

Effect of artificial chilling on the depth of endodormancy and vegetative and flower budbreak of peach and nectarine cultivars using excised shoots

Norberto Gariglio^a, Diego E. González Rossia^b, Marisa Mendow^a,
Carmina Reig^b, Manuel Agusti^{a,*}

^a *Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Kreder 2085, 3080 Esperanza, Santa Fe, Argentina*

^b *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain*

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Abstract

Stem cuttings were obtained from 12 peach and nectarine cultivars during leaf fall, placed in plastic bags at 3.0 ± 0.1 °C to simulate 0–800 h of chilling and forced to budbreak at 20.0 ± 1.0 °C for a period of 6 weeks. Some cultivars showed high blooming and leafing without exposure to chilling; chilling enhances leafing and blooming but the percentage increment was higher in leaf buds. In general, maximum budbreak was reached with less chilling accumulation (<100–200 h) in flower buds compared with leaf buds; excessive chilling caused a reduction of the percentage budbreak in flower but not in leaf buds. Additionally, chilling modified the proportion of blooming that occurred before leafing. In non-chilled shoots, blooming occurred earlier than leafing, except in cv. ‘San Pedro 16–33’ but the proportion of blooming before leafing decreased significantly with chilling in most cases. By studying the mean time to budbreak, we conclude that the flower bud generally has a lower intensity of rest; the intensity of rest declines at a slower rate in flower than in leaf buds with chilling; flower buds had greater heat requirements than leaf buds when the chilling requirement had been covered, so that each peach cultivar had a point of critical chilling accumulation below which blooming tended to occur earlier, and above which leafing tends to occur first. Flower and leaf buds had different depths of endodormancy but similar chilling requirements in the majority of peach and nectarine cultivars studied. Finally, different varieties with similar chilling requirements showed different responses to chilling. Therefore, the cutting test measuring the response of vegetative and floral buds provides considerable information on the characterisation of the variety, compared with the sole and traditional data of chilling requirements.

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1. Introduction

Dormancy is a phase of development that occurs annually in deciduous fruit trees and other temperate woody perennial (Saure, 1985). Although the biological basis of dormancy is still not clearly understood (Arora et al., 2003), it has long been known that the main factor in breaking bud endodormancy is low temperature (Balandier et al., 1993; Rouland and Arora, 1997) and that insufficient chilling causes abnormal patterns in budbreak and development in temperate zone fruit trees cultivated in warm climates (Bonhomme et al., 2005). The interest in understanding the mechanism of dormancy set and

release is mainly based on the necessity of manipulation of the dormant period in order to avoid spring frost damage, or to increase budbreak and obtain even flowering in areas of low chilling accumulation (Faust et al., 1997). Furthermore, in warm climates, where the cold requirements cannot be satisfied, an evaluation of the intensity of endodormancy is essential for determining when rest-breaking treatments are necessary (Dennis, 2003).

Precise determination of the temperature requirements for breaking dormancy is nearly impossible under field conditions, where solar radiation, diurnal fluctuations in temperature and other factors cannot be controlled (Dennis, 2003). Thus, the measurement of dormancy ‘depth’ and duration in diverse experimental contexts by isolation of buds as one-node cuttings (Balandier et al., 1993) or excised shoots (Dennis, 2003) is an appropriate method to advance the study of dormancy.

* Corresponding author. Tel.: +34 963879330; fax: +34 963877331.

E-mail address: magusti@prv.upv.es (M. Agusti).

The mean time required for bud-burst is used for comparisons of endodormancy in experimental or standard forcing conditions and its results are confirmed by biochemical tests (Crabbé and Barnola, 1996). The excised shoots method was mainly used to study the evolution of leaf bud endodormancy in winter, collecting twigs every 2 or 3 weeks (Balandier et al., 1993) and their response was paralleled to those observed in whole plants of sweet cherry (Arias and Crabbé, 1975). Balandier et al. (1993) used the excised shoot method not only to study the evolution of endodormancy in winter, but also to calculate the mean time to budbreak corresponding to the end point of endodormancy, exposing the excised shoot to low temperature conditions. Later, Citadin et al. (2001) exposed excised shoots to low temperatures to simulate two chilling accumulation treatments in order to calculate the heat requirements for blooming and leafing in peach. To our knowledge, they were among the first authors to observe the behaviour of leaf and flower buds using the excised shoots method. In this work, we studied the effect of artificial chilling on the depth of endodormancy and the percentage of floral and leaf budbreak, using the excised shoot method, in several peach and nectarine cultivars.

