



Communication

Could 101-14 Mgt Rootstock Affect Post-Spring Frost Vine Developing? Preliminary Findings

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Abstract: (1) Background: Spring frost damage is a common phenomenon that occurs in Southern Chile that considerably affects vine productivity and grape quality. (2) Methods: A field trial was conducted in order to study vine phenology and berry physicochemical parameters in Chardonnay, Sauvignon Blanc and Pinot Noir ungrafted and grafted (onto 101-14 Mgt rootstock) grapevines after a spring frost. This event killed the totality of primary bud shoots when the vines reached the phenological stage of unfolded leaves. (3) Results: From budburst, to flowering of secondary bud shoots, ungrafted Sauvignon Blanc grapevines presented an advanced phenology, whereas 101-14 Mgt rootstock tended to advance the maturity of Pinot Noir grapevines from flowering to ripening of berries. At harvest, berries from secondary buds of vines grafted onto 101-14 Mgt rootstock showed higher soluble solids than the ones from ungrafted Chardonnay and Sauvignon Blanc vines. High total phenolic content was found in berries from secondary buds of the grafted vines, compared to the ones from the ungrafted vines. Berry soluble solids variability tended to statistically decrease toward harvest in the studied plant materials, and the maximum coefficient of variation for soluble solids, berry weight, berry firmness and berry size reached 9.5%, 25.9%, 18.6% and 8.9%, respectively. (4) Conclusions: These preliminary results may be interesting for the Southern Chilean viticulturists since it seems that 101-14 Mgt rootstock could affect phenology and grape berry maturity of grapevines established in the Cautín Valley after spring frost damage.

Keywords: cold; grape; spring frost; viticulture; vine

1. Introduction

Chile's fruit production has shown a strong increase in recent decades, maintaining its position as one of the main exporter countries [1]. Grapes are an important export fruit in Chile, representing 18.8% of agricultural and livestock exports and 5.1% of non-copper exports. Climate change has affected not only vine productivity but also grapevine variety distribution in different wine-growing countries [2–6]. Some authors have suggested that

climate change will negatively affect vine cultivation in Mediterranean zones, while in the viticultural regions located toward the poles, it could benefit wine quality and also open new areas for viticulture [3,7].

Climate change has affected the distribution of varieties in all wine regions of the world, including Southern Chile, where the area of vineyards in the La Araucanía Region has increased from 10 to 105 ha since 2003 [8]. This tendency is in contrast to what occurred in the rest of the national viticultural zone, where vineyard surface area is decreasing [9]. Recent studies showed that agricultural land and meadows have decreased by 18 and 26%, respectively, between 1997 and 2013 in the La Araucanía region, mainly due to the expansion of forest plantations and urban areas [10]. In addition, the fruit crop area increased by 645% between the years 2000 and 2019 and will continue increasing to 2033 [10]. The authors confirmed that these changes have modified the relative weights of economic activities within the region, changing from cattle raising and marginal agriculture to more profitable activities such as fruit crops and forest plantations [10]. Despite this, the increase in the fruit crop surface area in Southern Chile depends considerably on the correct selection of plant species and planting site, and the adoption of frost control systems.

The viticulture in the La Araucanía Region is mostly located in the Malleco Valley (37–39° South Latitude), presenting a Mediterranean climate with hot and dry summers, and rainfall strongly concentrated in 4 months during the winter season [11]. The yield data registered by the viticulturists of this region have been reported to be considerably lower than those that occurred in the vineyards located in the central valleys of Chile [12], reaching close to 4 ton ha⁻¹. These low-yield phenomena may have different origins that address the specific edaphotopoclimatic conditions of the valley, scarce availability of specialists in the zone, low adoption of technologies for viticultural management, social and ethnic conflicts and the scarce availability of findings for scientific development [8,11,13]. In this fashion, the climate could be the most important factor that affects yield in this region, mostly due to the low temperatures and cloudiness during bloom, as well as the events of spring and summer rains, and the high incidence of spring and summer frost events [12].

Spring frost events increase the risk of long-term vine damage, compromising the industry's sustainability, mostly in viticultural regions that are planted using early budburst grapevine varieties [14]. Southern Chilean viticulture is based on the production of a limited number of grapevine varieties of early budburst time, such as Chardonnay, Sauvignon Blanc, Riesling, Gewürztraminer and Pinot Noir [11], since these varieties have low requirement of heat units to ripen grapes. The compound bud of the vine holds the current yield, and it is composed of different buds containing a primary and secondary organ, present with different levels of differentiation and productivity [14]. Spring frost could also affect secondary bud shoots and inflorescence formation [15], compromising the next season's yield.

An altered grape composition at harvest should be expected when spring frosts kill a significant number of primary shoots, due to their effects on vine balance [15]. Berry composition at harvest strongly influences the wine quality [16]. Ref. [2] Berry variability has usually been described as a negative parameter by winemakers, affecting fruit composition and wine quality. Moreover, this is a widespread phenomenon that is commonly accepted, and its evaluation is even omitted in several research trials published in the scientific literature. Berry heterogeneity within the bunch is a largely documented factor but there are few studies that have tried to understand and solve this phenomenon [17].

Several reports have demonstrated that rootstock can mitigate environmental stresses due to its tolerance against salinity, drought and cold [18,19]. Despite the aforementioned advantages with respect to the use of rootstocks, their adoption in Chilean viticulture is small, mainly due to the fact that phylloxera has not yet been detected in Chile. Generally, it is often claimed that geographical barriers such as the Atacama Desert and the Pacific Ocean act as unofficial border guards, and this is coupled with a two-year quarantine for imported plant material [20,21]. However, a large part of Chilean vineyards suffers from

root-feeding nematodes, which negatively affect vegetative growth, decreasing productivity and shortening the life expectancy of the vineyards [20]. The 101-14 Mgt rootstock is usually selected in the vineyard establishment under cold-climate sites in Chile to advance maturity [22]. However, to our knowledge, scarce scientific references can be found regarding the study of 101-14 Mgt rootstock's effects on scions after spring frost damage.

Therefore, the aim of this work was to study the effects of 101-14 Mgt rootstock on vine phenology and berry physicochemical parameters of Chardonnay, Sauvignon Blanc and Pinot Noir ungrafted and grafted grapevines after a spring frost event.

2. Materials and Methods

2.1. Study Sites, Plant Material and Experimental Design

The field study was carried out during the 2022–2023 season in an experimental vineyard belonging to the Universidad de La Frontera in Maquehue, Cautín Valley, La Araucanía Region. The vineyard of this trial was planted in 2016 using a vine spacing of 1.5×2.5 m, which corresponds to a planting density of 2.666 plants ha^{-1} . The vines were trained in a vertical shoot positioning system (VSP) and pruned in a cane system, leaving approximately 8 buds per linear meter of wire. The vines' rows were oriented north to south.

