agronomy

MDPI

Article

Relationship between Chilling Accumulation and Heat Requirement for Flowering in Peach Varieties of Different Chilling Requirements

Juan Yan [1], Zhixiang Cai [1], Zheng Chen [1], Binbin Zhang [1], Jiyao Li [1,2], Jianlan Xu [1], Ruijuan Ma [1], Mingliang Yu [1] and Zhijun Shen [1,*]

[1] Institute of Pomology, Jiangsu Academy of Agricultural Sciences/Jiangsu Key Laboratory for Horticultural Crop Genetic Improvement, No. 50 Zhongling Street, Nanjing 210014, China
[2] College of Horticulture, Nanjing Agricultural University, No. 1 Weigang, Nanjing 210095, China
[*] Correspondence: shenjaas@aliyun.com

Abstract: Previous studies have shown a negative correlation between chilling accumulation (CA) and heat requirements (HRs) in peaches (*Prunus persica* (L.) Batsch), consistent with findings in other plants in spring events. However, there is a lack of comparative research on the CA–HR relationship in peach varieties with different chilling requirements (CRs), and the specific impact of CA on HR reduction remains poorly described. To address this, we investigated the effects of CA on the days and HR for flowering in 54 peach varieties of differing CRs. Scanning electron microscopy was used to observe the effects of CA on the phenology of floral organ development in a selected peach variety. Our results indicate that, in general, peaches exhibit a reduced HR and accelerated flowering as the CA increases, but that the strength and trend of the CA–HR relationship is influenced by the CR and the variety. Low-CR varieties showed less sensitivity to CA increments, requiring higher relative increases in CA to significantly lower the HR, whereas high-CR varieties appeared to be more sensitive, with even modest changes leading to substantial reductions in HR. However, variations from this generality exist, even within varieties displaying the same rCA (the ratio of CA to CR). Additionally, we provide a summary of the relationship between the rCA and drHR in peaches of differing CRs, and identify several varieties exhibiting a strong response in the CA–HR relationship. This study also highlights the impact of CA on flower bud development, revealing slower progression under lower CA levels and accelerated growth with an increased CA. In particular, we identified the critical period of the enlargement and initiation of green scales as indicative of successful pollen grain formation. Finally, we present a schematic of the CA–HR relationship for flowering in peaches.

Keywords: peach (*Prunus persica* (L.) Batsch); chilling accumulation; heat requirement; flowering

check for updates

Citation: Yan, J.; Cai, Z.; Chen, Z.; Zhang, B.; Li, J.; Xu, J.; Ma, R.; Yu, M.; Shen, Z. Relationship between Chilling Accumulation and Heat Requirement for Flowering in Peach Varieties of Different Chilling Requirements. *Agronomy* **2024**, *14*, 1637. https://doi.org/10.3390/agronomy14081637

Academic Editor: Julián Cuevas González

Received: 28 June 2024
Revised: 15 July 2024
Accepted: 19 July 2024
Published: 26 July 2024

1. Introduction

The peach (*Prunus persica* (L.) Batsch) is predominantly found in temperate regions between the 30° and 45° north and south latitudes, ranking as the third most economically significant deciduous fruit tree globally after apples and pears, with an annual production of approximately 25 million tons (FAOSTAT, 2022). The onset of winter dormancy in peach trees requires exposure to a period of low temperatures (the chilling requirement, CR) to stimulate bud development, but also a period of warm temperatures (the heat requirement, HR) for normal flowering and growth [1–3], where the CR affects bud endodormancy and the HR influences the timing of bud break and flowering. Both of these processes are modulated by the climate [4–7].

It has long been recognized that the period of chilling accumulation (CA) can affect the HR for bud break in peaches [8–11] and other species, including apple, cherry and various forest tree species [12–16]. Decreased winter chilling elevates the need for spring forcing heat, while increased chilling fosters budburst under consistent forcing conditions [16–20].

The negative CA–HR relationship, also known as the tradeoff between chilling and forcing heat (Harrington and Gould, 2015), aids in the estimation of CR and HR sensitivity to CA for bud break across species [21,22], and has been integrated into numerous phenological models to project past or future phenological shifts [18,21,23,24].

The escalating trend of global climate warming has led to abnormal weather patterns, including mild winters, delayed springs and late frosts, which directly impact CA and subsequently affect HR, thereby influencing peach tree phenology and productivity. In response, the peach industry has extended peach cultivation to semi-tropical regions or to controlled greenhouse environments. Other possible solutions include the breeding of varieties with targeted CRs and HRs [7,25]. However, such breeding strategies require a better understanding of the CR, HR and the CA–HR relationships within varying climatic conditions [2,26,27]. Although the negative correlation between CA and HR in peaches has been re-examined relatively recently [4,12,14,15,28], the number of varieties tested was limited, and there remains a lack of empirical data describing the CA–HR relationship in varieties of different CRs. Our study utilized 54 peach varieties of differing CRs within a light- and temperature-controlled environment, to examine the impact of CA on the HR and days to flowering. Additionally, we utilized scanning electron microscopy (SEM) to observe the effects of CA on floral organ development in peaches with a CR of 400 h. This research marks the first exploration of the CA–HR relationship in peach varieties of differing CRs and provides initial insights into the phenological impact of an increased CA on floral bud development. These findings will assist in the adaptation of field cultivation strategies to climate change, and they can help guide more precise chilling and heat management in greenhouse cultivations of peach.

2. Materials and Methods

2.1. Temperature Data Collection

Temperature data used in the experiment were obtained from the weather station at the Jiangsu Academy of Agricultural Sciences, recording field temperatures every hour. Figure S1 displays the variation in temperature from 12 November 2023, to February 6, 2024 (the main period for CA in Nanjing, China), showing a maximum temperature of 23.14 °C, a minimum temperature of −9.11 °C and an average temperature of 5.46 °C. According to the 0–7.2 °C model [29], the beginning of CA was marked as November 12, when the average daily temperature for three consecutive days fell below 7.2 °C. The last field collection of twigs was conducted on February 6.

2.2. Materials

The peach varieties utilized were cultivated at the National Peach Germplasm Nursery in Nanjing (31°14′ N, 118°22′ E) between 2023 and 2024. All test trees were 6–7 years old, uniformly managed and cultivated in fertile soil with effective drainage and irrigation systems. A total of 54 peach varieties were utilized, consisting of six CR categories (200, 300, 400, 500, 600 and 700 h), each consisting of nine varieties (Table S1). The peach variety 'Jinxiazaoyoupan' was selected as a mid-range CR (400 h) for the electron microscope observation of flower bud development.

2.3. Peach Twig CA Treatment and Data Calculation

The cultivation of peach twigs and data collection were performed as described by Yan et al. [29] and Lin et al. [16]. Under field conditions, new branches from the last year's growing season (twigs) were exposed to a CA period equivalent to the variety's CR, before a sample of 5 twigs (apical 30–40 cm) were collected and transferred to growth chambers with their cut ends placed in water (ca. 3 cm) for cultivation. Further twig samples were collected from each variety every 100 or 200 h of additional CA until reaching a total of 1000 h (6 February 2024). The CA was calculated from the number of hours the twigs were exposed to temperatures between 0 and 7.2 °C [1]. The local temperature data (Figure S1) were obtained from the weather station at the Jiangsu Academy of Agricultural Sciences.

