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Chilling Requirement: A Main Step to Get Productive Response in Walnut and Pecan Cultivars in Catamarca (Argentina)

Eber Alexander Delgado[1,2]*, Dante Egardo Carabajal[1], Patricia Cecilia Flores[3], Neus Aletà Soler[4], Norberto Francisco Gariglio[5]

1 Agricultural Experiment Station Catamarca, National Institute of Agricultural Technology (INTA), Route 33, km 4.5, 4705 Sumalao, Valle Viejo, Catamarca, Argentina.
2 Regional Center for Energy and Environment for Sustainable Development (CREAS), National Scientific and Technical Research Council (CONICET) – National University of Catamarca (UNCA), Nuñez del Prado 366, San Fernando del Valle de Catamarca, 4700, Catamarca, Argentina.
3 National University of Rosario (UNR), J. Villarino Experimental Farm, C.C. 14 (2125) Zavalla, Santa Fe, Argentina.
4 Institute of Agrifood Research and Technology (IRTA.) Fruit Tree Program, Torre Marimon, 08140-Caldes de Montbui, Barcelona, Spain.
5 Institute of Agricultural Sciences of the Littoral (ICiAgro Litoral), National Scientific and Technical Research Council (CONICET) – National University of the Littoral (UNL), Faculty of Agricultural Sciences, Kreder 2805, 3080 Esperanza, Santa Fe, Argentina.

*Corresponding author's email: delgado.eber@inta.gob.ar

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ABSTRACT

The thermal requirements of deciduous fruit trees are a key factor in the expansion of walnut and pecan cultivation in Catamarca, a warm region in northwestern Argentina. This study assessed the chilling requirements of two Persian walnut (*Juglans regia* L.) cultivars ('Chandler' and 'Trompito INTA') and three pecan (*Carya illinoinensis* [Wangenh.] K. Koch) cultivars ('Stuart', 'Pawnee', and 'Western'). Well-lignified budsticks—one-year-old branches, 30–40 cm in length—were collected twice, during the autumns of 2018 and 2019, ensuring that both terminal buds (TB) and lateral buds (LB) remained intact. Samples were stored at 5 ± 0.5 °C to accumulate chilling hours (CH) across ten treatments (0–1000 CH, in 100 CH intervals) before forcing under greenhouse conditions. Budbreak was evaluated as the mean time to budbreak (MTB, days) and as the percentage of budbreak. Among pecan cultivars, 'Western' required the least chilling (300–400 CH), followed by 'Pawnee' (400–500 CH) and 'Stuart' (600–800 CH). Notably, in the first year of trials, both 'Pawnee' and 'Western' initiated budbreak without prior cold exposure. For walnuts, 'Trompito INTA' and 'Chandler' required approximately 500 CH and 800 CH, respectively, with budbreak occurring only after a minimum of 200 CH. The results indicate that pecan cultivars require fewer chilling hours to achieve 50% budbreak than to stabilize MTB. 'Trompito INTA' emerged as a low-chill Persian walnut cultivar, potentially well-suited to regions with mild winters. Its adaptability could contribute to climate change resilience in walnut production. Overall, these findings provide valuable guidance for cultivar selection and orchard expansion planning in the warm regions of Argentina.

Abbreviation: Chilling hours (CH), Growing degree days (GDD), Lateral bud (LB), Mean time to budbreak, One-year-old shoots (OYS), Terminal bud (TB)

Introduction

Pecans (*Carya illinoinensis* [Wangenh.] K. Koch) and walnuts (*Juglans regia* L.) are two nut species traditionally consumed in their regions of origin—the southern United States and Mexico for pecans, and Central Asia and Europe for walnuts (García-González et al., 2020; Hassani et al., 2020). Both are valued for their high protein content and frequent inclusion in healthy diets; walnuts, in particular, are classified as a nutraceutical product. They are notable for their high linoleic acid content—21% in pecans and 38% in walnuts—and for the substantial antioxidant content present in the kernel skin, which is always consumed along with the fruit. Pecans are also naturally sodium-free (Aletà and Abelló, 2020; Salas et al., 2023).

Global marketed pecan production averaged 150,000 t (kernel basis) over the five-year period 2019–2023, with almost 90% of total output originating from its natural production area. In 2021, Mexico and the United States accounted for approximately 63% and 38% of global exports, respectively (INC, 2021). In recent years, new producing countries have emerged, including South Africa, Australia, Israel, Brazil, Argentina, Peru, and Egypt (FAOSTAT, 2021; INC, 2022). Global pecan consumption remains low, averaging 30 g per person per year, although consumption in the United States reaches 225 g and exceeds 100 g in Mexico. The Netherlands and Israel are notable for per capita consumption exceeding 300 g (INC, 2022).

World walnut production is substantially higher, averaging 988,000 t (kernel basis) from 2019–2023. The leading producers are China (48%), the United States (29%), and Chile (7%), although these countries are not among the highest consumers. Global walnut consumption averages over 130 g per person annually, with particularly strong growth in Mediterranean countries that are also major producers, such as Spain, Italy, and Turkey. High consumption levels are also observed in countries with strong health-conscious markets, such as the Netherlands, Israel, and Germany, where per capita consumption can reach 500 g (INC, 2022). In Central Europe, Ukraine, Romania, Moldova, and France are significant producers and, more importantly, major exporters (FAOSTAT, 2021; INC, 2022).

Argentina contributes 0.2% of global pecan production and 0.6% of global Persian walnut production. Despite its modest share in world output, nut cultivation is economically significant in the country (CFFS, 2017). Over the past two decades, pecan cultivation has expanded markedly, now covering approximately 8,000 ha. It is the second most important nut crop in Argentina, following Persian walnut, which occupies 16,022 ha (CAPPECÁN, 2023; INDEC, 2018). Walnuts are traditionally consumed in Argentina at levels exceeding the global per capita average (150 g), and domestic demand appears to be met by local production, as the country is not among major importers. The recognized nutritional and health benefits of walnuts and pecans are contributing to increased demand both globally and within Argentina (Batlle et al., 2023).

Catamarca, located in northwestern Argentina, accounts for approximately 29% of the nation’s Persian walnut production (Cólica et al., 2023). In contrast, pecans represent an emerging nut crop in the region, increasingly cultivated in areas where, in recent years, walnut trees have exhibited unprecedented declines in productivity and adaptability (Trabichet, 2022). Within the province, traditional Persian walnut cultivation faces two primary limitations: water scarcity in high-altitude zones and insufficient winter chill accumulation in lower elevations. These constraints are expected to intensify under projected climate change scenarios, particularly due to reductions in chilling hours. The decline is predicted to be most pronounced in areas situated below 1,200 m above sea level, where high-chill cultivars such as ‘Chandler’ are likely to experience significant yield losses.

Addressing this challenge requires the identification of cultivars with lower chilling requirements and greater thermal adaptability to warmer conditions. In response, the National Institute of Agricultural Technology (INTA) in Catamarca conducted a 30-year evaluation of numerous Persian walnut genotypes. This effort culminated in the development of the ‘Trompito INTA’ cultivar, which has consistently demonstrated strong agronomic performance, producing nuts of comparable quality to ‘Chandler’ while exhibiting superior adaptation to areas with limited winter chill (Carabajal et al., 2021). These attributes position ‘Trompito INTA’ as a promising alternative for sustainable walnut cultivation under future climatic conditions. Meanwhile, pecan cultivars currently planted in Catamarca—specifically ‘Stuart’, ‘Western’, and ‘Pawnee’—are performing well in the region’s warm environments (Delgado & Carabajal, 2018).

