

Article

Endodormancy Release in Two Table Grape Cultivars with Contrasting Chilling Requirements: Linking Phenological Modeling with Biochemical Characterization

Yanli Sun ¹, Qian Qiu ¹ , Min Zhou ¹, Yang Hu ¹, Yusui Lou ², Lei Wang ^{1,*} and Shiping Wang ^{1,*} 

¹ Department of Plant Science, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China; syal0224@sjtu.edu.cn (Y.S.); qq9421@sjtu.edu.cn (Q.Q.); zhoumin509@sjtu.edu.cn (M.Z.); huyang5120@126.com (Y.H.)

² Institute of Horticultural Research, Henan Academy of Agricultural Sciences, Zhengzhou 450002, China; yusui86@126.com

* Correspondence: leiwang2016@sjtu.edu.cn (L.W.); fruit@sjtu.edu.cn (S.W.); Tel.: +86-18801966338 (L.W.); +86-021-34205956 (S.W.)

Abstract

Accurate determination of endodormancy release is essential for grapevine dormancy management. However, most phenological models are validated only against macroscopic budbreak dates, without physiological verification of predicted release dates. Here, we integrated phenological modeling with biochemical profiling to characterize endodormancy release in two table grape cultivars with contrasting chilling requirements: ‘Muscat Hamburg’ (*Vitis vinifera* L.) and ‘Shine Muscat’ (*Vitis labrusca* × *V. vinifera*). Endodormancy release dates were determined by forced budbreak assays, and chilling and heat requirements were estimated from 5 min temperature records using the Dynamic Model and Growing Degree Hours. ‘Muscat Hamburg’ released endodormancy on December 16 (10.95 Chill Portions), whereas ‘Shine Muscat’ released on January 6 (22.78 CP). Around these dates, coordinated biochemical changes occurred in buds, including starch depletion, hexose accumulation, ABA decline, GA₃ increase, and redox-related changes in H₂O₂ content and CAT activity. These changes were more pronounced in buds than in canes and were not identical across all biochemical indicators. Hydrogen cyanamide treatment induced biochemical changes similar to those observed during natural dormancy release, with cultivar-specific responses consistent across both conditions. These results indicate that experimentally determined endodormancy release dates are associated with population-level physiological changes, supporting the integration of phenological modeling with biochemical characterization in table grape production.

Keywords: grapevine; endodormancy release; phenological modeling; carbohydrate metabolism; ABA-GA balance; hydrogen cyanamide; table grape



Academic Editor: Sergio Ruffo Roberto

Received: 9 May 2026

Revised: 1 July 2026

Accepted: 2 July 2026

Published: 4 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Bud dormancy is a critical evolutionary survival strategy developed by temperate perennial woody plants to adapt to adverse winter conditions. By suspending visible growth and lowering metabolic activity, plants can safely overwinter and resume growth when environmental conditions become favorable in spring [1–3]. In deciduous fruit trees such as grapevine (*Vitis vinifera* L.), dormancy progression is closely associated with seasonal dynamics of cold hardiness, which is gradually acquired in autumn and progressively lost prior to spring budbreak [4]. Classically, winter dormancy is divided into two

sequential phases: endodormancy, characterized by growth suppression due to internal physiological factors despite favorable conditions, and ecodormancy, where internal inhibition is lifted but buds remain inactive due to adverse external temperatures [3,5,6]. The transition from endodormancy to ecodormancy, followed by budbreak, is primarily regulated by the accumulation of winter chilling and subsequent forcing [7,8]. Consequently, temperature is the principal environmental factor influencing grapevine winter-spring phenology [9]. The importance of defining temperature thresholds for grapevine growth responses has also long been recognized [10].

Accurate prediction of dormancy progression is essential for enhancing agricultural methodologies, especially in the controlled cultivation of table grapes, and provides a quantitative framework for understanding plant responses to climate warming [11–13]. However, many traditional phenological models rely on a sequential approach, in which chilling and forcing accumulation are treated as two temporally distinct phases [14,15]. Although unified models have been proposed to describe their potential overlap [16], many applications continue to depend on arbitrarily established transition points between the two phases [13]. This structural limitation overlooks increasing evidence that the effective temperature ranges for chilling and forcing substantially overlap during mid-winter, allowing a specific temperature to concurrently facilitate both processes [17]. Consequently, these models are highly sensitive to shifting climatic conditions, where reduced autumn chilling and accelerated spring forcing may drive spring phenology in opposite directions [18,19].

Besides this conceptual limitation, current grapevine modeling studies exhibit three main empirical gaps. Firstly, research has predominantly concentrated on wine grape cultivars such as ‘Cabernet Sauvignon’ and ‘Chardonnay’ [8,20,21], leaving a dearth of quantitative models for economically important table grape cultivars. Secondly, previous grapevine modeling studies have typically relied on daily or interpolated hourly meteorological data [9,21]; while adequate for numerous applications, such coarse temporal resolutions may limit the ability to resolve the rapid temperature fluctuations that drive the two-step chill accumulation mechanism underlying the Dynamic model [22], particularly in the mid-winter period when chilling and forcing temperature ranges coincide. Finally, and most critically, current research typically uses the macroscopic “bud break date” as the sole validation endpoint [12,21], without parallel tracking of the underlying biochemical states of the bud [23,24]. Consequently, the models can precisely replicate reported budbreak dates, although they do not indicate if their anticipated endodormancy release periods correspond to the actual biochemical condition of the bud.

Recent studies indicate that endodormancy release is not a passive consequence of accumulated temperature exposure, but an active physiological reprogramming driven by carbohydrate mobilization, hormonal rebalancing, and reactive oxygen species (ROS) signaling [3,25]. Starch reserves accumulated throughout winter are hydrolyzed into soluble sugars to support growth and enhance osmoprotection [23,26]. Concurrently, the antagonistic balance between abscisic acid (ABA) and gibberellins (GAs) shifts dramatically, with ABA levels declining and bioactive GA signaling increasing as chilling requirements are satisfied [27,28]. Other hormones such as auxin (IAA) and salicylic acid (SA) may also contribute to this transition [29,30]. This hormonal shift is frequently triggered by hydrogen peroxide (H_2O_2), a key redox signaling molecule whose temporary buildup correlates with a reduction in catalase (CAT) activity, thereby oxidatively disrupting the ABA-GA equilibrium to commence the budbreak process [24]. Notably, the commercial dormancy-breaking agent hydrogen cyanamide (HC) operates via this mechanism—transiently inhibiting CAT and elevating H_2O_2 levels—thereby artificially replicating the biochemical cascade of natural endodormancy release in buds whose chilling requirement has not yet been satisfied [24,31]. Monitoring the dynamics of sugar, hormones, and reactive oxygen species

during natural dormancy development and contrasting them with the trajectories induced by HC treatment provides a direct method to evaluate whether model-predicted endodormancy release dates align with the actual biochemical transitions in the bud.

‘Shine Muscat’ (*Vitis labrusca* × *V. vinifera*), a high-chill-requirement cultivar, and ‘Muscat Hamburg’ (*V. vinifera* L.), a low-chill-requirement cultivar, are economically important table grapes in East Asia. Cultivating both cultivars under the same environment minimized environmental confounding and enabled direct comparison of dormancy progression. Here, we integrated phenological modeling based on 5 min temperature records with synchronous measurements of sugars, hormones, H₂O₂, and CAT activity, and used hydrogen cyanamide (HC) treatment as an experimental contrast to natural dormancy release. Our objectives were to (1) determine endodormancy release dates by forced budbreak assays and estimate cultivar-specific chill and heat requirements from the temperature data, (2) test whether the experimentally determined endodormancy release dates corresponded to major biochemical transition phases in grapevine buds, and (3) compare natural versus HC-induced dormancy release. The cultivar-specific chilling and forcing requirements determined here provide a preliminary quantitative baseline for ‘Shine Muscat’ and ‘Muscat Hamburg’ and may inform the timing of dormancy-breaking treatments such as HC application in commercial production.

2. Materials and Methods

2.1. Plant Materials and Experimental Site

Two table grape cultivars with contrasting chilling requirements were used in this study: ‘Muscat Hamburg’ (*Vitis vinifera* L.), a low-chill-requirement cultivar, and ‘Shine Muscat’ (*Vitis labrusca* × *V. vinifera*), a high-chill-requirement cultivar. Seven-year-old own-rooted (ungrafted) vines of both cultivars (30 vines per cultivar) were grown in an unheated glass greenhouse at Shanghai Jiao Tong University, Shanghai, China (31°11′ N, 121°29′ E). No specific clonal designation was available for the planting material. The vines were trained on a T-shaped trellis and planted in a 1:1:1 (*v/v/v*) mixture of loam, organic fertilizer, and perlite, with plants spaced 100 cm apart and rows spaced 200 cm apart. Two drip irrigation lines were installed along each row for nutrient solution delivery. Both cultivars were grown under identical environmental conditions to minimize confounding effects and allow cultivar-specific differences in dormancy progression to be assessed.

2.2. Temperature Monitoring

Field temperature was continuously recorded at 5 min intervals from October 2025 to April 2026 using S11A remote temperature and humidity data loggers (Xuzhou Fara Electronics, Xuzhou, China) installed at canopy height in the greenhouse. Daily mean, minimum, and maximum temperatures were calculated from the 5 min records. Hourly temperature data were subsequently aggregated for use in phenological modeling.

2.3. Forced Budbreak Assay

To determine the timing of endodormancy release, the forced budbreak assay was conducted following established approaches for evaluating the physiological status of grapevine latent buds, with minor modifications [6,32]. Twenty one-year-old canes per cultivar were randomly selected from 30 available vines at each of the 17 sampling dates (8 October 2025, to 4 February 2026, at 5–10 d intervals). From each cane, segments were collected from the 4th to the 13th nodes counted from the base and brought to the laboratory. They were divided into two subsamples: 60 cuttings for the forcing test (10 cuttings per replicate, six replicates) and the remaining 140 for biochemical analyses (see Section 2.5). For the forcing test, cane segments were trimmed into single-node cuttings of approximately

10 cm in length, with one bud retained, and the cut surface at the top was sealed with paraffin. The cuttings were placed upright in containers filled with tap water, with the basal end submerged approximately 4–5 cm, and in a growth chamber under forcing conditions (25/20 °C day/night, 16 h light/8 h dark, PPFD 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The water in the containers was replaced every 3 d. Bud break, defined as the green-tip stage with visible green tissue at the bud tip [33], was recorded at 7, 14, 21, and 28 d of forcing. The bud break rate was calculated as the percentage of broken buds relative to the total number of incubated buds. The day-21 bud break rate was used as the final indicator of dormancy status. Endodormancy release was defined as the first sampling date at which the bud break rate exceeded 50%. This threshold was used as an operational criterion and has been widely adopted in dormancy research [34].