The growth chambers utilized a constant temperature of 22 °C, a light/dark cycle of 12/12 h, a light intensity of 2000 lux and a relative humidity of 60–70%. During twig cultivation, the water was changed every 3 days, with trimming of the cut ends (2–3 mm) to avoid wound sealing. Twigs were observed on a daily basis, and the date of the first flowering date (FFD) recorded.

The HR was calculated from the point of CR fulfillment until FFD and was expressed as the sum of growing degree hours (GDH; 4.5–25 °C) the buds were exposed to under both field (GDH_N; N for natural) and growth chamber (GDH_F; F for forcing) conditions [16] (Table S2). The ratio of CA to CR (rCA; =CA/CR) and the decrease ratio of HR_{CA} to HR_{CR} (drHR; = ($HR_{CR} - HR_{CA}$) × 100/HR_{CR} %) were then calculated for each variety's twigs on different collection dates.

2.4. Scanning Electron Microscopy of Peach Buds

The variety 'Jinxiazaoyoupan', which displays a CR of 400 h, was selected. Twigs were collected at CA levels of 0, 200, 400, 600 and 800 h (20 each) from the field and transferred to growth chambers, as described above, until the first flower bloomed or for a maximum of 21 days. Prior to electron microscopy, 10 healthy buds were randomly selected every 4–7 days (the sampling information is given in Table S3), and the average and standard deviation of the flower bud diameters were recorded.

Fresh tissue blocks of flower buds were cut using a sharp blade quickly within 1–3 min, then washed in phosphate-buffered solution and fixed in 2.5% glutaraldehyde for 2 h at room temperature, then transferred to 4 °C for dehydration in a series consisting of ethanol followed by isoamyl acetate for 15 min. The samples were then dried with a critical point dryer (Quorum K850, Laughton, UK) and sputter-coated with gold (Hitachi MC1000, Tokyo, Japan) for 30 s. Images were acquired with a scanning electron microscope (Hitachi SU8100, Tokyo, Japan).

3. Results

3.1. Effect of Increasing CA on HR for Flowering in Peach Varieties of Different CRs

3.1.1. Effect of Increasing CA on FFD in Peach Varieties of Different CRs

Figure 1 illustrates that in the peach varieties tested, when the CA equals the varietal CR, the days from sampling to FFD ranged from 16 to 21, indicating variations in flowering HR among varieties. With further increases in CA, the FFD was observed to show a general decrease. However, the decrease in FFD with CA differed in magnitude between the CRs and varieties.

Peaches with lower CRs (200–400 h types) typically flowered within 6–10 days when the CA reached 1000 h (Figure 1a–c), while those with higher CRs (500–700 h types) required 10–15 days (Figure 1d–f). However, the magnitude of the decrease in FFD with increases in CA differed between varieties, and the general negative trend was not obviously linear in most. Just a few varieties exhibited a linear sensitivity to CA, including Vars. 13, 17, 49 and 50. For example, with Var. 17 'Chaowuyuehuo', each 100 h CA increment reduced the number of flowering days by two (a 200 h increase in the last stage).

3.1.2. Impact of Increasing CA on HR in Peaches of Different CRs

As shown in Figure 1, a general decrease in FFD was observed with increases in CA above the varietal CR, indicating that extending the CA to above the CR will generally decrease the HR. The effect of CA on HR was therefore examined with tests of significance between the HR observed at the CR (HR_{CR}) and where CA > CR (HR_{CA}). The results (Figure 2) indicated significant decreases in HR_{CA} with increasing the CA over the CR for all experimental varieties. However, varieties with a higher CR (600 and 700 h) appeared more responsive to an increasing CA, showing significant HR_{CA} reduction with 100 CA h, whereas low-CR varieties (CRs of 300–500 h) required a CA of 200 h, and varieties with a CR of 200 h required a CA of 300 h to achieve significant effects. In addition, it was noted that the HR_{CA} did not decrease linearly with an increasing CA, especially in low-CR varieties, which showed smaller decreases with a higher CA.

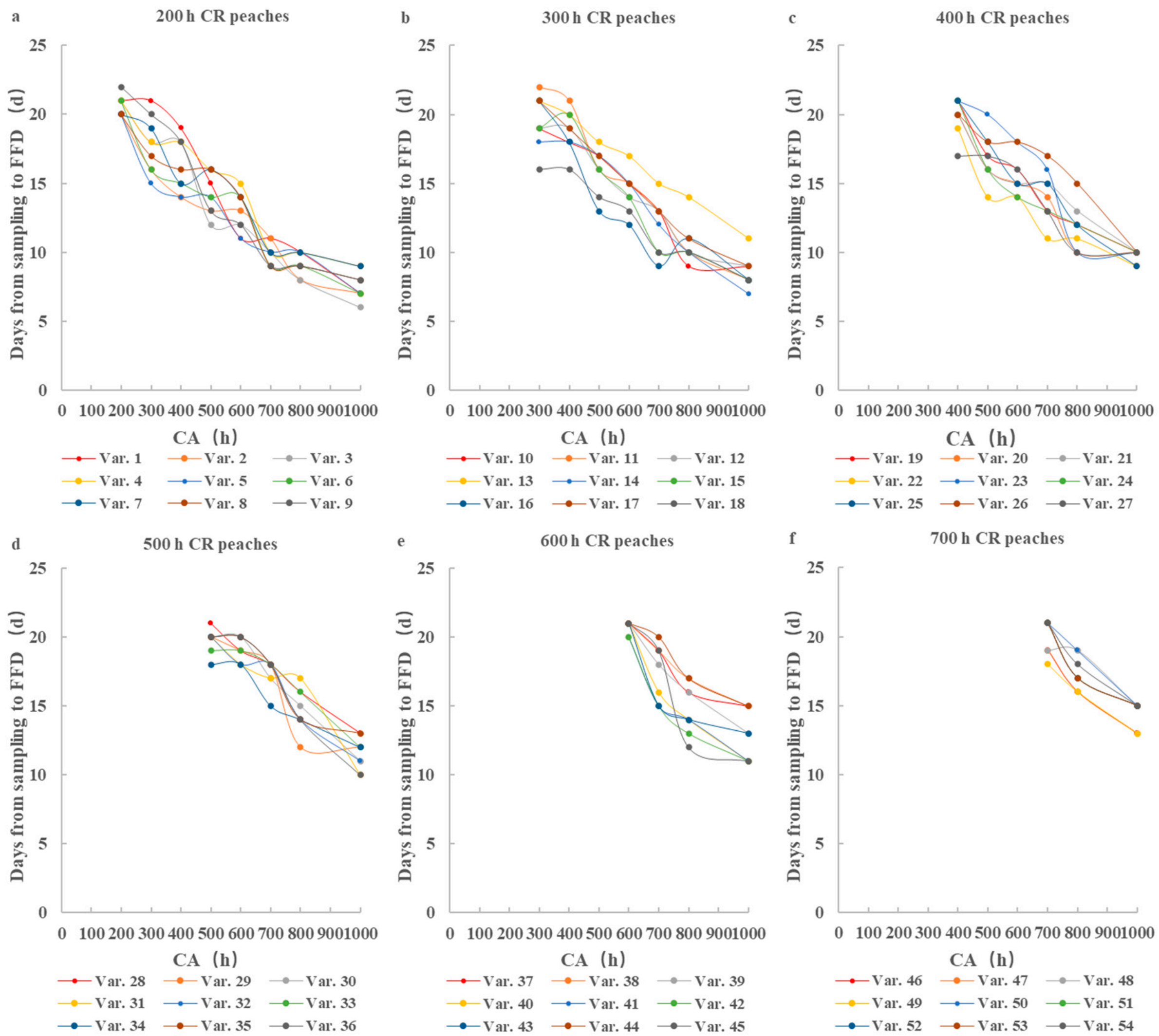

Figure 1. Effect of CA on days to first flowering (FFD) after transfer in varieties of differing CRs. Note: In (**f**), there is overlap in trends for Vars. 46 and 47, as well for Vars. 51, 52 and 53, resulting in obscured data points.

