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Phenological Performance, Thermal Demand, and Qualitative Potential of Wine Grape Cultivars Under Double Pruning

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Abstract: The production of winter wines in Southeastern Brazil represents a relatively recent but expanding viticultural approach, with increasing adoption across diverse wine-growing regions. This system relies on the double-pruning technique, which allows for the harvest of grapes during the dry and cooler winter season, favoring a greater accumulation of sugars, acids, and phenolic compounds. This study aimed to characterize the phenological stages, thermal requirements, yield, and fruit quality of the fine wine grape cultivars 'Sauvignon Blanc', 'Merlot', 'Tannat', 'Pinot Noir', 'Malbec', and 'Cabernet Sauvignon' under double-pruning management in a subtropical climate. The vineyard was established in 2020, and two production cycles were evaluated (2022/2023 and 2023/2024). Significant differences in the duration of phenological stages were observed among cultivars, ranging from 146 to 172 days from pruning to harvest. The accumulated thermal demand was higher in the first cycle, with a mean of 1476.9 growing degree days (GDD) across cultivars. The results demonstrate the potential of *Vitis vinifera* L. cultivars managed with double pruning for high-quality wine production under subtropical conditions, supporting the viability of expanding viticulture in the state of São Paulo. 'Cabernet Sauvignon' and 'Sauvignon Blanc' showed the highest yields, reaching 3.03 and 2.75 kg per plant, respectively, with productivity values of up to 10.8 t ha^{-1}. 'Tannat' stood out for its high sugar accumulation (23.4 °Brix), while 'Merlot' exhibited the highest phenolic (234.9 mg 100 g^{-1}) and flavonoid (15.3 mg 100 g^{-1}) contents. These results highlight the enological potential of the evaluated cultivars and confirm the efficiency of the double-pruning system in improving grape composition and wine quality in non-traditional viticultural regions.

Keywords: grape wine; phenology; subtropical viticulture; winter wine production; bioactive compounds; grape quality

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1. Introduction

Grapes (*Vitis* spp.) are among the most economically important fruit crops worldwide, encompassing a wide diversity of species and cultivars used for fresh consumption (ta-

ble grapes), raisin production, juice, and winemaking [1]. Brazilian viticulture occupies approximately 75,000 hectares of vineyards [2], with the states of Rio Grande do Sul, São Paulo, Pernambuco, Santa Catarina, Paraná, Bahia, and Minas Gerais standing out as the main wine-growing areas. In terms of fine wine production, a 9.3% increase was recorded in 2021 and 2022, with a notable growth of 19.5% in red wine production, while volumes of white and rosé wines remained virtually stable [3]. According to IBGE estimates, grape production in Brazil reached 1,450,805 tons in 2022.

In southeastern Brazil, an emerging viticulture has shown significant potential for high-quality fine wine production, driven by the implementation of the double-pruning technique [4–6]. This management strategy has enabled the cultivation of *Vitis vinifera* L. cultivars in regions previously considered unsuitable for premium wine production [7]. The double-pruning technique modifies the phenological cycle of the grapevine, allowing for harvest to occur during the dry winter season—a period characterized by mild daytime temperatures, lower nighttime temperatures, and minimal rainfall. This strategy enables the harvest of grapes under optimal health and ripening conditions, resulting in berries with improved physicochemical and biochemical attributes, and consequently, wines of superior intrinsic quality [8].

For the production of fine wines in new viticultural regions, it is essential to assess the adaptability of grapevine cultivars in relation to the specific characteristics of each terroir. The double-pruning management enhances sugar accumulation and phenolic ripening, promoting superior sensory attributes and improving wine quality [9]. In addition to their commercial relevance, grapes are rich in polyphenols, resveratrol [10], vitamins (such as C and K), and dietary fiber [11], which have been associated with antioxidant, anti-inflammatory, and cardioprotective effects. These nutritional and functional properties have contributed to the growing interest in grape consumption and value-added products [10,11].

Although winter wine production in southeastern Brazil began only in 2004, in southern Minas Gerais, the system has already expanded to over 35 distinct wine-producing regions, with a growing community of winegrowers [12]. This expansion underscores the need to evaluate the agronomic and qualitative performance of grapevine cultivars under the specific conditions imposed by double pruning in subtropical climates. Within this context, double pruning has emerged as a promising strategy for advancing viticulture in the state of São Paulo [13]. Among the *Vitis vinifera* L. cultivars grown in the region, the red cultivars 'Cabernet Sauvignon' and 'Merlot' and the white cultivar 'Sauvignon Blanc' are particularly notable due to their recognized enological potential [14].

The double-pruning technique modifies the phenological cycle of the grapevine, allowing harvest to occur during the dry winter season, a period characterized by mild daytime temperatures, lower nighttime temperatures, and minimal rainfall [14]. Such conditions favor the accumulation and preservation of sugars, organic acids, and phenolic compounds, leading to musts with superior technological and sensory quality [8]. Moreover, recent studies have demonstrated that double pruning reduces disease pressure, enhances grape balance, and increases vineyard sustainability in tropical and subtropical regions.

Winter wine production has expanded considerably since its introduction in 2004 in southern Minas Gerais, with over 35 producing regions now adopting this model [13]. Notable examples include the production of high-quality 'Syrah', 'Tempranillo', and 'Chardonnay' wines with enhanced color, acidity, and aromatic profiles. The alignment of grape ripening with cooler and drier conditions also lowers chemical inputs and production costs. Among the cultivars used in subtropical regions of Brazil, 'Cabernet Sauvignon', 'Merlot', and 'Sauvignon Blanc' stand out for their high enological potential and commercial value [13,14]. These varieties are widely used both for table grape and wine markets,

especially red blends and varietal whites. Their adaptation across diverse Brazilian terroirs reinforces their economic relevance for the industry.

Given the increasing interest in double-pruning management and the expansion of vineyards in non-traditional wine-growing areas, the present study aims to evaluate the phenological development, thermal requirements, ripening pattern, yield, physicochemical composition, and biochemical quality of the cultivars Sauvignon Blanc, Merlot, Tannat, Pinot Noir, Malbec, and Cabernet Sauvignon subtropical conditions. The findings are expected to guide grape growers and winemakers in cultivar selection and vineyard planning, contributing to the sustainable development of the fine wine industry in southeastern Brazil.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted from February 2023 to August 2024 in a vineyard located at Santa Lúcia do Tietê Farm, in the municipality of Mineiros do Tietê, São Paulo State, Brazil (22°32′25″ S, 48°24′13″ W; altitude: 580 m). The experimental area is situated in a transitional zone between the tropical montane Atlantic Forest and the Cerrado biome, and is characterized by a humid subtropical climate (Cfa) according to the Köppen–Geiger classification, with hot summers and evenly distributed rainfall throughout the year.

2.2. Climatic Data

According to the climatic data obtained during the two production cycles (Figure 1), the area exhibits climatic characteristics similar to those of other winter wine-producing regions in southeastern Brazil, such as in the states of Minas Gerais and São Paulo [15,16].

Climatic conditions favorable to grape cultivation for winter wine production include reduced rainfall during the harvest period (June to August), low relative humidity, high thermal amplitude (below 30 °C), and high solar radiation incidence. These factors contribute to a slower ripening process, resulting in the greater accumulation of phenolic compounds, aroma compounds, and aroma precursors. Such conditions are essential for the production of both red and white wines with high enological potential [5,17].

During the 2023 growing cycle, total annual rainfall was 2135.4 mm, with an average temperature of 23.4 °C and a relative humidity of 76%. In 2024, a reduction in these climatic indices was observed, with total precipitation of 989.0 mm, a mean annual temperature of 19.6 °C, and relative humidity of 75.5%. During the berry ripening period, meteorological data collection was intensified and conducted daily in both production cycles (Figure 1a,b).

2.3. Vineyard Establishment and Growing Conditions

The establishment of the vineyard began in 2020, with soil preparation, liming, and corrective fertilization based on agrochemical analyses. A vertical shoot positioning (VSP) trellis system with wooden posts was installed, with a spacing of 3.0 m between rows and 1.0 m between vines. Certified grapevine seedlings were planted in November 2020.

The vines were managed under a double-pruning system over two production cycles. Training pruning was performed on 17 August 2022, and 13 August 2023, while production pruning was carried out on 12 February 2023, and 17 February 2024, aiming for harvest during the dry winter period typical of southeastern Brazil. Following pruning, hydrogen cyanamide (Dormex®, BASF, Ludwigshafen am Rhein, Germany) was applied at a 5% concentration, combined with 0.1% silicon-based surfactant, to promote uniform budbreak.

Pruning was carried out following the long pruning system, characterized by the maintenance of canes with three active buds. This type of pruning aims to promote a balance between vegetative vigor and fruit production, resulting in a more homogeneous distribution of clusters throughout the canopy and allowing for improved aeration and

light interception. The decision to retain three buds seeks to optimize bud break and fruit load, particularly in cultivars that exhibit good fertility in basal to median buds. Moreover, long pruning is a recommended practice for young vines still in formation or in regions where climatic conditions may impair the sprouting of distal buds, thereby ensuring greater production consistency. This strategy also contributes to vineyard sustainability by reducing the need for corrective interventions throughout the vegetative cycle [18].