In natural settings, each genotype develops evolutionary strategies to withstand adverse conditions such as winter dormancy (Fallah et al., 2022). This adaptive process involves the accumulation of both chilling and heat units to ensure budbreak occurs at an optimal time (Aslamarz et al., 2010). During this period, any plant structure containing a meristem undergoes a temporary reduction in activity (Zhuang et al., 2016). Recently, a negative impact on reproductive phenology has been documented in numerous temperate woody species, including Persian walnut (*J. regia* L.), resulting in reduced productivity and vegetative growth (Aletà et al., 2021). In Catamarca, monitoring indicates that these effects are likely linked to a reduction in winter chill, which has severely affected walnut trees in certain areas. However, the precise chilling and heat requirements for many species and cultivars remain poorly defined, underscoring the importance of understanding cultivar-specific dormancy-breaking needs (Benmoussa et al., 2017).

Given the projected impacts of climate change—particularly in temperate and cold subtropical regions where nut production is concentrated—selecting genotypes well adapted to local conditions is essential (Luedeling et al., 2009; El Yaacoubi et al., 2016). In Argentina, winter chill availability is expected to decline (Cabré & Nuñez, 2020), potentially compromising the productivity of high-chill cultivars such as 'Chandler' (Del Barrio et al., 2021). Consequently, promoting low-chill cultivars such as 'Trompito INTA' is critical for ensuring the adaptation and long-term sustainability of walnut cultivation in the warm valleys of Catamarca and similar environments.

From a physiological perspective, endodormancy represents a phase of true dormancy that persists regardless of otherwise favorable environmental conditions (Lang et al., 1987). The release from endodormancy is triggered by exposure to low temperatures (Zhuang et al., 2016), which restore the plant's growth potential but do not immediately induce active growth (Fadón & Rodrigo, 2018). Following this stage, if ambient temperatures remain suboptimal, the plant enters ecodormancy (Lang et al., 1987). Ecodormancy is ultimately broken when sufficient heat accumulation occurs, leading to bud sprouting and flowering. The specific heat requirement for this process varies among species and cultivars (Fadón & Rodrigo, 2018).

Numerous recent studies have documented the adverse effects of insufficient winter chill accumulation in both walnut and pecan. In these species, inadequate chilling can result in irregular, delayed, or incomplete budbreak; weak and uneven shoot elongation; limited leaf expansion; and an overall decline in vegetative vigor (Fadón et al., 2020; Luedeling & Gassner, 2012). Such symptoms are frequently accompanied by reduced floral induction and abnormal development of reproductive structures (Kumar et al., 2024).

In dichogamous species such as walnut and pecan, chilling deficiency is particularly detrimental because it disrupts the synchronization between male and female flowering phases, thereby reducing cross-pollination efficiency (Aletà et al., 2021; Ajamgard et al., 2017; Hassankhah et al., 2017). This desynchronization can lead to reduced fruit set, premature nut drop, and diminished yield, as well as lower final nut quality (Benmoussa et al., 2017). Insufficient chilling has also been associated with prolonged pollen release and an extended pistillate flowering period, further complicating cross-pollination in commercial orchards (Shinwari et al., 2025). These disruptions have additionally been linked to higher incidences of poorly developed or empty fruits ("stick-tights") and to adverse impacts on yield in subsequent seasons (Kumar et al., 2024). These physiological consequences underscore the importance of selecting cultivars with chilling requirements suited to local climatic conditions and of accurately monitoring winter chill accumulation as a key component of orchard management (Campoy et al., 2019; Luedeling et al., 2013). Chilling requirements are quantified in terms of chilling hours and/or chilling units, which reflect the cumulative duration that buds are exposed to specific low-temperature thresholds (Richardson et al., 1974; Weinberger, 1950).

To determine whether a cultivar's chilling requirement has been met, researchers commonly assess parameters such as the stabilization of the mean time to budbreak (MTB) in response to cold (Gholizadeh et al., 2017) or the chilling threshold necessary to achieve over 50% bud sprouting (Guo et al., 2014). MTB also represents the "heat requirement" that each bud type and cultivar must fulfill to initiate budbreak (Gariglio et al., 2006). However, precise quantification of chilling hours or units under field conditions is challenging due to continual variation in solar radiation, daily temperature fluctuations, and other environmental factors (Guo et al., 2014; Rodríguez et al., 2021).

To address these challenges, standardized methodologies have been developed to assess the depth and progression of dormancy in deciduous fruit species. These include both model-based approaches—such as chill models (Luedeling et al., 2009)—and biological methods applied to one-year-old shoots (OYS) or isolated buds exposed to natural and/or artificial cold treatments under controlled conditions (Balandier et al., 1993). Biological assessments have been employed in walnut by Aslamarz et al. (2009) in Iran and Del Barrio et al. (2022) in northern Patagonia, Argentina.

The objective of the present study was to monitor the effect of chilling accumulation on the depth of endodormancy and on budbreak in one-year-old shoots of two walnut cultivars ('Trompito INTA' and 'Chandler') and three pecan cultivars ('Pawnee', 'Stuart', and 'Western').

Materials and Methods

Site and plant material description

Plant materials were obtained from two experimental plots located at the Agricultural Experiment Station Catamarca (EEA) of the National Institute of Agricultural Technology (INTA), Valle Viejo, Catamarca, Argentina (28°28′13″ S; 65°43′41″ W; 514.58 m.a.s.l.). The region's climate is classified as semi-arid, with an average annual temperature of 20.9 °C, a frost-free period of approximately 308 days, and annual precipitation ranging from 430 mm in the south to 630 mm in the far north (Trabichet, 2022). Maximum and minimum temperatures, as well as accumulated winter chill, were recorded using an INTA meteorological station and are presented in Figure S1. Winter chilling accumulation was quantified using the Chilling Hours Model (Weinberger, 1950).

The orchards are established on sandy loam soils with a deep agricultural horizon. Walnut trees were planted at a spacing of 10 × 10 m, and pecan trees at 8 × 8 m. Irrigation was applied by flooding, and the soil surface was maintained under vegetative ground cover. The rootstocks used were seedling plants of each respective species: *Juglans regia* for walnut and *Carya illinoinensis* for pecan. At the time of sampling, the walnut cultivars ‘Chandler’ and ‘Trompito INTA’ were 10 years old, while the pecan cultivars ‘Stuart’, ‘Pawnee’, and ‘Western’ were 7 years old. These ages ensured that the tissues analyzed originated from trees in full production, exhibiting stable phenological and physiological behavior.

Each cultivar was selected for its agronomic relevance, phenological diversity, and adaptation to the semi-arid conditions of the region. ‘Chandler’ (*Juglans regia*) is a late-fruiting cultivar with high chilling requirements, widely recognized as the production standard in Argentina. It is characterized by strong lateral fruiting, high nut yield, and excellent kernel quality, although it is sensitive to spring frosts due to its late budbreak. ‘Trompito INTA’ (*Juglans regia*), a national cultivar developed by INTA, exhibits earlier budbreak and greater adaptability to areas with low winter chill. It is particularly well suited to warm, semi-arid regions such as Catamarca and maintains good productivity under these conditions. ‘Stuart’ (*Carya illinoinensis*) is a traditional cultivar with late budbreak, moderate chilling requirements, and adaptability to a wide range of environments, producing stable yields and commonly planted in long-established orchards. ‘Pawnee’ (*Carya illinoinensis*) is an early- to mid-season cultivar with low chilling requirements, early nut maturation, and excellent kernel quality, making it particularly suitable for warm areas with mild winters. ‘Western’ (*Carya illinoinensis*) is a mid-season cultivar with intermediate chilling requirements and good tolerance to heat and drought, making it a viable option for semi-arid production systems, and is valued in the market for its desirable nut characteristics.