2. Material and methods

During leaf fall (8 November in Valencia, Spain, and 3 April in Esperanza, Argentina) and before chilling accumulation, 50 twigs were randomly collected from 10 adult trees of each variety of peach and nectarine (*Prunus persica* L. Batsch).

Cultivars studied were 'Flordaprince', 'Flordastar', 'Earlygrande', 'San Pedro 16–33', 'Flordaking', 'Tropic Snow', 'Hermosillo' and 'Maycrest' (from Esperanza, Argentina), and 'Baby Gold', 'Spring Belle', 'Sun Red' (or 'Rubro sol'), 'September Free' and 'Armking' from an experimental orchard situated near the town of Llíria, Spain (39°39,281'N–000°38,344'W; 180 m above sea-level). In the Spanish orchard, the trees were planted at 6 m × 4 m (416 trees/ha) in a sandy-loamy soil, and grafted onto 'GF 677' rootstock, while in the Argentinean orchard (31°26'S–60°56'W; 40 m above sea-level), the trees were planted at 5 m × 5 m (400 trees/ha) in a silty-loamy soil, and grafted onto 'Cuaremsillo' rootstock.

Twigs were cut into segments 15 cm long obtaining 400 stem cuttings for each cultivar. Only three axillary buds from a typical node with one central leaf bud and two flower buds at 2 cm from their apical were conserved, while the other buds were eliminated. A group of five cuttings were then placed in plastic bags and exposed to a low temperature (3.0 ± 0.1 °C) (Balandier et al., 1993) to simulate different chilling accumulation treatments: 0, 100, 200, 300, 400, 500, 600, 700 and 800 h. Treatments represent 0–800 chilling hours, according to Weinberger (1950).

After chilling, the shoots were placed with their basal tip in water and forced in a phytotron at 8-h photoperiod ($22.5 \mu\text{mol}/(\text{m}^2 \text{ s})$) (Citadin et al., 2001) and 20.0 ± 1.0 °C for 6 weeks. The basal ends of the shoots were cut (Balandier et al., 1993; Citadin et al., 1998) weekly and water was replaced daily. Flower and vegetative budbreak were observed three times a week. The number of buds that reached the balloon or green tip

Table 1
Effects of chilling accumulation at 3.0 ± 0.1 °C on the percentage of vegetative budbreak of excised shoots of different peach and nectarine (*Prunus persica* L. Batsch) cultivars forced at constant temperature (20 °C) for 45 days

Cultivar	CH H ^a	Vegetative budbreak (%) ^b								
		Chilling accumulation at 3.0 ± 0.1 °C								
		0	100	200	300	400	500	600	700	800
Flordaprince	150 ^c	33cB	46bA	93aA	100aA	100aA	100aA	100aA	92aA	86aA
Flordastar	250 ^c	0cC	13bB	100aA	100aA	95aA	90aA	93aA	94aA	100aA
Earlygrande	260 ^c	26cB	40bA	100aA	93aA	100aA	100aA	93aA	90aA	87aA
Tropic Snow	250 ^c	24cB	27cB	79bB	100aA	94aA	88aA	88aA	88aA	88aA
Sun Red	250 ^{d,f}	50aA	50aA	50aC	40aC	30aB	30aC	30aC	32aB	35aB
San Pedro 16–33	297 ^c	44dA	57cA	71bB	72bB	100aA	100aA	100aA	100aA	92aA
Hermosillo	350 ^c	0cC	20bB	83aB	93aA	93aA	100aA	100aA	100aA	100aA
Flordaking	450 ^c	0dC	13cB	47bC	93aA	93aA	90aA	100aA	100aA	93aA
Maycrest	600 ^c	0eC	0eC	6eD	20dD	26dB	45cC	66bB	85aA	100aA
Spring Belle	650 ^g	0cC	0cC	0cD	0cE	0cC	35bC	50bB	82aA	100aA
Armking	600 ^{e,f}	0dC	0dC	0dD	20cD	30cB	70bB	100aA	100aA	91aA
Baby Gold	850 ^{d,f,h}	0dC	0dC	0dD	0dE	0dC	40cC	66bB	91aA	100aA
September Free	850 ^f	0cC	0cC	0cD	40bC	100aA	100aA	100aA	100aA	90aA

Means followed by different lower case letters in the same line and by the different capital letters in the same column differ significantly ($P < 0.05$).