2.4. Phenological Modeling

All phenological modeling was performed using hourly temperature data as inputs via the ‘chillR’ package in R (version 4.3.1). Chill accumulation was calculated using the Dynamic Model [20] and expressed as Chill Portions (CP). The chilling requirement (CR) for each cultivar was defined as the cumulative CP reached at its experimentally determined endodormancy release date. Heat accumulation was quantified as Growing Degree Hours (GDH) and calculated using the Asymmetric Curvilinear Model [35] implemented in ‘chillR’. The cardinal temperatures for the GDH model were set to the default parameters of 4 °C (base temperature, below which no effective heat accumulation occurs), 25 °C (optimum temperature, at which forcing effectiveness is maximal), and 36 °C (critical high temperature, above which the forcing contribution declines to zero). These parameters were originally established by Anderson et al. [35] and have been widely adopted in grapevine phenological modeling studies [8,12,21]. These default values were retained because cultivar-specific GDH parameterizations are not currently available for ‘Muscat Hamburg’ and ‘Shine Muscat’. For each cultivar, GDH accumulation was initiated from its respective endodormancy release date. The heat requirement (HR) was defined as the cumulative GDH from the endodormancy release date to the date on which 50% of the buds of the corresponding cultivar in the greenhouse had broken bud. Sensitivity analysis was performed to evaluate the reliability of the chilling requirement calculations. The bud break threshold used to define endodormancy release was varied from 40% to 63%. To evaluate the reliability of the chilling required calculations, the bud break threshold used to define endodormancy release was varied from 40% to 63%. For intermediate thresholds (e.g., 50% and 60%) that occurred between two consecutive empirical sampling dates, the release date was estimated by linear interpolation of the bud break rate between the two flanking sampling dates.

2.5. Sample Collection for Biochemical Analysis

As described in Section 2.3, samples remaining after the forced budbreak assay were used for biochemical analyses. Winter buds and approximately 1 cm of adjacent cane tissue above and below each bud node were sampled simultaneously. For each cultivar at each sampling date, approximately 140 buds and the associated cane segments were available for biochemical analyses. From these materials, approximately 80 buds were used for biochemical assays, while the remaining samples were retained as backup material. The 80 buds were divided before grinding into four composite subgroups, with each subgroup containing approximately 20 buds. This subdivision was necessary because the grinding procedure required paired tubes for balance. Cane segments from the same sampling pool were also divided into four composite subgroups and processed independently using the same grinding procedure. Each bud or cane subgroup was independently ground to a fine

powder in liquid nitrogen and stored at -80°C . Each independently processed subgroup was available as one composite biological replicate at the subgroup level. The number of composite biological replicates used differed among assays. Hormone analysis was performed using all four composite subgroups ($n = 4$). Soluble sugar, starch, H_2O_2 , and CAT analyses were performed using three composite subgroups ($n = 3$). For soluble sugar analysis, each composite replicate was measured in duplicate as technical replicates, and duplicate values were averaged before statistical analysis. Because each replicate consisted of pooled material from multiple buds or cane segments, the biochemical data represent composite-sample-level averages rather than single-bud or single-cane measurements.

2.6. Determination of Soluble Sugar and Starch Contents

Soluble sugar contents were determined following the extraction procedure of Moing et al. [36] with modifications. Freeze-dried samples were defatted with petroleum ether and subsequently extracted with 80% (v/v) ethanol at 65°C for 20 min. The extracts were concentrated by vacuum rotary evaporation until only the aqueous phase remained, adjusted to a final volume of 5 mL with ultrapure water, and purified using C18 solid-phase extraction (SPE) cartridges. Glucose, fructose, and sucrose were quantified by high-performance liquid chromatography (LC3000 Semi-preparative Isocratic HPLC System; CXTH, Beijing, China) equipped with a differential refractive index detector (KNAUER, Germany) and an amino column (250×4.6 mm, $5\text{ }\mu\text{m}$). The mobile phase was acetonitrile:water:concentrated ammonia (750:245:5, $v/v/v$), delivered at 1.0 mL/min at 30°C . The injection volume was 20 μL . Quantification was performed using external standard calibration curves prepared from authentic glucose, fructose, and sucrose standards. Stock solutions of the mixed standards were prepared at 5 mg/mL and serially diluted before analysis. For starch determination, the residue remaining after sugar extraction was gelatinized in a boiling water bath and hydrolyzed with 9.2 mol/L perchloric acid. Starch content was determined using the anthrone-sulfuric acid colorimetric method. Absorbance was measured at 630 nm, and concentrations were calculated from a standard curve.

2.7. Determination of Endogenous Hormone Contents

Endogenous hormones (ABA, GA_3 , IAA, and SA) were extracted using a modified organic solvent method adapted from Li et al. [37]. Freeze-dried samples were ground in liquid nitrogen and extracted with ethyl acetate. The supernatant was evaporated to dryness under a nitrogen stream, reconstituted in 50% methanol, and filtered through a 0.22 μm organic membrane prior to analysis. Hormones were separated and quantified by HPLC on an XBridge C18 column (250×4.6 mm, $5\text{ }\mu\text{m}$; Waters, Milford, MA, USA). The injection volume was 20 μL . The mobile phase consisted of (A) 0.1% formic acid in methanol and (B) 0.1% formic acid in water, with the following gradient program: 0–4 min, 20% A; 4–8 min, 20–50% A; 8–20 min, 50–80% A; 20–22 min, 80% A; 22–22.2 min, 80–20% A; and 22.2–30 min, 20% A. The flow rate was 0.8 mL/min, the column temperature was 25°C , and the detection wavelength was 254 nm. Quantification was performed using external standard calibration curves prepared from authentic standards of ABA, GA_3 , IAA, and SA; internal standards were not used. GA_3 was identified by comparison of retention time with an authentic GA_3 standard analyzed under the same chromatographic conditions. Method validation parameters for this HPLC-based hormone quantification method, including LOD and LOQ, were reported by Li et al. [37]. In the present study, the calibration curves showed good linearity for all four hormones, with R^2 values greater than 0.998 (Table S5).

2.8. Determination of H_2O_2 Content and CAT Activity

H_2O_2 content and CAT activity were determined using commercial micro-assay kits (Solarbio, Beijing, China) according to the manufacturer's instructions. H_2O_2 content was measured by colorimetry, and CAT activity was determined by the rate of H_2O_2 decomposition at 240 nm.

2.9. Hydrogen Cyanamide (HC) Treatment

To provide an experimental contrast to natural dormancy release, HC treatment was applied on 13 October 2024. At that time, vegetative growth had ceased, canes were fully lignified, and winter buds were tightly closed. Daily minimum temperatures remained above 10 °C, with minimal effective chilling accumulation; therefore, the buds were considered to be in an early dormant state before deep endodormancy. A split-vine design was used on T-shaped trellised vines to minimize inter-vine variability, with one cordon assigned to HC treatment and the opposite cordon serving as a control. Buds on the treated arm were swabbed with 2.5% (v/v) Dormex solution (50% active hydrogen cyanamide; Ningxia Darong Chemical Products Co., Ltd., Shizuishan, Ningxia, China) plus 2% (v/v) Tween 80 until runoff, while control buds were mock-treated with distilled water. No rainfall occurred within one week after application. Bud and cane samples were collected at 0, 5, 12, and 18 d after treatment, with sampling intervals adjusted according to bud break progression. At each time point, at least 200 buds per cultivar per treatment were collected. Samples were pooled by treatment, rapidly frozen in liquid nitrogen, homogenized, and used for downstream assays with three biological replicates per assay. Biochemical analyses followed Sections 2.5–2.8.

2.10. Statistical Analysis

All statistical analyses were performed using R (version 4.3.1). Because sampling was destructive, the same buds or canes were not measured repeatedly across dates. Biological replicates at each sampling date therefore represented independent sets of sampled material. First, temporal changes across the 17 sampling dates were analyzed for each biochemical indicator. Analyses were performed independently for each cultivar and tissue type. One-way analysis of variance (ANOVA) was used, followed by Tukey's honestly significant difference (HSD) test for multiple comparisons. The analysis was implemented using the 'tukey_hsd()' function from the 'rstatix package' (version 0.7.2). Although pairwise comparisons across all 17 sampling dates were computed, interpretation focused on comparisons with the experimentally determined endodormancy release date of each cultivar. The release dates were December 16 for 'Muscat Hamburg' and January 6 for 'Shine Muscat'. These comparisons were used to identify sampling dates showing significant biochemical differences relative to the release date. For soluble sugar data, shaded intervals were used to highlight periods around the release dates during which significant differences were observed. Second, inter-cultivar differences between 'Muscat Hamburg' and 'Shine Muscat' at each sampling date were assessed using Student's t-test. Tests were performed independently for each tissue type and biochemical indicator. The complete results of inter-cultivar comparisons are provided in Tables S2 and S3. For graphical clarity, significance levels were presented using a simplified two-tier notation: $p < 0.05$ and $p < 0.001$. In Figures 2–5, asterisks indicate significant comparisons for 'Muscat Hamburg', and open circles indicate significant comparisons for 'Shine Muscat'. For heatmap visualization, mean values of each biochemical indicator at each sampling date were standardized using Z-scores within each indicator and cultivar. All values are presented as means $\pm$ standard error (SE). The same statistical framework was applied to all biochemical indicators, including sugars, hormones, and oxidative stress markers.

3. Results

3.1. Temperature Dynamics and Sequential Endodormancy Release

Temperature dynamics during the overwintering period (October 2025 to April 2026) exhibited typical seasonal fluctuations, providing sufficient chilling for both grapevines (Figure 1a). Daily mean temperature declined from approximately 30 °C in early October to 5–10 °C during late December to early January, then gradually increased to about 20 °C by April. The diurnal temperature range was considerable, with daily maximum-to-minimum differences of up to 15–20 °C throughout the overwintering period.

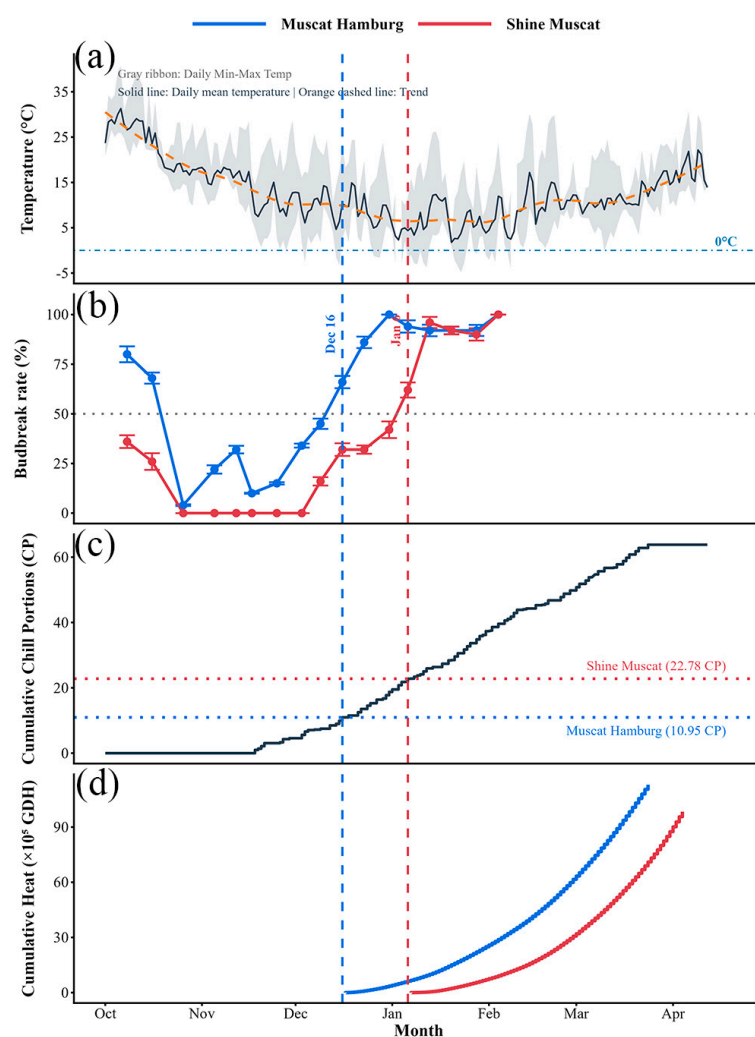


Figure 1. Temperature dynamics and endodormancy release in 'Muscat Hamburg' and 'Shine Muscat' grapevines. (a) Daily temperature fluctuations during the overwintering period (October 2025–April 2026). The gray ribbon represents the daily minimum–maximum temperature range, the solid line indicates the daily mean temperature, and the orange dashed line shows the LOESS-smoothed trend. The blue dash-dot line represents the 0 °C baseline. (b) Bud break rates of single-node cuttings under forcing conditions (25/20 °C, 16 h light/8 h dark). The gray horizontal dotted line marks the 50% bud break threshold used as the criterion for endodormancy release. Values are means $\pm$ SE. (c) Cumulative Chill Portions (CP) calculated using the Dynamic Model. The blue and red horizontal dotted lines indicate the chilling requirements for 'Muscat Hamburg' (10.95 CP) and 'Shine Muscat' (22.78 CP), respectively. (d) Cumulative Growing Degree Hours (GDH, $\times 10^5$; base temperature 4 °C). Heat accumulation for each cultivar was calculated based on its respective endodormancy release date. Vertical dashed lines across all panels indicate the experimentally determined endodormancy release dates for 'Muscat Hamburg' (blue) and 'Shine Muscat' (red).

