring frost, the amount of PBS present in the canopy at harvest is indeed directly correlated to yield, LA/Y changes, and, consequently, grape sugar loading. If the current season crop is pursued, then an altered fruit composition should be expected and harvest time should be carefully planned based on periodical monitoring of ripening kinetics. Further studies are needed to investigate the best postfrost canopy management strategies.

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

Supplementary Figure 1. No significant correlation was found between the number of secondary bud shoots (SBS,) or suckers (SK,) per vine vs. total vine yield (a) or grape TSS (b). *Supplementary Figure 2.* No significant correlation was found between vine LA/Y and secondary bud shoots (SBS,) grape TSS (a) or total anthocyanins (b). (*Supplementary Materials*)

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