^a Chilling hours: chilling requirement of the cultivar.

^b Measured as the percentage of excised shoots that reached the green tip stage.

^c Valentini (2002).

^d Barbosa et al. (1997).

^e Powel (1998).

^f Okie (1998).

^g Adjusted according to its behaviour in Valencia, Spain.

^h Agustí (2004).

Table 2

Effects of chilling accumulation at 3.0 ± 0.1 °C on the percentage of flower budbreak of excised shoots of different peach and nectarine (*Prunus persica* L. Batsch) cultivars forced at constant temperature (20 °C) for 45 days

Cultivar	CH H ^a	Floral budbreak (%) ^b								
		Chilling accumulation at 3.0 ± 0.1 °C								
		0	100	200	300	400	500	600	700	800
Flordaprince	150 ^c	46cB	46cC	86aA	80aA	66bB	68bB	66bB	62bC	60bB
Flordastar	250 ^c	53bB	67bB	87aA	47bB	33cC	38cC	40cC	35cD	40cC
Earlygrande	260 ^c	53cB	93aA	93aA	86aA	86aA	80aA	70bB	65cC	60cB
Tropic Snow	250 ^c	72bA	82aA	100aA	76bA	52cB	59cB	58cC	55cC	53cC
Sun Red	250 ^{d,f}	62cB	60cB	75bB	77bA	75bA	85aA	100aA	70bB	50cC
San Pedro 16–33	297 ^c	85aA	88aA	100aA	85aA	80aA	81aA	80aA	71bB	65bB
Hermosillo	350 ^c	26cC	36cC	86aA	93aA	66bB	65bB	63bB	61bB	60bB
Flordaking	450 ^c	46cB	60bB	100aA	100aA	93aA	85aA	85aA	87aA	93aA
Maycrest	600 ^c	0dE	0dD	40cC	46cB	66bB	75bB	80aA	91aA	100aA
Spring Belle	650 ^g	0dE	0dD	37cC	48cB	87aA	81aA	71bB	68bB	67bB
Armking	600 ^{c,f}	12dD	31cC	87aA	100aA	91aA	89aA	87aA	60bB	25cD
Baby Gold	850 ^{d,f,h}	0cE	0cD	0cD	0cC	12bD	20bC	37aC	38aD	40aC
September Free	850 ^f	12cD	13cC	12cC	50bB	71aB	81aA	75aB	71aB	68aB

Means followed by different lower case letters in the same line and by the different capital letters in the same column differ significantly ($P < 0.05$).

^a Chilling hours: chilling requirement of the cultivar.

^b Measured as the percentage of excised shoots that reached the green tip stage.

^c Valentini (2002).

^d Barbosa et al. (1997).

^e Powel (1998).

^f Okie (1998).

^g Adjusted according to its behaviour in Valencia, Spain.

^h Agustí (2004).

stage was recorded (Citadin et al., 2001). Results were expressed as the percentage of budbreak or as mean time to budbreak (MTB) in days (arithmetic mean of each four group of five excised shoots) (Balandier et al., 1993).

Trials were conducted in a complete randomized design with four replicates of five cuttings per treatment and cultivar. We also calculated the standard error or the ANOVA for each treatment using the Tuckey test for means separation. Percentages were analysed after arcsine transformation.

3. Results

Leaf budbreak was highly affected by chilling accumulation (Table 1). ‘Flordaprince’, ‘Earlygrande’, ‘Tropic Snow’, ‘Sun Red’ and ‘San Pedro 16–33’ showed leaf budbreak even without chilling accumulation, with a high percentage of leafing ($\approx 50\%$) in the last two varieties (Table 1). Except in ‘Sun Red’, leaf budbreak increased significantly with chilling (Table 1). ‘Flordaprince’, ‘Flordastar’, ‘Earlygrande’ and ‘Hermosillo’ did not show a significant increase in the percentage of budbreak after 200 h of chilling; ‘Tropic Snow’ and ‘Flordaking’ after 300 h; ‘San Pedro 16–33’ and ‘September Free’ after 400 h; ‘Armking’ after 600 h and, ‘Maycrest’, ‘Spring Belle’ and ‘Baby Gold’ after 700 h (Table 1).