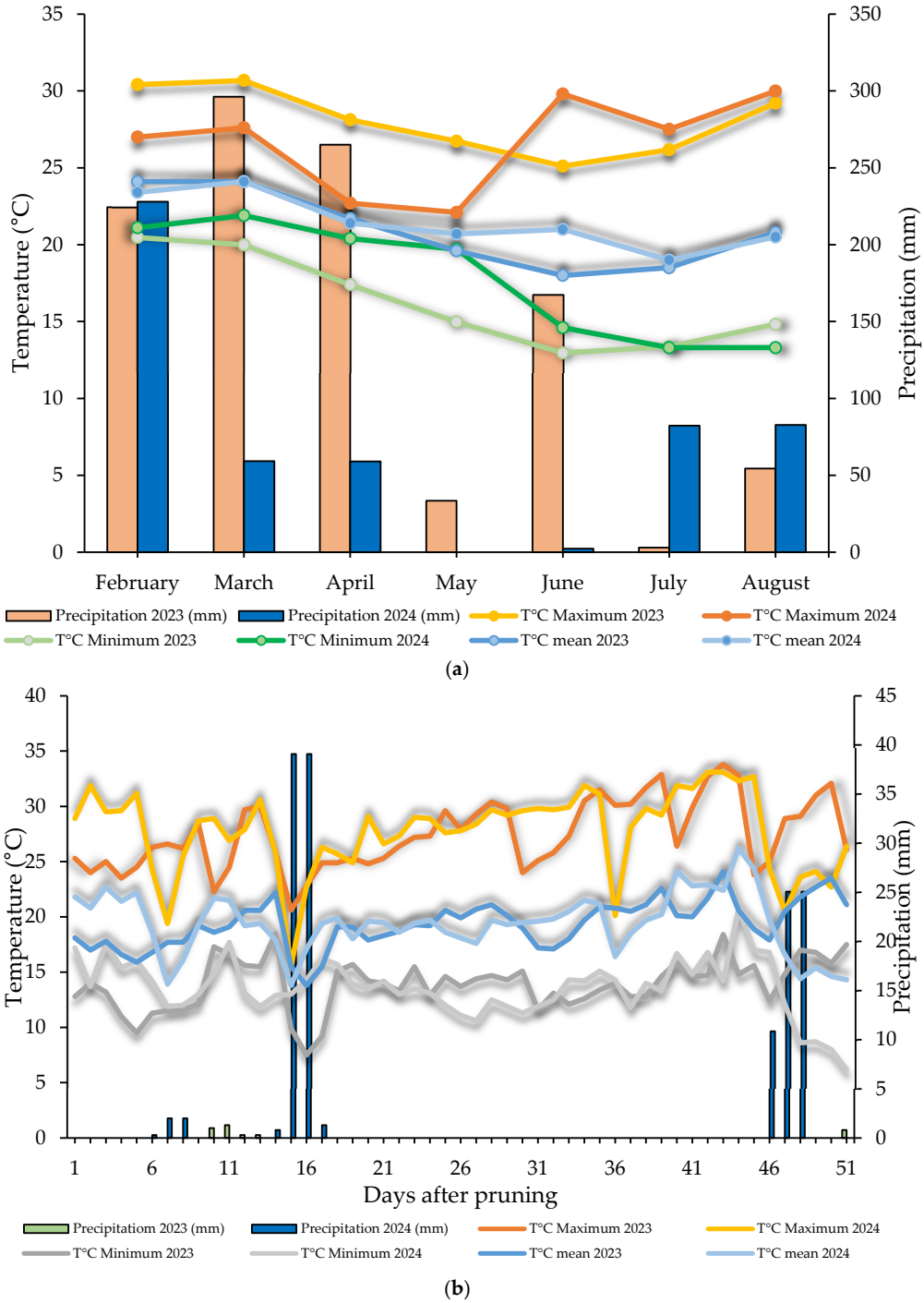


Figure 1. Annual climatic data for maximum, minimum, and mean temperatures and accumulated rainfall (**a**); and climatic data after the onset of ripening, including maximum, minimum, and mean temperatures and accumulated precipitation (**b**), during the 2023 and 2024 growing seasons in Mineiros do Tietê, São Paulo, Brazil.

Canopy management practices included the removal of leaves from the basal part of the branch, shoot thinning, tipping, tying, and berry thinning (40–70%). Top-dressing fertilizations were performed based on soil analysis and the recommendations of Bulletin 100 from the Agronomic Institute of Campinas [19]. At the onset of berry ripening (veraison), protective nets with 10% shading were installed to guard against hail, birds, and bees.

2.4. Experimental Design and Treatments

The experimental design was a randomized complete block design (RCBD), with six treatments and eight blocks, totaling 48 experimental plots. Each plot consisted of five grapevines, spaced 3.0 m between rows and 1.0 m between plants, resulting in a total area of 15 m^2 per plot. A buffer space of 1.5 m was maintained between adjacent plots to reduce edge effects and minimize interference between treatments. The treatments corresponded to six *Vitis vinifera* L. cultivars: 'Sauvignon Blanc', 'Merlot', 'Tannat', 'Pinot Noir', 'Malbec', and 'Cabernet Sauvignon'.

2.5. Evaluated Variables

2.5.1. Duration of Phenological Stages

Phenological stages were characterized according to the phenology scale by Eichhorn and Lorenz [20]. Evaluations were performed twice a week until flowering and weekly thereafter until harvest. The phenological stages observed were:

- Budburst (BB) (4): 50% of buds with visible young leaves;
- Flowering (FL) (23): 50% at full open flowering stage;
- Fruit Set (FS) (27): berries with a diameter greater than 2 mm;
- Verasion (V) (35): 50% of berries showing color change and softening;
- Harvest (H) (38): 100% of berries with appropriate color and physiological ripeness.

2.5.2. Thermal/Temperature Condition

Thermal/temperature condition was estimated using the accumulation of growing degree days (GDD), following the method proposed by Winkler [21], using the equation

$$GDD = \Sigma\ (T_m - 10\ ^\circ C) \times DAP$$

where T_m represents the daily mean temperature (°C), 10 °C is the base temperature for grapevine development, and DAP refers to days after pruning.

2.5.3. Ripening (Maturation) Dynamics

Grape ripening was monitored weekly (precisely every 7 days) from the onset of berry ripening. Soluble solids (SSs, °Brix) were determined using a digital refractometer (Reichert r2i300) (Buffalo, NY, USA); titratable acidity (TA, % tartaric acid) was measured by titrating 5 mL of must diluted in 100 mL of distilled water with 0.1 N sodium hydroxide solution, using phenolphthalein as an indicator. The results were expressed as a percentage of tartaric acid, with 0.1 N NaOH until pH 8.2–8.4; pH was assessed by direct reading using a digital pH meter (Tecnal) (Piracicaba, Brazil); and the maturity index (SS/TA) was calculated. For each sampling, 16 bunches were randomly collected per plot, and six berries were sampled from each cluster (two from each third).

2.5.4. Quantitative and Qualitative Parameters

Harvest was carried out when clusters reached physiological maturity. At this stage, all clusters were counted and weighed to determine the total number of clusters and yield per vine, and to estimate productivity in tons per hectare (t ha^{-1}).

From a sample of 15 clusters per plot, the fresh weight, length, and width of clusters, berries, and rachis were evaluated, as was the number of berries per cluster. For the physicochemical analyses of the must, 100 berries per experimental plot were used. The must was obtained by the manual pressing of the berries.

2.5.5. Bioactive Compounds and Antioxidant Activity

Biochemical Characteristics of Berries

Biochemical analyses were carried out at the Laboratory of Plant Chemistry and Biochemistry, UNESP/Botucatu. Ten berries per cluster were collected (three from the top, four from the middle, and three from the bottom). The berries were halved, immediately frozen in liquid nitrogen, and ground using mortar and pestle. The resulting samples were stored at −20 °C until analysis, which was performed in triplicate.

For supernatant extraction, 300 mg of pulp was mixed with 5 mL of acidified methanol (80%). The mixture was homogenized for 30 s in a vortex and subjected to an ultrasonic bath for 20 min. It was then centrifuged at 6000 rpm for 10 min at 5 °C. The supernatant was collected, and the extraction procedure was repeated once more. The combined extracts were stored in amber vials for further analysis.

Total Phenolic Compounds

Total phenolic content was quantified using the Folin–Ciocalteu reagent, according to Singleton and Rossi [22]. The results are expressed as mg of gallic acid equivalents per kg of fresh matter (mg GAE/kg FM). To achieve this, 0.5 mL of the extract was mixed with 0.5 mL of distilled water, 0.5 mL of diluted Folin–Ciocalteu reagent (1:4), and 2.5 mL of 4% sodium carbonate solution. The mixture was vortexed and kept in the dark for one hour before reading absorbance at 725 nm using a spectrophotometer. A gallic acid standard curve was also established.

Total Flavonoids

Total flavonoid content was determined using aluminum chloride ($AlCl_3$) and a benchtop spectrophotometer. Four milliliters of the extract were homogenized and left in the dark for 30 min, followed by centrifugation at 6000 rpm for 25 min at 5 °C. Absorbance was read at 510 nm, and the results are expressed as mg quercetin equivalent per 100 g fresh matter, according to Popova et al. [23].

Total Anthocyanin Content

Total anthocyanins (TAC) were quantified by the pH differential method proposed by Giusti and Wrolstad [24]. An aliquot of the extract was diluted in two buffer solutions—0.025 M potassium chloride (pH 1.0) and 0.4 N sodium acetate (pH 4.5). Absorbance readings were taken at 510 nm (cyanidin-3-*O*-glucoside peak) and 700 nm (turbidity correction) using a spectrophotometer. The results are expressed as mg cyanidin-3-*O*-glucoside equivalents per 100 g of whole berry grape sample.

Antioxidant Activity

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, according to Brand-Williams et al. [25]. The results were calculated using a Trolox standard curve ($y = 23.606x + 2.5805$; $R^2 = 0.9961$), and are expressed as μg Trolox equivalents per 100 g of sample.

Additionally, the FRAP (Ferric Reducing Antioxidant Power) method was applied according to Brand-Williams et al. [25], using a mixture of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ (in 40 mM HCl), and 2.5 mL of $FeCl_3$. Absorbance was read at

595 nm, and the results are expressed as mmol $FeSO_4$ equivalents per 100 g of sample ($y = 0.007 + 0.0044$; $R^2 = 0.9973$).

2.6. Statistical Analysis

Data were subjected to tests for normality and homogeneity of variances. A two-factor ANOVA was performed in a split-plot design over time, with cultivars as the main plots and production cycles as the subplots. The experimental design was a randomized complete block with eight replicates and five plants per plot. Means were compared using Tukey's test at a 5% significance. Ripening curve data were analyzed by polynomial regression. All statistical analyses were performed using SISVAR 5.6 software [26].

3. Results and Discussion

3.1. Duration of Phenological Stages

Significant variations were observed in the duration of phenological stages between the 2023 and 2024 production cycles. In 2024, budburst occurred on average 13.1 days after pruning, and harvest took place at 128.0 days, while in 2023 these events occurred at 12.4 and 146.5 days, respectively. Thermal demand was also lower in 2024, with an accumulated total of 1207.6 degree days, in contrast to 1746.3 degree days recorded in the previous cycle, reflecting differences in climatic conditions and management practices between years.