Under forcing conditions, the progression of bud break revealed that the two cultivars differed markedly in the timing of endodormancy release (Figure 1b). ‘Muscat Hamburg’ exhibited an initial bud break rate (80%) in early October, which declined sharply to a minimum (4%) by early November suggesting that buds had entered a deep endodormant state by that time. Following continued chilling accumulation, the bud break rate recovered progressively and first exceeded 50% at the December 16 sampling date (observed rate: 63%). ‘Shine Muscat’ entered a deeper and more prolonged endodormancy, with bud break rates declining to near 0% by early November and remaining at this level until mid-December. The bud break rate did not exceed 50% until the January 6 sampling date (observed rate: 63%), roughly three weeks later than ‘Muscat Hamburg’.

Based on the Dynamic Model, the chilling requirements (CR) at the experimentally determined endodormancy release dates were quantified (Figure 1c). ‘Muscat Hamburg’ required a relatively low chill accumulation of 10.95 CP to break endodormancy, whereas ‘Shine Muscat’ required 22.78 CP, approximately 2.1-fold higher. Sensitivity analysis was conducted across alternative bud break thresholds (40%, 50%, and 60%) (Table S1). Although varying the threshold shifted the exact dates and CP values, ‘Shine Muscat’ consistently required greater chill accumulation than ‘Muscat Hamburg’. Based on the interval between the endodormancy release dates and the bud break dates observed under forcing conditions (‘Muscat Hamburg’: 23 March 2026; ‘Shine Muscat’: 31 March 2026), the heat requirements were determined to be 10,813 GDH for ‘Muscat Hamburg’ and 12,062 GDH for ‘Shine Muscat’ (Figure 1d). The relatively comparable heat requirement thresholds indicate that the cultivar difference in heat demand was substantially smaller than in chilling demand.

3.2. Dynamic Changes in Soluble Sugar Contents During Dormancy

The three soluble sugars showed distinct temporal patterns in both buds and canes, with marked changes occurring around the experimentally determined endodormancy release dates (Figure 2). In buds, glucose and fructose exhibited highly similar dynamics (Figure 2a,c). Both sugars remained at relatively low levels during early dormancy, declined to minima around mid-November, and then increased gradually from late November onward. In both cultivars, hexose accumulation became more apparent from December 23 and reached high levels around mid-January. In ‘Muscat Hamburg’, glucose and fructose increased sharply after the endodormancy release date (December 16), whereas in ‘Shine Muscat’, the increase was more gradual and reached lower absolute levels. Similar overall trends were observed in canes, although absolute levels were lower during early dormancy (Figure 2b,d).

Sucrose dynamics differed from those of glucose and fructose (Figure 2e,f). In buds, ‘Shine Muscat’ generally maintained higher sucrose levels than ‘Muscat Hamburg’ during much of the dormancy period ($p < 0.001$, Table S2). Both cultivars showed fluctuating sucrose levels during mid-dormancy. In ‘Muscat Hamburg’, sucrose declined after December 16, concurrent with the rapid increase in glucose and fructose, and then increased again from mid-January. In ‘Shine Muscat’, sucrose changed markedly from late December to January, but its timing did not consistently match the hexose accumulation pattern. In canes, sucrose in both cultivars declined to near-zero levels around their respective release periods (< 0.3 mg/g), followed by a rapid rebound, with ‘Shine Muscat’ reaching 8.13 mg/g by January 20 (Figure 2f).

Overall, glucose and fructose accumulation was closely associated with the endodormancy release period in both cultivars. In contrast, sucrose dynamics did not show a consistent temporal relationship with hexose accumulation. Sucrose declined concur-

rently with hexose accumulation in ‘Muscat Hamburg’, whereas in ‘Shine Muscat’, sucrose changes occurred before the main phase of hexose accumulation.

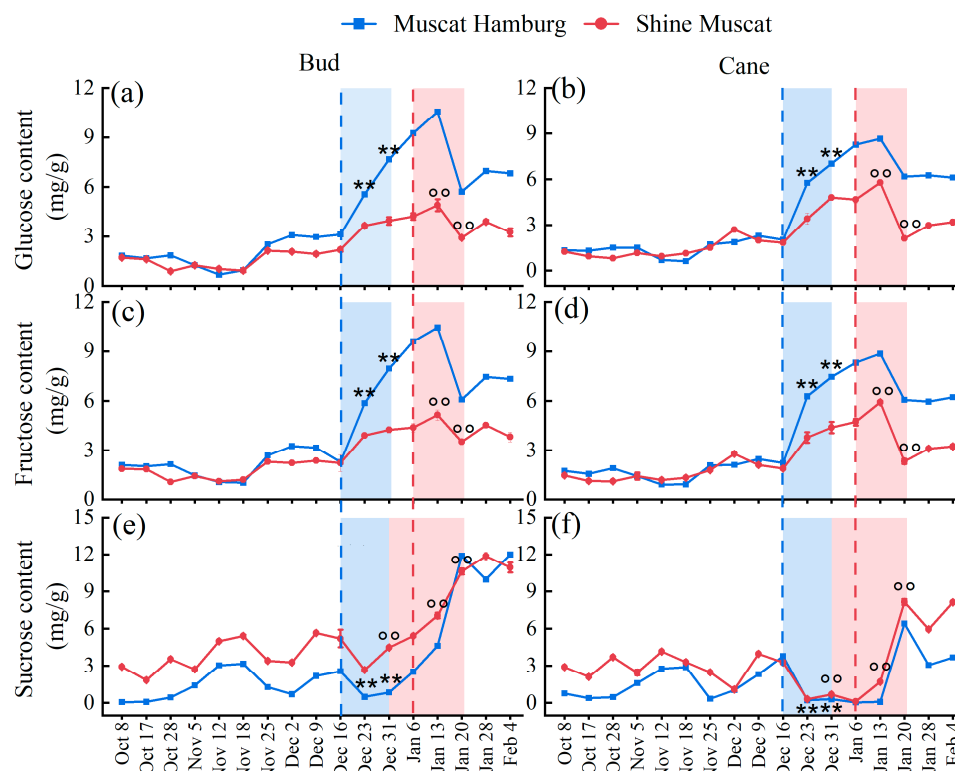


Figure 2. Dynamic changes in soluble sugar contents in buds and canes of ‘Muscat Hamburg’ and ‘Shine Muscat’ grapevines during dormancy (8 October 2025–4 February 2026). (a,b) Glucose, (c,d) fructose, and (e,f) sucrose contents in buds (left column) and canes (right column). Vertical dashed lines indicate the experimentally determined endodormancy release dates for ‘Muscat Hamburg’ (Dec 16, blue) and ‘Shine Muscat’ (Jan 6, red). The light blue and light red shaded bands indicate intervals around the experimentally determined endodormancy release dates during which significant soluble sugar changes were observed for ‘Muscat Hamburg’ and ‘Shine Muscat’, respectively. Asterisks indicate significant differences for ‘Muscat Hamburg’ relative to its release date (Dec 16) (* or °, $p < 0.05$; ** or °°, $p < 0.001$; Tukey’s HSD). Values are presented as means $\pm$ SE of three composite biological replicates ($n = 3$), each measured in duplicate; technical duplicates were averaged before statistical analysis.

3.3. Dynamic Changes in Starch Content During Dormancy

Starch content showed tissue-specific dynamics during dormancy and was generally inversely associated with the soluble sugar changes described above (Figure 3). In buds, both cultivars accumulated starch during early dormancy and showed marked depletion around their respective endodormancy release dates (Figure 3a, Table S2). In ‘Muscat Hamburg’, starch peaked around December 9 and then declined sharply at its release date on December 16 ($p < 0.001$), followed by partial recovery in mid-January. In ‘Shine Muscat’, starch reached a higher peak around December 16 and decreased significantly around its release date on January 6 ($p < 0.001$), stabilizing thereafter. These decreases coincided with the rapid accumulation of glucose and fructose described above, suggesting starch remobilization during endodormancy release. In canes, starch dynamics were less pronounced than in buds (Figure 3b). ‘Muscat Hamburg’ showed a gradual reduction post-release, while ‘Shine Muscat’ maintained relatively stable levels through most of dormancy, with a significant decrease detected in late January. Overall, starch depletion

was more pronounced in buds than in canes, consistent with the tissue-specific patterns of soluble sugar accumulation described above.

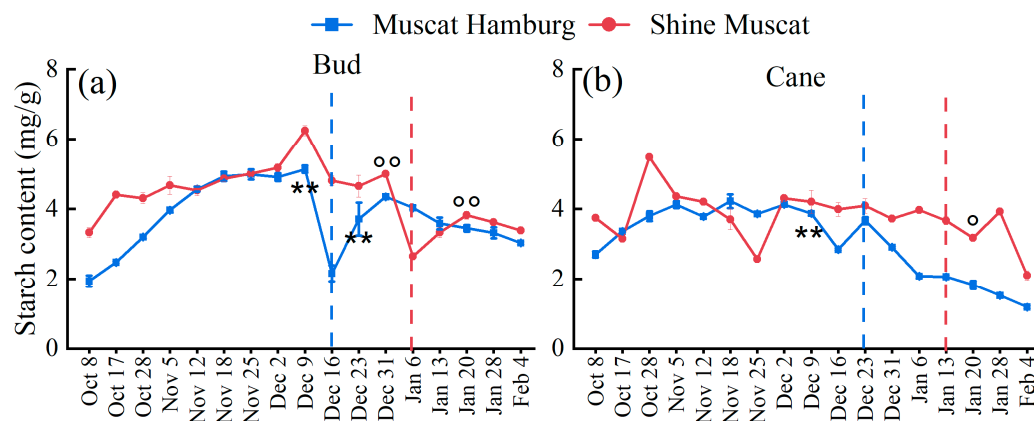


Figure 3. Dynamic changes in starch content in buds and canes of ‘Muscat Hamburg’ and ‘Shine Muscat’ grapevines during dormancy (8 October 2025–4 February 2026). Starch content in buds (a) and canes (b). Vertical dashed lines indicate the experimentally determined endodormancy release dates for ‘Muscat Hamburg’ (December 16, blue) and ‘Shine Muscat’ (January 6, red). Symbols indicate significant differences between each sampling date and the experimentally determined endodormancy release date within each cultivar. Asterisks indicate comparisons for ‘Muscat Hamburg’, and open circles indicate comparisons for ‘Shine Muscat’ (* or °, $p < 0.05$; ** or °°, $p < 0.001$; Tukey’s HSD). Values are presented as means $\pm$ SE ($n = 3$ composite biological replicates).