At planting, the grapevines were displayed in a randomized block design, accounting for different treatments, where three of them corresponded to Pinot Noir, Chardonnay and Sauvignon Blanc grapevines grafted onto the 101-14 Mgt rootstock, and the other three corresponded to the same ungrafted vines. Thus, six treatments were displayed randomized in the experimental vineyard at planting moment. Four replications were planted along the experimental vineyard, accounting for a total of 20 vines per treatment (5 vines per replicate), randomly distributed in plots of 5 vines.

To ensure the vine's water needs, the vineyard was equipped with a drip irrigation system using two lines with 2 L h^{-1} drippers separated by 50 cm. The vines were irrigated when the leaf water potential reached between 1.0 and 1.2 MPa. Since there is a high risk of frost damage in the vineyard, an automatic frost control system by sprinkler irrigation was installed at the moment of planting, using 32 L h^{-1} drippers covering 3.5 m per wetting side.

To characterize the vineyard climatic conditions in terms of temperature and precipitation during the season, an automatic weather station (AWS) located 100 m from the experimental vineyard was utilized. The vineyard location reached an average annual precipitation of 1126 mm which was accumulated mainly in the rainy season (autumn and winter). The average, maximum and minimum temperatures reached during the growing season (from October to April) are presented in Table 1. During this period, the amount of precipitation accumulated was around 281 mm. The highest amounts of water naturally fell in the vineyard in the months of October, March and April, respectively (Table 1). The Growing Degree Days (GDD) were calculated based on the daily data obtained from a meteorological station located in the vineyard (Table 1). The accumulated GDD during the growing season was 967.2 heat units (>10 °C), and the highest accumulation was recorded in January (Table 1).

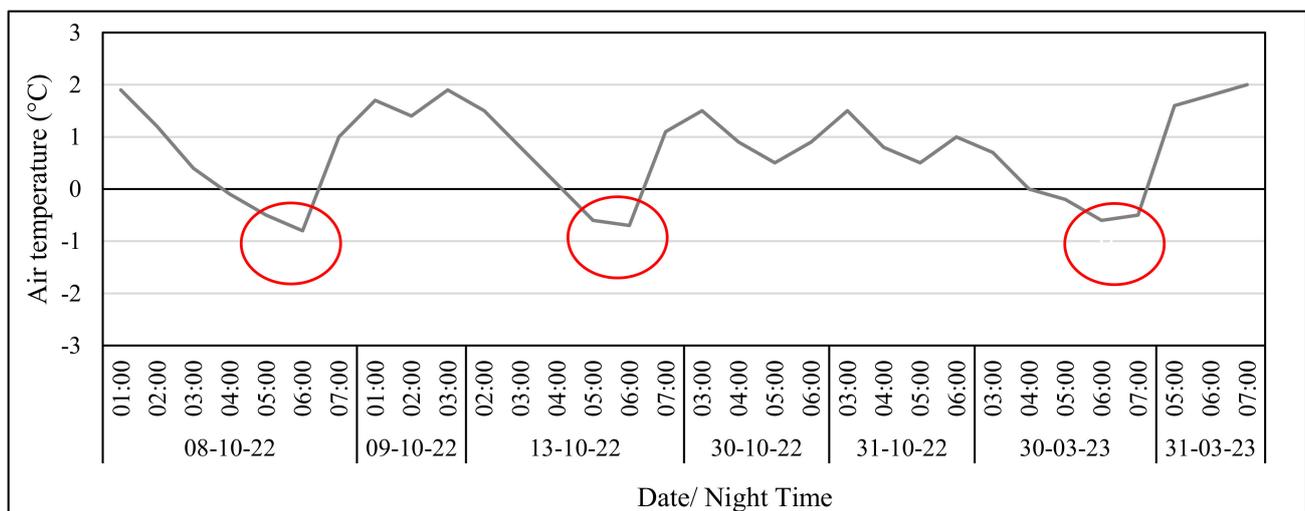
Figure 1 presents the air temperature and frost events registered throughout the growing season. The lowest temperatures were reached on 8 and 13 October, with 0.8 and 0.7 °C, respectively.

The vineyard soil is Andisol and it was formed from volcanic ash. The soil texture is loam, presenting 39.6, 39.8 and 20.6% of sand, silt and clay, respectively. Soil bulk density is 0.87 g cc^{-1} with a moisture retention of 34.43 at 15 atm. The organic matter of the soil was 8% and pH in water was 6.15. Soil reached 18 mg kg^{-1} of N, 6 mg kg^{-1} of P and 78 mg kg^{-1} of K. Aluminum saturation reached 0.24% and the cation exchange capacity was $8.44 \text{ cmol}^+ \text{ kg}^{-1}$.

Table 1. Meteorological and bioclimatic data accounting for temperatures and precipitations during the growing season (from October to April) in Maquehue, La Araucanía Region in the 2022–23 season.

	2022–2023
Growing season temperature	
Minimum average (°C)	8.1
Mean average (°C)	14.8
Maximum average (°C)	21.5
Mean temperature of the warmest month (°C)	17
Maximum temperature of the warmest month (°C)	25.6
Growing degree days accumulation (heat units)	967.2
Growing season precipitation	
October (mm)	116.5
November (mm)	38.2
December (mm)	14
January (mm)	5.7
February (mm)	2.2
March (mm)	56.4
April (mm)	48
Total accumulated precipitation (mm)	281

The warmest month corresponds to February in Maquehue, La Araucanía Region.

**Figure 1.** Records of frost events occurring during the 2022–2023 season in Maquehue, La Araucanía Region, Chile. Red circles correspond to air temperatures below 0 °C.

Furthermore, between the phenological stages of budburst and flowering (October 30), a frost event occurred, killing all primary bud shoots (BBCH scale 11) (Figure 2). The death of the primary bud shoots was established by visual determination, quantifying the damage at the vineyard level. The rest of the subsequent days with frost temperatures were controlled using the frost control system. The estimated production of the Pinot Noir, Chardonnay and Sauvignon Blanc grapevines ungrafted and grafted onto the 101-14 Mgt rootstock was calculated by weighing the harvest bunches of thirty vines. Based on this, the estimated production was 2026, 1751 and 1893 kg ha⁻¹; and 1733, 1893 and 1895 kg ha⁻¹ in Pinot Noir, Chardonnay, and Sauvignon Blanc grapevines ungrafted and grafted onto the 101-14 Mgt rootstock, respectively.