Material collection and test planning

The study was conducted on well-lignified budsticks, specifically one-year-old shoots (OYS) collected twice—in autumn (May, Southern Hemisphere) of 2018 and 2019. Samples were obtained randomly, prior to the onset of cold accumulation (Aslamarz et al., 2009; Gariglio et al., 2006), from six trees per cultivar of both species. Each shoot measured 30–40 cm in length and 1.5–2 cm in diameter. The terminal bud (TB) and one lateral bud (LB) were retained, while all other buds were removed. For each cultivar and year, 220 samples were collected and subsequently disinfected using a fungicide mixture of carbendazim and thiram (Aslamarz et al., 2009). Ten lots of 20 OYS per cultivar were prepared. Each lot was placed in a 10 L non-toxic polyethylene bag containing moist coniferous sawdust to prevent dehydration (Ruiz et al., 2018), and then stored in a controlled-temperature chamber. Budsticks from the reference treatment (0 CH; no chilling accumulation) were transferred directly to the greenhouse to induce budbreak.

Chilling treatments

The OYS-containing bags were placed in a cold chamber maintained at 5 ± 0.5 °C, where ten artificial chilling treatments were simulated. At 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1,000 chilling hours (CH), one bag per cultivar was removed from the chamber (Weinberger, 1950). The shoots from the control treatment (0 CH) were placed in the greenhouse immediately after field collection to force budbreak. Inside the cold chamber, the photoperiod was set to 8 h, with a light intensity of 100 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) (Gariglio et al., 2006). An iButton DS1921G Thermochron Logger (2 kB capacity, −40 to +85 °C range) was installed inside the chamber to continuously monitor temperature.

Budbreak in the greenhouse

Following the completion of cold treatments, excised shoots were arranged into sets of five OYS and placed in 500 mL plastic containers, with their basal ends submerged in a 5% sucrose solution (Hajinia et al., 2021). To prevent occlusion of conducting vessels, the basal ends were re-cut weekly, and the solution was replaced daily with fresh water (Dennis Jr., 2003). The plant material was forced to sprout in a greenhouse under natural photoperiod conditions, maintaining day/night temperatures of 25/15 °C, respectively.

Budbreak progression for both lateral buds (LB) and terminal buds (TB) was monitored three times per week. Observations for each shoot were concluded when the buds reached the phenological stages of balloon or green tip—stage “Cf” in Persian walnut (Germain & Lespinasse, 1999) and stage “V4” in pecan (Frusso, 2007). Accumulated growing degree days (GDD) were calculated (Richardson et al., 1974) from the date OYS were transferred to the greenhouse until 50% of the buds reached balloon or green tip. GDD was determined as the sum of daily (Tm − Tb) values, where Tm is the daily mean temperature (average of daily minimum and maximum temperatures; Aslamarz et al., 2009) and Tb is the base temperature for growth (10 °C for both walnut and pecan; Del Barrio et al., 2022; Trabichet, 2022). Observations continued for up to 140 days from the onset of the forcing period.

Experimental design and statistical analysis

The experiment followed a completely randomized design with four replications (containers) of five OYS per treatment and cultivar. Results were expressed either as mean time to budbreak (MTB, in days; calculated as the arithmetic mean of the four groups of five OYS) or as the percentage of budbreak (Gariglio et al., 2006). Analysis of variance (ANOVA) was used to assess differences in MTB and budbreak percentage, and means were separated using Tukey's test at the 5% significance level ($P < 0.05$). Percentage data were arcsine transformed prior to analysis. Statistical analyses were conducted using the InfoStat program (Di Rienzo et al., 2011). Linear regression analysis was performed to evaluate correlations between the chilling requirement of the cultivars and their corresponding MTB values.

Results

Chilling effect on MTB

In Persian walnut, the cultivars 'Chandler' and 'Trompito INTA' exhibited no sprouting in the treatments with 0 and 100 CH in either year. As a result, MTB values could not be determined for these treatments. In contrast, pecan cultivars demonstrated budbreak even in the absence of chilling accumulation (0 CH), although in the first year, 'Stuart' also failed to sprout under 0 CH conditions (Table 1). Across all cultivars, MTB values decreased consistently as chilling hours increased. For Persian walnut, in which budbreak was absent at 0 and 100 CH, the highest MTB value was observed in the 200 CH treatment (Table 1).

Table 1. Effect of different artificial chilling accumulation (CH) at 5 ± 0.5 °C on the mean time to budbreak (MTB, in days) in terminal (TB) and lateral (LB) vegetative buds of excised shoots from two Persian walnut cultivars, 'Chandler' and 'Trompito INTA', and three pecan cultivars, 'Stuart', 'Pawnee' and 'Western', forced to budbreak at 25/15 °C (day/night) for 140 d.

	Year 2018																					
Cultivar	**Chilling hours**																					
	0		100		200		300		400		500		600		700		800		900		1000	
	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB
Chandler	-	-	-	-	125^{a}	132^{a}	121^{a}	125^{b}	113^{b}	116^{c}	107^{b}	112^{d}	99^{c}	105^{e}	80^{d}	84^{f}	68^{e}	72^{h}	66^{e}	67^{h}	65^{e}	68^{h}
Trompito INTA	-	-	-	-	107^{a}	110^{a}	102^{b}	107^{a}	94^{c}	96^{b}	71^{d}	76^{c}	65^{e}	67^{d}	65^{e}	68^{d}	64^{e}	66^{d}	65^{e}	68^{d}	66^{e}	68^{d}
Stuart	-	-	99^{a}	107^{a}	87^{b}	94^{b}	84^{c}	91^{c}	76^{d}	84^{d}	63^{e}	68^{e}	53^{f}	56^{f}	54^{f}	56^{f}	54^{f}	55^{f}	53^{f}	53^{f}	53^{f}	53^{f}
Pawnee	109^{a}	114^{a}	96^{b}	101^{b}	83^{c}	90^{c}	70^{d}	76^{d}	61^{e}	64^{e}	48^{f}	53^{f}	47^{f}	51^{f}	47^{f}	53^{f}	47^{f}	51^{f}	48^{f}	51^{f}	49^{f}	52^{f}
Western	94^{a}	99^{a}	75^{b}	79^{b}	57^{c}	60^{c}	34^{d}	37^{d}	35^{d}	37^{d}	34^{d}	36^{d}	34^{d}	37^{d}	34^{d}	35^{d}	33^{d}	36^{d}	33^{d}	34^{d}	33^{d}	34^{d}
	Year 2019																					
Chandler	-	-	-	-	141^{a}	149^{a}	139^{a}	140^{b}	131^{b}	133^{c}	118^{c}	122^{d}	111^{d}	114^{e}	88^{e}	94^{f}	71^{f}	75^{g}	68^{f}	76^{g}	69^{f}	76^{g}
Trompito INTA	-	-	-	-	116^{a}	119^{a}	104^{b}	110^{b}	95^{c}	102^{c}	68^{d}	71^{d}	70^{d}	70^{d}	69^{d}	70^{d}	69^{d}	71^{d}	70^{d}	72^{d}	70^{d}	71^{d}
Stuart	104^{a}	114^{a}	94^{b}	100^{b}	84^{c}	90^{c}	79^{d}	84^{d}	74^{e}	77^{e}	69^{f}	73^{f}	65^{g}	68^{g}	59^{h}	65^{g}	45^{i}	47^{h}	46^{i}	47^{h}	45^{i}	46^{h}
Pawnee	86^{a}	89^{a}	74^{b}	80^{b}	65^{c}	70^{c}	49^{d}	54^{d}	36^{e}	38^{e}	36^{e}	38^{e}	37^{e}	39^{e}	35^{e}	39^{e}	35^{e}	37^{e}	36^{e}	39^{e}	37^{e}	38^{e}
Western	82^{a}	87^{a}	74^{b}	78^{b}	62^{c}	66^{c}	51^{d}	56^{d}	34^{e}	36^{e}	35^{e}	37^{e}	35^{e}	37^{e}	35^{e}	37^{e}	36^{e}	36^{e}	35^{e}	36^{e}	35^{e}	35^{e}

Means followed by different letters in the same row differ significantly according to Tukey's test ($P \leq 0.05$).