3.4. Dynamic Changes in Endogenous Hormone Contents During Dormancy

The four endogenous hormones showed distinct temporal patterns, with the most pronounced changes occurring in bud tissues around the experimentally determined endodormancy release dates (Figure 4). In buds, ‘Shine Muscat’ maintained higher ABA levels than ‘Muscat Hamburg’ throughout dormancy (Table S3, Figure 4a). In ‘Muscat Hamburg’, ABA fluctuated during early dormancy, peaking at 52.3 ng/g on Nov 25, and then declined to a minimum at the release date (Dec 16). In ‘Shine Muscat’, ABA declined gradually and reached its lowest level on Jan 6 and then increased rapidly thereafter. In canes, ABA levels were much lower in both cultivars, and only ‘Shine Muscat’ showed a significant late-season decline (Figure 4b).

GA dynamics revealed a substantial difference between cultivars in buds (Figure 4c). ‘Shine Muscat’ consistently showed higher GA₃ than ‘Muscat Hamburg’, with both cultivars reaching a peak on Dec 2. GA in ‘Muscat Hamburg’ declined around the release date, whereas in ‘Shine Muscat’ dropped to a minimum on Jan 6 and then increased sharply. In canes, GA₃ levels were markedly lower, and no significant temporal changes were detected around the release dates for either cultivar (Figure 4d).

In buds, IAA remained relatively stable throughout dormancy in both cultivars, with a significant post-release decline in ‘Muscat Hamburg’ but no significant change around the release date in ‘Shine Muscat’ (Figure 4e). In canes, IAA levels were lower overall, with a transient peak in ‘Shine Muscat’ followed by a sharp decrease (Figure 4f). SA in buds showed a general declining trend during dormancy in both cultivars, accompanied by a post-release increase in ‘Shine Muscat’, whereas ‘Muscat Hamburg’ showed no significant changes around the release date (Figure 4g). SA concentrations in canes remained very low throughout the sampling period (Figure 4h).

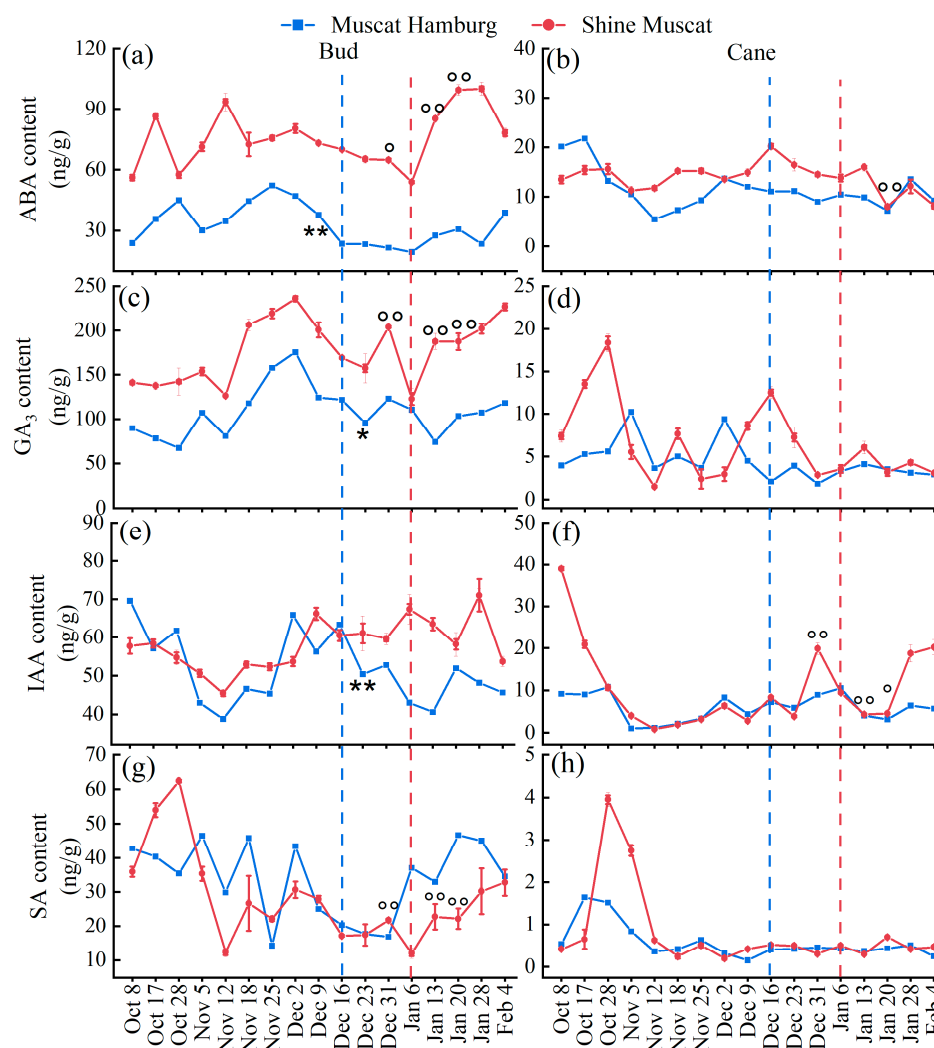


Figure 4. Dynamic changes in endogenous hormone contents in buds and canes of ‘Muscat Hamburg’ and ‘Shine Muscat’ grapevines during dormancy (8 October 2025–4 February 2026). (a,b) ABA, (c,d) GA₃, (e,f) IAA, and (g,h) SA contents in buds (left column) and canes (right column). Vertical dashed lines indicate the experimentally determined endodormancy release dates for ‘Muscat Hamburg’ (Dec 16, blue) and ‘Shine Muscat’ (Jan 6, red). Symbols indicate significant differences between each sampling date and the experimentally determined endodormancy release date within each cultivar. Asterisks indicate comparisons for ‘Muscat Hamburg’, and open circles indicate comparisons for ‘Shine Muscat’ (* or °, $p < 0.05$; ** or °°, $p < 0.001$; Tukey’s HSD). The complete results of inter-cultivar comparisons at each sampling date are shown in Table S3. Values are presented as means $\pm$ SE ($n = 4$).

3.5. Dynamic Changes in CAT Activity and H₂O₂ Content During Dormancy

In buds, CAT activity increased progressively from early October to early winter in both cultivars (Figure 5a). In ‘Muscat Hamburg’, CAT activity peaked sharply on December 9 and then declined significantly around its endodormancy release date (December 16). In ‘Shine Muscat’, CAT activity showed a similar overall increase but peaked later, reaching its highest values around December 31, followed by a decline before its release in early January. Inter-cultivar differences in bud CAT activity were evident at multiple sampling dates (Supplementary Table S4), with ‘Shine Muscat’ maintaining higher CAT activity than ‘Muscat Hamburg’ during much of the pre-release period and again after late December.

Bud H₂O₂ content also showed marked temporal changes (Figure 5c). In ‘Muscat Hamburg’, H₂O₂ content increased to a transient maximum on December 9 and declined thereafter. In ‘Shine Muscat’, H₂O₂ levels were generally higher throughout the sampling

period and reached their highest values between December 16 and December 23, before decreasing significantly by late December. Differences between cultivars were most evident from mid-December to early January (Table S4), when ‘Shine Muscat’ showed substantially higher H_2O_2 content than ‘Muscat Hamburg’.

In canes, both CAT activity and H_2O_2 content showed substantially weaker temporal variation than in buds (Figure 5b,d). Cane CAT activity fluctuated within a relatively narrow range and did not exhibit a clear peak during the release period. Cane H_2O_2 content generally declined from October to December in both cultivars, with a minor transient increase in ‘Shine Muscat’ around early January. Overall, redox-related changes were less pronounced in canes than in buds.

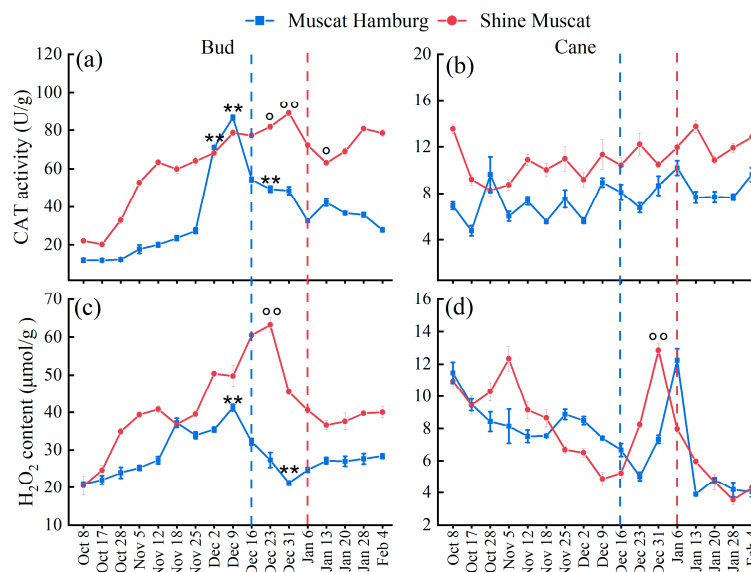


Figure 5. Dynamic changes in catalase (CAT) activity and hydrogen peroxide (H_2O_2) content in buds and canes of ‘Muscat Hamburg’ and ‘Shine Muscat’ grapevines during dormancy (8 October 2025–4 February 2026). (a,b) CAT activity and (c,d) H_2O_2 content in buds (left column) and canes (right column). Vertical dashed lines indicate the experimentally determined endodormancy release dates for ‘Muscat Hamburg’ (Dec 16, blue) and ‘Shine Muscat’ (Jan 6, red). Symbols indicate significant differences between each sampling date and the experimentally determined endodormancy release date within each cultivar. Asterisks indicate comparisons for ‘Muscat Hamburg’, and open circles indicate comparisons for ‘Shine Muscat’ (* or °, $p < 0.05$; ** or °°, $p < 0.001$; Tukey’s HSD). Values are presented as means $\pm$ SE ($n = 3$).

3.6. Biochemical Responses to Hydrogen Cyanamide Treatment

To further evaluate whether the biochemical transitions identified around the experimentally determined endodormancy release dates reflected physiologically meaningful processes, hydrogen cyanamide (HC) was applied as an independent dormancy-breaking agent, and the resulting biochemical changes were compared with those observed during natural dormancy release. HC treatment markedly accelerated bud break in both cultivars, although the response differed in timing and magnitude (Figure 6a,b). In ‘Muscat Hamburg’, bud break exceeded 50% by 12 d and reached approximately 92% by 18 d, whereas bud break in the control remained low throughout the experimental period. ‘Shine Muscat’ responded more gradually, with bud break remaining below 20% at 12 d and exceeding 50% only at 18 d.