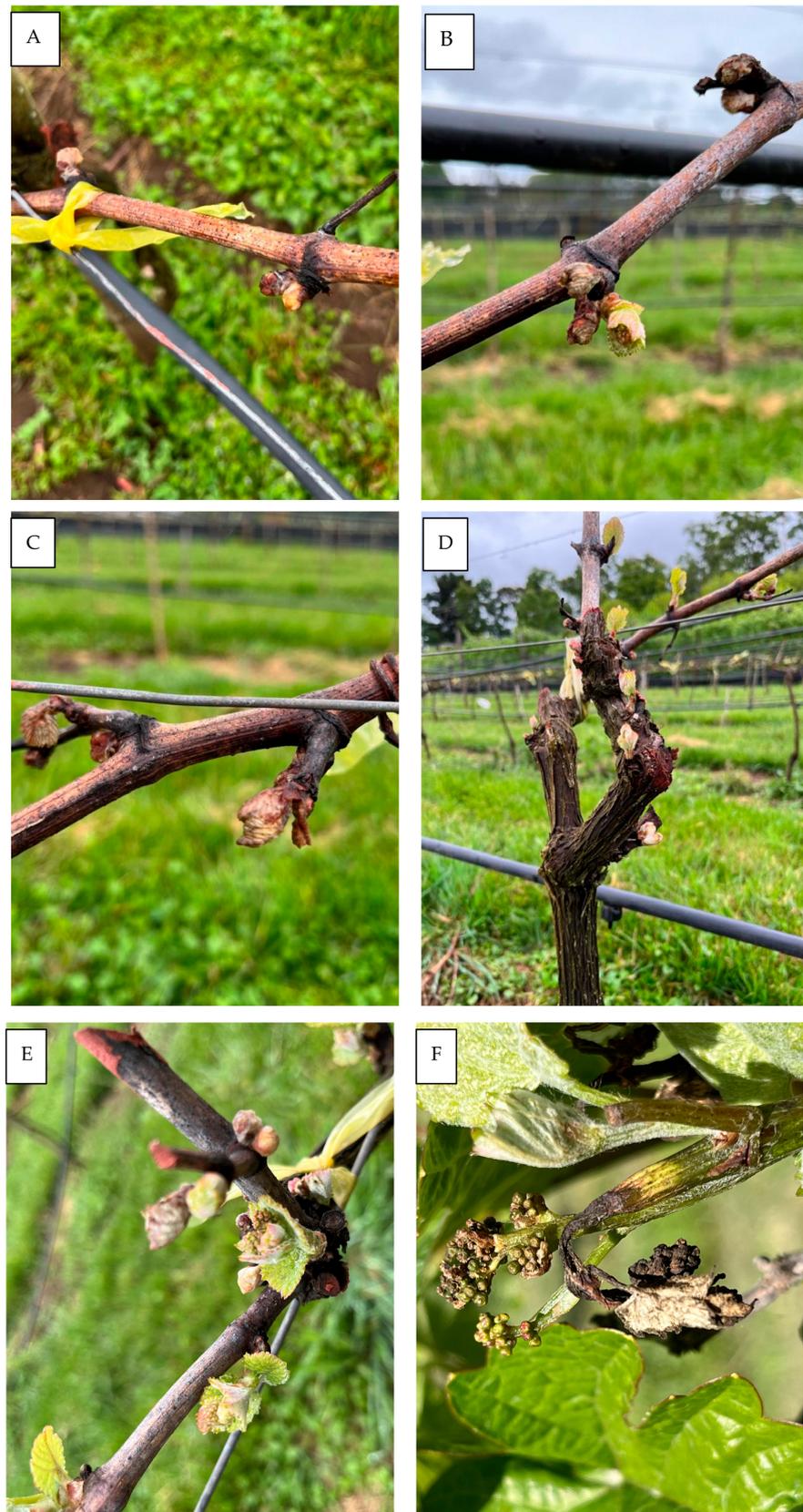


Figure 2. Frost damage occurred in the experimental vineyard at shoot level in the phenological stage of budburst (05 BBCH scale) (A,B). (C,D) correspond to the damage of the vineyard at the phenological stage of the first unfolded leaf (11 BBCH scale) and (E,F) represent the damage of the frost event on October 30.

2.2. Determination of Phenological Stages

Phenological stages of vines were defined according to the BBCH general scale. The record of each phenological stage was made in five vines per replicate for each treatment. Phenological stage was determined by visual estimation when more than 50% of the buds reached the corresponding stage in a selected branch. The vine bud development was checked twice a week from the beginning of September 2022.

2.3. Determination of Physicochemical Parameters of Berries Throughout Ripening and Harvest

The soluble solids, berry size, firmness and weight of berries were determined at the 81, 83, 85 and 89 stages of the BBCH scale to evaluate the development and variability of these parameters throughout ripening. In this way, the beginning of ripening corresponds to the 81 stage; berries developing color corresponds to the 83 stage; softening of berries corresponds to the 85 stage; and berries ripe for harvest corresponds to 89 BBCH. Due to climate conditions (Table 1), harvest was performed when the vine phenological stage reached the 89 BBCH stage and considering a soluble solids content between 18 and 20 °Brix. Subsequently, the individual vines were harvested, and the yield per vine by each replication was registered.

Berry sampling for the aforementioned determinations was performed, obtaining four replicates of 50 berries by repetition and treatment; thus, a total of 200 berries per repetition were analyzed at each of the studied ripening stages. The sampling method consisted of the random collection of the grape berries from different parts of the cluster, and the determinations were carried out in the individual berry samples. Based on this, by each stage of sampling, a total of 1200 berries were individually analyzed by treatment and phenological stage. Subsequent to sampling, the picked berries were transferred to a laboratory of La Frontera University to evaluate the individual grape berries.

Soluble solids (°Brix) were determined using a digital refractometer (ATAGO, Saitama, Japan), whereas the berry weight was analyzed using an analytical balance (Cubis Precision Balance, Sartorius, Göttingen, Germany). The pH was determined using a digital pH meter at harvest as the aforementioned determinations, and total acidity was determined by the volumetric titration method with sodium hydroxide (0.1 N) using an automatic titrator (HANNA Mod. HI-84532, Woonsocket, RI, USA). The fruit firmness and berry size were determined individually on 50 fruits by a texture meter (FirmPro, Happyvolt, Santiago, Chile).

2.4. Analysis of Low Molecular Weight Organic Acid Composition in Berries

At harvest, a sample of 50 berries was collected from each replicate per treatment (varieties and 101-14 Mgt rootstock or ungrafted combination). These berries were frozen and freeze-dried to determine the low molecular weight of organic acids compositions in berries, according to Medeiros et al. [23]. Briefly, samples were subjected to methanol extraction centrifuged at 14,000 and the supernatant was recovered, which it was dried and re-dissolved in 800 µL of deionized sterile water and filtered (0.22 µm) for HPLC injection. The chromatographic separation was analyzed by high-performance liquid chromatography (HPLC) using an HPLC system (model LC-Net II/ADC, Jasco, Tokyo, Japan) equipped with a detector diode array (DAD, model MD 2015 Plus, Jasco, Tokyo, Japan), according to Millaleo et al. [24]. Briefly, the analysis was conducted with a 250 × 4 mm reverse phase column (LiChrospher 100 RP-18, 5 mm particle size, Merck, Darmstadt, Germany). The mobile phase was H₂O–CH₃OH buffered with 200 mM orthophosphoric acid at pH 2.1 with an isocratic gradient. The flow rate was 1 mL min⁻¹, and injected samples were detected at a wavelength of 210 nm. The organic acid anions (oxalate, malate, citrate, succinate, fumarate and tartaric) were identified by comparing the retention times and adding standards for each organic acid anion.