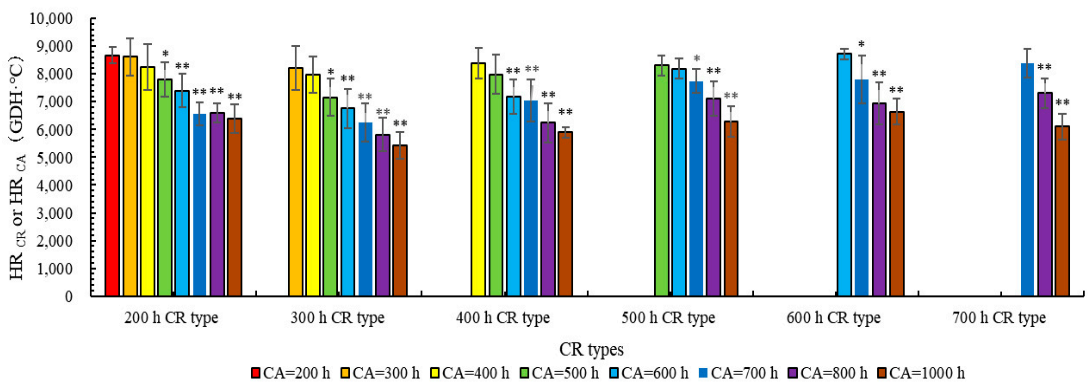

Figure 2. Effect of CA on HR_{CA} in peach varieties with differing CRs. Note: Asterisks indicate significant differences relative to varietal HR_{CR} (*, $p < 0.05$; **, $p < 0.01$).

3.1.3. Relationship between rCA and drHR of Peaches with Different CRs

To further examine the CA-HR relationship, the CA and HR were transformed to rCA, and drHR (see Section 2), to better reflect their proportional differences to values observed at the varietal CR (Figure 3). In general, increases in rCA were accompanied by relatively sharp increases in drHR. However, a few varieties (e.g., Vars. 14, 17 and 28) exhibited a more gradual increase, and Var. 8 showed a minimal drHR (0.8%) at a lower rCA (1.50), which is notably lower than that observed in other varieties. Exceptionally, the drHR of Var. 16 remained relatively stable (35.0–38.1%) after an initial increase with an increasing rCA (2.50–5.00).

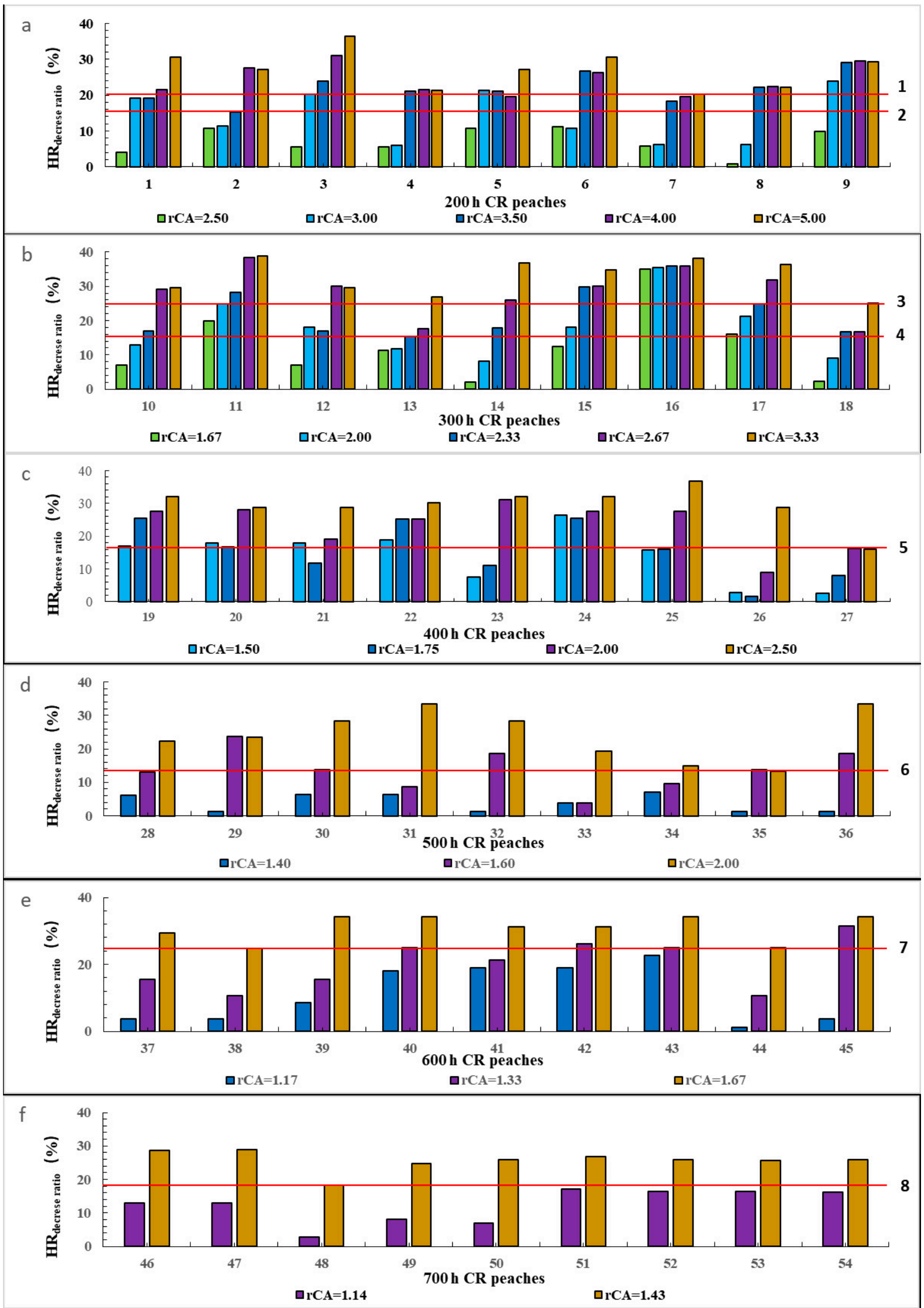

Figure 3. Relationship between rCA and drHR of peach varieties of different CRs. (**a**–**f**) Red lines 1 and 2 represent rCA values of 5.00 and 3.50 in the 200 h CR type, res. Red lines 3 and 4 represent rCA values of 3.33 and 2.33 in the 300 h CR type; red lines 5–8 represent rCA values of 2.50, 2.00, 1.67 and 1.43 in the 400, 500, 600 and 800 h CR types, respectively.