The biochemical changes associated with HC-induced bud break generally paralleled those observed during natural endodormancy release. Starch content declined progressively in both cultivars under HC treatment, whereas the controls showed only limited variation over time. Significant decreases were detected from 12 d onward in ‘Muscat

Hamburg' and following a similar but slightly more gradual pattern in 'Shine Muscat' (Figure 6c,d). Concurrently, fructose accumulated significantly from 12 d in both cultivars, while little change was observed in the controls (Figure 6e,f), consistent with the carbohydrate transition observed around the natural release dates. Hormonal changes showed a similar pattern. ABA declined sharply under HC treatment, decreasing markedly by 12 d in 'Muscat Hamburg' and progressively through 18 d in 'Shine Muscat', while the controls exhibited relatively minor changes (Figure 6g,h). Conversely, GA₃ content increased significantly in both cultivars, rising rapidly and peaking at 12 d in 'Muscat Hamburg', and increasing more gradually but remaining elevated at 12 and 18 d in 'Shine Muscat' (Figure 6i,j). These changes resembled the ABA-GA shift observed during natural endodormancy release. Moreover, the cultivar-specific differences in HC response were consistent with their contrasting natural dormancy characteristics. 'Muscat Hamburg', which exhibited lower chilling requirements and earlier natural release, also showed faster and stronger biochemical responses to HC. 'Shine Muscat', with higher chilling requirements and later natural release, responded more gradually. Together, these results indicate that the biochemical transitions identified around the experimentally determined release dates reflect physiologically meaningful changes associated with endodormancy release.

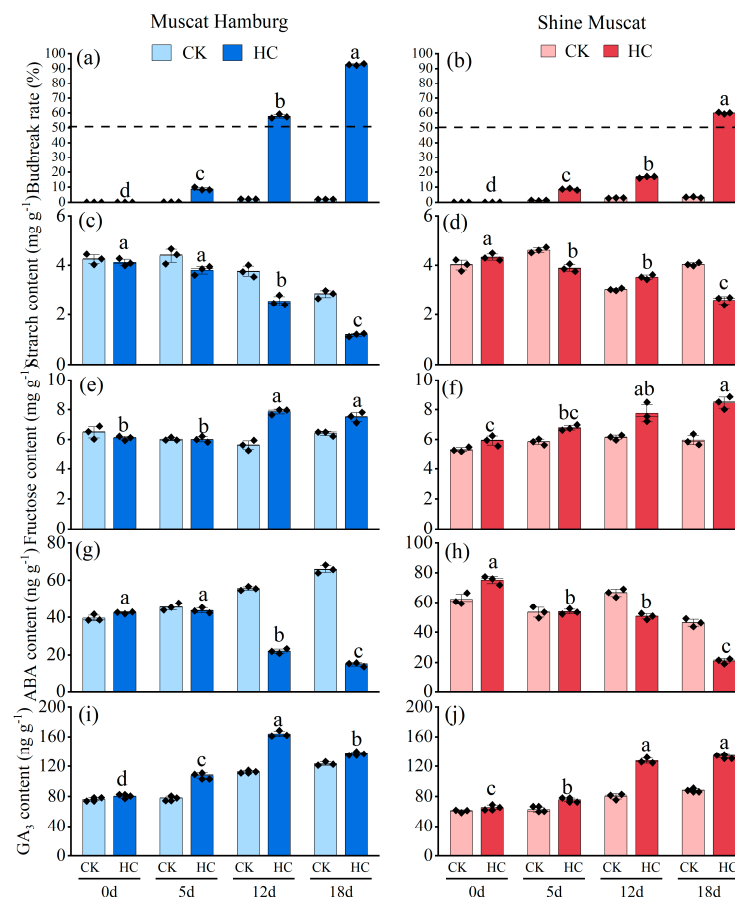


Figure 6. Effects of hydrogen cyanamide (HC) treatment on bud break rate and key biochemical markers in buds of 'Muscat Hamburg' and 'Shine Muscat'. Bud break rate (a,b), starch content (c,d), glucose content (e,f), ABA content (g,h), and GA₃ content (i,j) were measured at 0, 5, 12, and 18 days after treatment. The left column shows 'Muscat Hamburg' and the right column shows 'Shine Muscat'. Light-colored bars represent the distilled water control (CK), and dark-colored bars represent HC-treated buds. The dashed line in (a,b) indicates the 50% bud break threshold. Different lowercase letters denote significant differences among time points within the HC treatment group (Tukey's HSD, $p < 0.05$). Values are presented as means $\pm$ SE ($n = 3$), with individual biological replicates overlaid as diamonds.

3.7. Integrated Temporal Patterns of Biochemical Indicators During Natural Dormancy

To provide an integrated view of biochemical dynamics during dormancy progression, Z-score-normalized values of all measured indicators were visualized as a heatmap for each cultivar (Figure 7). In ‘Muscat Hamburg’, concurrent changes in multiple biochemical indicators were evident around the experimentally determined ED release date (December 16). Within carbon metabolism, starch remained relatively high during early dormancy and declined markedly from mid-December onward, whereas glucose and fructose stayed low during the early phase and increased sharply around and after the release date. Sucrose showed cultivar-dependent dynamics and did not consistently precede the main increase in glucose and fructose. Within hormone signaling, ABA was elevated during early to mid-dormancy and declined around the release date, whereas GA showed a reciprocal pattern, remaining low during deep dormancy and increasing from mid-December onward. In contrast, IAA and SA displayed more variable temporal profiles without a clear transition centered on the release date. Within redox metabolism, both H₂O₂ content and CAT activity also showed clear shifts around the release period, supporting the view that oxidative regulation formed part of the broader physiological transition.

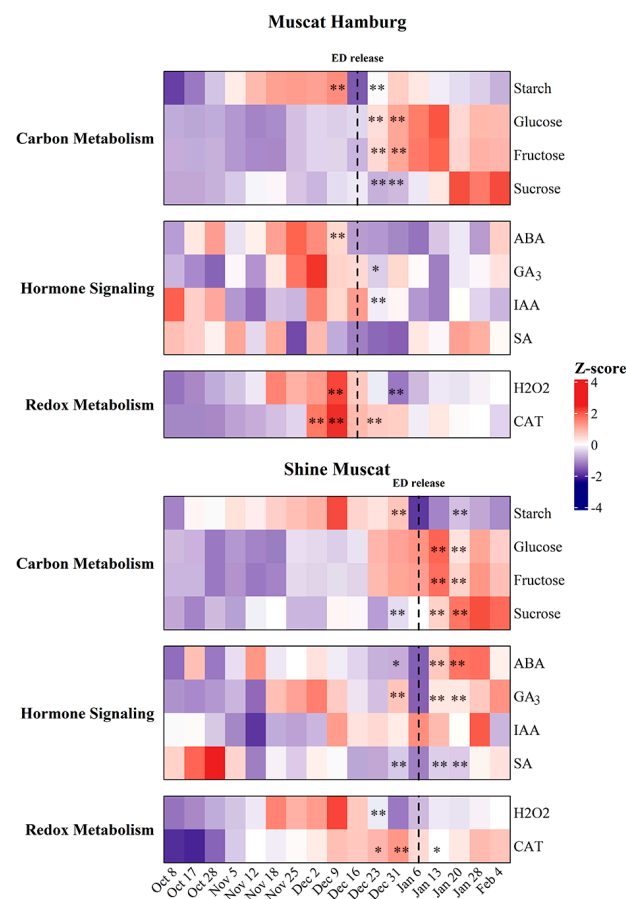


Figure 7. Heatmap of temporal dynamics of key biochemical indicators in buds during natural dormancy progression in ‘Muscat Hamburg’ and ‘Shine Muscat’. Indicators are grouped into carbon metabolism (starch, glucose, fructose, and sucrose), hormone signaling (ABA, GA, IAA, and SA), and redox signaling (H₂O₂ and CAT). Values were standardized within each indicator and cultivar and are presented as Z-scores. The dashed vertical line indicates the experimentally determined endodormancy (ED) release date for each cultivar (December 16 for ‘Muscat Hamburg’; January 6 for ‘Shine Muscat’). Red indicates values above the mean, and blue indicates values below the mean. Asterisks indicate significant differences between each sampling date and the experimentally determined endodormancy release date within each cultivar, based on the original non-normalized data (* $p < 0.05$; ** $p < 0.001$; Tukey’s HSD).

In ‘Shine Muscat’, the overall pattern was broadly similar but shifted later in time, consistent with its later ED release date (January 6). Starch remained relatively high through December and declined from late December into January, whereas glucose and fructose increased mainly around and after the release date. Sucrose also changed during this period, but its timing did not consistently match the glucose and fructose pattern. For hormone signaling, ABA remained elevated longer than in ‘Muscat Hamburg’ and declined from late December through January, while GA increased from late December onward. Some biochemical changes, particularly in ABA, were already detectable before the January 6 release date. Redox-related indicators showed a similar but later pattern, with elevated H_2O_2 and CAT levels extending into late December and early January, broadly consistent with the delayed timing of release in this cultivar.

Overall, both cultivars exhibited marked biochemical changes around their respective endodormancy release dates. The timing of these changes differed between cultivars. In ‘Muscat Hamburg’, changes were more closely clustered around the release date, whereas in ‘Shine Muscat’, several indicators changed over a broader period surrounding release. These patterns involved carbohydrate metabolism, hormone signaling, and redox-related responses. Together, the results indicate that the experimentally determined release dates were associated with coordinated but not identical biochemical changes across indicators and cultivars.

4. Discussion

4.1. Cultivar-Specific Chilling Requirements and the Value of High-Resolution Temperature Data

The forced bud break assay revealed a substantial difference in chilling requirement between ‘Muscat Hamburg’ and ‘Shine Muscat’ (10.95 vs. 22.78 CP), whereas their heat requirements were relatively similar (10,813 vs. 12,062 GDH, Figure 1c,d). This result indicates that the primary source of cultivar divergence in spring phenology lies in the endodormancy release phase rather than in the subsequent ecodormancy forcing phase, consistent with previous findings in other deciduous fruit species where chilling requirement varies more widely than heat requirement among cultivars [7,13]. Grapevine cultivars are also known to differ markedly in chilling requirements, yet quantitative estimates based on the Dynamic Model remain scarce for table grape cultivars [4,38]. The approximately 2.1-fold difference in CP between the two cultivars provides a useful baseline for these economically important cultivars. This distinction is particularly important under climate warming, because insufficient winter chill under warm winter conditions may first impair the completion of endodormancy release, thereby leading to irregular or delayed bud break and altered spring phenology [9,39,40]. However, these estimates are based on a single dormancy season and should be confirmed through multi-year observations under different climatic conditions.