2.5. Analysis of Total Phenols and Phenolic Composition in Berries

Total phenols were determined by the Folin–Ciocalteu method in ethanolic extracts according to [25]. Absorbance was measured at 765 nm. Chlorogenic acid was used as standard. Phenolic compounds analysis was carried out based on 12 biological repetitions or berries per treatment, which were freeze-dried. Firstly, 150 mg was extracted with 1 mL of the extraction solvent ethanol. The phenol composition of fruit extracts was analyzed by high-performance liquid chromatography (HPLC), using a HPLC system (model LC-Net II/ADC, Jasco, Tokyo, Japan) equipped with a detector diode array (DAD, model MD 2015 Plus, Jasco, Tokyo, Japan) and a reverse-phase column (RP-18, Kromasil 250 × 4.6 mm; Waters, Milford, MA, USA). Phenolic acids were analyzed according to the method proposed by Ruhland and Day [26] with a flow rate of 1.0 mL min⁻¹. The phenolic caffeic, gallic and chlorogenic acids were used as standards (Sigma Chemical Co., St. Louis, MO, USA). Signals were detected at 320 nm. Acidified water (phosphoric acid 10%) (A) and 100% acetonitrile (B) composed the mobile phase. The eluent gradient was: 0–9 min of 100% A, 9.1–19.9 min of 81% A and 19% B, and 20–25 min of 100% B [27].

2.6. Statistical Analysis

Since the objective of this study is to preliminarily evaluate the behavior of grapevines after a spring frost, the effect of the treatments (differences between the ungrafted and grafted grapevines by cultivar) on the variables analyzed was evaluated independently at harvest. Standard error was used to define the variability of each measured parameter throughout ripening in the individual treatments. Thus, the coefficient of variation was determined as the ratio of the standard deviation to the mean. The statistical analysis of physicochemical parameters and the rest of the determined variables was performed using a one-way analysis of variance (ANOVA) at a significance level of 95% (*p*-value < 0.05). If there were statistically significant differences, the means were compared using a Duncan test. The statistical software INFOSTAT (version 2017, Argentina) was used for all data analyses.

3. Results

3.1. Phenological Development of Vines

Figure 3 shows the phenological development of the ungrafted and grafted Chardonnay, Pinot Noir and Sauvignon Blanc grapevines from the “wool stage” (05 stage of BBCH scale) to berries developing color (83 stage of BBCH scale), in which nineteen different evaluations were performed after the frost event.

After the 4th and 25th of November, ungrafted Sauvignon Blanc showed a more advanced phenological development than the ungrafted and grafted Chardonnay vines. Despite these differences, the studied plant material reached a similar stage of phenology (~53 stage of BBCH scale: inflorescences clearly visible) on the 1st of December compared to the rest of the evaluated plant materials. Based on these results, from bud development to inflorescence-emerging phenological period, ungrafted Sauvignon Blanc presented a more advanced phenology than the rest of the studied grapevines.

After the 6th of December, ungrafted and grafted Pinot Noir tended to show a more advanced phenological development than the rest of the studied grapevines, which showed varied behavior in terms of their phenological development. In addition, Pinot Noir grafted onto 101-14 Mgt reached the 77, 81 and 83 BBCH scales (stage 77: berries beginning to touch; stage 81: beginning of ripening; berries begin to develop variety-specific color; and stage 83: berries developing color) first, compared to the rest of plant materials evaluated.

Based on these results, 101-14 Mgt rootstock may statistically advance the maturity of Pinot Noir grapevines from flowering to berry ripening.

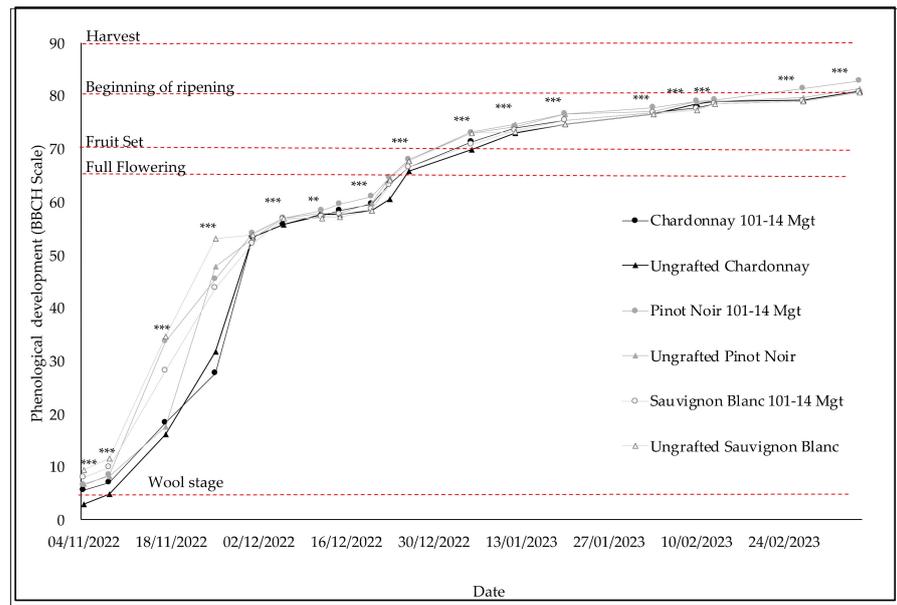


Figure 3. Phenological development of grapevines after frost from “wool stage” (05 BBCH scale) to the beginning of ripening (81 BBCH scale). ** Indicated statistical differences ($p < 0.01$). *** Indicated statistical differences ($p < 0.001$).

3.2. Berry Parameters Evolution Throughout Ripening

The measurement of soluble solids ($^{\circ}$ Brix), weight (g), firmness (g mm^{-1}) and size (mm) in berry samples at the 81, 83, 85 and 89 stages of the BBCH scale in ungrafted and grafted Chardonnay, Pinot Noir and Sauvignon Blanc grapevines, post-development of the secondary buds after a spring frost damage, are shown in Figure 4.