The initial MTB values for each species and cultivar were directly correlated with the respective cultivar's chilling requirement (Fig. 1). In Persian walnut, the initial mean MTB across both years was higher in 'Chandler' (136 d) than in 'Trompito INTA' (113 d). Similarly, in pecan, 'Stuart' exhibited the highest initial mean MTB (106 d), followed by 'Pawnee' (99 d) and 'Western' (90 d).

For the 'Chandler' cultivar, MTB values showed no significant differences beyond 800 CH in either year, indicating saturation in the response to additional chilling (Table 1). In 'Trompito INTA', the stabilization point varied slightly by year, occurring after 600 CH in 2018 and after 500 CH in 2019 (Table 1). At stabilization, MTB values were approximately 65 days for 'Trompito INTA' and 75 days for 'Chandler', with no statistical differences between cultivars. Although the initial MTB was 17% higher in 'Chandler' at the onset of dormancy, the rate of MTB decline with chilling accumulation was faster in 'Trompito INTA', allowing it to meet its chilling requirement with 200–300 fewer CH than 'Chandler'.

Among pecan cultivars, 'Stuart' exhibited the highest MTB values, exceeding those of 'Pawnee' and 'Western' by 20% and 35%, respectively (Table 1). In 'Stuart', MTB stabilization occurred above 600 CH in 2018 and above 800 CH in 2019. The cultivar 'Pawnee' reached stability above 500 CH in 2018 and above 400 CH in 2019. 'Western' had the lowest chilling requirement, with MTB stabilization occurring at 300 CH in 2018 and 400 CH in 2019 (Table 1).

Chilling effect on budbreak percentage

In all cultivars, budbreak percentage increased with accumulated chilling. 'Trompito INTA' exhibited a low budbreak percentage when chilling accumulation was below 400 CH in both years (Table 2). In contrast, in 'Chandler', low budbreak percentages were observed at chilling levels below 600 CH in one year (e.g., 2018) and below 700 CH in the other (e.g., 2019). For pecan cultivars, 'Stuart', 'Pawnee', and 'Western' exhibited budbreak even in the absence of chilling accumulation, except for 'Stuart' in the first year of the experiment.

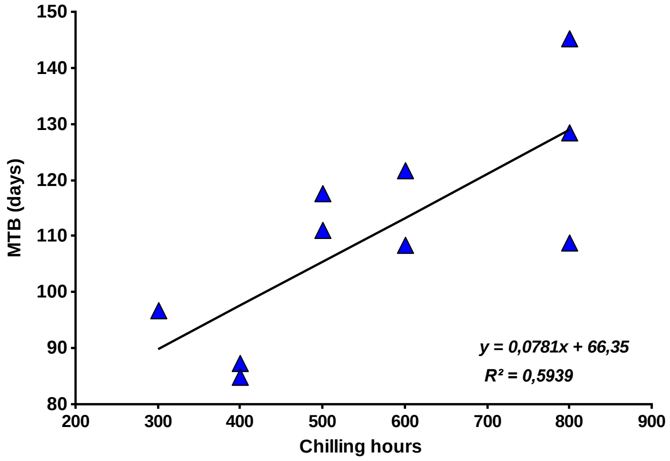

Fig. 1. Relationship between the chilling requirement (chilling hours) of different Persian walnut and pecan cultivars and their maximum mean time to budbreak (MTB; days).

The 'Trompito INTA' cultivar consistently required 500 CH in both years to achieve 50% budbreak. In contrast, 'Chandler' exhibited year-to-year variability, requiring between 700 and 800 CH. For pecan cultivars, treatments with either 0 or 100 CH resulted in budbreak rates exceeding 50% for both 'Pawnee' and 'Western'. Notably, 'Western' achieved 100% budbreak with as few as 100 CH. In the case of 'Stuart', a chilling requirement of 200–300 CH was sufficient to reach approximately 50% budbreak in both years (Table 2).

Response of terminal versus lateral buds

The mean time to budbreak (MTB) for lateral buds was consistently longer than that of terminal buds—by at least 4% in Persian walnut (Fig. 2A) and 6% in pecan (Fig. 2B). The MTB for both bud types stabilized at the same chilling hour thresholds.

Walnut vs. pecan response to chilling

A differential response to chilling accumulation was observed between the two species with respect to both mean time to budbreak (MTB) and the chilling requirement for 50% budbreak. In walnut, similar amounts of chilling were necessary to stabilize MTB and achieve 50% budbreak. In contrast, pecan required fewer CH to reach 50% budbreak than to stabilize MTB, suggesting that some buds can sprout earlier with limited chilling, whereas greater chilling is required for uniform budbreak (Table 3).

Table 2. Effect of different artificial chilling accumulation (CH) at 5 ± 0.5 °C on the budbreak percentage (%) of terminal (TB) and lateral (LB) vegetative buds of excised shoots from two Persian walnut cultivars, ‘Chandler’ and ‘Trompito INTA’, and three pecan cultivars, ‘Stuart’, ‘Pawnee’ and ‘Western’, forced at 25/15 °C (day/night) for 140 d.

	Year 2018																					
	Chilling hours																					
Cultivar	0		100		200		300		400		500		600		700		800		900		1000	
	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB	TB	LB
Chandler	0^{c}	0^{d}	0^{c}	0^{d}	0^{c}	0^{d}	0^{c}	0^{d}	0^{c}	0^{d}	0^{c}	0^{d}	15^{b}	0^{d}	95^{a}	76^{c}	100^{a}	94^{b}	100^{a}	100^{a}	100^{a}	100^{a}
Trompito INTA	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	5^{c}	7^{c}	31^{b}	32^{b}	95^{a}	95^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Stuart	0^{e}	0^{e}	14^{d}	0^{e}	54^{c}	44^{d}	66^{b}	57^{c}	95^{a}	70^{b}	100^{a}	96^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Pawnee	10^{d}	14^{d}	34^{c}	14^{d}	67^{b}	54^{c}	96^{a}	92^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Western	30^{b}	34^{b}	96^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
	Year 2019																					
Chandler	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	63^{b}	41^{b}	96^{a}	96^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Trompito INTA	0^{c}	0^{d}	0^{c}	0^{d}	0^{c}	0^{d}	5^{c}	0^{d}	41^{b}	24^{c}	97^{a}	94^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Stuart	5^{d}	14^{e}	44^{c}	26^{d}	72^{b}	54^{c}	79^{b}	84^{b}	100^{a}	94^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Pawnee	60^{c}	50^{c}	90^{b}	86^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Western	86^{b}	58^{c}	97^{a}	74^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Means followed by different letters in the same row differ significantly according to Tukey’s test ($P \leq 0.05$).