‘Muscat Hamburg’ appeared to have entered a deep endodormant state by early November, as indicated by the decline in forced budbreak rate to a minimum at that time. For ‘Shine Muscat’, endodormancy appeared to be already established at or before the first sampling date in early October, as the forced budbreak rate was below 50% from the outset. The reliability of these estimates is further strengthened by the use of 5 min temperature records, which were subsequently aggregated to hourly data. This method, in contrast to the daily mean temperatures or interpolated hourly values commonly used in previous grapevine modeling studies [9,21], more effectively retains short-term thermal fluctuations during winter, which are relevant to chill accumulation in the Dynamic Model [22]. This is especially important under greenhouse conditions, where large diurnal temperature amplitudes may make the duration and sequence of temperature exposure more informative than coarse daily summaries. Baumgarten [41] experimentally showed that dormancy release was more strongly affected by chilling duration than by absolute

chilling temperature, and that effective chilling could occur across a broader temperature range (-2 to 10 °C) than traditionally assumed. Although we did not directly compare release date estimates across temporal resolutions, high-resolution temperature data likely reduced information loss and improved the reliability of linking release dates to subsequent biochemical transitions.

4.2. Biochemical Changes Associated with Endodormancy Release

The experimentally determined endodormancy release dates were accompanied by coordinated but not identical changes in carbohydrate metabolism, hormone balance, and redox status. These patterns indicate that dormancy release was associated with broader metabolic adjustment rather than a single simultaneous shift in all biochemical indicators. This interpretation is consistent with the view that bud dormancy is a dynamic and quantitative process. Cooke [42] proposed that dormancy in tree buds represents a continuum of states, with dormancy depth changing quantitatively in response to temperature conditions before, during, and after chilling. Baumgarten [41] showed that forcing requirement and budbreak percentage changed gradually with increasing chilling exposure, rather than exhibiting a sharp transition between endodormancy and ecodormancy. Similar gradual physiological changes have also been reported in apple, where bud weight and water content changed incrementally under forcing conditions [43]. However, because the present study was based on discrete sampling dates and composite tissues, these data should be interpreted as population-level biochemical patterns rather than as proof of a gradual transition within every individual bud. Asynchronous progression among individual buds within the sampled population therefore remains possible.

In terms of carbon metabolism, starch depletion and hexose accumulation were the most consistent carbohydrate changes associated with the endodormancy release period in both cultivars (Figures 2 and 3). Starch content declined around the experimentally determined release dates, particularly in buds. This decline was accompanied by subsequent increases in glucose and fructose. This pattern is consistent with reserve carbohydrate remobilization during dormancy progression and release. By contrast, sucrose showed more cultivar-dependent dynamics and did not consistently precede hexose accumulation. In ‘Muscat Hamburg’, sucrose declined after the release date, concurrent with the rapid increase in glucose and fructose. In ‘Shine Muscat’, sucrose changes occurred during the period surrounding release. However, its timing did not provide a consistent early signal relative to glucose and fructose. Overall, sucrose dynamics were less consistent than the starch–hexose pattern and were therefore interpreted separately.

The association between starch depletion, hexose accumulation, and dormancy progression is also supported by previous studies. Signorelli [44] reported that latent buds of *Vitis vinifera* cv. Cabernet Sauvignon showed low levels of hexoses and phosphorylated sugars during deep dormancy. In the same study, fructose exhibited a strong inverse correlation with dormancy depth. Similarly, in our study, glucose and fructose remained at low levels during deep endodormancy and increased around the release period (Figure 2a,c). Similar starch dynamics have also been reported in ‘Muscat de Hamburg’, where cane starch content was highest at dormancy onset in November and declined during winter [45]. Together, these observations support an association between carbohydrate remobilization and grapevine dormancy progression. In this study, starch depletion and hexose accumulation represented the most consistent carbohydrate changes observed around endodormancy release.

Hormonal changes also showed marked temporal shifts around the release period in both cultivars, with ABA declining in both cultivars and GA₃ showing cultivar-specific dynamics (Figure 4a,c). Previous studies have shown that ABA plays a central role in

dormancy establishment and maintenance. In grapevine, its endogenous levels increase markedly at the onset of dormancy and then decline gradually toward dormancy release [28], with similar trends reported in other deciduous fruit species such as peach [46], pear [47], and sweet cherry [48]. In grapevine, Noriega [49] showed that bud release from endodormancy was accompanied by the downregulation of ABA biosynthesis genes, including *VvNCED1*. This suggests that ABA decline during dormancy release may involve reduced ABA biosynthesis in addition to enhanced catabolism. GA accumulation is generally linked to growth resumption and bud activation, and chilling-induced GA biosynthesis has been implicated in restoring symplastic connectivity during bud break in poplar [27]. However, the temporal dynamics of GA did not fully conform to this generalized framework in both cultivars. In ‘Muscat Hamburg’, GA₃ content peaked on December 2, approximately two weeks before the endodormancy release date, and declined around the release period (Figure 4c). In ‘Shine Muscat’, GA showed a decrease prior to the release date followed by an increase after January 6. These results suggest that GA dynamics may exhibit cultivar-specific temporal patterns during dormancy progression. One possible explanation is that GA accumulation represents a transient regulatory phase during the transition toward dormancy release. Similar variability in GA responses during dormancy has also been reported in other woody species [50]. Overall, while ABA decline was a consistent feature of the release period in both cultivars, GA dynamics were more variable, suggesting that the ABA-GA framework may require cultivar-specific interpretation. Redox-related indicators also showed marked temporal changes around the release period (Figure 5), although the observed patterns were more complex than the classical CAT-inhibition/H₂O₂-accumulation model. In the classical model, CAT inhibition leads to H₂O₂ accumulation, which triggers downstream signaling for dormancy release [24]. However, in the present study, both CAT activity and H₂O₂ content increased concurrently during the pre-release period and declined thereafter. This simultaneous increase suggests that CAT upregulation may represent a compensatory antioxidant response to elevated H₂O₂ production. This pattern differs from the simple inverse relationship typically described between CAT activity and H₂O₂ content. Beauvieux et al. [25] proposed that ROS dynamics during dormancy are regulated by the balance between ROS production and scavenging. The concurrent increases in CAT activity and H₂O₂ content observed here extend this concept to natural endodormancy release in table grape buds, suggesting that redox regulation during natural release involves a more complex adjustment than the acute CAT inhibition induced by HC treatment.

By contrast, IAA and SA did not show consistent changes around the experimentally determined release dates (Figure 4e,g). This suggests that their association with endodormancy release was less consistent than that of ABA and GA₃ in this study. The two cultivars also differed in the timing and magnitude of biochemical changes. In ‘Muscat Hamburg’, most changes were more closely clustered around December 16, whereas in ‘Shine Muscat’, several indicators changed over a broader period surrounding January 6 (Figure 7). This pattern suggests that the difference between the two cultivars was not only a matter of earlier versus later release date, but also reflected cultivar-dependent differences in the timing of release-associated biochemical changes. Similar cultivar-dependent differences in the timing of dormancy-related processes have been reported in other fruit species, including sweet cherry [51] and apple [43]. The gradual decline of ABA around the release period is consistent with previous reports showing that ABA levels decrease progressively during late dormancy in grapevine and other fruit species [45,46].

These results suggest that a single forced-budbreak threshold provides a useful operational criterion for identifying endodormancy release, but may not capture all associated biochemical changes. Signorelli [44] showed that metabolic markers, including sugar profiles, can differentiate dormancy states and may serve as proxies for dormancy status. A similar metabolite-based approach has also been reported in sweet cherry [51], where specific compounds helped distinguish dormancy phases beyond phenological observation alone. Our results are consistent with these studies in showing that biochemical profiling can complement forced budbreak assays. Integrating forced budbreak assays with multi-indicator biochemical characterization may therefore improve the physiological interpretation of dormancy progression in grapevine.

4.3. Tissue-Specific Responses: Buds Versus Canes

The biochemical changes associated with endodormancy release were consistently more pronounced in buds than in canes. Starch depletion, hexose accumulation, and hormonal shifts were all more clearly expressed in bud tissue, while cane responses were generally attenuated and, in some cases, lacked significant temporal changes around the release dates (Figures 2–5). This tissue specificity is consistent with the understanding that buds are the primary site of dormancy regulation [41]. This aligns with prior studies indicating that carbohydrate partitioning between buds and adjacent tissues differs markedly during the dormancy period [52]. Recent studies in grapevine further support the view that dormancy status is regulated mainly at the bud level. Velappan [53] showed that grapevine latent buds can remain dormant despite relatively high metabolic activity, indicating that dormancy status is not simply a passive reflection of overall metabolic activity in surrounding tissues. De Rosa [54] further demonstrated that winter sugar dynamics in grapevine buds, including hexose and raffinose accumulation, were closely linked to both dormancy progression and freezing tolerance. Collectively, these studies support the view that dormancy progression is closely linked to bud-level metabolic regulation. Our findings further show that these alterations were more pronounced in buds than in adjacent cane tissues.

4.4. HC Treatment as Supporting Evidence for Natural Dormancy Release Patterns

HC treatment was used here to provide supporting evidence for the biochemical patterns identified during natural dormancy release. HC-treated buds showed starch depletion, hexose accumulation, ABA decline, and GA elevation, closely paralleling the biochemical signature observed during natural dormancy release (Figure 6). HC-induced dormancy release in both cultivars was accompanied by ROS-mediated signaling and ABA reprogramming, consistent with findings reported by Pérez [24], Ophir [55], Zhang [56]. Although HC was applied before deep endodormancy, the consistency between HC-induced and naturally occurring changes indicates that the biochemical patterns identified around the release dates are physiologically meaningful. Moreover, cultivar-specific differences in HC response paralleled those observed under natural conditions. ‘Muscat Hamburg’ responded faster and more strongly, whereas ‘Shine Muscat’ responded more gradually, consistent with their contrasting chilling requirements and natural release timing (Figure 6a). The consistency between natural and HC-induced release suggests that the experimentally determined release dates reflect real physiological transitions rather than simply operational thresholds. It should be noted that the HC experiment was conducted in the 2024–2025 season, whereas the natural dormancy observations were performed in the 2025–2026 season. Differences in autumn and winter temperatures between the two years may have affected the initial dormancy depth and the rate of biochemical responses to HC treatment. Nevertheless,

HC-treated buds in both cultivars showed directional changes similar to those observed during natural dormancy release, including starch depletion, hexose accumulation, ABA decline, and GA increase. In addition, ‘Muscat Hamburg’ responded faster than ‘Shine Muscat’ under both conditions, consistent with its lower chilling requirement. These results provide supporting evidence for the physiological association between the identified biochemical transitions and dormancy release, although direct causal interpretation is limited by the cross-season comparison.

4.5. Integrating Phenological Modeling with Biochemical Validation

Many current phenological models for grapevine are validated against observed budbreak dates or other macroscopic phenological stages [8,12,21]. Recent modeling frameworks such as PhenoFlex [57], which has also been applied to grapevine [58], represent important advances in dormancy prediction, yet they still rely on macroscopic budbreak as their calibration endpoint. Although budbreak is a convenient and clearly identifiable endpoint, it occurs well after endodormancy release and reflects the combined outcome of chilling and forcing. As a result, a model may predict budbreak date reasonably well while still misestimating the timing of endodormancy release. This limitation highlights the need for validation approaches that extend beyond macroscopic phenology alone. Our study provides such an approach by combining experimentally determined release dates from forced budbreak assays with independent biochemical characterization within the bud. Carbohydrate, hormonal, and redox changes all occurred around the release dates in both cultivars. Combined with the supporting evidence from HC treatment, these results indicate that the experimentally determined dates have clear physiological significance. Similar metabolite-based validation strategies have also been proposed in other woody species, including sweet cherry, where specific metabolites were shown to help identify endodormancy release and subsequent developmental transitions [51]. In grapevine, metabolic markers such as sugar profiles have likewise been suggested as indicators of dormancy state [44]. Our results extend this idea by showing that these biochemical transitions can be temporally linked to experimentally determined release dates.