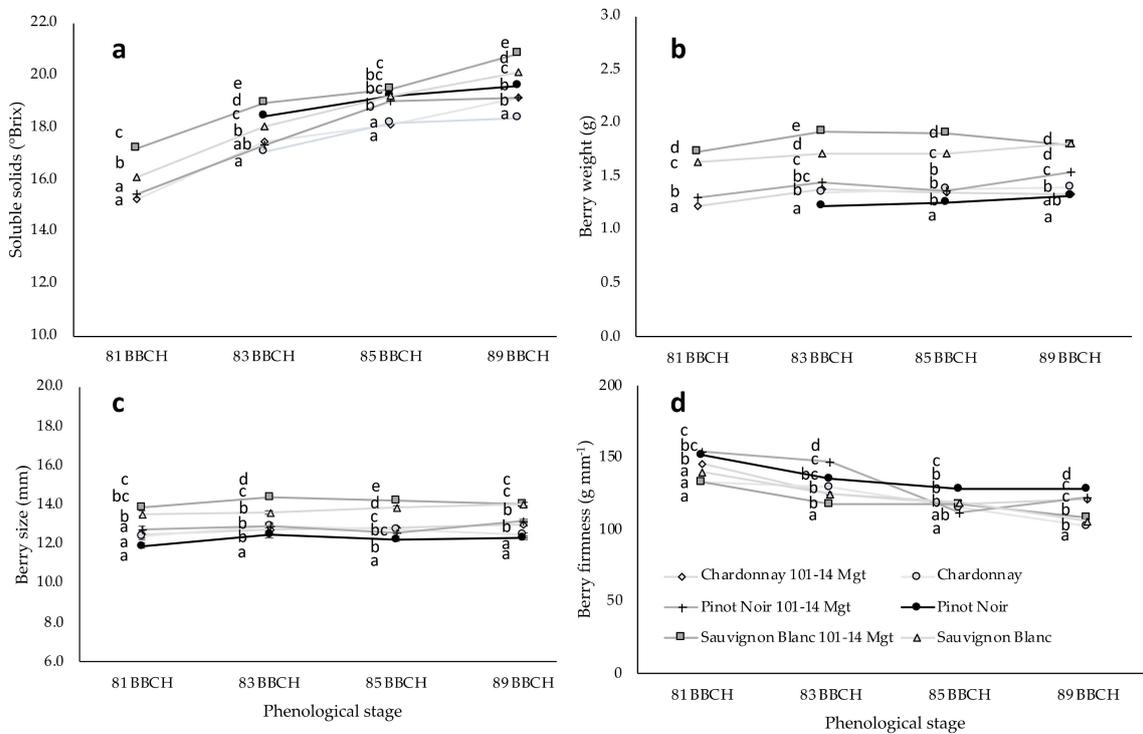


Figure 4. Soluble solids (a), berry weight (b), berry size (c) and berry firmness (d) in grafted onto 101-14 Mgt and ungrafted Chardonnay, Pinot Noir and Sauvignon Blanc vines; measured in the 81, 83, 85 and 89 stages of the BBCH phenological scale. Different letters within the same row indicate significant differences ($p < 0.05$).

Berries from Sauvignon Blanc grapevines grafted onto 101-14 Mgt presented the highest soluble solids at 81 (beginning of ripening: berries begin to develop variety-specific color), 83 (berries developing color) and 89 (berries ripe for harvest) stages as well as the highest weight and size at the 81, 83 and 85 (softening of berries) stages. Sauvignon Blanc berries from grafted grapevines showed the lowest firmness at the 83 stage. Berries from ungrafted Pinot Noir grapevines showed the highest firmness at the 85 and 89 stages; the lowest size at most of the measured phenological stages; and the lowest weight at 83 and 85 stages. Berries from Pinot Noir grapevines grafted onto 101-14 Mgt presented the highest firmness at the beginning of the vine ripening at 83 stage, whereas berries from ungrafted Chardonnay grapevines showed the lowest firmness and soluble solids at berries ripe to ripening in the 89 stage.

Soluble solids concentration increased from the beginning of ripening to berries ripe for harvest by approximately 20 to 25%. At harvest, the grafted and ungrafted Sauvignon Blanc grapevines presented higher concentrations of soluble solids than the rest of the evaluated plant materials (20.60 °Brix and 19.97 °Brix, respectively). In contrast, grafted and ungrafted Pinot Noir and Chardonnay grapevines presented lower soluble solids values than Sauvignon Blanc grapevines (19.13 and 19.55, and 19.11 and 18.34 °Brix, respectively). Berry weight and berry size did not statistically vary throughout the berry ripening. At harvest, the grafted and ungrafted Sauvignon Blanc grapevines presented higher berry weights than the rest of the evaluated plant materials (1.78 and 1.81 g, respectively). In contrast, grafted and ungrafted Pinot Noir and Chardonnay grapevines presented lower berry weights than Sauvignon Blanc grapevines (1.53 and 1.32, and 1.33 and 1.40 g, respectively).

At harvest, the grafted and ungrafted Sauvignon Blanc grapevines presented higher berry sizes than the rest of the evaluated plant materials (14.01 and 14.04 mm, respectively). In contrast, grafted and ungrafted Pinot Noir and Chardonnay grapevines presented lower berry sizes than Sauvignon Blanc grapevines (13.16 and 12.30, and 12.46 and 13.01 mm, respectively). Berry firmness decreased across berry ripening in all the studied plant materials. At harvest, the ungrafted and grafted Pinot Noir as well as grafted Chardonnay presented higher berry firmnesses than ungrafted and grafted Sauvignon Blanc as well as ungrafted Chardonnay (128.94, 122.76 and 121.31; and 106.66, 108.40 and 102.89 g mm⁻¹, respectively).

3.3. Variability in Berry Weight, Soluble Solids, Firmness and Size

The coefficients of variation (CVs) in the analysis of soluble solids, weight, firmness and size of berries from grafted onto 101-14 Mgt and ungrafted Chardonnay, Pinot Noir and Sauvignon Blanc vines; measured at 81, 83, 85 and 89 stages of the BBCH phenological scale, post-development of the secondary buds after a spring frost damage; are shown in Table 2.

The CV of soluble solids statistically decreased in most of the berries of the evaluated plant material, with the exception of ungrafted Pinot Noir as the vine phenology advanced from 81 to 89 stages. In this latter phenological stage, the berries from grafted Chardonnay showed a higher CV of soluble solids than the grafted Pinot Noir vines.

The CV of berry weight showed scarce differences in the samples as the vine phenology advanced from 81 to 89 stages. The CV of berry weight decreased only in the ungrafted Pinot Noir from 85 to 89 stages (26.38 to 19.32%, respectively). In contrast, the CV of berry weight increased from 81 to 89 BBCH (18.55 to 25.92%, respectively) in grafted Sauvignon Blanc. At 89 BBCH, grafted Sauvignon Blanc berries presented a higher CV of berry weight than the rest of the ungrafted grapevine varieties.