The total duration from pruning to harvest varied among cultivars—Sauvignon Blanc (146 days), Merlot (159 days), Tannat (156 days), Pinot Noir (146 days), Malbec (158 days), and Cabernet Sauvignon (142 days) (Table 1). The duration of the phenological stages varied significantly among grapevine cultivars and production cycles, highlighting the influence of genetic characteristics and environmental conditions on the developmental cycle. The stages of budburst, flowering, fruit set, onset of ripening, and harvest exhibited notable differences, directly affecting vineyard management and harvest planning.

Table 1. Duration of phenological stages (days after pruning) of the grape cultivars 'Sauvignon Blanc', 'Merlot', 'Pinot Noir', 'Tannat', 'Malbec', and 'Cabernet Sauvignon' during the 2023 and 2024 production cycles under subtropical conditions in São Paulo, Brazil.

Cultivar	Production Cycle	BB	FL	FS	V	HV	GDD
Sauvignon Blanc	I	12.3 ± 0.71 Abb *	44.3 ± 2.12 Ca	47.1 ± 2.59 Ca	87.9 ± 0.64 Ba	130.9 ± 0.00 Ca	1700.3 ± 5.89 Ca
	II	13.4 ± 0.52 ABa	31.6 ± 7.44 Ab	34.8 ± 1.49 Bb	85.8 ± 4.27 Ca	121.0 ± 0.00 Cb	1122.7 ± 14.3 Db
Merlot	I	11.9 ± 0.83 Ba	48.3 ± 4.50 Ba	53.5 ± 3.25 Ba	93.8 ± 3.33 Aa	171.0 ± 0.00 Aa	1758.9 ± 1.55 Ba
	II	12.5 ± 0.76 BCa	32.1 ± 0.83 Ab	35.8 ± 2.12 ABb	90.6 ± 2.07 Bb	128.0 ± 0.00 Bb	1218.0 ± 10.1 Bb
Tannat	I	13.0 ± 0.53 Aa	53.9 ± 6.20 Aa	58.4 ± 5.53 Aa	97.0 ± 3.33 Aa	148.0 ± 0.00 Ba	1785.5 ± 2.25 Aa
	II	13.5 ± 0.53 ABa	35.0 ± 1.51 Ab	39.0 ± 0.76 Ab	95.3 ± 1.04 Aa	128.0 ± 0.00 Bb	1204.7 ± 8.45 Cb
Pinot Noir	I	12.8 ± 1.04 Aba	45.3 ± 3.45 BCa	48.1 ± 3.68 Ca	88.3 ± 0.71 Bb	130.0 ± 0.00 Da	1702.8 ± 4.39 Ca
	II	13.5 ± 1.07 ABa	32.9 ± 0.83 Ab	37.0 ± 1.69 ABb	90.8 ± 4.50 Ba	121.0 ± 0.00 Cb	1119.9 ± 18.4 Db
Malbec	I	11.8 ± 0.89 Bb	43.3 ± 1.83 Ca	45.9 ± 1.64 Ca	94.7 ± 1.39 Aa	171.0 ± 0.00 Aa	1769.8 ± 0.49 Ba
	II	13.6 ± 0.52 Aa	32.0 ± 0.76 Ab	35.6 ± 1.77 ABb	93.9 ± 2.03 Aba	128.0 ± 0.00 Bb	1202.7 ± 8.18 Cb
Cabernet Sauvignon	I	12.6 ± 1.06 Aba	43.9 ± 1.55 Ca	46.6 ± 1.77 Ca	94.0 ± 0.00 Aa	129.0 ± 0.00 Eb	1760.3 ± 0.96 Ba
	II	12.3 ± 0.46 Ca	32.0 ± 0.76 Ab	35.8 ± 1.58 ABb	92.8 ± 2.66 Aba	142.0 ± 0.00 Aa	1377.8 ± 5.46 Ab
CV(%)	I	5.95	7.06	6.17	2.54	2.93	0.56
	II	6.11	6.47	5.84	2.82	2.01	0.62
Mean		12.7	39.5	43.1	92.1	137.3	1476.90

* Means followed by different uppercase letters in columns and lowercase letters in rows differ significantly according to Tukey's test at a 5% probability level ($p > 0.05$). Values are expressed as mean ± standard deviation (n = 8). Abbreviations: BB—budburst; FL—full flowering; FS—fruit set; V—verasion; HV—harvest; GDD—growing degree days. CV: coefficient of variation.

The observed variations in the duration of phenological stages between the 2023 and 2024 production cycles under double-pruning management align with findings from recent studies conducted in Brazil. For instance, research on the 'BRS Isis' grape cultivar in São Paulo demonstrated that the duration from pruning to harvest varied significantly across

different harvest seasons, ranging from 137 to 168 days, influenced by climatic conditions and rootstock combinations. The thermal demand also fluctuated, with accumulated degree days between 1651 and 1841, highlighting the impact of environmental factors on grapevine development [27]. Similarly, studies on the 'BRS Núbia' seedless grape in São Manuel, SP, reported that the total cycle duration from pruning to harvest was 155 days, with thermal requirements of 1806.4 degree days [28]. These findings underscore the importance of understanding the phenological behavior and thermal requirements of grape cultivars under double-pruning systems to optimize vineyard management and harvest planning in subtropical regions of Brazil [29,30].

The duration of phenological stages in grapevines subjected to double pruning varies considerably between production cycles and cultivars, being influenced by both genetic factors and environmental conditions. In subtropical climates, the complete cycle from pruning to harvest has been reported to range from approximately 146 to 172 days, depending on the grape cultivar and the specific climatic conditions of each growing season [31]. For instance, in 'Syrah' grapevines cultivated under the double-pruning system in Southeastern Brazil, researchers observed that the summer cycle was shorter than the winter cycle. This was primarily due to a more rapid maturation period during the summer, which led to variations in the duration of stages such as budburst, flowering, veraison, and harvest [11]. These results are consistent with observations that the thermal time requirement (expressed in growing degree days, GDD) also differs between production years, reflecting variability in climate and management practices.

Such fluctuations in the phenological timing have direct implications for vineyard management strategies and harvest scheduling. Therefore, understanding the dynamics of phenological development across cultivars and growing seasons is essential for optimizing the quality of grapes and wine produced under winter viticulture conditions in subtropical regions [16,31].

3.1.1. Budburst

Budburst showed little variation between the production cycles—an average of 12.4 days in 2023 and 13.1 days in 2024. The shortest period was recorded for 'Merlot' (11.9 $\pm$ 0.83 days) and 'Malbec' (11.8 $\pm$ 0.89 days) in the first cycle, while the longest was observed for 'Malbec' in the second cycle (13.6 $\pm$ 0.52 days). This phenological stage marks the beginning of the vegetative cycle and is crucial for the overall development rate. Early budburst may accelerate the cycle but increases the risk of damage from late frosts in regions with unstable climates [32].

3.1.2. Full Flowering

Marked differences were observed between production cycles. The period from pruning to flowering was longer in 2023 (46.5 days) compared to 2024 (32.6 days), due to higher temperatures in the latter (Figure 1), which accelerated phenological development. The cultivars 'Tannat' (53.9 $\pm$ 6.20 days) and 'Merlot' (48.3 $\pm$ 4.5 days) exhibited the longest pruning-to-flowering periods. In contrast, 'Sauvignon Blanc' had the shortest (37.9 days). Early flowering, as observed in 'Sauvignon Blanc' and 'Malbec', may be advantageous in regions requiring shorter production cycles.

This reduction is likely attributable to higher average temperatures in 2024, which accelerated phenological development. These findings are consistent with previous research conducted by Júnior and Hernandes [33], who evaluated the phenology and maturation dynamics of 'Syrah' grapes cultivated under a double pruning system during the winter season. Their study reported that the period from pruning to flowering can vary depending on climatic conditions, particularly temperature, which plays a crucial role in modulating

the timing of phenological events. In our study, cultivars such as 'Tannat' and 'Merlot' showed extended durations (53.9 and 48.3 days, respectively), while 'Sauvignon Blanc' flowered earlier (37.9 days), indicating genotypic variability in developmental response. As earlier flowering may be beneficial in subtropical regions where compressed production cycles are desirable to avoid adverse weather conditions later in the season [34].

3.1.3. Fruit Set

The duration from pruning to fruit set was shorter in 2024 (36.3 days) than in 2023 (49.9 days). 'Tannat' consistently exhibited the longest cycle in both years (58.4 ± 5.53 days in 2023 and 39.0 days in 2024), while 'Sauvignon Blanc' showed the shortest durations (47.1 days in 2023 and 34.8 ± 1.49 days in 2024). This stage is highly sensitive to environmental factors such as water deficit and temperature [35], and cultivars with shorter cycles may exhibit greater resilience under climatic stress.

3.1.4. Verasion

The onset of ripening occurred slightly earlier in 2024 (91.5 days) compared to 2023 (92.1 days). 'Tannat' exhibited the latest ripening (97.0 ± 3.33 days in 2023 and 95.3 ± 1.04 days in 2024), whereas 'Sauvignon Blanc' was the earliest (87.9 ± 0.64 days in 2023 and 85.8 ± 4.27 days in 2024). This stage directly influences wine quality as it defines the balance between sugars and acids. Early ripening, as seen in 'Sauvignon Blanc', is often associated with fresher wines with higher acidity [27,36].

3.1.5. Harvest

In the 2023 production cycle, the period from pruning to harvest ranged from 121 to 185 days, with 'Pinot Noir' and 'Sauvignon Blanc' being the earliest cultivars and 'Cabernet Sauvignon' the latest. In 2024, all cultivars presented shorter cycles, with 'Sauvignon Blanc' again showing the shortest duration (121 days), while 'Cabernet Sauvignon' remained the last to be harvested (142 days).

The shortened cycle in 2024 was mainly attributed to climatic conditions between February and June, with average temperatures of 23.4 °C and 21 °C, respectively. Shorter cycles can reduce the level of biotic stress caused by pathogens, favoring early-ripening cultivars in regions with short summers [37,38]. Recent studies have highlighted that under tropical and subtropical climates, early harvests supported by short-cycle cultivars contribute significantly to reducing fungal outbreaks and ensuring technological maturity at harvest [39]. Cycle duration influences both sensory attributes and vineyard management feasibility, and is thus critical for production planning.