To facilitate practical applications, below we summarize the general relationship between rCA and drHR for each peach CR type and indicate varieties that are highly sensitive to increases in CA. For 200 h CR-type peaches, at rCA = 3.50, the drHR can reach 15% or above, with a maximum of 29.0% (Var. 9); at rCA = 5.00, the drHR can reach 20% or above, with a maximum of 36.4% (Var. 3). For 300 h CR-type peaches, at rCA = 2.33, the drHR can reach 15% or above, with a maximum of 35.0% (Var. 16); at rCA = 3.33, the drHR can decrease by 20% or above, with Var. 11 decreasing up to 38.8%. For 400 h CR-type peaches, at rCA = 2.50, the drHR can decrease by 15% or above, with Var. 25 decreasing up to 36.9%. For 500 h CR-type peaches, at rCA = 2.00, the drHR can decrease by 13% or above, with Vars. 31 and 36 decreasing up to 33.4%. For 600 h CR-type peaches, at rCA = 1.67, the drHR can decrease by 24% or above, with Vars. 39, 40, and 45 decreasing up to 34.2%. For 700 h CR-type peaches, at rCA = 1.43, the drHR can decrease by 18% or above, with Var. 47 decreasing up to 29.0%.

3.2. SEM Analysis of the Effect of CA and GDH_N on the Developmental Phenology of Flower Buds

The effects of CA and GDH_N on the phenology of flower bud development were studied by scanning electron microscopy (SEM). The mid-range (400 h) CR-type variety 'Jinxiazaoyoupan' was selected for this purpose. The results are presented in Figure 4 and summarized in Table 1.

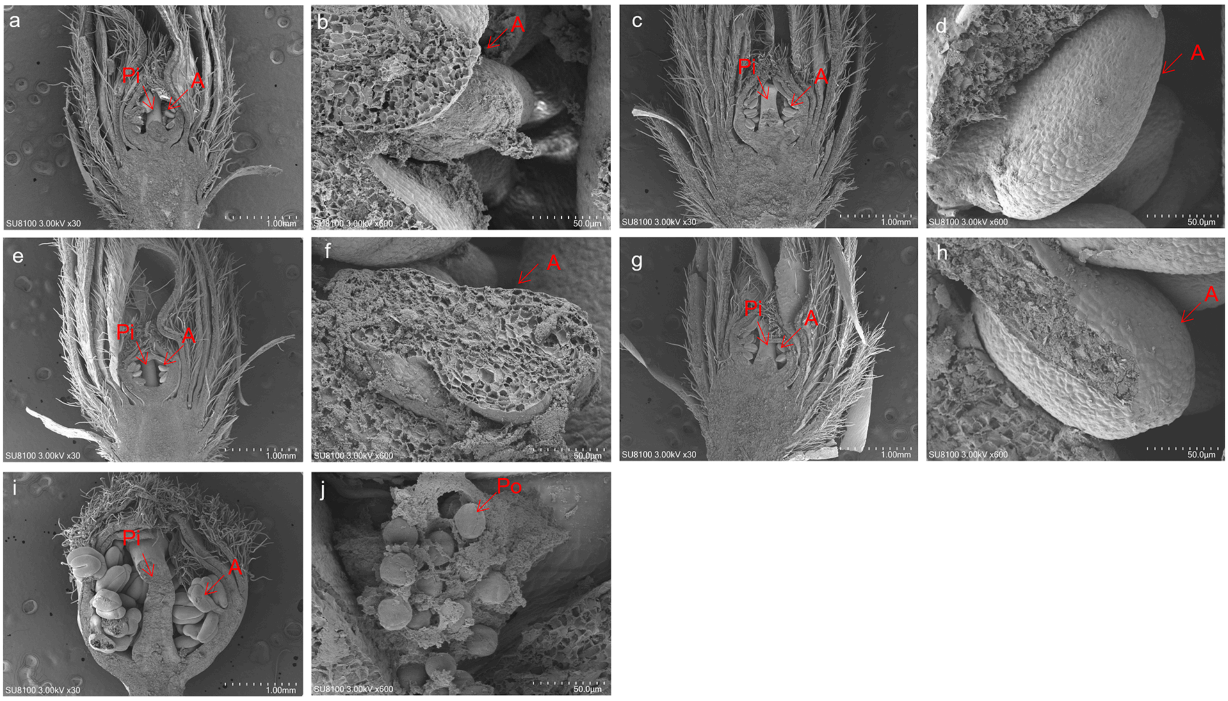

Figure 4. *Cont.*

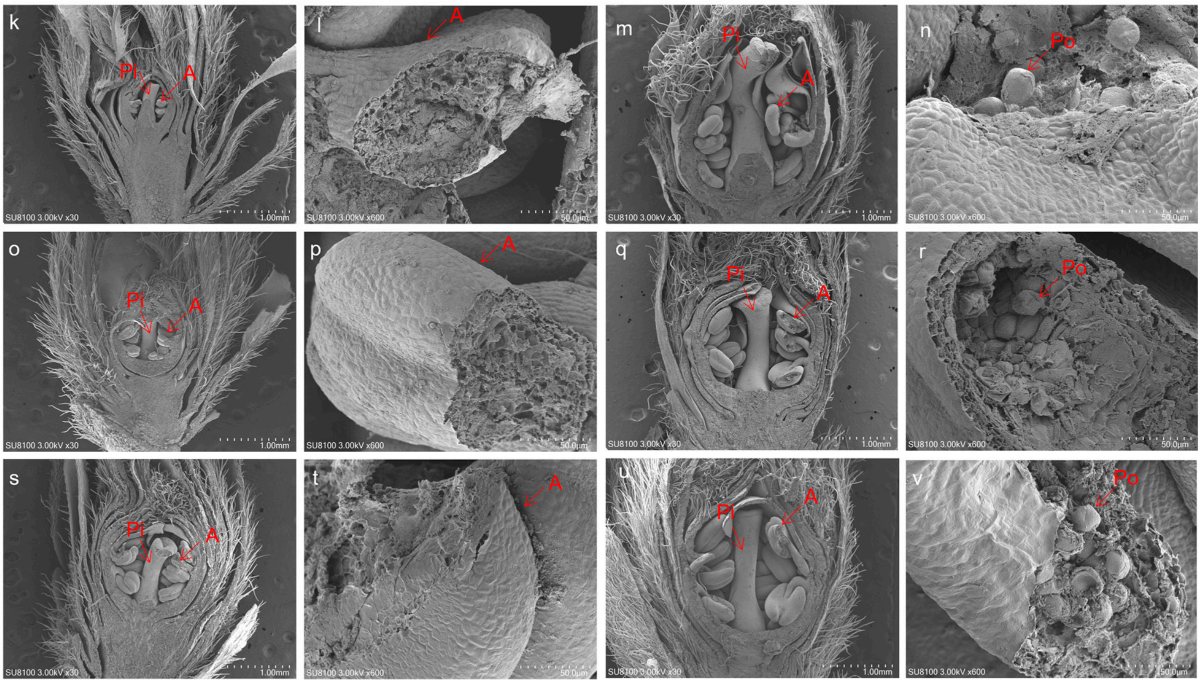

Figure 4. SEM of peach variety 'Jinxiazaoyoupan' (CR = 400 h) flower bud development under different CAs and GDHs during greenhouse cultivation. Note: (**a**–**d**) Morphological and cross-sectional views of flower buds and anthers cultivated in the greenhouse for 0 and 21 days for CA = 0 h, GDH_N = 0 GDH·°C. The morphology of the flower buds, anthers and pollen grains is depicted in panels (**e**–**v**), where (**e**–**j**) represent 0, 12 and 21 days of cultivation for CA = 200 h, GDH_N = 0 GDH·°C; (**k**–**n**) represent 0 and 12 days of cultivation for CA = 400 h, GDH_N = 0 GDH·°C; (**o**–**r**) represent 0 and 7 days of cultivation for CA = 600 h, GDH_N = 603 GDH·°C; and (**s**–**v**) represent 0 and 5 days of cultivation for CA = 800 h, GDH_N = 1343 GDH·°C. Key: A: anther; Pi: pistil; Po: pollen. The magnification (30× or 600×) and scale (1 mm or 50 μm = ten grid lengths) are given on the bottom left and right of each panel, respectively.