The CV of berry firmness showed a statistical decrease in most of the evaluated plant materials, except for grafted Sauvignon Blanc grapevines, with a different behavior. The CV of berry firmness decreased from 81 to 89 BBCH in grafted Chardonnay (19.13 to 11.6%, respectively) and ungrafted Pinot Noir (18.26 to 9.97%, respectively) grapevines. The CV of berry firmness decreased from 81 to 85 BBCH in ungrafted Chardonnay (21.86 to 13.43%, respectively) grapevines. The CV of berry firmness decreased from 83 to 89 BBCH in

grafted Pinot Noir (23.47 to 13.48%, respectively) and ungrafted Sauvignon Blanc (21.07 to 13.48%, respectively) grapevines. At the 89 BBCH, ungrafted Chardonnay presented the highest CV of berry weight.

Table 2. Coefficient of variation (%) of soluble solids, weight, firmness and size in grafted onto 101-14 Mgt and ungrafted Chardonnay, Pinot Noir and Sauvignon Blanc vines measured in the 81, 83, 85 and 89 stages of the BBCH phenological scale.

Determination	Plant Material	81 BBCH	83 BBCH	85 BBCH	89 BBCH
Soluble solids (°Brix)	Grafted Chardonnay	14.66 ± 2.87 b,A	15.06 ± 0.69 b,B	9.29 ± 2.16 a,BC	9.48 ± 2.23 a,B
	Ungrafted Chardonnay	NC	15.93 ± 0.90 c,B	11.90 ± 0.11 b,C	8.45 ± 0.97 a,AB
	Grafted Pinot Noir	14.52 ± 2.85 b,A	8.38 ± 2.92 a,A	6.18 ± 0.39 a,A	6.71 ± 0.41 a,A
	Ungrafted Pinot Noir	NC	8.57 ± 0.72 a,A	8.99 ± 1.03 a,AB	8.06 ± 0.82 a,AB
	Grafted Sauvignon Blanc	13.49 ± 4.29 b,A	6.55 ± 1.26 a,A	7.84 ± 1.01 a,AB	6.80 ± 1.42 a,AB
	Ungrafted Sauvignon Blanc	10.52 ± 0.84 b,A	8.91 ± 0.84 ab,A	9.25 ± 2.54 ab,BC	6.97 ± 1.75 a,AB
Berry weight (g)	Grafted Chardonnay	22.09 ± 3.87 a,AB	20.16 ± 2.81 a,AB	22.41 ± 1.33 a,BC	23.10 ± 1.50 a,BC
	Ungrafted Chardonnay	NC	24.72 ± 1.65 a,CD	23.19 ± 2.63 a,BC	20.12 ± 3.94 a,AB
	Grafted Pinot Noir	23.55 ± 3.48 a,B	21.63 ± 0.82 a,BC	24.30 ± 2.15 a,C	22.00 ± 3.05 a,BC
	Ungrafted Pinot Noir	NC	25.76 ± 4.39 ab,D	26.38 ± 3.57 b,C	19.32 ± 1.58 a,AB
	Grafted Sauvignon Blanc	18.55 ± 1.96 a,AB	17.32 ± 1.48 a,A	20.14 ± 3.09 a,AB	25.92 ± 3.58 b,C
	Ungrafted Sauvignon Blanc	16.46 ± 1.54 a,A	18.01 ± 2.52 a,AB	18.23 ± 0.66 a,A	16.55 ± 1.90 a,A
Firmness (g mm ⁻¹)	Grafted Chardonnay	19.13 ± 2.68 b,A	19.12 ± 2.86 b,AB	12.47 ± 0.53 a,A	11.66 ± 1.12 a,AB
	Ungrafted Chardonnay	21.86 ± 3.01 b,A	19.79 ± 3.99 b,AB	13.43 ± 1.09 a,A	18.55 ± 3.80 ab,C
	Grafted Pinot Noir	21.89 ± 4.51 ab,A	23.47 ± 6.11 b,B	26.10 ± 3.31 b,B	13.48 ± 2.95 a,AB
	Ungrafted Pinot Noir	18.26 ± 1.33 b,A	18.06 ± 2.57 b,AB	12.00 ± 0.63 a,A	9.97 ± 0.91 a,A
	Grafted Sauvignon Blanc	16.79 ± 2.67 a,A	14.49 ± 1.73 a,A	13.14 ± 2.38 a,A	13.83 ± 1.21 a,AB
	Ungrafted Sauvignon Blanc	18.66 ± 0.90 ab,A	21.07 ± 3.76 b,AB	14.10 ± 2.55 a,A	14.38 ± 2.49 a,B
Size (mm)	Grafted Chardonnay	7.63 ± 1.62 a,AB	6.98 ± 1.34 a,A	7.77 ± 0.80 a,BC	6.92 ± 0.79 a,AB
	Ungrafted Chardonnay	9.03 ± 0.18 c,B	8.14 ± 0.40 bc,A	7.75 ± 0.54 ab,BC	6.78 ± 0.90 a,AB
	Grafted Pinot Noir	9.29 ± 0.59 a,B	7.58 ± 0.91 a,A	9.08 ± 0.58 a,C	8.37 ± 1.46 a,BC
	Ungrafted Pinot Noir	9.71 ± 2.11 a,B	8.84 ± 2.04 a,A	19.75 ± 1.72 b,D	7.60 ± 0.63 a,ABC
	Grafted Sauvignon Blanc	6.31 ± 0.60 a,A	6.02 ± 0.42 a,A	6.78 ± 0.77 a,AB	8.94 ± 0.98 b,C
	Ungrafted Sauvignon Blanc	5.65 ± 0.85 a,A	6.55 ± 0.71 a,A	6.24 ± 0.29 a,A	6.07 ± 0.34 a,A

Different lowercase letters within a row represent significant differences among the phenological stages, and different uppercase letters within a column represent significant differences among the plant material in the same phenological stage (p -value < 0.05). NC: not calculated.

The CV of berry size showed scarce differences in the samples as the vine phenology advanced from 81 to 89 stages. The CV of berry size decreased from 81 to 89 BBCH in ungrafted Chardonnay (9.03 to 6.78%, respectively) grapevines, whereas it increased at this same phenological period in grafted Sauvignon Blanc (6.31 to 8.94%, respectively) grapevines. At 89 BBCH, ungrafted Sauvignon Blanc grapevines presented a lower CV of berry size than the grafted Pinot Noir and Sauvignon Blanc (6.07 to 8.37 and 8.94, respectively) samples.

3.4. Yield Per Vine, Berry Weight, Physicochemical Parameters, Organic Acids and Phenolic Compounds in Berries at Harvest

Yield per vine, berry weight, physicochemical parameters, organic acids and phenolic acids in samples from ungrafted and grafted Chardonnay, Pinot Noir and Sauvignon Blanc grapevines determined at harvest, post-development of the secondary buds after a spring frost damage, are shown in Table 3.