3.2. Thermal Demand of Grapevines

In 2023, the average thermal requirement was higher (1476.9 GDD), reflecting greater vine demand to complete the cycle. 'Tannat' showed the highest accumulation (1785.5 ± 2.25 GDD), followed by 'Malbec' (1769.8 ± 0.49) and 'Cabernet Sauvignon' (1760.3 ± 0.96). In contrast, the 2024 cycle required less thermal accumulation, with 'Cabernet Sauvignon' needing 1377.8 ± 5.46 GDD. A base temperature of 10 °C was used for GDD calculations, below which no vegetative development occurs [40]. In comparison with other regions, 'Cabernet Sauvignon' requires around 1200 GDD in Santa Catarina (Epagri, 2005) [41], and up to 1625 GDD in semiarid regions [42]. Thermal demand directly affects the pace of development, with higher temperatures shortening the intervals between phenological stages [43]. Understanding thermal requirements allows for the prediction of phenological progression and enables better harvest scheduling, optimizing both management practices and labor organization.

These results are consistent with previous research on grapevines grown under double-pruning or double-cropping systems in subtropical regions. Koyama et al. [29], for instance, reported thermal requirements of 1744.1 GDD for the summer crop and 1845.0 GDD for the off-season crop of the hybrid seedless cultivar BRS Melodia under a double-cropping system. These findings highlight the role of seasonal and environmental variations in shaping phenological dynamics and thermal accumulation patterns.

Further evidence is provided by Monteiro et al. [28], who observed that the cultivar 'BRS Núbia' required an average of 1,807.5 GDD across two production cycles, demonstrating the relative consistency in thermal requirements of some cultivars under similar environmental conditions. In addition, Sánchez et al. [27] examined the 'BRS Isis' grapevine grafted onto different rootstocks and reported thermal demands ranging from 1651 to 1841 GDD, showing how both climatic factors and rootstock genotype influence grapevine development in subtropical climates. Taken together, these studies reinforce the importance of characterizing the thermal requirements of each cultivar under specific growing conditions, particularly when innovative management systems such as double pruning are employed. Understanding these thermal patterns is critical for optimizing vine performance, harvest scheduling, and grape quality under changing climatic conditions.

3.3. Yield Components and Physical Characteristics

Significant differences were observed in the physical characteristics of clusters, berries, and rachis among cultivars (Table 2), with variations attributed to varieties and climatic conditions. 'Sauvignon Blanc' showed consistency across cycles, with cluster weights of 87.9 g (Cycle I) and 84.5 g (Cycle II), and an increase in cluster length (8.29 cm in Cycle II). 'Tannat' exhibited a marked increase in cluster weight (from 125.4 g to 143.0 g) and overall size.

In contrast, 'Pinot Noir' and 'Malbec' showed reduced cluster weight in the second cycle. Cluster width increased across all cultivars in Cycle II compared to Cycle I, while length varied, with decreases observed only in 'Sauvignon Blanc' and 'Cabernet Sauvignon'. Berry size also varied, with 'Malbec' showing notable increases in both length and width. Smaller berries (with a mass below 2.0 g) are favorable for the extraction of phenolic compounds, which are essential for red wine production [42]. The number and size of berries directly affect cluster mass [43]. Cultivars such as 'Tannat' produce robust clusters suitable for full-bodied wines, while 'Pinot Noir' tends to yield lighter, more aromatic wines.

These findings align with previous studies that have reported cultivar-specific responses to double pruning. For example, Favero et al. [17] observed that 'Syrah' grapevines under double pruning in Southeastern Brazil produced higher yields and improved fruit composition during the winter season compared to the summer, highlighting the influence of seasonal conditions on grape development. Similarly, Tecchio et al. [44] reported that 'Cabernet Franc' and 'Syrah' cultivars grafted onto 'IAC 766' rootstock exhibited superior yield performance and berry quality under subtropical conditions, emphasizing the role of rootstock-scion combinations in determining vine productivity and fruit characteristics.

Furthermore, the increase in cluster width across all cultivars in Cycle II, despite variations in cluster length, suggests an adaptive morphological response to environmental factors. Berry size also varied, with 'Malbec' showing significant increases in both length and width, which could be attributed to favorable climatic conditions or improved assimilate partitioning during the second cycle. These observations underscore the importance of considering both genetic and environmental factors in vineyard management practices.

Table 2. Physical characteristics of clusters, berries, and rachis of wine grape cultivars grown under double-pruning management in a subtropical climate during the 2023 (I) and 2024 (II) growing seasons in São Paulo, Brazil.

Cultivar	Production Cycle	FWC	CL	CW	CL/CW	FWB	BL	BW	BL/BW	NBC	FWR
Sauvignon Blanc	I	87.9 ± 16.9 bcA *	7.90 ± 1.07 Ba	4.67 ± 0.37 BCb	1.69 ± 0.16 Bca	1.41 ± 0.10 Ba	1.45 ± 0.05 ABa	1.28 ± 0.03 Ba	1.13 ± 0.02 ABa	59.3 ± 12.3 Ba	4.27 ± 0.78 Ca
	II	84.5 ± 4.05 BCa	8.29 ± 0.54 Ca	6.75 ± 0.61 BCa	1.24 ± 0.12 BCb	1.36 ± 0.10 Ca	1.42 ± 0.03 BCa	1.27 ± 0.03 Ca	1.11 ± 0.03 Aba	58.8 ± 5.61 Ba	5.00 ± 0.59 Ba
Merlot	I	56.6 ± 8.47 Da	8.25 ± 0.83 Bb	5.25 ± 0.63 BCb	1.58 ± 0.12 Ca	1.09 ± 0.04 Cb	1.14 ± 0.14 Cb	1.10 ± 0.03 Db	1.03 ± 0.14 Ba	49.4 ± 8.29 Ba	3.01 ± 0.48 Da
	II	68.0 ± 10.9 CDa	10.3 ± 0.82 Ba	7.00 ± 0.85 Ba	1.49 ± 0.08 Aba	1.27 ± 0.05 CDa	1.31 ± 0.19 Ca	1.35 ± 0.03 Ba	0.98 ± 0.15 Ba	50.8 ± 7.08 ABa	3.50 ± 0.69 Ca
Tannat	I	125.4 ± 20.4 Ab	10.3 ± 1.98 Ab	14.1 ± 1.17 Ab	0.72 ± 0.10 Da	1.41 ± 0.12 Bb	1.37 ± 0.04 Bb	1.26 ± 0.03 BCb	1.09 ± 0.02 ABa	83.2 ± 8.75 Aa	7.38 ± 0.54 Ab
	II	143.0 ± 19.8 Aa	15.3 ± 1.98 Aa	18.1 ± 1.82 Aa	0.84 ± 0.04 Da	1.64 ± 0.15 Ba	1.57± 0.06 Aba	1.39 ± 0.08 Ba	1.13 ± 0.04 Aa	83.0 ± 8.61 Aa	8.20 ± 0.36 Aa
Pinot Noir	I	66.1 ± 10.7 CDa	6.88 ± 0.74 Ba	4.22 ± 0.74 Cb	1.67 ± 0.27 BCa	1.09 ± 0.14 Ca	1.40 ± 0.20 ABa	1.19 ± 0.03 Ca	1.18 ± 0.19 Aa	58.3 ± 8.88 Ba	2.63 ± 0.80 Da
	II	46.3 ± 8.16 Db	6.18 ± 0.59 Da	5.52 ± 0.33 CDa	1.12 ± 0.11 Cb	1.21 ± 0.14 CDa	1.30 ± 0.03 Cb	1.19 ± 0.04 Da	1.09 ± 0.02 Aba	26.9 ± 7.60 Db	2.21 ± 0.60 Da
Malbec	I	137.2 ± 28.6 Aa	10.5 ± 1.21 Aa	5.53 ± 0.52 Bb	1.90 ± 0.21 Ba	2.11 ± 0.18 Aa	1.55 ± 0.06 Ab	1.45 ± 0.05 Ab	1.07 ± 0.03 ABa	62.0 ± 11.4 Ba	6.19 ± 1.54 Ba
	II	97.0 ± 22.8 Bb	11.3 ± 1.34 Ba	7.65 ± 0.83 Ba	1.48 ± 0.17 ABb	2.13 ± 0.11 Aa	1.67 ± 0.05 Aa	1.50 ± 0.03 Aa	1.12 ± 0.02 Aba	43.6 ± 8.88 CDb	3.75 ± 0.76 Cb
Cabernet Sauvignon	I	99.9 ± 11.0 Ba	10.2 ± 1.10 Aa	4.14 ± 0.20 Ca	2.48 ± 0.27 Aa	1.26 ± 0.09 BCa	1.32 ± 0.02 Ba	1.22 ± 0.02 BCa	1.07 ± 0.02 ABa	75.7 ± 6.27 Aa	4.60 ± 0.49 Ca
	II	56.0 ± 11.3 Db	7.61 ± 0.88 CDb	4.70 ± 0.70 Da	1.63 ± 0.05 Ab	1.10 ± 0.03 Db	1.30 ± 0.01 Ca	1.19 ± 0.00 Da	1.09 ± 0.00 ABa	47.9 ± 8.13 BCDb	2.97 ± 0.80 CDb
CV(%)	I	21.5	17.1	14.6	13.1	9.38	8.92	3.75	2.53	16.6	16.7
	II	16	8.86	9.15	10.2	7.2	2.53	2.50	2.48	12.9	19.1
Média		88.9	9.41	7.31	1.49	1.42	1.40	1.28	1.10	50.1	4.47

* Means followed by different uppercase letters in columns and lowercase letters in rows differ significantly according to Tukey's test at a 5% probability level ($p > 0.05$). Values are expressed as mean ± standard deviation (n = 8). Abbreviations: FWC: fresh weight of clusters; CL: cluster length; CW: cluster width; CL/CW: cluster length-to-width ratio; FWB: fresh weight of berries; BL: berry length; BW: berry width; BL/BW: berry length-to-width ratio; NBC: number of berries per cluster; FWR: fresh weight of rachis. CV: coefficient of variation.