Under the conditions of CA at 0 h (GDH_N = 0 GDH·°C), no significant differences in the morphology or size of the reproductive organs (pistil and anthers) were observed over 21 days, and only slight differences in the overall bud size were detected on day 21 (Table 1, Figure 4a–d). At CA = 200 h (0.5 × CR h and a GDH_N = 0 GDH·°C), the bud diameter was seen to increase to 2.7 mm on day 14 (Table 1), with a slight enlargement of the stigma and anthers, but no pollen grains were detected. After 21 days of cultivation, a significant enlargement of the buds (to a diameter of 4.0 mm) was observed, accompanied by a noticeable development of the pistil and anthers, along with the presence of pollen grains (Table 1, Figure 4e–j). After a CA period of 400 h (CA = CR h; GDH_N = 0 GDH·°C), a slight bud enlargement was observed (to a diameter of 2.6 mm) on day 7, followed by a significant bud enlargement on day 12 (to a diameter of 3.7 mm), with a conspicuous development of the pistil and anthers, along with the presence of pollen grains (Table 1, Figure 4k–n). After a CA treatment of 600 h (1.5 × CR; GDH_N = 603 GDH·°C), a slight enlargement of the flower buds and an increase in the reproductive organs were observed on day 0 relative to CA = 0, 200 and 400 h. After 7 days of cultivation, a significant enlargement of the flower buds (with a diameter of 4.0 mm) and a conspicuous development of the pistil and anthers were observed, along with the presence of pollen grains (Table 1, Figure 4o–r). For CA = 800 h (2 × CR; GDH_N = 1343 GDH·°C), the flower buds exhibited a significant development on day 0, with a noticeable enlargement and larger reproductive organs compared to flower buds exposed to a CA of 600 h. After 5 days of cultivation, a further enlargement of the flower buds was observed (to 3.4 mm), along with a conspicuous development of the pistil and anthers, and the presence of pollen grains (Table 1, Figure 4s–v).

Table 1. The phenotypic state of 400 h CR peach 'Jinxiazaoyoupan' flower buds under different CA and GDH_N conditions during greenhouse cultivation.

CA (h)/GDH_N (GDH °C)	Flower Bud Collection Date	Days of Bud Cultivation	Bud Diameter (mm)	Phenotypic State Description
0/0	12 November 2023	0	2.2 ± 0.2	--
	19 November 2023	7	2.2 ± 0.3	No significant change
	26 November 2023	14	2.4 ± 0.1	No significant change
	3 December 2023	21	2.8 ± 0.3	Slight enlargement of flower buds was noted relative to the preceding state
200/0	5 December 2023	0	2.1 ± 0.2	No significant difference relative to day 0 of CA = 0 h
	12 December 2023	7	2.3 ± 0.2	No significant changes were observed relative to the preceding state
	19 December 2023	14	2.7 ± 0.1	Slight enlargement of flower buds relative to the preceding state
	26 December 2023	21	4.0 ± 0.4	Significant enlargement of flower buds and conspicuous development of the reproductive organs, with >25% flower buds showing green scales
400/0	25 December 2023	0	2.3 ± 0.2	No significant changes were observed relative to day 0 of CA = 0 h and 200 h
	2 January 2024	7	2.6 ± 0.4	Slight enlargement of flower buds relative to the preceding state
	7 January 2024	12	3.7 ± 0.4	Significant enlargement of the flower buds and conspicuous development of the reproductive organs, with >25% flower buds showing green scales
	12 January 2024	17	4.2 ± 0.3	>25% flower buds showing red scales
	15 January 2024	21	5.3 ± 0.6	The first flower bloomed
600/603	8 January 2024	0	2.7 ± 0.3	Significant enlargement of the flower buds and conspicuous development of the reproductive organs were observed compared to day 0 of CA = 0 h, 200 h and 400 h
	15 January 2024	7	4.0 ± 0.5	Significant enlargement of the flower buds and conspicuous development of the reproductive organs relative to the preceding state
	19 January 2024	11	4.3 ± 0.5	>50% flower buds showing green scales
	24 January 2024	16	5.7 ± 0.9	The first flower bloomed
800/1343	24 January 2024	0	3.1 ± 0.3	Significant enlargement of the flower buds and conspicuous development of the reproductive organs relative to day 0 of CA = 600 h
	29 January 2024	5	3.8 ± 0.2	Significant enlargement of the flower buds and conspicuous development of the reproductive organs, with >25% flower buds showing red scales
	5 February 2024	12	5.6 ± 0.7	The first flower bloomed

The results indicate that when the CA is lower than the CR, flower bud development is significantly delayed under greenhouse heating. For a CA of 0 h, no flower bloom was obtained after 21 days of cultivation, and 21 days were required for pollen grain formation at a CA of 200 h. As the CA increases, the development rate of the flower buds and their reproductive organs accelerates, resulting in faster pollen grain formation and earlier flowering. At the point of CA = CR (400 h), 12 days were required for pollen formation, and the first flower bloom was noted after 21 days. For CAs of 600 and 800 h, pollen grain formation required only 7 and 5 days, respectively, and the first flower bloom was observed after 16 and 12 days of cultivation, respectively. In addition, the period of significant bud enlargement and the onset of green scales was observed to be coincident with pollen grain formation, occurring at 21 days of cultivation at CA = 200 h, 12 days at CA = 400 h, 7 days at CA = 600 h and 5 days at CA = 800 h.

4. Discussion

The spring plant phenologies of flowering and leaf-out are influenced by three main factors: winter chilling, spring forcing (temperature and HR) and photoperiod. These cues individually or collectively impact budburst and leaf-out, with chilling and forcing having the most pronounced joint effect [18]. Studies of peaches have highlighted a negative correlation between CA and HR for bud break [12,14,15]. For example, Gariglio et al. [12] studied mostly low-CR cultivars and found that an increased CA led to a reduction in the time to bud break. Varieties with similar CR levels exhibited different responses to chilling. However, their cuttings were single bud segments, which may not behave as do buds on intact shoots. Okie et al. [14] demonstrated that as the chilling exposure increases in higher-CR peach varieties (650–950 chill units), there was an exponential decrease in the time and heat accumulation needed for floral bud break. Conversely, varieties of lower CRs show a curvilinear relationship with CA, where more heat is required for bud break. Tan et al. [15] found HR to be affected by the duration of chilling exposure in low-CR peach varieties. Under conditions of insufficient chilling exposure (CA < CR), the bud burst rate was severely reduced and the HR tended to infinity. When the exposure to chilling was equal to or exceeded the CR, the HR was reduced. In our research, higher increases in the CA over the CR were shown to result in greater decreases in FFD and HR in all 54 peach varieties. Also, SEM observations of the variety 'Jinxiazaoyoupan' (CR of 400 h) indicated that reducing the CA to less than that of the varietal CR results in extremely slow flower bud development, whereas under conditions where the CA exceeds the CR, the development rate of the flower buds and their reproductive organs is accelerated, leading to faster pollen grain formation and earlier flowering. Our findings are in agreement with previous research indicating a negative CA–HR relationship and a complex interplay between these factors in influencing peach phenology [14,15].