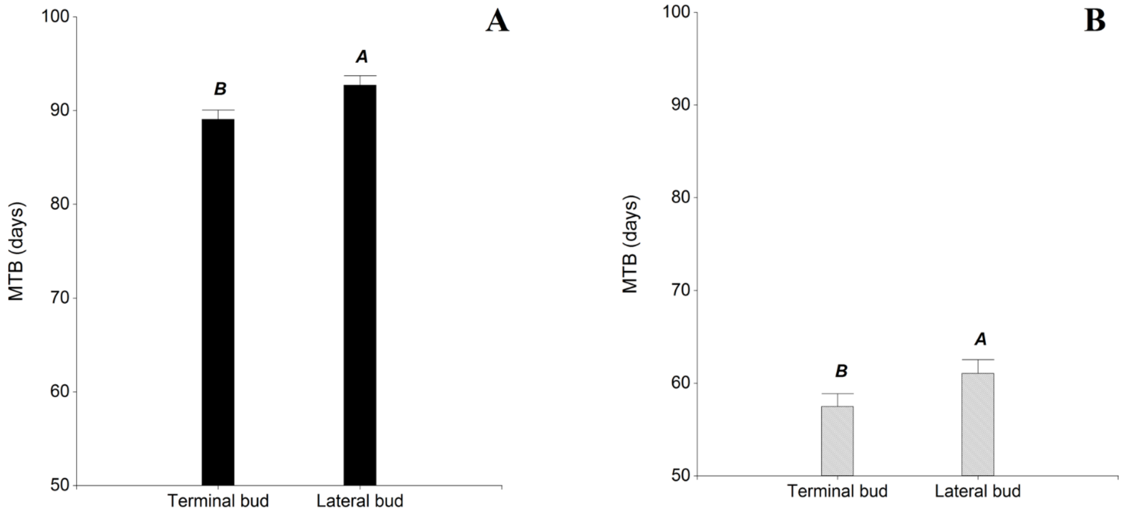

Fig. 2. Mean time to budbreak (MTB, days) of terminal and lateral buds in excised shoots of (**A**) Persian walnut and; (**B**) pecan. Different letters indicate significant differences according to Tukey's test ($P \leq 0.05$).

Table 3. Chilling requirements for one-year-old shoots of various Persian walnut cultivars ('Chandler' and 'Trompito INTA') and pecan cultivars ('Stuart', 'Pawnee', and 'Western'): assessment based on two criteria indicating fulfilled chilling requirement to break dormancy-mean time to budbreak (MTB, in days) stability and budbreak percentage over 50%. Data encompass two study years (2018 and 2019).

Cultivar	Year	Fulfilled chilling requirement criteria (h)		Range of chilling accumulation with budbreak >50%
		MTB (d) stabilization	[a]50% budbreak	
Chandler	2018	800	700	700 - 1.000
	2019	800	800	800 - 1.000
Trompito INTA	2018	600	500	500 - 1.000
	2019	500	500	500 - 1.000
Stuart	2018	600	300	300 - 1.000
	2019	800	200	200 - 1.000
Pawnee	2018	500	200	200 - 1.000
	2019	400	100	100 - 1.000
Western	2018	300	100	100 - 1.000
	2019	400	100	100 - 1.000

a: 50% budbreak of terminal and lateral buds at 90 d of observation.

Heat requirements

It was observed that the mean heat requirement for initiating budbreak increased with the chilling requirement of the cultivar. In cases of very deep dormancy, budbreak did not occur even when substantial heat units were provided, as seen in Persian walnut (cv. 'Chandler' and 'Trompito INTA') at 0 and 100 CH. Conversely, heat requirements decreased markedly with greater chilling accumulation. Significant differences in heat requirements, expressed as growing degree days (GDD), were detected between terminal and lateral buds across the walnut and pecan cultivars examined (Fig. 3A, B). The only exception was the lateral buds of 'Pawnee' and 'Western', which exhibited low heat requirements and did not differ significantly (Fig. 3B).

In the 'Chandler' cultivar, terminal buds (TB) and lateral buds (LB) required 680 and 720 GDD, respectively, in 2018 to break dormancy, whereas in 2019 these values increased to 705 and 745 GDD, respectively (Fig. 3B). In contrast, the 'Trompito INTA' cultivar exhibited lower thermal requirements, ranging from 652 to 675 GDD for TB and 673 to 712 GDD for LB across both years—approximately 5% less than those of 'Chandler' (Fig. 3A, B). Significant differences in GDD required for budbreak were observed among the cultivars. In both years, 'Chandler' showed the highest thermal requirements, followed by 'Trompito INTA', 'Stuart', and 'Pawnee', while 'Western' had the lowest, requiring only about 50% of the total thermal accumulation needed by 'Chandler' (Fig. 3A, B).