3.4. Fertility

The number of clusters per plant varied significantly among cultivars. 'Cabernet Sauvignon' recorded the highest number in the second cycle (11.8 ± 0.99 clusters plant^{-1}), while 'Pinot Noir' showed the lowest value in the first cycle (4.88 ± 1.36 clusters plant^{-1}) (Table 3). The increased yield observed in Cycle II reflects favorable environmental conditions, such as temperature and water availability, which contributed to greater biomass accumulation. This trend was consistent across cultivars, suggesting that the second cycle was more suitable for maximizing productivity.

Significant differences ($p < 0.05$) between cycles and among cultivars confirm the influence of double-pruning management under subtropical conditions. The superior performance in Cycle II may be associated with a greater number of fruitful shoots and increased bud fertility. These results indicate a strong genotype × environment interaction. 'Cabernet Sauvignon' stood out for its high yield and productivity, demonstrating good adaptability to the double-pruning system, whereas 'Malbec' and 'Pinot Noir' showed lower performance, indicating greater sensitivity to the growing conditions.

Similarly, 'Pinot Noir' has been studied under double-cropping systems—conceptually similar to double pruning—in which a second crop is forced within the same season. These studies have shown that the cultivar can respond positively, but yield components such as the number of clusters per plant and berry size may be significantly influenced by the timing of bud forcing and prevailing environmental conditions. The cultivar has been shown to exhibit a certain degree of sensitivity to modifications in phenological timing, which may impact its reproductive efficiency under non-traditional management strategies [45].

Table 3. The yield, productivity, and number of clusters per plant of wine grape cultivars grown under double-pruning management in a subtropical climate during the 2023 (I) and 2024 (II) growing seasons in Mineiros do Tietê, São Paulo, Brazil.

Cultivar	Production Cycle	Yield	Productivity	Number of Clusters per Vine
		(kg Vine^{-1})	(t ha^{-1})	(un)
Sauvignon Blanc	I	2.13 ± 0.29 Ab *	7.59 ± 1.02 Ab	9.25 ± 5.09 Ab
	II	2.75 ± 0.15 Bb	9.82 ± 0.53 Ba	11.6 ± 5.18 Aba
Tannat	I	0.88 ± 0.24 Bb	3.12 ± 0.85 Bb	5.63 ± 1.41 BCb
	II	1.09 ± 0.01 Ca	3.89 ± 0.05 Ca	7.38 ± 1.06 Ca
Pinot Noir	I	0.70 ± 0.09 BCb	2.50 ± 0.33 BCb	4.88 ± 1.36 Cb
	II	0.89 ± 0.01 CDa	3.18 ± 0.04 CDa	7.63 ± 1.19 Ca
Malbec	I	0.50 ± 0.13 Cb	1.79 ± 0.48 Cb	6.37 ± 1.41 BCb
	II	0.78 ± 0.03 Da	2.77 ± 0.11 Da	10.0 ± 1.20 Ba
Cabernet Sauvignon	I	2.13 ± 0.28 Ab	7.59 ± 0.99 Ab	7.38 ± 1.06 Bb
	II	3.03 ± 0.06 Aa	10.8 ± 0.20 Aa	11.8 ± 0.99 Aa
CV(%)	I	11.6	11.6	39.5
	II	10.6	10.7	5.2
Mean		1.49	5.3	8.2

* Means followed by different uppercase letters in columns and lowercase letters in rows differ significantly according to Tukey's test at a 5% probability level ($p > 0.05$). Values are expressed as mean ± standard deviation (n = 8). CV: coefficient of variation.

Regarding 'Malbec', late shoot pruning (LSP)—a practice related to double pruning—has been investigated as a strategy to shift phenological phases and improve grape quality. While LSP has been associated with reductions in the number of clusters and total yield, it can simultaneously enhance berry composition, particularly in terms of phenolic content. This trade-off underscores the importance of aligning management techniques with specific production goals, such as favoring quality over quantity in premium wine production [46]. These findings collectively highlight the relevance of genotype × environment interactions under double-pruning systems. They also reinforce the need for cultivar-specific management strategies to maximize both yield potential and grape quality in subtropical viticultural regions.

3.5. Yield and Productivity

Grape yield varied significantly among cultivars and between production cycles. The cultivars 'Cabernet Sauvignon' and 'Sauvignon Blanc' showed the highest yields in the second cycle (3.03 ± 0.06 kg plant^{-1} and 2.75 ± 0.15 kg plant^{-1}, respectively), whereas 'Malbec' exhibited the lowest performance (0.50 ± 0.13 kg plant^{-1} in Cycle I), likely due to poor adaptation to the local conditions. According to Steduto et al. [32], the number of clusters per plant has a direct influence on grapevine yield under this management system. All cultivars showed increased yield in Cycle II compared to Cycle I, which can be attributed to a greater formation of fruitful shoots.

These results suggest that double-pruning management under subtropical conditions benefited cultivars such as 'Sauvignon Blanc' and 'Cabernet Sauvignon', which demonstrated better adaptation and higher productivity. 'Sauvignon Blanc' has shown high productivity in different Brazilian regions, particularly when managed with the double-pruning technique for winter wine production. In contrast, cultivars such as 'Malbec' may require specific management adjustments to optimize their performance under these conditions. By specific management, we refer to tailored viticultural practices such as modifications in pruning type and timing, shoot and cluster thinning, canopy management, irrigation scheduling, and fertilization strategies, all adjusted to the physiological

responses and phenological behavior of the cultivar within the regional climatic context. These interventions aim to improve fruit quality and yield stability, ensuring the cultivar reaches its full productive and enological potential.

According to Rizzon et al. [47], the factors influencing grapevine productivity can be grouped into permanent and cultural components. Permanent factors include imposed elements such as climate, soil, and biological environment, as well as selected factors such as cultivar, rootstock, planting density, and row orientation. Cultural factors encompass training systems, pruning strategies, irrigation methods, fertilization, and phytosanitary management. Additionally, Pedro junior et al. [48] emphasized that grape production is influenced by multiple factors that determine both fruit quality and productivity. The production of fine wine grapes demands extensive technical knowledge to perform the cultural practices required for high-quality grapes, as well as significant labor input for manual vineyard operations, which raises the overall production costs [49]. The cultivar 'Merlot' did not produce enough grapes to allow for the evaluation of physical characteristics under double-pruning management in subtropical conditions. This contrasts with southern Brazil, where it is considered one of the most important cultivars.

3.6. Berry Ripening

On average, berry ripening began 93 days after pruning (DAP) during the 2023 cycle, with a thermal accumulation of 1745.3 growing degree days. The cultivars 'Sauvignon Blanc', 'Merlot', 'Tannat', 'Pinot Noir', 'Malbec', and 'Cabernet Sauvignon' began ripening at 88, 94, 97, 88, 95, and 94 DAP, respectively. The ripening phenology stage showed significant variation in duration among cultivars ($p > 0.05$), although a consistent pattern of uniformity was observed within each variety. Ripening in the 2023 season began on 16 May (93 DAP), with 'Sauvignon Blanc' and 'Pinot Noir' showing early ripening (11 May 88 DAP), while 'Malbec' and 'Tannat' exhibited the latest onset at 95 and 97 DAP, respectively.

Analyses of the ripening curve revealed significant differences among cultivars for soluble solids (°Brix), pH, titratable acidity (TA), and maturity index (MI). Over the course of the production cycle, a typical ripening trend was observed—increases in SS and pH and a decrease in acidity, reflecting progressive ripening (Figures 2–4). Quadratic models provided a good fit for the variables as a function of days after the onset of ripening. In Figures 2–5, * denotes a statistically significant difference at the 5% level ($p < 0.05$), while ** indicates a highly significant difference at the 1% level ($p < 0.01$).

3.6.1. Soluble Solids Content

Soluble solids (SSs) content, expressed in °Brix, is one of the main indicators of grape maturity and grape quality, as it reflects sugar accumulation in the berries. Among the evaluated cultivars, 'Tannat' reached the highest value, with 23.4 °Brix at 128 DAP, indicating a high potential alcohol content for winemaking. This result reinforces its good adaptation to the double-pruning system in a subtropical climate, which favors sugar synthesis [50,51]. 'Pinot Noir' and 'Sauvignon Blanc' also showed consistent increases in SS content, reaching ideal levels for wines with balanced acidity and alcohol content. As noted by Falcão et al. [52], although °Brix is not a direct measurement of sugars, its increase directly reflects the physiological maturity of the grape and the quality of the must.

High SS values are directly related to the sensory profile and complexity of wines, as they influence the synthesis of phenolic compounds, aromatic precursors, and organic acids [53,54]. According to Brazilian regulations, wine grapes must reach 18–21 °Brix for optimal harvest [55]. All cultivars in this study achieved values within this range, indicating appropriate technological maturity. 'Tannat', in particular, stood out with 23.4 °Brix at