This approach is particularly relevant under climate change. As winter temperatures rise, it will become increasingly important to estimate chilling fulfillment and endodormancy release timing more accurately [9]. Starch depletion, hexose accumulation, and ABA decline identified in this study could serve as physiological reference points for evaluating model-predicted release dates. In practice, such markers may help refine the physiological interpretation of phenological model outputs by indicating whether predicted release dates coincide with release-associated biochemical changes in buds. This information could support management decisions in table grape production, including the timing of hydrogen cyanamide application, initiation of forcing practices after sufficient dormancy release, and cultivar-specific production scheduling. Overall, integrating phenological modeling with biochemical characterization may improve the physiological interpretation of dormancy progression in grapevine. A schematic summary of these coordinated physiological changes is presented in Figure 8.

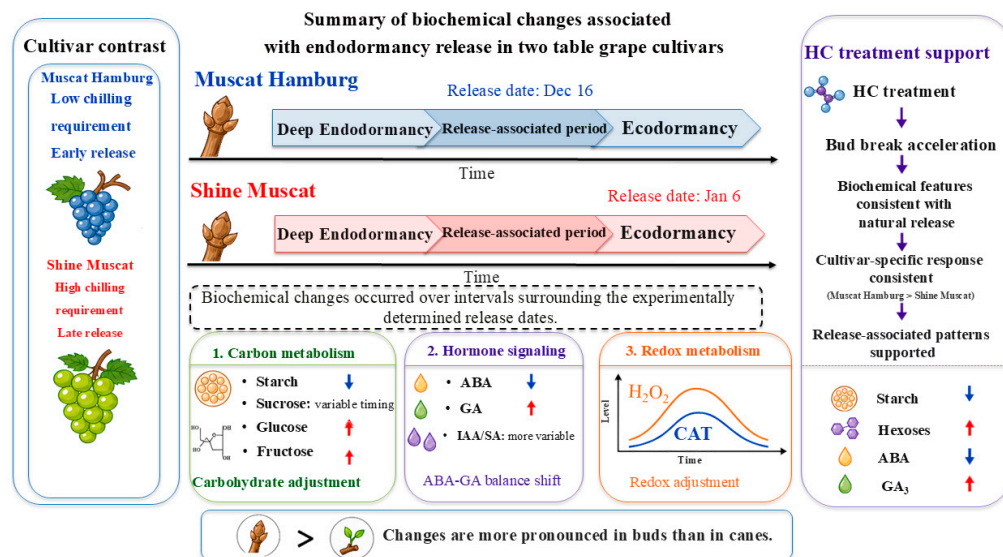


Figure 8. Summary of biochemical changes associated with endodormancy release in two table grape cultivars. The upper section compares dormancy progression in ‘Muscat Hamburg’ and ‘Shine Muscat’. ‘Muscat Hamburg’ had a lower chilling requirement and an earlier release date (December 16), whereas ‘Shine Muscat’ had a higher chilling requirement and a later release date (January 6). The diagram illustrates a release-associated period between deep endodormancy and ecodormancy. The central panel summarizes three major categories of biochemical changes observed around the release period. Carbon metabolism was characterized by starch depletion, glucose and fructose accumulation, and variable sucrose timing. Hormone signaling was characterized by ABA decline and GA₃ increase, with IAA and SA showing more variable patterns. Redox metabolism was characterized by transient changes in H₂O₂ content and CAT activity. The right panel shows that hydrogen cyanamide (HC) treatment induced biochemical changes directionally consistent with natural dormancy release. Cultivar-specific response patterns under HC treatment were also consistent with those observed under natural conditions. These results support the physiological relevance of the observed release-associated patterns. The bottom panel indicates that biochemical changes were more pronounced in buds than in canes.

5. Conclusions

This study showed that experimentally determined endodormancy release dates in grapevine were associated with coordinated biochemical changes in carbohydrate metabolism, hormone balance, and redox status. These changes were not identical across all indicators, suggesting broader metabolic adjustment at the population level. In both cultivars, starch depletion and glucose/fructose accumulation were the most consistent carbohydrate changes around the release period, whereas sucrose showed more cultivar-dependent dynamics. The two cultivars differed markedly in chilling requirement and in the timing and magnitude of biochemical changes. These changes were generally more pronounced in buds than in canes. HC treatment induced directionally consistent biochemical responses, supporting the physiological relevance of the observed release-associated changes. Because the natural dormancy observations were conducted over a single season and based on composite samples, multi-year studies with finer-scale sampling are needed to assess the generalizability of these patterns. These findings support the integration of phenological modeling with biochemical characterization for dormancy research and management in table grape production.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12070819/s1>, Table S1: Sensitivity of estimated chilling requirements to the bud burst threshold used to define endodormancy release; Table S2:

Significance of cultivar differences in soluble sugar and starch contents between ‘Muscat Hamburg’ and ‘Shine Muscat’ at each sampling date; Table S3: Significance of cultivar differences in endogenous hormone contents in buds and shoots between ‘Muscat Hamburg’ and ‘Shine Muscat’ at each sampling date; Table S4: Significance of cultivar differences in CAT activity and H₂O₂ content in buds and shoots between ‘Muscat Hamburg’ and ‘Shine Muscat’ at each sampling date. Table S5: External standard calibration parameters for endogenous hormone quantification by HPLC.

Author Contributions: Conceptualization, Y.S. and S.W.; methodology, Y.S.; software, Y.S.; validation, Y.S., Q.Q., and M.Z.; formal analysis, Y.S.; investigation, Y.S.; resources, L.W.; data curation, Y.S.; writing—original draft preparation, Y.S.; writing—review and editing, L.W. and Y.L.; visualization, Y.S.; supervision, Y.H.; project administration, S.W.; funding acquisition, L.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Shanghai Agricultural Science and Technology Innovation Project (Grant No. Hu Nong Ke [A2025012]), the Fujian Province Science and Technology Plan Project (Grant No. 2024S0051), the Ningbo Science and Technology Development Special Fund (Grant No. 2024S018), and the China Agriculture Research System (Grant No. CARS-29-zp-7).

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Acknowledgments: During the preparation of this manuscript, the authors used ChatGPT for the purposes of language editing and polishing. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Lang, G.A.; Early, J.D.; Martin, G.C.; Darnell, R.L. Endo-, para-, and ecodormancy: Physiological terminology and classification for dormancy research. *HortScience* **1987**, *22*, 371–377. [[CrossRef](#)]
- Rohde, A.; Bhalerao, R.P. Plant dormancy in the perennial context. *Trends Plant Sci.* **2007**, *12*, 217–223. [[CrossRef](#)] [[PubMed](#)]
- Considine, M.J.; Considine, J.A. On the language and physiology of dormancy and quiescence in plants. *J. Exp. Bot.* **2016**, *67*, 3189–3203. [[CrossRef](#)] [[PubMed](#)]
- Kovaleski, A.P. Woody species do not differ in dormancy progression: Differences in time to budbreak due to forcing and cold hardiness. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2112250119. [[CrossRef](#)] [[PubMed](#)]
- Arora, R.; Rowland, L.J.; Tanino, K. Induction and release of bud dormancy in woody perennials: A science comes of age. *HortScience* **2003**, *38*, 911–921. [[CrossRef](#)]
- Pouget, R. Considérations générales sur le rythme végétatif et la dormance des bourgeons de la vigne. *Vitis* **1972**, *11*, 198–217.
- Benmoussa, H.; Ghrab, M.; Ben Mimoun, M.; Luedeling, E. Chilling and heat requirements for local and foreign almond (*Prunus dulcis* Mill.) cultivars in a warm Mediterranean location based on 30 years of phenology records. *Agric. For. Meteorol.* **2017**, *239*, 34–46. [[CrossRef](#)]
- García de Cortázar-Atauri, I.; Brisson, N.; Gaudillere, J.P. Performance of several models for predicting budburst date of grapevine (*Vitis vinifera* L.). *Int. J. Biometeorol.* **2009**, *53*, 317–326. [[CrossRef](#)] [[PubMed](#)]
- Luedeling, E. Climate change impacts on winter chill for temperate fruit and nut production: A review. *Sci. Hortic.* **2012**, *144*, 218–229. [[CrossRef](#)]
- Pouget, R. Nouvelle conception du seuil de croissance chez la vigne. *Vitis* **1968**, *7*, 201–205.
- Swanepoel, J.; De Villiers, F.; Pouget, R. Predicting the date of bud burst in grapevines. *S. Afr. J. Enol. Vitic.* **1990**, *11*, 46–49.
- Zapata, D.; Salazar-Gutierrez, M.; Chaves, B.; Keller, M.; Hoogenboom, G. Predicting key phenological stages for 17 grapevine cultivars (*Vitis vinifera* L.). *Am. J. Enol. Vitic.* **2017**, *68*, 60–72. [[CrossRef](#)]
- Fernandez, E.; Whitney, C.; Luedeling, E. The importance of chill model selection—A multi-site analysis. *Eur. J. Agron.* **2020**, *119*, 126103. [[CrossRef](#)]
- Egea, J.; Ortega, E.; Martínez-Gómez, P.; Dicenta, F. Chilling and heat requirements of almond cultivars for flowering. *Environ. Exp. Bot.* **2003**, *50*, 79–85. [[CrossRef](#)]
- Harrington, C.A.; Gould, P.J.; St. Clair, J.B. Modeling the effects of winter environment on dormancy release of Douglas-fir. *For. Ecol. Manag.* **2010**, *259*, 798–808. [[CrossRef](#)]
- Chuine, I. A unified model for budburst of trees. *J. Theor. Biol.* **2000**, *207*, 337–347. [[CrossRef](#)] [[PubMed](#)]