Furthermore, we observed variations in the magnitude and patterns of decreased days and HR to flowering with an increasing CA among different CRs and varieties. Low-CR varieties were less responsive to CA increases and required more CA to significantly reduce the HR, whereas high-CR varieties exhibited greater sensitivity to CA changes, with even small increases leading to significant reductions in HR. The study by Okie et al. [14] using high-CR varieties also supported the exponential CA–HR relationship, showing how increasing the CA dramatically reduced the time and heat accumulation needed for peach floral bud break. Previous studies have indicated that the HR decreases with an increasing CA, resulting in gradual changes in peach bud-flowering rates [15]. Our results indicated the reduction in FFD and HR with an increasing CA differed among the peach varieties, suggesting a diverse response profile. This underscores the CR type- and variety-specific nature of the CA–HR relationship in peaches, just like the viewpoint proposed by [30], emphasizing the need to consider these factors when studying phenological diversity within cultivars across different environmental conditions and locations, especially amidst climatic changes.

It is commonly believed that two distinct temperature responses mark the end of dormancy in perennial species: a CR, fulfilled by cool conditions, and an HR (forcing), satisfied by warmer temperatures. However, during the dormant period, it is challenging to distinguish clearly between the chill and heat accumulation phases [30]. Some viewpoints suggest that, despite a tradeoff between the amount of forcing and chilling, plants may accumulate both chilling and forcing units simultaneously during dormancy [31]. Most current chilling models have considered the dormancy break a sequential process: after appropriate chilling is received, a fixed amount of heat accumulation will produce bloom. Thus, the initiation of CA marks the entry into endodormancy, while the breaking of endodormancy (and subsequent transition to ecodormancy) signals the onset of heat accumulation [7,32–34]. Our SEM observations with 'Jinxiazaoyoupan' support the rationality of differentiating between the chill and heat accumulation phases. With CA values of 0, 200 and 400 h, there were no apparent morphological differences in the flower buds, indicating that under conditions where the CR is unsatisfied (CA < CR), even with substantial heat accumulation (2722–3988 GDH·°C), flower bud development is aborted or is minimal and the HR cannot be evaluated. The question arises as to what extent the CA influences the HR for flowering. Early suggestions proposed that when more than half of the necessary cold hours for a variety have been accumulated, the tree might exhibit delayed blooming and that each additional day of cold beyond the minimum development stage reduces the lag in flower bud development exposed to warmth by a time corresponding roughly with the duration of the cold received [14]. This implies that the CA must exceed half of the CR to affect the HR. Our SEM observations indicated that CA = 1/2 CR (200 h) promoted pollen grain development at 21 days, while at CA = 0 h, only a slight swelling of the flower buds and reproductive organs was observed. Subsequent increases in the CA led to accelerated flower bud development, earlier pollen grain appearance and accelerated flowering.

It would be of interest to determine if there is a functional threshold value for the HR, above which increasing the CA no longer reduces the HR. Tan et al. [15] first highlighted the possible existence of an optimal effective CA (> CR) that minimizes the total time required for buds to transition from dormancy to budding. In this research, we noted that for peach varieties with a CR of 200 h, the HR remained relatively constant once the CA reached 700 h, representing 3.5 times the CR increase. For peach varieties with CRs of 300 h and 400 h, the HR ceased to exhibit significant changes after the CA surpassed 800 h. This supports the possibility of a limit to the optimization of the CA ($CA_{optimum}$), but also suggests that this limit may be dependent on the CR. These findings are supported by the earlier research of Scalabrelli and Couvillon [11] with the 'Redhaven' peach (950 chill units), who reported that while initial extensions to the artificial chilling duration (CA) enhanced the floral bud break and reduced the required HR, further increases in CA only slightly enhanced the bud break without affecting the HR. Our research also suggests that the CA–HR relationship can differ between varieties of the same CR. Variety 16 indicated that some varieties may achieve a stable HR after exposure to relatively short periods of CA.

Accurate predictions of the changes in spring plant phenology with climate change are essential for projections of fruit output from growing seasons. However, progress towards prediction has been slow, because the major cues known to drive phenology generally co-vary in nature and may interact, making accurate predictions of plant responses to climate change complex and non-linear [18]. For peach trees, their dormancy release and growth phenology are primarily influenced by CA and heat accumulation. Therefore, clarifying the relationship between the two can better serve phenological predictions. While the impact of CA on HR may vary among different CRs and varieties, we have observed a general pattern for the tested variety, which is the following: For 200 h CR-type peaches at rCA = 3.50, the drHR can reach 15% or above; at rCA = 5.00, the drHR can reach 20% or above. For 300 h CR-type peaches, at rCA = 2.33, the drHR can reach 15% or above; at rCA = 3.33, the drHR can decrease by 20% or above. For 400 h CR-type peaches, at rCA = 2.50, the drHR can decrease by 15% or above. For 500 h CR-type peaches, at rCA = 2.00, the drHR can decrease by 13% or above. For 600 h CR-type peaches, at rCA = 1.67, the drHR can decrease by 24% or above. For 700 h CR-type peaches, at rCA = 1.43, the drHR can decrease by 18% or above.

We also identified 11 varieties that exhibit a strong CA–HR relationship, for which phenological predictions within the context of climate change can be made with greater confidence.

Research into the phenology of peach bud development during dormancy has been relatively limited. Hernandez et al. [35,36] conducted systematic observations on the gametophyte development of low- and high-CR peaches, revealing that the differentiation process of anthers for pollen grain formation occurs during ecodormancy. This study, for the first time, observed the morphological and organ development of flower buds under different CAs, indicating that under an insufficient CA and with zero heat accumulation under natural conditions, the initial morphology of flower buds at CA = 0, 200 and 400 h is essentially static. With the provision of heat, pollen grains can still form, but take a significantly longer time to develop. However, as the CA increases, the rate of organ development accelerates, leading to a notable reduction in the time required for pollen grain formation. We report for the first time that pollen grain formation primarily occurs during the period of significant bud enlargement and the initiation of green scale formation. These findings will provide valuable guidance in future theoretical research and for production practices.

Based on our observations and the previous findings discussed above, we discuss the non-linear CA–HR relationship in peaches (Figure 5). Firstly, when the bud enters endodormancy, under conditions of no chilling exposure (CA = 0), the HR tended to infinity. Secondly, during endodormancy and ecodormancy, under conditions of increasing chilling exposure (0 < CA < $CA_{optimum}$), the HR tends to decrease in a non-linear fashion, which is CR- and variety-specific. Thirdly, under conditions of sufficient chilling exposure (CA > $CA_{optimum}$), the HR is no longer reduced. In addition, the limit to $CA_{optimum}$ may be dependent on the CR and variety.