128 DAP (Figure 4), demonstrating excellent sugar accumulation and strong potential for producing structured wines.

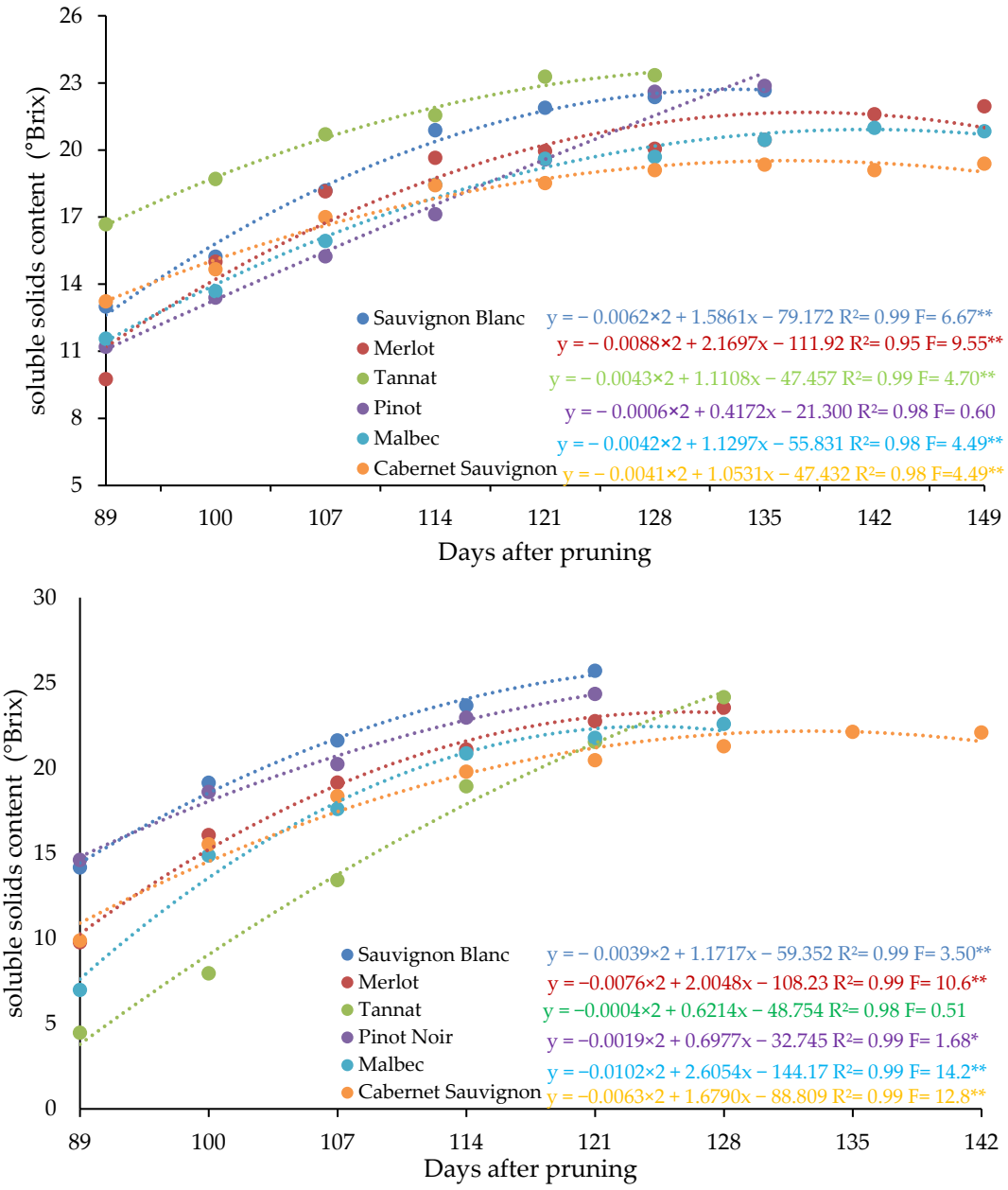


Figure 2. Evolution of soluble solids content (°Brix) in the must of wine grapes as a function of days after pruning, in grapevines managed under the double-pruning system. Mineiros do Tietê, São Paulo, Brazil.

According to Falcão et al. [52], monitoring the progression of berry ripening through variables such as soluble solids and titratable acidity is essential to characterize the chemical composition up to the point of physiological maturity. The primary criterion for determining the optimal harvest time is sugar content, measured in degrees Brix, which represents the total soluble solids—approximately 90% of which are sugars [56].

The decision on the harvest date is one of the most critical for grape growers, as it involves factors beyond physicochemical parameters, such as flavor, berry and seed integrity, sanitary status, and the intended wine style. Moreover, research has demonstrated that the timing of harvest can significantly influence the aromatic composition of wines. A study by Allamy et al. [57] highlighted that delaying harvest dates in Merlot and Cabernet-

Sauvignon grapes led to increased concentrations of compounds associated with cooked fruit aromas, thereby altering the wine's sensory profile. These findings underscore the importance of integrating sensory evaluations with traditional physicochemical analyses to make informed harvest decisions that align with the intended wine style.

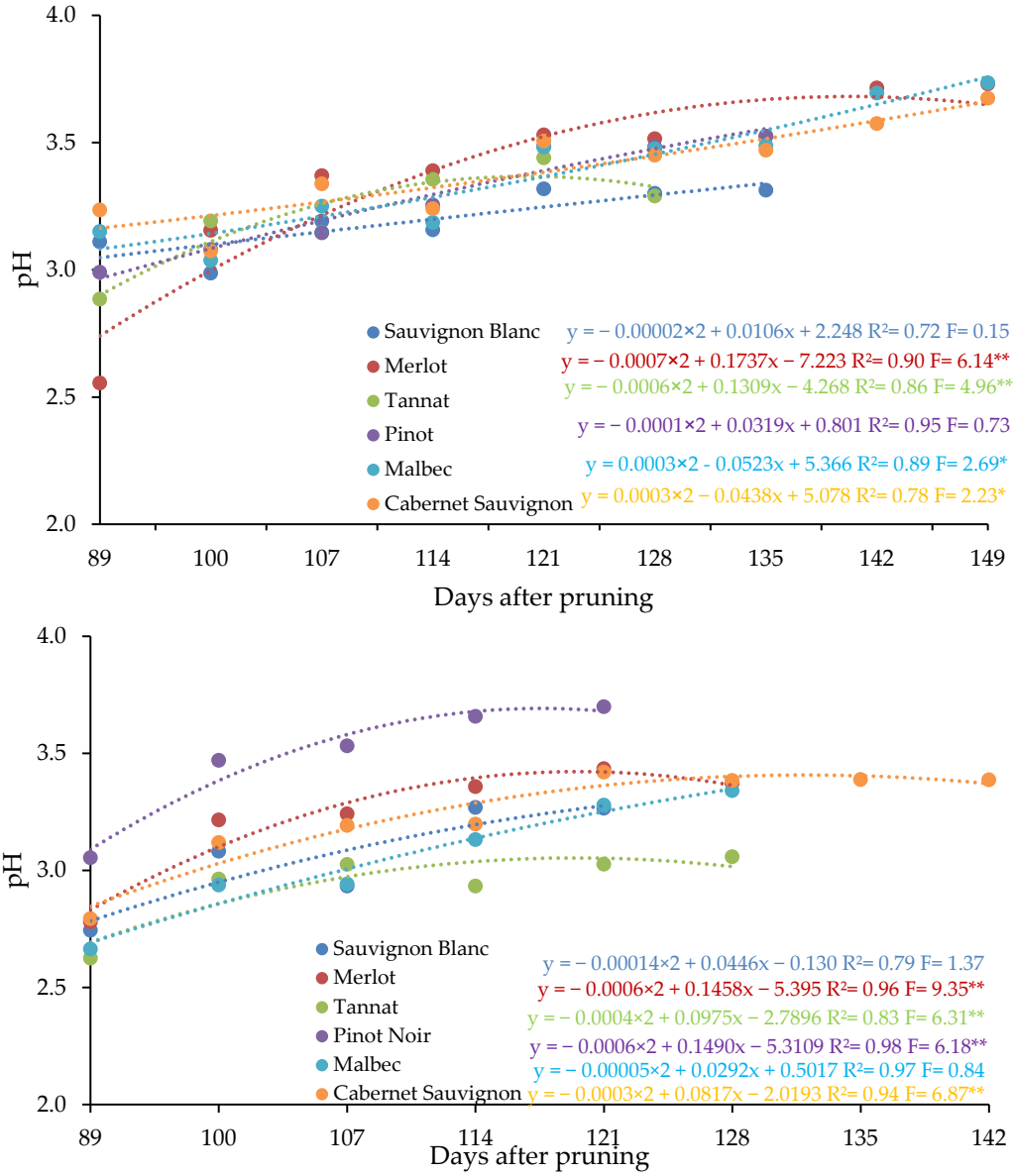


Figure 3. Evolution of must pH in wine grapes as a function of days after pruning in grapevines managed under the double-pruning system. Mineiros do Tietê, São Paulo, Brazil.

The cultivars 'Tannat', 'Pinot Noir', and 'Sauvignon Blanc' showed a quadratic increase in soluble solids over days after pruning, reflecting efficient sugar accumulation and favorable SS/TA ratios, which contribute to enological balance, similarly reported in previous studies [58,59]. For red wine production, Júnior et al. [60] recommend a minimum of 21 °Brix. Although °Brix is an indirect measure of sugars—since it also includes vitamins, phenolics, pectins, and organic acids [61], sugars still account for up to 90% of the total soluble solids.