Yield per vine was statistically similar in all the studied plant materials, reaching mean values of 722.96, 600.00 and 709.33 g for ungrafted grapevines and 709.33, 691.43 and 742.86 g in the grafted grapevines. Berry weight affected physicochemical parameters in berries at harvest considerably. In this sense, berries from ungrafted Chardonnay vines showed a higher berry weight and lower soluble solids content than the ones from grafted vines, as well as similar pH and total acidity. Berries from ungrafted Pinot Noir vines

showed higher soluble solids and a lower berry weight and total acidity than the ones from grafted vines, as well as similar pH. Berries from ungrafted Sauvignon Blanc vines presented lower soluble solids than the ones from grafted vines, and a similar berry weight, pH and total acidity.

Table 3. Berry weight, physicochemical parameters, organic acids and phenolic acids in berries from ungrafted and grafted grapevines onto 101-14 Mgt of Chardonnay, Pinot Noir and Sauvignon Blanc varieties determined at harvest.

	Ungrafted Chardonnay	Chardonnay 101-14 Mgt	Ungrafted Pinot Noir	Pinot Noir 101-14 Mgt	Ungrafted Sauvignon Blanc	Sauvignon Blanc 101-14 Mgt
Yield						
Yield per vine (g)	722.96 ± 292.24 a	709.33 ± 299.14 a	600.00 ± 329.85 a	691.43 ± 301.87 a	709.33 ± 319.13 a	742.86 ± 302.37 a
Physicochemical parameters						
Berry weight (g)	1.42 ± 0.34 b	1.33 ± 0.31 a	1.32 ± 0.26 a	1.54 ± 0.34 c	1.81 ± 0.33 d	1.79 ± 0.46 d
Soluble solids (°Brix)	18.18 ± 1.94 a	19.07 ± 1.73 b	19.50 ± 1.61 c	19.12 ± 1.28 b	19.97 ± 1.80 d	20.84 ± 1.56 e
pH	2.93 ± 0.03 b	2.89 ± 0.03 ab	2.90 ± 0.10 ab	2.95 ± 0.00 b	2.84 ± 0.05 a	2.86 ± 0.03 ab
Total acidity *	10.10 ± 1.10 cd	8.52 ± 0.55 bc	5.48 ± 0.32 a	7.58 ± 0.92 b	9.36 ± 0.28 cd	10.48 ± 1.39 d
Organic acids						
Tartaric acid **	20.88 ± 1.49 ab	40.13 ± 2.41 d	18.01 ± 1.31 a	24.80 ± 1.12 b	24.45 ± 1.12 b	33.33 ± 1.86 c
Malic acid **	267.66 ± 13.23 b	683.78 ± 30.07 c	273.77 ± 16.07 b	220.67 ± 9.17 a	295.27 ± 16.49 b	281.29 ± 14.43 b
Citric acid **	3.35 ± 0.25 a	19.46 ± 0.69 d	18.24 ± 0.83 d	6.86 ± 0.61 bc	5.156 ± 0.38 ab	8.146 ± 0.53 c
Oxalic acid **	14.57 ± 1.00 a	17.99 ± 1.48 a	15.32 ± 0.52 a	16.13 ± 0.92 a	17.96 ± 0.89 a	18.7 ± 0.80 a
Succinic acid **	5.25 ± 0.41 a	9.43 ± 0.54 a	6.75 ± 0.34 a	9.34 ± 2.55 a	11.86 ± 0.56 a	6.11 ± 0.31 a
Fumaric acid **	0.12 ± 0.01 a	n.d.	0.22 ± 0.02 b	0.14 ± 0.01 a	n.d.	n.d.
Lactic acid **	0.20 ± 0.01 a	n.d.	n.d.	n.d.	n.d.	0.18 ± 0.00 a
Phenolic acids						
Gallic acid ***	77.09 ± 4.10 a	61.04 ± 1.98 a	144.80 ± 10.61 c	132.39 ± 5.46 bc	114.49 ± 9.27 b	112.82 ± 5.19 b
Chlorogenic acid ***	n.d.	n.d.	38.79 ± 2.98 a	50.41 ± 3.82 b	n.d.	n.d.
Caffeic acid ***	n.d.	16.03 ± 1.19 c	15.35 ± 0.94 c	11.14 ± 0.76 b	4.82 ± 0.37 a	n.d.
Total phenolic content ****	4.99 ± 0.14 ab	7.92 ± 0.34 d	7.07 ± 0.15 c	13.27 ± 0.34 e	4.53 ± 0.16 a	5.22 ± 0.11 b

Different lowercase letters within a row represent significant differences among the plant material (Tukey's test, $p < 0.05$). * g L^{-1} of tartaric acid, ** $\mu\text{mol g}^{-1}$ DW, *** $\mu\text{g g}^{-1}$ FW, **** mg g^{-1} FW. nd: not detected.

Malic acid was the major organic acid in terms of $\mu\text{mol g}^{-1}$ of dry weight in all varieties at harvest (Table 3). Berries from grafted Chardonnay grapevines presented the highest tartaric acid (2-fold) and malic acid content (2.6-fold), as well as high levels of citric acid (5.8-fold), compared to ungrafted Chardonnay. The ungrafted grapevines presented lower levels of tartaric acid than the grafted plant materials, by variety. Berries from ungrafted Chardonnay grapevines presented the lowest citric acid content of the plant materials and varieties. The contents of oxalic acid and succinic acid in berries were similar in the studied plant materials. Fumaric acid was not detected in berries from Sauvignon Blanc and grafted Chardonnay grapevines, and its content was higher in ungrafted Pinot Noir samples than the rest of the evaluated plant materials. Lactic acid was only detected in berries from ungrafted Chardonnay and grafted Sauvignon Blanc, and its content was statistically similar between these studied plant materials (Table 3).

With respect to the phenolic acids detected, gallic acid was the most abundant in terms of $\mu\text{g g}^{-1}$ of fresh weight. This phenolic acid was lower in the berries from Chardonnay grapevines (2-fold) than in the rest of the studied plant materials, whereas, as expected, berries from Pinot Noir grapevines showed the highest levels of gallic acid (Table 3). Chlorogenic acid was only detected in Pinot Noir samples and higher levels (1.3-fold) of this acid were showed in berries from the grafted grapevines. Berries from ungrafted Sauvignon Blanc grapevines presented the lowest caffeic acid and this acid was not detected in berries from ungrafted Chardonnay, and grafted Sauvignon Blanc.

By variety, grafted grapevines presented higher total phenolic content than ungrafted grapevines.