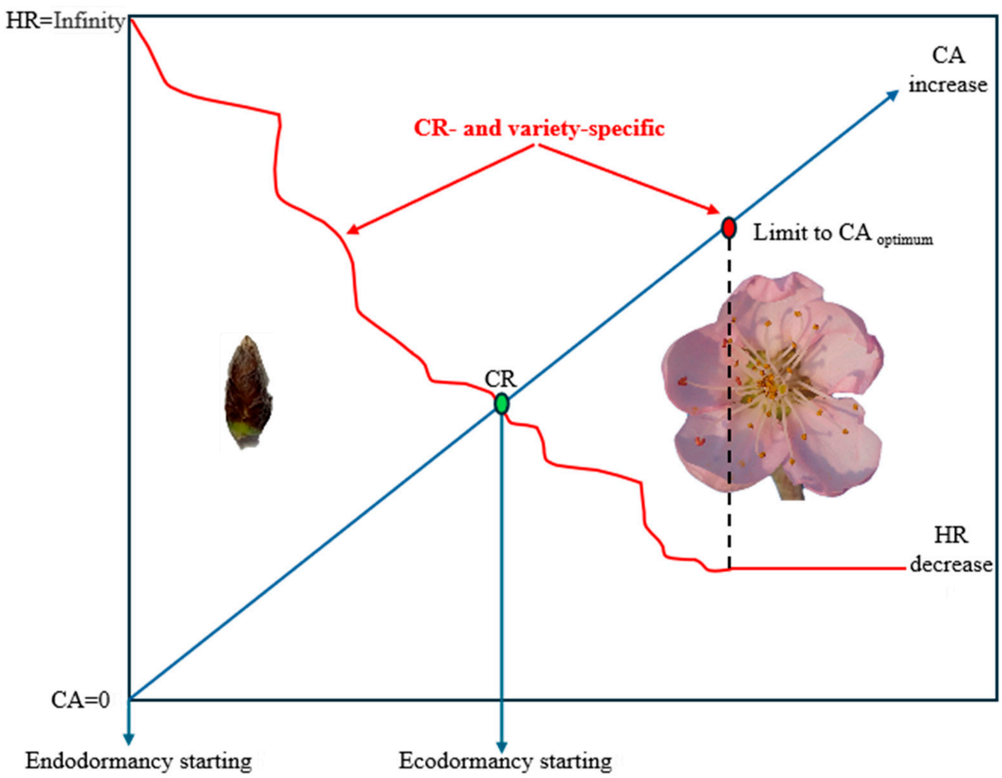

Figure 5. Schematic of the proposed CA–HR relationship for flowering in peaches. Note: CA: chilling accumulation; HR: heat requirement; CR: chilling requirement.

5. Conclusions

In this study, we investigated the effects of CA on the days and HR for flowering across 54 peach varieties differing in CRs, as well as the effects of CA and GDH on the phenology of flower bud development in the 'Jinxiazaoyoupan' variety (400 h CR). In general, the peaches exhibited reduced days and HR for flowering with an increasing CA, as reflected in the rate of developmental progression of their flower buds and reproductive organs. However, the CA–HR relationship was found to be influenced by the CR and variety. We proposed a direct yet non-linear tradeoff between CA and HR, where the relative reductions in HR in peach varieties with higher CRs are greater than those of lower CRs. We introduced this to discuss a non-linear CA–HR relationship in peaches. Additionally, we provided a summary of the relationship between the rCA and drHR across peaches of various CRs, and identified several varieties exhibiting a strong CA–HR relationship, thus facilitating phenological predictions. These research findings can aid in predicting differences in flowering times among varieties within their current environments, as well as anticipating how each variety and genotype may respond to climate changes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14081637/s1, Figure S1: The daily temperatures of the peach germplasm nurseryfrom November 2023 to February 2024; Table S1: Selected peach varieties and their chilling requirements (CR); Table S2: Collected twigchilling requirement (CR) and chilling accumulation (CA) treatments; Table S3: Sampling information for electron microscope observation offlower buds in Var. 'Jinxiazaoyoupan' with 400 h CR.

Author Contributions: Conceptualization, J.Y. and Z.S.; methodology, J.Y.; software, Z.C. (Zhixiang Cai); validation, J.Y., Z.C. (Zhixiang Cai) and Z.C. (Zheng Chen); formal analysis, B.Z.; investigation, Z.C. (Zheng Chen); resources, Z.S.; data curation, J.L.; writing—original draft preparation, J.Y.; writing—review and editing, R.M. and J.X.; supervision, M.Y.; project administration, Z.S.; funding acquisition, Z.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the China Agriculture Research System (CARS-30); Species Conservation Project of the Ministry of Agriculture and Rural Affairs (19210895); National Crop Germplasm Resources Infrastructure in China (NHGRC2020-NH16); The Open Competition Project of Seed Industry Revitalization of Jiangsu Province (Grant no. JBGS(2021)016); Accurate Identification of Peach and Strawberry Germplasm Resources in Southern China (19230697); Peach and Strawberry (Southern) Germplasm Safe Preservation (19240424); and Species Conservation Project of Crop Germplasm Resources (Peach and Strawberry) in Jiangsu Province (2023-SJ-011).

Data Availability Statement: No new data were created.

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

References

1. Yan, J.; Zhang, M.; Cai, Z.; Shen, Z.; Ma, R.; Xu, Z.; Su, Z.; Yu, M. Effects of chilling and heat requirement differences between peach floral bud and leaf bud on their phenological process. *J. Plant Genet. Resour.* **2021**, *22*, 1281–1292.
2. Yan, J.; Zhao, B.; Sun, M.; Song, H.; Cai, Z.; Li, J.; Su, Z.; Zhang, M.; Shen, Z.; Xu, J.; et al. Adaptability of peach under air temperature change based on chilling requirement. *Acta Hortic. Sin.* **2023**, *50*, 724–736.
3. Douglas, G.B.; Gasic, K. Peach [*Prunus persica* (L.) Batsch] cultivars differ in apparent base temperature and growing degree hour requirement for floral bud break. *Front. Plant Sci.* **2022**, *13*, 801606.
4. Pawasut, A.; Fujishige, N.; Yamane, K.; Yamaki, Y.; Honjo, H. Relationships between chilling and heat requirement for flowering in ornamental peaches. *Engei Gakkai Zasshi* **2004**, *73*, 519–523. [CrossRef]
5. Razavi, F.; Hajilou, J.; Tabatabaei, S.J.; Dadpour, M.R. Comparison of chilling and heat requirement in some peach and apricot cultivars. *Res. Plant Biol.* **2011**, *1*, 40–47.
6. Zhao, X.; Han, X.; Wang, Q.; Wang, X.; Chen, X.; Li, L.; Fu, X.; Gao, D. Early bud break 1 triggers bud break in peach trees by regulating hormone metabolism, the cell cycle, and cell wall modifications. *J. Exp. Bot.* **2020**, *71*, 3512–3523. [CrossRef] [PubMed]
7. Atagul, O.; Calle, A.; Demirel, G.; Lawton, J.M.; Bridges, W.C.; Gasic, K. Estimating heat requirement for flowering in peach germplasm. *Agronomy* **2022**, *12*, 1002. [CrossRef]
8. Rom, R.C.; Harrington, E.H. The effect of varying temperature regimes on degree-days to bloom in the Elberta peach. *Proc. Am. Soc. Hortic. Sci.* **1966**, *88*, 239–244.