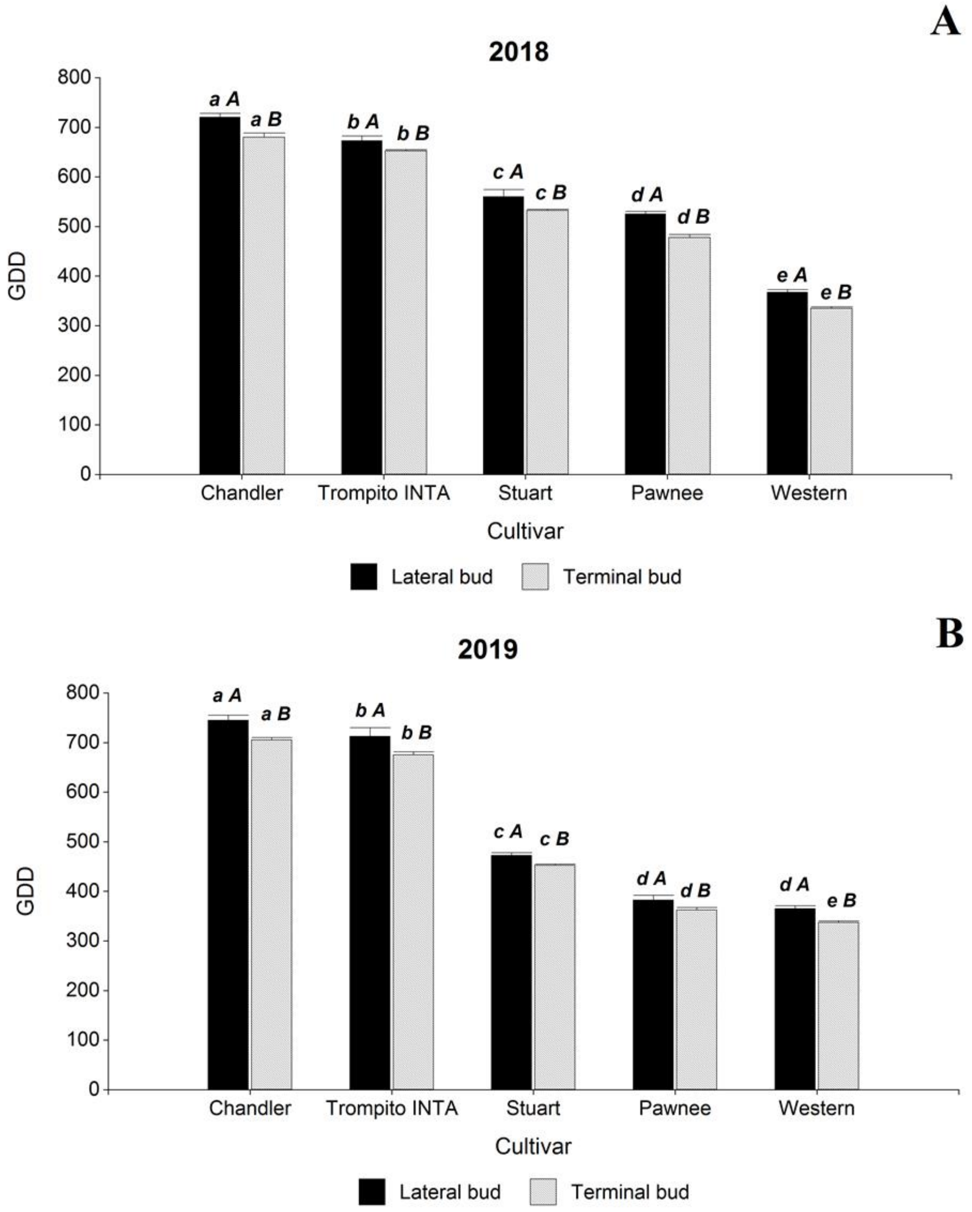

Fig. 3. Heat requirements of terminal buds (TB) and lateral buds (LB) of one-year-old shoots to attain the phenological stage "Cf" in Persian walnut and "V4" in pecan, in two consecutive years, (**A**) 2018 and; (**B**) 2019. Mean separation between cultivars (lowercase letters) and between bud types within cultivars (uppercase letters) according to Tukey's test ($P \leq 0.05$). Vertical bars represent standard error.

Discussion

Excised buds from Persian walnut cultivars exposed to fewer than 100 chilling hours (CH) exhibited dehydration symptoms after 140 days of forcing, preventing budbreak. This phenomenon, previously reported in peach [*Prunus persica* (L.) Batsch] (Gariglio et al., 2006), underscores the necessity of fulfilling chilling requirements to avoid prolonged dormancy and ensure successful budbreak. Notably, excised buds from both Persian walnut and pecan were able to withstand optimal forcing conditions for an extended observation period. However, the absence of budbreak under insufficient chilling suggests that, even in optimal environmental conditions, a lack of required chilling can impose physiological stress and prolong dormancy, as observed in the walnut cultivars studied.

The mean time to budbreak (MTB) reached its highest values under low or zero chilling accumulation, coinciding with the absence of budbreak—except in pecan cultivars. In pecan, budbreak occurred even without chilling, although MTB generally decreased as chilling increased. This trend is consistent with observations in apricot [*Prunus armeniaca* L.] by Campoy et al. (2011), where high MTB values were recorded just before chilling accumulation began. As in apricot, the elevated MTB values in the present study may indicate a well-established dormancy state, as described by Fadón & Rodrigo (2018).

Previous studies have shown a positive correlation between the chilling requirements of cultivars and the MTB at dormancy onset in deciduous fruit species such as peach (Gariglio et al., 2006) and apricot (Campoy et al., 2011). In *Prunus* species, dormancy induction is typically triggered by a reduction in day length, although it is also associated with decreasing minimum temperatures (Heide, 2008; Maurya & Bhalerao, 2017). In contrast, dormancy induction in apple and pear is more closely linked to exposure to low temperatures (<12 °C) (Heide & Prestrud, 2005), leading to an increase in MTB during early dormancy stages as temperatures decline (Castro et al., 2017).

The results obtained for walnut and pecan cultivars suggest a pattern of dormancy induction and progression similar to that of peach and apricot, characterized by a continuous decrease in MTB with increasing chilling accumulation (Table 1). This pattern may indicate a role of photoperiod in dormancy induction for both species. A modeling study by Charrier (2022) supports this hypothesis, demonstrating that photoperiod, rather than low temperatures, exerts a significant influence on dormancy induction in the Persian walnut cultivar ‘Franquette’.

In 2019, walnut cultivars exhibited a greater initial dormancy depth (higher MTB values), whereas pecan cultivars showed lower values compared with the previous year. This variation may be influenced by multiple factors during the growing season, including light exposure (Hernández et al., 2021), plant nutrition (Fadón et al., 2018), and temperature conditions (El Yaacoubi et al., 2016). Such factors can alter chilling requirements from one year to the next (Gholizadeh et al., 2017; Tominaga et al., 2022). In this study, MTB values at dormancy onset in walnut and pecan cultivars displayed an inverse relationship with temperature during leaf drop under field conditions at the EEA Catamarca. Specifically, MTB values were lower in the colder year at the time of leaf drop—a pattern that was consistent across different walnut and pecan cultivars grown at four locations in Catamarca Province (unpublished data). It is notable that leaf drop generally occurs about 15 days later in pecan than in walnut. In 2019, MTB values at dormancy onset were 9–16 days greater for walnut cultivars and 12–23 days fewer for pecan cultivars relative to the previous year (Table 1). These changes correlated with higher temperatures during leaf drop in walnuts and lower temperatures in pecans in 2019 compared with 2018 (Fig. S1). This suggests that temperature conditions during leaf drop play a crucial role in determining dormancy depth and that species may respond differently within the same year due to differences in leaf drop timing.

Chilling requirements are generally considered fulfilled when MTB stabilizes in response to additional chilling accumulation (Campoy et al., 2019) or when budbreak percentage exceeds 50% under forcing conditions (Gariglio et al., 2006). Based on these criteria, chilling requirements for the walnut cultivars ‘Chandler’ and ‘Trompito INTA’ in this study ranged from 500 to 800 CH. These findings align with earlier work by Aslamarz et al. (2009), who reported chilling requirements of 400–1500 CH for walnut cultivars. More recent estimates suggest that ‘Chandler’ requires 800–1000 CH (Hajinia et al., 2021), while Del Barrio et al. (2020) place its requirement at approximately 900 CH. The latter authors also note that walnut cultivars requiring fewer than 800 CH are uncommon and typically lack significant commercial value.

Nevertheless, exceptions exist. For instance, the Iranian cultivar ‘Damavand’ (formerly ‘Z30’) has an estimated requirement of 650 CH and has been proposed for cultivation in regions with mild winters (Aslamarz et al., 2009; Hassani et al., 2020). Other commercially valuable low-chill cultivars include ‘Sunland’ (550 CH) and ‘Serr’ (650 CH) (Aslamarz et al., 2009; Hassankhah et al., 2017). In this context, the ‘Trompito INTA’ cultivar is noteworthy for its low chilling requirement (Table 3), placing it in the low-chill category according to Aslamarz et al. (2009). Furthermore, ‘Trompito INTA’ matches or exceeds ‘Chandler’ in fruit quality in the Catamarca Province, Argentina (Carabajal et al., 2021), offering high oil yield and superior quality compared with USDA National Nutrient Database standards (Cittadini et al., 2020). Importantly, it also matures approximately one month earlier than ‘Chandler’ (Carabajal et al., 2021), emphasizing the value of low-chill cultivars in sustaining productivity under projected declines in winter chill associated with climate change.

‘Trompito INTA’ emerges as a strategic cultivar, offering potential both to mitigate the impacts of global climate change and to support the global expansion of walnut cultivation. In Catamarca Province, for instance, ‘Trompito INTA’ plays a pivotal role in increasing walnut production (Carabajal et al., 2021). Under projected climate change scenarios, where winter chill accumulation is expected to decline (Rodríguez et al., 2021; Salama et al., 2021), the chilling requirement of ‘Chandler’ (950–1110 h, Utah Model) (Hassankhah et al., 2017) could become a limiting factor for cultivation in warm regions such as Catamarca. Our findings confirm that under low-chill conditions, as observed in Catamarca, cultivars with high chilling requirements face limitations in budbreak, and this challenge is likely to intensify with global warming. In this context, the identification and use of low-chill cultivars such as ‘Trompito INTA’ will be essential for maintaining the productivity of deciduous fruit trees in marginal environments.