17. Flynn, D.; Wolkovich, E. Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytol.* **2018**, *219*, 1353–1362. [[CrossRef](#)] [[PubMed](#)]
18. Chamberlain, C.J.; Cook, B.I.; García de Cortázar-Atauri, I.; Wolkovich, E.M. Rethinking false spring risk. *Glob. Change Biol.* **2019**, *25*, 2209–2220. [[CrossRef](#)] [[PubMed](#)]
19. Fernandez, E.; Mojahid, H.; Fadón, E.; Rodrigo, J.; Ruiz, D.; Egea, J.A.; Ben Mimoun, M.; Kodad, O.; El Yaacoubi, A.; Ghrab, M. Climate change impacts on winter chill in Mediterranean temperate fruit orchards. *Reg. Environ. Change* **2023**, *23*, 7. [[CrossRef](#)]
20. Caffarra, A.; Eccel, E. Increasing the robustness of phenological models for *Vitis vinifera* cv. Chardonnay. *Int. J. Biometeorol.* **2010**, *54*, 255–267. [[CrossRef](#)] [[PubMed](#)]
21. Camargo-Alvarez, H.; Salazar-Gutiérrez, M.; Keller, M.; Hoogenboom, G. Modeling the effect of temperature on bud dormancy of grapevines. *Agric. For. Meteorol.* **2020**, *280*, 107782. [[CrossRef](#)]
22. Fishman, S.; Erez, A.; Couvillon, G. The temperature dependence of dormancy breaking in plants: Computer simulation of processes studied under controlled temperatures. *J. Theor. Biol.* **1987**, *126*, 309–321. [[CrossRef](#)]
23. Rubio, S.; Dantas, D.; Bressan-Smith, R.; Pérez, F.J. Relationship between endodormancy and cold hardiness in grapevine buds. *J. Plant Growth Regul.* **2016**, *35*, 266–275. [[CrossRef](#)]
24. Pérez, F.J.; Vergara, R.; Rubio, S. H₂O₂ is involved in the dormancy-breaking effect of hydrogen cyanamide in grapevine buds. *Plant Growth Regul.* **2008**, *55*, 149–155. [[CrossRef](#)]
25. Beauvieux, R.; Wenden, B.; Dirlewanger, E. Bud dormancy in perennial fruit tree species: A pivotal role for oxidative cues. *Front. Plant Sci.* **2018**, *9*, 657. [[CrossRef](#)] [[PubMed](#)]
26. Kaufmann, H.; Blanke, M. Changes in carbohydrate levels and relative water content (RWC) to distinguish dormancy phases in sweet cherry. *J. Plant Physiol.* **2017**, *218*, 1–5. [[CrossRef](#)] [[PubMed](#)]
27. Rinne, P.L.H.; Welling, A.; Vahala, J.; Ripel, L.; Ruonala, R.; Kangasjärvi, J.; van der Schoot, C. Chilling of dormant buds hyperinduces FLOWERING LOCUS T and recruits GA-inducible 1,3-beta-glucanases to reopen signal conduits and release dormancy in Populus. *Plant Cell* **2011**, *23*, 130–146. [[CrossRef](#)] [[PubMed](#)]
28. Zheng, C.; Halaly, T.; Acheampong, A.K.; Takebayashi, Y.; Jikumaru, Y.; Kamiya, Y.; Or, E. Absciscic acid (ABA) regulates grape bud dormancy, and dormancy release stimuli may act through modification of ABA metabolism. *J. Exp. Bot.* **2015**, *66*, 1527–1542. [[CrossRef](#)] [[PubMed](#)]
29. Aloni, R.; Baum, S.F.; Peterson, C.A. The role of cytokinin in sieve tube regeneration and callose production in wounded coleus internodes. *Plant Physiol.* **1990**, *93*, 982–989. [[CrossRef](#)] [[PubMed](#)]
30. Howe, G.T.; Horvath, D.P.; Dharmawardhana, P.; Priest, H.D.; Mockler, T.C.; Strauss, S.H. Extensive transcriptome changes during natural onset and release of vegetative bud dormancy in Populus. *Front. Plant Sci.* **2015**, *6*, 989. [[CrossRef](#)] [[PubMed](#)]
31. Vergara, R.; Rubio, S.; Pérez, F.J. Hypoxia and hydrogen cyanamide induce bud-break and up-regulate hypoxic responsive genes (HRG) and VvFT in grapevine buds. *Plant Mol. Biol.* **2012**, *79*, 171–178. [[CrossRef](#)] [[PubMed](#)]
32. Pouget, R. Recherches physiologiques sur le repos végétatif de la vigne (*Vitis vinifera* L.): La dormance des bourgeons et le mécanisme de sa disparition. *Ann. Amélior. Plant.* **1963**, *13*, 1–247.
33. Lorenz, D.H.; Eichhorn, K.W.; Bleiholder, H.; Klose, R.; Meier, U.; Weber, E. Growth stages of the grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*)-Codes and descriptions according to the extended BBCH scale. *Aust. J. Grape Wine Res.* **1995**, *1*, 100–103. [[CrossRef](#)]
34. Dennis, F.G., Jr. Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. *HortScience* **2003**, *38*, 347–350. [[CrossRef](#)]
35. Anderson, J.; Richardson, E.; Kesner, C. Validation of chill unit and flower bud phenology models for ‘Montmorency’ sour cherry. In *Proceedings of the I International Symposium on Computer Modelling in Fruit Research and Orchard Management*; International Society for Horticultural Science: Leuven, Belgium, 1985; Volume 184, pp. 71–78.
36. Moing, A.; Carbonne, F.; Rashad, M.H.; Gaudillère, J.P. Carbon fluxes in mature peach leaves. *Plant Physiol.* **1992**, *100*, 1878–1884. [[CrossRef](#)] [[PubMed](#)]
37. Li, J.; Liu, B.; Li, X.; Li, D.; Han, J.; Zhang, Y.; Li, L.; Wang, H.; Wang, H.; Wang, J.; et al. Exogenous absciscic acid mediates berry quality improvement by altered endogenous plant hormone levels in ‘Ruiduhongyu’ grapevine. *Front. Plant Sci.* **2021**, *12*, 739964. [[CrossRef](#)] [[PubMed](#)]
38. Anzanello, R.; Fialho, F.B.; dos Santos, H.P. Chilling requirements and dormancy evolution in grapevine buds. *Ciênc. Agrotec.* **2018**, *42*, 364–371. [[CrossRef](#)]
39. Campoy, J.A.; Ruiz, D.; Egea, J. Dormancy in temperate fruit trees in a global warming context: A review. *Sci. Hortic.* **2011**, *130*, 357–372. [[CrossRef](#)]
40. Salama, A.-M.; Ezzat, A.; El-Ramady, H.; Alam-Eldein, S.M.; Okba, S.K.; Elmenofy, H.M.; Hassan, I.F.; Illés, A.; Holb, I.J. Temperate fruit trees under climate change: Challenges for dormancy and chilling requirements in warm winter regions. *Horticulturae* **2021**, *7*, 86. [[CrossRef](#)]

41. Baumgarten, F.; Zohner, C.M.; Gessler, A.; Vitasse, Y. Chilled to be forced: The best dose to wake up buds from winter dormancy. *New Phytol.* **2021**, *230*, 1366–1377. [[CrossRef](#)] [[PubMed](#)]
42. Cooke, J.E.K.; Eriksson, M.E.; Junttila, O. The dynamic nature of bud dormancy in trees: Environmental control and molecular mechanisms. *Plant Cell Environ.* **2012**, *35*, 1707–1728. [[CrossRef](#)] [[PubMed](#)]
43. Milyaev, A.; Born, U.; Sprich, E.; Hagemann, M.; Flachowsky, H.; Luedeling, E. Identifying indicators of apple bud dormancy status by exposure to artificial forcing conditions. *Tree Physiol.* **2024**, *44*, tpa112. [[CrossRef](#)] [[PubMed](#)]
44. Signorelli, S.; Velappan, Y.; Hermawaty, D.; Feil, R.; Couture, C.; Considine, J.A.; Lunn, J.E.; Considine, M.J. Seasonal changes in the physiology and metabolism of grapevine latent buds. *J. Exp. Bot.* **2026**, *77*, 1756–1772. [[CrossRef](#)] [[PubMed](#)]
45. Călugăr, A.; Cordea, M.I.; Babeș, A.; Fejer, M. Dynamics of starch reserves in some grapevine varieties (*Vitis vinifera* L.) during dormancy. *Bull. UASVM. Hortic.* **2019**, *76*, 185–192. [[CrossRef](#)] [[PubMed](#)]
46. Wang, D.; Gao, Z.; Du, P.; Xiao, W.; Tan, Q.; Chen, X.; Li, L.; Gao, D. Expression of ABA metabolism-related genes suggest similarities and differences between seed dormancy and bud dormancy of peach (*Prunus persica*). *Front. Plant Sci.* **2016**, *6*, 1248. [[CrossRef](#)] [[PubMed](#)]
47. Li, J.; Xu, Y.; Niu, Q.; He, L.; Teng, Y.; Bai, S. Absciscic acid (ABA) promotes the induction and maintenance of pear (*Pyrus pyrifolia* White Pear Group) flower bud endodormancy. *Int. J. Mol. Sci.* **2018**, *19*, 310. [[CrossRef](#)] [[PubMed](#)]
48. Chmielewski, F.M.; Gotz, K.; Homann, T.; Huschek, G.; Rawel, H. Identification of endodormancy release for cherries (*Prunus avium* L.) by abscisic acid and sugars. *J. Hortic.* **2017**, *4*, 210. [[CrossRef](#)]
49. Noriega, X.; Pérez, F.J. ABA biosynthesis genes are down-regulated while auxin and cytokinin biosynthesis genes are up-regulated during the release of grapevine buds from endodormancy. *J. Plant Growth Regul.* **2017**, *36*, 814–823. [[CrossRef](#)]
50. Sabir, I.A.; Hu, X.; Khan, I.; Qin, Y. Regulatory Mechanisms of Bud Dormancy: Environmental, Hormonal, and Genetic Perspectives. *Int. J. Mol. Sci.* **2025**, *26*, 2517. [[CrossRef](#)] [[PubMed](#)]
51. Götz, K.-P.; Chmielewski, F.-M. Metabolites that confirm induction and release of dormancy phases in sweet cherry buds. *Metabolites* **2023**, *13*, 231. [[CrossRef](#)] [[PubMed](#)]
52. Bonhomme, M.; Rageau, R.; Lacomte, A.; Gendraud, M. Influences of cold deprivation during dormancy on carbohydrate contents of vegetative and floral primordia and nearby structures of peach buds (*Prunus persica* L. Batch). *Sci. Hortic.* **2005**, *105*, 223–240. [[CrossRef](#)]
53. Velappan, Y.; Chabikwa, T.G.; Considine, J.A.; Agudelo-Romero, P.; Foyer, C.H.; Signorelli, S.; Considine, M.J. The bud dormancy disconnect: Latent buds of grapevine are dormant during summer despite a high metabolic rate. *J. Exp. Bot.* **2022**, *73*, 2061–2076. [[CrossRef](#)] [[PubMed](#)]
54. De Rosa, V.; Falchi, R.; Moret, E.; Vizzotto, G. Insight into carbohydrate metabolism and signaling in grapevine buds during dormancy progression. *Plants* **2022**, *11*, 1027. [[CrossRef](#)] [[PubMed](#)]
55. Ophir, R.; Pang, X.; Halaly, T.; Venkateswari, J.; Lavee, S.; Galbraith, D.; Or, E. Gene-expression profiling of grape bud response to two alternative dormancy-release stimuli expose possible links between impaired mitochondrial activity, hypoxia, ethylene-ABA interplay and cell enlargement. *Plant Mol. Biol.* **2009**, *71*, 403–423. [[CrossRef](#)] [[PubMed](#)]
56. Zhang, Z.; Zhuo, X.; Zhao, K.; Zheng, T.; Han, Y.; Yuan, C.; Zhang, Q. Transcriptome profiles reveal the crucial roles of hormone and sugar in the bud dormancy of *Prunus mume*. *Sci. Rep.* **2018**, *8*, 5090. [[CrossRef](#)] [[PubMed](#)]
57. Luedeling, E.; Schiffrers, K.; Fohrmann, T.; Urbach, C. PhenoFlex—An integrated model to predict spring phenology in temperate fruit trees. *Agric. For. Meteorol.* **2021**, *307*, 108491. [[CrossRef](#)]
58. Kamimori, M.; Hosomi, A. Predicting budbreak dates for ‘Delaware’ grape considering chilling and heat requirements using PhenoFlex. *Ecol. Model.* **2025**, *501*, 110985. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.