4. Discussion

Spring frost damage is one of the most important threats to developing the wine industry in the austral new zones [4,28]. Under these conditions, rootstocks are not only used to treat vine diseases and pests but also to provide the scion with the capacity to tolerate abiotic stress conditions, including cold tolerance [29]. Rootstocks can improve the internal and external quality of scion varieties, regulating grape maturity and increasing yield under abiotic stress conditions [19,21]. The risk of spring frost increases considerably as bud development progresses, and once buds reach the stage of “cotton buds”, they became more sensitive to freezing [30]. Spring frost damage in this trial affected the vines in a more advanced phenology than those exposed by these authors (BBCH 11: first leaf unfolded and spread away from shoot), killing 100% of primary shoots. In the Marquette variety, when the spring freezing temperatures killed over 80% of primary shoots, secondary buds restored a full canopy with a yield that was about 60% of a standard year [14]. Similar results were found in this trial in which spring freezing temperatures affected close to 56% of the yield of a standard season. Despite this, yield per vine at harvest was statistically similar in all the studied plant materials ranging from 600.00 to 742.86 g (in ungrafted Pinot Noir and Sauvignon Blanc grafted onto 101-14 Mgt), respectively. To our knowledge, there are no available studies published in relation to the effect of rootstock on vineyard productivity after a frost event affecting 100% of primary shoots.

Some rootstocks can advance or delay the grape harvest time, but their behavior on the scion is highly site-specific [19]. Scarce information about their effects on the vine's development after spring frost damage and berry maturity from the secondary bud shoots is available. Our preliminary results showed that from the budburst to flowering of the secondary buds after a spring frost, ungrafted Sauvignon Blanc presented a more advanced phenology than the rest of the studied grapevines, whereas 101-14 Mgt rootstock tended to advance the maturity of Pinot Noir grapevines from flowering to ripening of berries (Figure 3). Moreover, berries from Sauvignon Blanc and Chardonnay grapevines grafted onto 101-14 Mgt rootstock showed higher soluble solids than ungrafted grapevines at harvest (Figure 4). In this way, advanced maturation was shown in Verdejo Negro grapevines grafted onto 101-14 Mgt growing in Cangas del Narcea in Northwestern Spain [22]. Similarly, a higher maturity for berries was shown in the Frontenac variety grafted on 101-14 Mgt and 3309C than the own-rooted vines [18]. Despite this, the cited findings were obtained in trials studying shoots from primary buds. To our knowledge, there is no information about the effects of 101-14 Mgt rootstock on the development and behavior of vines after spring frost damage.

As the phenology of the grapevine advanced, the maturity variability behavior of the berries from secondary bud shoots in this trial was similar to those observed in other reports in berries from primary bud shoots [17,31,32]. The coefficient of variation (CV) indicated that berry soluble solids variability tended to statistically decrease toward harvest in most of the evaluated plant materials, and 101-14 Mgt rootstock does not seem to influence this behavior (Table 2). The results exposed in this trial only confirmed the statistical decrease in the variability of berry maturation for soluble solids and, to a lesser extent, on berry firmness and berry size but not on berry weight. To our knowledge, this is the first result that confirmed heterogeneous maturity in soluble solids in berries from secondary bud shoots. In all the measured samples, soluble solids content at harvest in the berries studied in this trial ranged from 6.3 to 26.0 °Brix. In this way, the soluble solids variation in Garnacha and Syrah berries from primary bud shoots reached between 9.0 and 11.5 °Brix, which confirms a lower heterogeneous maturity of berries in terms of sugar accumulation compared to the ones from secondary bud shoots [32]. Spring frost damage can vary from total desiccation of the shoot to partial injury, preserving the viability of some shoot parts [33]. Spring frost events killed over 80% of the shoots arising from primary buds in the Marquette variety, while secondary buds were almost unaffected [14]. Some authors have reported similar findings in different wine-growing countries. Del Zozzo et al. [15] reported that the number of primary bud shoots killed by a severe frost event was related

to the number of secondary bud shoots and suckers developed. Secondary bud shoots had low fertility after a frost event, producing smaller and looser bunches (−28% mass and −27% compactness, respectively) than the ones collected from the primary bud shoots [15]. Moreover, Frioni et al. [14] showed variability in the delay in the phenology of secondary shoot buds and suggested that sugar accumulation may be related to potential interactions between leaf age and cluster microclimate. These authors reported that secondary shoot buds had younger leaves than primary shoot buds and a potential for higher photosynthetic activity later in the season. Since each berry develops individually, different levels of soluble solids are reached in a bunch. Based on these findings, it is possible to suggest that the variability in berry maturity at harvest is higher in berries from secondary shoot buds than in the ones from primary shoot buds. On the other hand, the weight and size variability of the berries from secondary bud shoots at harvest was higher in the grafted Sauvignon Blanc than in the ungrafted grapevines, and it increased toward harvest (Table 2). In addition, there were no statistical differences in the CV of these parameters in the rest of the plant materials evaluated throughout the ripening of berries (Table 2). Contrary to these findings, berries obtained from primary bud shoots in grapevines cultivated under cold climate conditions showed a high variability in berry diameter at véraison in Cabernet Franc and Concord and subsequently, it was reduced toward harvest by as much as 11% in Cabernet Franc [34]. The differences showed in these trials could be related to fruit set dynamics that could be different in the primary and secondary bud shoots.

Berry parameter results differed between varieties, and it is possible to suggest more of an interaction between rootstock and variety than a determined effect of the rootstock itself. Berries from secondary bud shoots in grafted vines showed higher levels of tartaric acid and total phenolic content by variety compared to the ungrafted vines, despite the differences in berry weights among the samples (Table 3). Similar results, in total phenolic content, were found in berries from primary bud shoots in Merlot grapevines grafted onto 101-14 Mgt, compared to ungrafted grapevines [20]. In addition, it has been reported that 101-14 Mgt rootstock increases flavanol content in berries and in the skins and seeds of Petit Verdot, compared to other plant materials [35]. The 101-14 Mgt rootstock has become widely planted in the cold viticultural zones and is currently one of the most popular rootstocks. This plant material generally induces moderate to low vigor to the scion and is more vigorous when it is planted on fertile soils with water availability to irrigate, inducing low yield-to-pruning ratios and probably enhancing the accumulation of phenolic compounds in the grapes. In this way, it was shown that grafting not only increased the transcription levels of stilbene, anthocyanin, proanthocyanidins and flavonol synthesis genes but also affected the expression of numerous transcription factors, suggesting that grafting can promote phenolic compound accumulation in grape berry skin during development [36]. In this way, future studies should consider different analyses related to gene transcription to deepen the knowledge about the effects of rootstocks on scion behavior.

Climate trends indicated an increased risk of frost in the Cautín Valley, in which it has increased with eight more frost events from 1985 to 2015 [3]. Late spring frost is a severe risk for the sustainability of grape production, which results in important economic damage to the national wine industry [37]. Therefore, it seems that 101-14 Mgt could be an interesting rootstock to modulate phenology and berry maturity in the short-vegetative cycle varieties established under the cold climate conditions of the Cautín Valley in Southern Chile.

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