Pecan cultivars exhibited chilling requirements ranging from 300 to 800 CH. While precise thresholds are not well established in the literature, some studies have suggested a range of 400–800 CH (Kuden et al., 2013) and even up to 1,000 CH (Grageda Grageda et al., 2013). It is possible that chilling requirements in pecan are less critical than in Persian walnut and other deciduous fruit trees. Our results support this hypothesis, showing that pecan cultivars achieved adequate budbreak even without fully meeting their estimated chilling requirements, or in some cases, without any chilling accumulation. This finding suggests a greater degree of thermal flexibility in pecan, which could have significant implications for its cultivation in regions with mild winters.

The results obtained with excised buds from Persian walnut and pecan closely matched those recorded in potted plants (unpublished data), with only minor differences for the ‘Western’ cultivar (Table S1). This indicates that the biological method using isolated shoots is a reliable approach for studying cold response and dormancy evolution in both species. Dennis Jr. (2003) cautioned that single-node cuttings are not suitable for assessing the cold response of whole trees, as bud development in intact plants is influenced by correlational inhibition, including apical dominance. However, previous research has demonstrated that excised shoots of sweet cherry exhibit a cold response comparable to that of whole plants (Benmoussa et al., 2017).

In all Persian walnut and pecan cultivars, budbreak percentage increased significantly with chilling accumulation. These results are consistent with previous findings in walnut cultivars and genotypes (Aslamarz et al., 2009; Hassankhah et al., 2017) and in apricot, where increased chilling also resulted in higher budbreak rates (Campoy et al., 2011). This pattern contrasts with that observed in peach and pear cultivars, where increased chilling reduced budbreak percentage in low-chill cultivars once a maximum threshold was reached (Gariglio et al., 2006).

For Persian walnut cultivars, chilling requirements were consistent regardless of whether they were determined using MTB stabilization or a 50% budbreak criterion. In pecan, however, the estimated chilling requirement varied depending on the criterion applied: less chilling was needed to achieve 50% budbreak than to reach MTB stabilization. This suggests that pecan does not display symptoms of insufficient chilling under either controlled or field conditions, which may explain its strong agronomic performance across a wide range of chilling accumulations, in contrast to Persian walnut (Table 3). A similar pattern has been reported in low-chill apple cultivars (Castro et al., 2017).

Nevertheless, the present study does not address the potential effects of insufficient chilling on flower quality or fruit set. In many temperate fruit trees, winter chilling is critical for completing floral differentiation, particularly in the sexual organs (Guo et al., 2014; Salama et al., 2021). Adequate chilling also affects flower fertility, which may partly explain the excessive flower and fruit drop often observed in years with low winter chill (Hajinia et al., 2021; Penso et al., 2020). Consequently, our results do not account for the potential impacts of insufficient chilling on floral fertility, fruit set, or final harvest yield in pecan.

It is well established that terminal buds generally require less chilling than lateral buds to overcome dormancy (Gholizadeh et al., 2017). In the present study, however, both terminal and lateral buds reached MTB stabilization with similar chilling accumulation, indicating comparable chilling requirements. This finding suggests that, although both bud types achieve MTB stabilization under the same chilling conditions, the principal distinction lies in the depth of dormancy. Specifically, lateral buds exhibit a deeper dormancy (>MTB) than terminal buds. Similar differences in dormancy depth between terminal and lateral buds have been documented in other fruit trees, such as apricot and peach (Campoy et al., 2011; Penso et al., 2020).

The stabilization of MTB in response to chilling accumulation also reflects the accumulation of heat, expressed as GDD, required to overcome ecodormancy and initiate growth (Gariglio et al., 2006). In the walnut and pecan cultivars studied, the stabilized MTB ranged from 70 d in walnut to 35 d in pecan (Table 1). These values are substantially higher than those reported for other fruit tree species, such as 3–18 d in peach (depending on the cultivar) (Balandier et al., 1993; Gariglio et al., 2006), 12–18 d in apricot (Campoy et al., 2011), and 10–20 d in fig (*Ficus carica* L.) (Oukabli and Mekaoui, 2012). This relatively high thermal requirement in Persian walnut and pecan likely contributes to their later budbreak compared with other temperate fruit tree crops.

The results also show a marked decrease in heat requirements once chilling requirements have been fully satisfied, consistent with observations by Salama et al. (2021). This reduction is evident in the studied cultivars, where GDD accumulation is minimized after the chilling threshold is reached. Such a pattern underscores the interdependence of chilling and heat requirements in the phenological development of walnut and pecan. Once optimal chilling is achieved, buds appear more physiologically prepared to progress toward budburst with a reduced heat requirement. This mechanism plays a critical role in synchronizing phenological events, helping to ensure that budburst occurs at a favourable time and reducing the risk of damage from late frosts.

These findings provide important insights into the behaviour of these cultivars under varying climatic conditions, supporting the refinement of orchard management strategies and the selection of cultivars best suited to specific environments to optimize production. Variations in GDD among the evaluated cultivars indicate that terminal and lateral buds respond differently to thermal accumulation, influencing their capacity to initiate growth under different climates. Similar differences in heat requirements between terminal and lateral buds have been reported in peach (Penso et al., 2020) and in walnut (Aslamarz et al., 2009).

Conclusion

The biological method based on isolated shoots has proven to be a valuable tool for investigating dormancy in Persian walnut and pecan. The principal findings can be summarized as follows: (i) walnut cultivars exhibit similar chilling requirements when evaluated using common criteria such as MTB stabilization or achieving >50% budbreak, whereas pecan cultivars display lower chilling requirements (100 to 300 CH less than walnut cultivars) for 50% budbreak, indicating greater ecological adaptability; (ii) stabilized MTB values in Persian walnut (65–70 d) exceed those observed in pecan (35–45 d), and both species show comparatively high thermal requirements to overcome ecodormancy relative to other fruit tree crops; (iii) the cultivar ‘Trompito INTA’ emerges as a promising option for mitigating the effects of global climate change due to its lower chilling requirements.

Under projected climate change scenarios—characterized by shorter and warmer winters—the study region may experience reduced winter chill accumulation, potentially compromising budburst in cultivars with high chilling demands. In this context, the identification of genotypes with lower chilling requirements and greater plasticity in thermal response, as demonstrated in this study, becomes especially relevant. From a practical standpoint, growers in regions at risk of insufficient winter chilling should prioritize cultivars such as ‘Trompito INTA’, which combine reduced chilling requirements with stable budburst performance. Furthermore, pecan cultivars with low chilling thresholds may serve as a strategic alternative or complement to walnut cultivation in these environments. Overall, these results can inform orchard planning, cultivar selection, and the anticipation of adaptive management strategies, thereby supporting sustained productivity under increasing climatic variability.