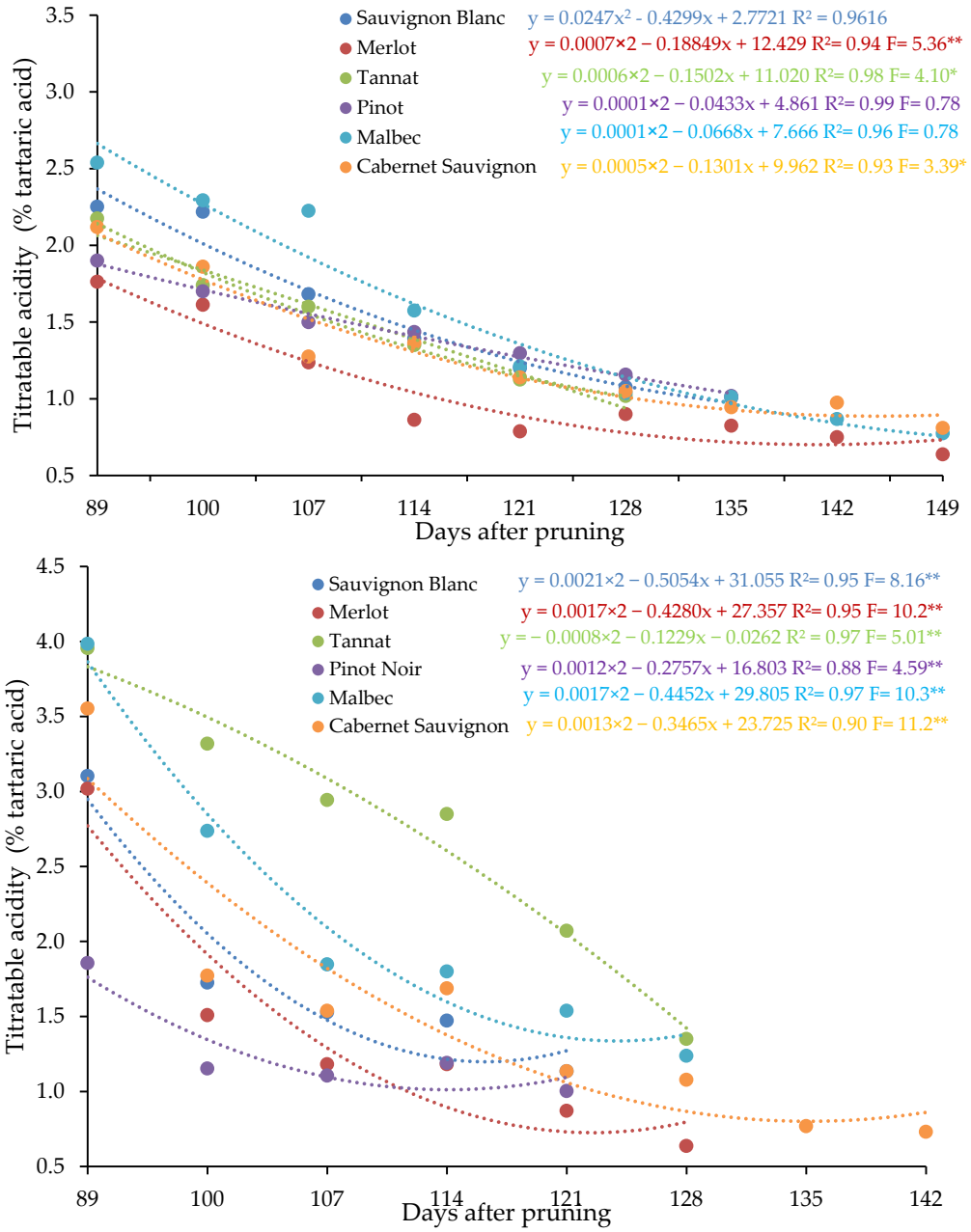


Figure 4. Evolution of titratable acidity in the must of wine grapes as a function of days after pruning in grapevines managed under the double-pruning system. Mineiros do Tietê, São Paulo, Brazil.

Harvesting for fine red wines should be performed at the peak of both technological and phenolic ripeness, with soluble solids ranging from 21 to 26 °Brix and titratable acidity between 5 and 6.5 g L^{-1} [61,62]. °Brix is also critical for estimating potential alcohol content, as higher values indicate greater fermentable sugar levels [63]. Harvest was performed when soluble solids and titratable acidity values stabilized between two consecutive samplings, as described by Falcão et al. [52]. The cultivars 'Tannat', 'Pinot Noir', and 'Sauvignon Blanc' reached satisfactory SS levels, with 'Tannat' standing out by reaching 23.4 °Brix at 128 DAP, indicating a high potential for producing wines with greater alcohol content and complexity (Figure 4).

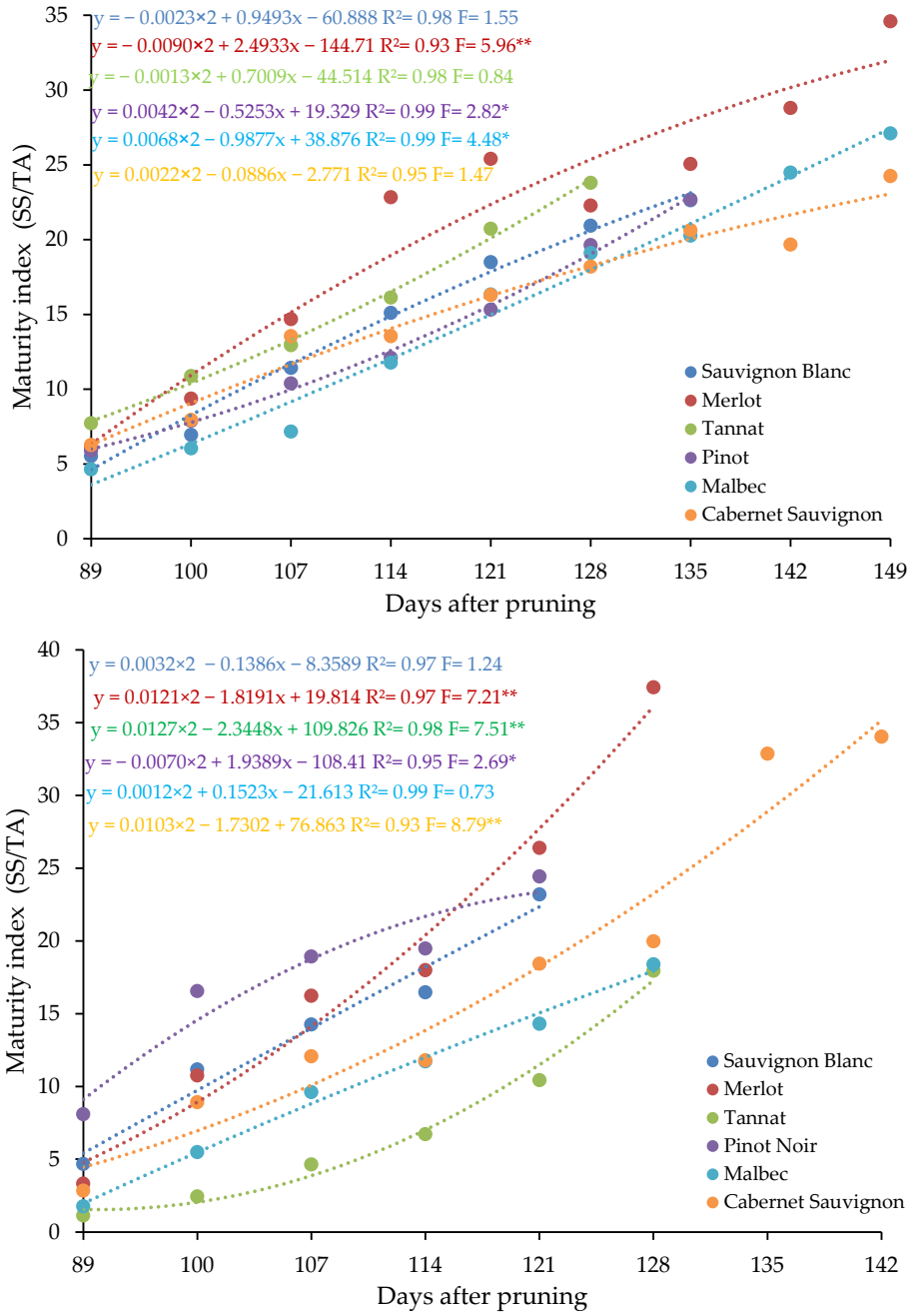


Figure 5. The evolution of the maturity index in the must of wine grapes as a function of days after pruning in grapevines managed under the double-pruning system. Mineiros do Tietê, São Paulo, Brazil.

3.6.2. pH

The analysis of pH evolution throughout the 2023 and 2024 production cycles revealed significant differences among cultivars. Statistical models fitted to the data indicated a linear trend for 'Sauvignon Blanc' and 'Pinot Noir', and a quadratic trend for 'Tannat', 'Malbec', 'Merlot', and 'Cabernet Sauvignon', reflecting distinct ripening dynamics.

In the 2023 cycle, pH values remained relatively stable, with 'Sauvignon Blanc' and 'Cabernet Sauvignon' standing out for their consistency. 'Tannat' exhibited a progressive increase in pH, indicating advanced ripening, while 'Pinot Noir' remained close to levels considered ideal for winemaking. In 2024, differences among cultivars became more pronounced. 'Cabernet Sauvignon' showed a greater increase in pH compared to the previous year, reflecting a higher accumulation of compounds associated with maturity. 'Merlot' and

'Malbec' also exhibited elevated pH values, while 'Sauvignon Blanc' maintained similar behavior to that observed in the previous cycle, demonstrating good consistency.

Quadratic pH behavior peaked at 128 days after pruning (DAP), ranging from 3.2 ('Tannat') to 3.7 ('Malbec'). 'Merlot' recorded the highest pH value at the end of ripening (3.72), indicating an advanced stage of maturity. The increase in pH, together with the reduction in titratable acidity, suggests optimal technological ripening, favoring wines with a smoother and more balanced sensory profile [54]. pH has a direct influence on wine quality, affecting microbial stability, color, and sensory characteristics Gerra and Pereira [8], reported that wines produced in southeastern Brazil from cultivars such as 'Cabernet Sauvignon', 'Malbec', and 'Merlot' usually present pH values between 3.2 and 3.5. Values between 3.2 and 3.4 are considered ideal, as they are associated with anthocyanin stability and color intensity [64].

During ripening, acidity reduction is mainly due to the degradation of malic acid via cellular respiration, which leads to an increase in pH [65,66]. Thus, double-pruning management under subtropical conditions proved effective in achieving pH levels suitable for quality wine production, ensuring a balance between freshness, acidity, and stability. The pH is an indicator directly related to berry acidity and showed significant variation among the evaluated cultivars, reaching its maximum at 128 days after pruning (DAP). Values ranged from 3.2 for 'Tannat' to 3.7 for 'Malbec' (Figure 5).

The evolution of pH throughout the ripening cycle provides relevant information regarding the degree of maturation and the acid–base characteristics of the must, being a decisive parameter for wine quality. pH affects flavor, microbial stability, and wine color, directly influencing the sensory profile [52]. Among the cultivars, 'Merlot' showed the greatest variation, reaching 3.72 at 142 DAP, indicating a sharp decrease in acidity and a more advanced stage of ripening. Elevated pH values are associated with lower perceived acidity, favoring wines with a smoother mouthfeel.

According to Guerra and Pereira [8], wines produced in southeastern Brazil from red cultivars such as 'Cabernet Sauvignon', 'Malbec', 'Merlot', 'Pinot Noir', and the white 'Sauvignon Blanc' generally present pH values ranging from 3.2 to 3.5, which supports the findings of the present study. Secondly, Rojas-Lara and Morrison [67], reported that pH tends to increase linearly during ripening, whereas total acidity decreases exponentially, mainly due to malic acid degradation. In environments with higher water availability, pH tends to be lower for a given level of acidity, highlighting the influence of environmental conditions on grape chemical composition.

3.6.3. Titratable Acidity (TA)

Titratable acidity (TA), expressed as tartaric acid equivalents, showed a quadratic decline throughout the ripening process in all cultivars. The highest acidity values were observed in 'Pinot Noir' and 'Tannat', which stood out compared to the other cultivars (Figure 4). This reduction in TA during ripening is essential for achieving a balanced wine taste. High acidity levels can result in wines with a more astringent mouthfeel, while controlled acidity contributes to freshness and sensory stability.