9. Couvillon, G.A.; Hendershott, C.H. A characterization of the "After-Rest" period of flower buds of two peach cultivars of different chilling requirements. *J. Am. Soc. Hortic. Sci.* **1974**, *99*, 23–26. [CrossRef]
10. Couvillon, G.A.; Erez, A. Influence of prolonged exposure to chilling temperatures on bud break and heat requirement for bloom of several fruit species. *J. Am. Soc. Hortic. Sci.* **1985**, *110*, 47–50. [CrossRef]
11. Scalabrelli, G.; Couvillon, G.A. The effect of temperature and bud type on rest completion and the GDHC requirement for budbreak in 'Redhaven' peach. *J. Am. Soc. Hortic. Sci.* **1986**, *111*, 537–540. [CrossRef]
12. Gariglio, N.; González Rossia, D.E.; Mendow, M.; Reig, C.; Agusti, M. Effect of artificial chilling on the depth of endodormancy and vegetative and flower budbreak of peach and nectarine cultivars using excised shoots. *Sci. Hortic.* **2006**, *108*, 371–377. [CrossRef]
13. 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]
14. Okie, W.R.; Blackburn, B. Increasing Chilling Reduces Heat Requirement for Floral Budbreak in Peach. *HortScience* **2011**, *46*, 245–252. [CrossRef]
15. Tan, Y.; Li, L.; Li, D.; Chen, D.; Leng, C.; Gao, D. Relationship between Chilling and Heat in Budburst Regulation of Peaches for Protected Cultivation. *Chin. J. Appl. Environ. Biol.* **2012**, *18*, 728–733. [CrossRef]
16. Lin, S.; Wang, H.; Ge, Q.; Hu, Z. Effects of chilling on heat requirement of spring phenology vary between years. *Agric. For. Meteorol.* **2022**, *312*, 108718. [CrossRef]
17. Polgar, C.A.; Primack, R.B. Leaf-out phenology of temperate woody plants: From trees to ecosystems. *New Phytol.* **2011**, *191*, 926–941. [CrossRef] [PubMed]
18. Flynn, D.F.B.; Wolkovich, E.M. Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytol.* **2018**, *219*, 1353–1362. [CrossRef]
19. Du, Y.J.; Pan, Y.Q.; Ma, K.P. Moderate chilling requirement controls budburst for subtropical species in China. *Agric. For. Meteorol.* **2019**, *278*, 107693. [CrossRef]
20. Wang, H.; Wang, H.; Ge, Q.; Dai, J. The interactive effects of chilling, photoperiod, and forcing temperature on flowering phenology of temperate woody plants. *Front. Plant Sci.* **2020**, *11*, 443. [CrossRef]
21. Morin, X.; Lechowicz, M.J.; Augspurger, C.; O'keefe, J.; Viner, D.; Chuine, I. Leaf phenology in 22 North American tree species during the 21st century. *Glob. Chang. Biol.* **2009**, *15*, 961–975. [CrossRef]
22. Polgar, C.A.; Gallinat, A.; Primack, R.B. Drivers of leaf-out phenology and their implications for species invasions: Insights from Thoreau's Concord. *New Phytol.* **2014**, *202*, 106–115. [CrossRef]
23. Bennie, J.; Kubin, E.; Wiltshire, A.; Huntley, B.; Baxter, R. Predicting spatial and temporal patterns of bud-burst and spring frost risk in north-west Europe: The implications of local adaptation to climate. *Glob. Change Biol.* **2010**, *16*, 1503–1514. [CrossRef]
24. Clark, J.S.; Melillo, J.; Mohan, J.; Salk, C. The seasonal timing of warming that controls onset of the growing season. *Glob. Chang. Biol.* **2014**, *20*, 1136–1145. [CrossRef]
25. Demirel, G.; Calle, A.; Lawton, J.M.; Atagul, O.; Fu, W.; Gasic, K. Ppe.CR.1 DNA test for predicting chilling requirement in peach. *Sci. Rep.* **2023**, *13*, 987. [CrossRef]
26. Parker, L.E.; Abatzoglou, J.T. Warming winters reduce chill accumulation for peach production in the Southeastern United States. *Climate* **2019**, *7*, 94. [CrossRef]
27. Li, Y.; Cao, K.; Li, N.; Zhu, G.; Fang, W.; Chen, C.; Wang, X.; Guo, J.; Wang, Q.; Ding, T.; et al. Genomic analyses provide insights into peach local adaptation and responses to climate change. *Genome Res.* **2021**, *31*, 592–606. [CrossRef]
28. Citadin, I.; Raseira, M.C.B.; Herter, F.G.; Silva, J.D. Heat requirement for blooming and leafing in peach. *HortScience* **2001**, *36*, 305–307. [CrossRef]
29. Yan, J.; Zhang, M.H.; Peng, B.; Su, Z.; Xu, Z.; Cai, Z.; Yang, J.; Ma, R.; Yu, M.; Shen, Z. Predicting chilling requirement of peach floral buds using electronic nose. *Sci. Hortic.* **2021**, *290*, 110517. [CrossRef]
30. Luedeling, E.; Guo, L.; Dai, J.; Leslie, C.; Blanke, M.M. Differential responses of trees to temperature variation during the chilling and forcing phases. *Agric. For. Meteorol.* **2013**, *181*, 33–42. [CrossRef]
31. Harrington, C.A.; Gould, P.J. Tradeoffs between chilling and forcing in satisfying dormancy requirements for pacific northwest tree species. *Front. Plant Sci.* **2015**, *6*, 120. [CrossRef]
32. Campoy, J.A.; Ruiz, D.; Cook, N.; Allderman, L.; Egea, J. High temperatures and time to budbreak in low chill apricot 'Palsteyn'. towards a better understanding of chill and heat requirements fulfillment. *Sci. Hortic.* **2011**, *129*, 649–655. [CrossRef]
33. Ruiz, D.; Egea, J.; Salazar, J.A.; Campoy, J.A. Chilling and heat requirements of Japanese plum cultivars for flowering. *Sci. Hortic.* **2018**, *242*, 164–169. [CrossRef]
34. Montazeran, A.; Khadivi, A.; Khaleghi, A. The first report: Chilling and heat requirements of seedless barberry (*Berberis vulgaris* L. var. asperma). *Sci. Hortic.* **2018**, *231*, 188–193. [CrossRef]

35. Hernandez, J.A.; Diaz-Vivancos, P.; Acosta-Motos, J.R.; Alburquerque, N.; Martínez, D.; Carrera, E.; García-Bruntón, J.; Barba-Espín, G. Interplay among antioxidant system, hormone profile and carbohydrate metabolism during bud dormancy breaking in a high-chill peach variety. *Antioxidants* **2021**, *10*, 560. [CrossRef]
36. Hernandez, J.A.; Diaz-Vivancos, P.; Martinez-Sanchez, G.; Alburquerque, N.; Martínez, D.; Barba-Espín, G.; Acosta-Motos, J.R.; Carrera, E.; García-Bruntón, J. Physiological and biochemical characterization of bud dormancy: Evolution of carbohydrate and antioxidant metabolisms and hormonal profile in a low chill peach variety. *Sci. Hortic.* **2021**, *281*, 109957. [CrossRef]