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Author contributions

Conceptualization: EAD and NFG; Methodology: EAD and NFG; Formal analysis: EAD, DEC, and NFG; Investigation: EAD; Resources: DEC; Data curation: EAD; Writing—original draft: EAD; Writing—review and editing: EAD, DEC, PCF, NAS, and NFG; Supervision: DEC, PCF, NAS, and NFG; Project administration: DEC; Funding acquisition: DEC. All authors have read and approved the final version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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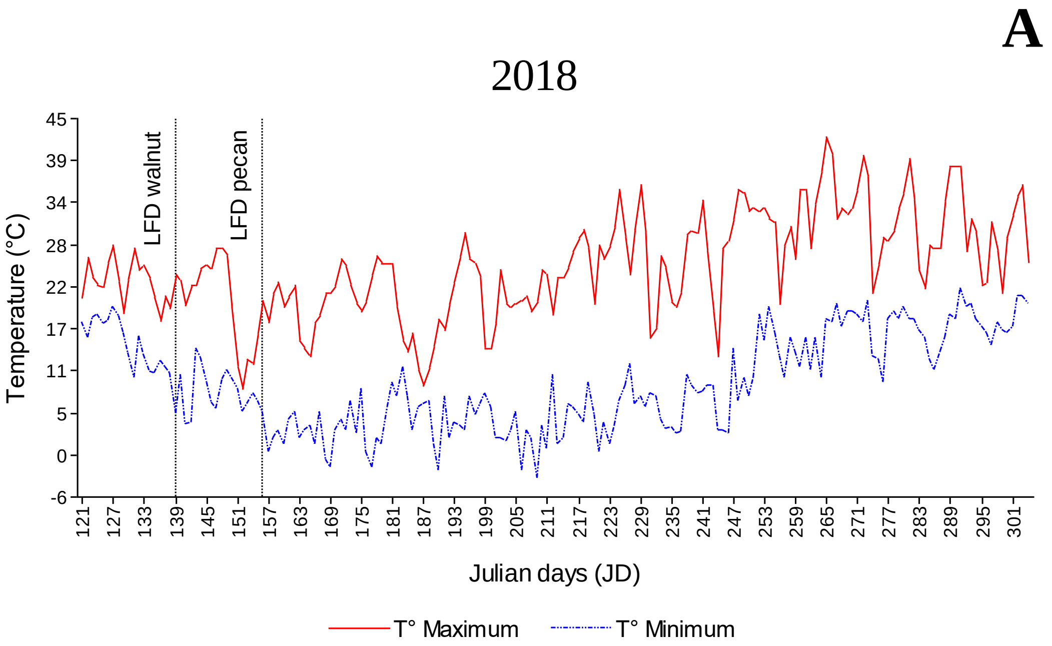
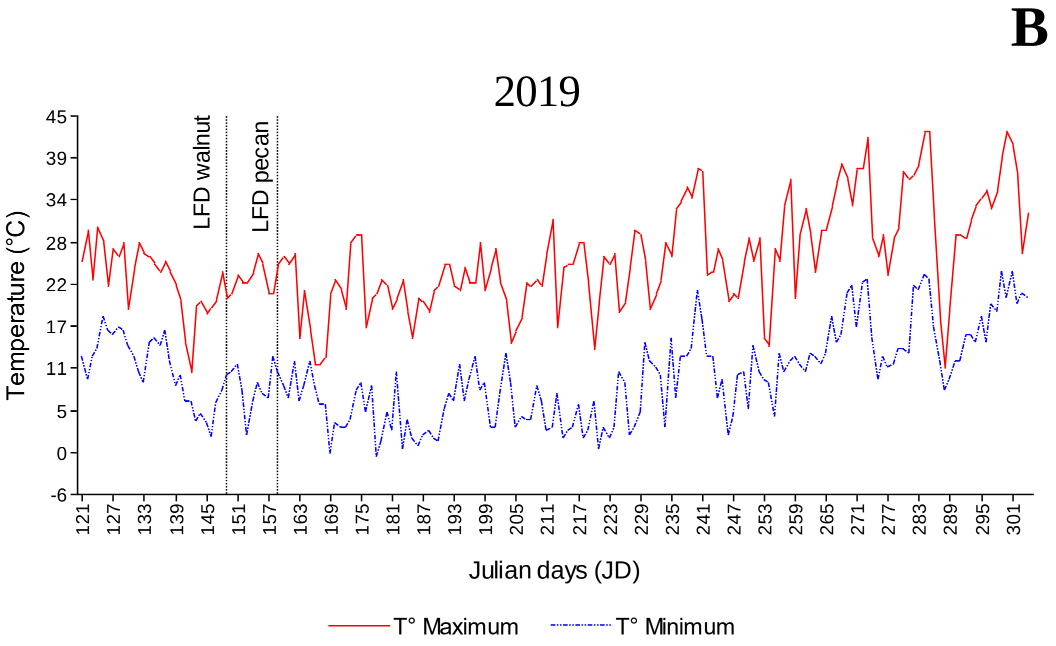
Supplementary Materials





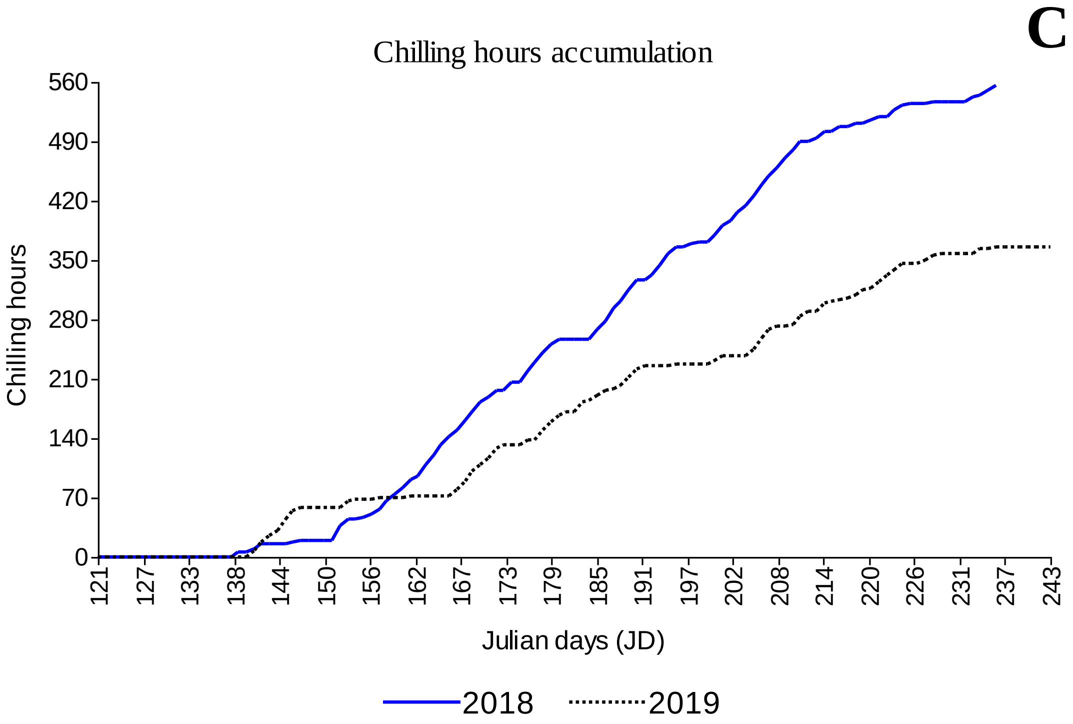


Figure S1. Leaf fall date (LFD) of Persian walnut and pecan, along with daily records of maximum and minimum temperatures during the years (**a**) 2018; (**b**) 2019 and; (**c**) chilling hours accumulation evolution at the Agricultural Experimental Station of INTA Catamarca, Argentina.

Table S1. Chilling requirement for potted plants of various European walnut cultivars ('Chandler' and 'Trompito INTA') and pecan cultivars ('Stuart', 'Pawnee', and 'Western'): Assessment bases on two criteria indicating fulfilled chilling requirement to break dormancy — Mean time to budbreak (MTB) stability and budbreak percentage over 50%. Data encompass two study years (2018 and 2019).

Cultivar	**Years**	**Plants**	
		TMB	**50%**
Chandler	2018	>800	800
	2019	>800	600
Trompito INTA	2018	600	600
	2019	600	400
Stuart	2018	600	400
	2019	>800	200
Pawnee	2018	600	400
	2019	400	200
Western	2018	600	200
	2019	400	200