Cooler temperatures during the production cycle help preserve berry acidity, which in turn favors the retention of aroma compounds and enhances wine liveliness, as reported by Esteban et al. [68]. According to Brazilian regulations, acceptable acidity levels for fine wines range from 40 to 130 meq L^{-1}, with the ideal interval being between 55 and 130 meq L^{-1} [58]. The cultivars 'Pinot Noir' and 'Tannat' remained within this range, ensuring enological quality and an appropriate structure for wines with greater aging potential.

Titratable acidity (TA), expressed as tartaric acid equivalents, showed a declining trend during ripening across all evaluated cultivars, with a more pronounced reduction

during the final stages of the cycle. In contrast, soluble solids content increased as ripening progressed due to sugar accumulation. This inverse relationship between SS and TA was consistent across both production cycles (Figure 4).

The cultivars 'Pinot Noir' and 'Tannat' maintained the highest acidity levels throughout the period, reflecting their genetic characteristics and greater potential for producing premium wines with aging capacity. The combined evaluation of these variables is essential for determining the optimal harvest point, as technological maturity—necessary for fine wine production—is achieved when physicochemical parameters are balanced [69]. The acidity values observed are in accordance with Brazilian regulations for fine wines, which stipulate acceptable limits between 40 and 130 meq L^{-1}, with the ideal range between 55 and 130 meq L^{-1} [58]. The high acidity recorded in some cultivars may be attributed to cooler temperatures in high-altitude regions, which slow the degradation of organic acids such as malic acid [70].

Previous studies, such as that of Brighenti et al. [71], reported similar trends for the cultivar 'Sauvignon Blanc' in high-altitude regions of Brazil, reinforcing the present findings. Several factors contribute to the reduction in TA, including physiological, genetic, and environmental variables [72–74]. The decline in acidity is mainly attributed to the respiratory degradation of malic acid, dilution resulting from berry growth, and the salt formation of organic acids [75].

3.6.4. Maturity Index (SS/TA)

The maturity index (MI), calculated as the ratio between soluble solids content and titratable acidity (SS/TA), showed significant variation among cultivars, reaching values of 20.6 for 'Cabernet Sauvignon' and 28.8 for 'Merlot' at 142 days after pruning.

This indicator is widely used to assess the balance between sugar and acidity and is fundamental in defining the wine style to be produced. According to Ribéreau-Gayon [76], determining the ideal harvest time involves integrating technological (SS/TA), aromatic, and phenolic parameters to maximize enological potential.

The highest MI values observed in 'Merlot' indicate a favorable balance between sugars and acidity, supporting the production of wines with a smoother and rounder profile. In contrast, 'Cabernet Sauvignon' presented a lower MI, a feature that may impart greater freshness and structure to the wine—desirable attributes for age-worthy red wines.

According to Caceres-Mella [77], the ratio between soluble solids and titratable acidity (SS/TA) is widely used as an indicator of grape ripeness and enological quality and potential of varieties in experimental years. However, this index should be interpreted with caution, as the increase in SS does not always occur in inverse proportion to the reduction in TA. Nonetheless, the SS/TA ratio can serve as a reliable indicator of the sugar–acid balance for a given cultivar and region, especially when compared to reference vintages with established enological benchmarks. In this study, maturity index values at 142 days after pruning ranged from 20.6 ('Cabernet Sauvignon') to 28.8 ('Merlot') (Figure 5), highlighting significant differences among cultivars regarding the ideal harvest balance point.

3.7. Biochemical Composition and Antioxidant Potential of Grapes

The biochemical characterization of grapes—including total phenolics, flavonoids, anthocyanins, and antioxidant activity—is essential for determining wine sensory quality, stability, and aging potential. The cultivars 'Merlot' and 'Tannat' exhibited the highest contents of total phenolics and flavonoids in grape must (Table 4), indicating a higher antioxidant potential and, consequently, greater aging capacity and complexity. Phenolic compounds such as anthocyanins and proanthocyanidins are products of the grapevine's

secondary metabolism and are directly associated with the color, body, bitterness, and astringency of red wines, particularly under water deficit conditions [78].

Table 4. The bioactive compounds and antioxidant activity of wine grape cultivars grown under double-pruning management in a subtropical climate during the 2024 growing season, São Paulo, Brazil.

Cultivar	Bioactive Compounds and Antioxidant Activity				
	Phenols	Flavonoids	Anthocyanins	DPPH	FRAP
	(mg 100 g^{-1})			(µg Trolox g^{-1})	(mmol $FeSO_4$ g^{-1})
Sauvignon Blanc	101.3 c *	10.1 c	18.7 cd	12.2 a	3.49 a
Merlot	234.9 a	15.3 a	76.1 a	11.1 cd	3.34 cd
Tannat	216.6 a	14.7 a	64.6 ba	11.6 bc	3.41 bc
Pinot Noir	151.2 b	12.3 b	33.8 bc	11.7 ab	3.42 ab
Malbec	209.9 a	14.5 a	77.7 a	11.1 d	3.33 d
Cabernet Sauvignon	206.3 a	14.4 a	14.4 d	11.6 bcd	3.41 bcd
DMS	1.22	2.07	111.5	0.55	1.91
CV (%)	8.65	27.9	22.4	2.39	3.49
Mean	186.69	13.5	47.5	11.6	3.40

* Lowercase letters in rows differ significantly according to Tukey's test at a 5% probability level ($p > 0.05$). CV: coefficient of variation.

The double-pruning technique promotes ripening under ideal climatic conditions, enhancing the accumulation of polyphenols and antioxidants, which improves both the sensory and enological quality of wines [17]. This not only adds value to the final product but also enhances the health benefits associated with moderate wine consumption. The 'Merlot' cultivar, with the highest contents of phenolics and flavonoids, is highly promising for the production of age-worthy wines due to its oxidative stability and aromatic persistence. In contrast, 'Sauvignon Blanc', with lower levels of these compounds, is better suited for fresh, early-consumption wines. The high anthocyanin content observed in 'Malbec' and 'Merlot' contributes to the intense coloration of wines, a desirable trait in more robust products (Table 4).

In red wines, phenolic compound concentrations typically range from 1000 to 4000 mg L^{-1}, while in white wines they range from 200 to 300 mg L^{-1} [78]. The color of red wines is determined by multiple factors, including pre-fermentation conditions. The interaction between tannins and anthocyanins—especially malvidin-3-*O*-glucoside, the most abundant pigment in red grapes—plays a key role in defining color intensity and stability [79]. Antioxidant activity, assessed by DPPH and FRAP assays, confirmed the superior performance of 'Merlot' and 'Tannat' in terms of phenolic stability and suitability for producing complex wines. Interestingly, 'Sauvignon Blanc' and 'Pinot Noir' also showed high antioxidant activity regardless of the method used, with 'Pinot Noir' standing out in the DPPH assay. Despite having lower total phenolic content, these cultivars demonstrated excellent antioxidant performance, with similar average values.

It is worth noting that the methods employed yielded distinct sensitivities: the DPPH assay proved more responsive to the presence of antioxidant compounds than the FRAP method. Among all samples, 'Sauvignon Blanc' recorded the highest antioxidant potential, with performance comparable to red cultivars, despite its lower anthocyanin content—compounds that are exclusive to red grapes. The average antioxidant activity measured by DPPH across all cultivars was 11.6, underscoring the suitability of the evaluated grapes for high-quality wine production. The results demonstrate that, beyond their enological potential, these cultivars represent a viable and technically distinct alternative for sustainable wine production in subtropical regions.

A study conducted by Rockenbach et al. [80] evaluated the phenolic content and antioxidant activity of grape pomace from various *Vitis vinifera* L. cultivars widely produced in Brazil. The research focused on red grape varieties such as 'Cabernet Sauvignon', 'Merlot', and 'Bordeaux'. The findings revealed that 'Cabernet Sauvignon' grape pomace exhibited the highest total phenolic content, measuring 74.75 mg of gallic acid equivalents per gram. Correspondingly, this variety also demonstrated superior antioxidant activity, with DPPH and ABTS radical scavenging capacities of 505.52 and 485.42 μmol Trolox equivalents per gram, respectively. The FRAP assay further confirmed its potent antioxidant potential, recording a reducing power of 249.46 μmol Trolox equivalents per gram. These results underscore the significant role of grape variety in determining the phenolic composition and antioxidant capacity of grape by-products. The study highlights the potential of utilizing grape pomace, particularly from 'Cabernet Sauvignon', as a valuable source of natural antioxidants for food and nutraceutical applications.

4. Conclusions

The cultivars evaluated in this study exhibited distinct phenological, physicochemical, and productive performances under double pruning in a subtropical environment. Among them, 'Sauvignon Blanc', 'Merlot', 'Tannat', and 'Cabernet Sauvignon' stood out for their favorable agronomic behavior and fruit composition. The cultivation of 'Cabernet Sauvignon' and 'Sauvignon Blanc' is particularly recommended for winter wine production under double pruning, given their high yield potential and desirable technological attributes. Red cultivars, especially 'Tannat' and 'Merlot', demonstrated elevated phenolic content and antioxidant activity, reinforcing their value for premium red wine production. Double pruning proved to be an effective management strategy for enhancing grape quality in sub-tropical regions, although it requires precise technical execution throughout the production cycle. The results support informed decision-making for cultivar selection and vineyard planning, and they contribute to expanding viticulture into non-traditional areas of Brazil. Further studies are encouraged across multiple production cycles and locations to validate these findings, as well as to explore the enological potential of each cultivar and the long-term viability and economic impact of adopting the double pruning system.

This study demonstrates the relevance of double pruning as a viable strategy for optimizing grapevine performance and improving fruit quality under subtropical conditions. By shifting the production cycle to the winter season, the technique benefits from more favorable climatic conditions, contributing to the production of high-quality grapes. Given the limited research on Vitis vinifera cultivars managed under double pruning, especially in emerging terroirs such as the State of São Paulo, this work provides valuable insights into cultivar adaptability and productivity. Understanding these responses is essential for developing targeted management practices and strengthening the scientific foundation of winter viticulture. Such knowledge is crucial for advancing Brazil's position in the global wine industry and unlocking the potential of São Paulo as a competitive and distinctive wine-producing region.

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