Flower budbreak showed differences compared with leaf budbreak in its response to chilling (Table 2). High flower budbreak ($>45\%$) was observed in shoots not exposed to chilling in seven cultivars (‘Flordaprince’, ‘Flordastar’, ‘Earlygrande’, ‘Tropic Snow’, ‘Sun Red’, ‘San Pedro 16–33’ and ‘Flordaking’), and only three varieties (‘Maycrest’, ‘Spring Belle’ and ‘Baby

Gold’) did not show flower budbreak for non-chilling treatment (Table 2). Except in the case of ‘San Pedro 16–33’, a significant increase in flower budbreak was observed in response to chilling but, generally, maximum budbreak was reached with less chilling accumulation (<100 – 200 h) compared to leaf buds (Table 1). High chilling caused a significant reduction in the percentage of flower budbreak (Table 2), but not in vegetative budbreak. As shown in Table 2, flower budbreak diminished significantly in shoots chilled for 300 h or more in ‘Flordastar’ and ‘Tropic Snow’; ≥ 400 h in ‘Flordaprince’ and ‘Hermosillo’; ≥ 600 h in ‘Earlygrande’ and ‘Spring Belle’ and for ≥ 700 h in ‘Sun Red’, ‘San Pedro 16–33’ and ‘Armking’. The exceptions were ‘Flordaking’, ‘Maycrest’, ‘Baby Gold’ and ‘September Free’ (Table 2) for which no significant reduction in flower budbreak caused by high chilling was observed. This phenomenon was not observed in vegetative budbreak (Table 1).

Chilling also modified the proportion of blooming that occurred before leafing (Table 3). In non-chilled shoots, blooming occurred earlier than leafing, except in ‘San Pedro 16–33’, which showed a similar proportion of blooming both before and after leafing. As the chilling exposure increased, the proportion of shoots that showed blooming before leafing decreased significantly. In ‘San Pedro 16–33’, ‘Tropic Snow’, ‘Armking’, ‘Spring Belle’ and ‘September free’ in shoots chilled for 600 h or more, blooming always occurred after leafing (Table 3).

‘San Pedro 16–33’ had the lowest MTB value, both for leaf and flower buds, when shoots did not receive chilling (Fig. 1a; Table 4). Furthermore, it was the only cultivar in which the MTB for leaf buds was not higher than the MTB for flower buds (Fig. 1; Table 4). Chilling reduced MTB values both in leaf and flower

Table 3

Effect of chilling accumulation at 3.0 ± 0.1 °C on the proportion of blooming that occurred before leafing, measured from excised shoot of different cultivars of peach and nectarine (*Prunus persica* L. Batsch) forced at constant temperature (20 °C) for 45 days

Cultivar	Chilling accumulation at 3.0 ± 0.1 °C ^a								
	0	100	200	300	400	500	600	700	800
Flordaprince	100 ± 0	71 ± 3	69 ± 4	25 ± 3	1 ± 0.2	12 ± 1	10 ± 1	13 ± 1	11 ± 1
Flordastar	100 ± 0	100 ± 0	46 ± 2	42 ± 2	20 ± 1	19 ± 1	22 ± 1	25 ± 2	33 ± 2
Earlygrande	100 ± 0	89 ± 4	42 ± 3	23 ± 2	22 ± 2	14 ± 1	8 ± 1	15 ± 1	18 ± 1
Sun Red	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	83 ± 1	53 ± 2	0 ± 0
San Pedro 16–33	52 ± 3	40 ± 3	5 ± 1	18 ± 1	20 ± 1	8 ± 1	0 ± 0	0 ± 0	0 ± 0
Tropic Snow	100 ± 0	83 ± 4	21 ± 2	15 ± 1	11 ± 1	10 ± 1	0 ± 0	0 ± 0	0 ± 0
Hermosillo	100 ± 0	100 ± 0	69 ± 4	7 ± 0	20 ± 1	19 ± 1	20 ± 1	18 ± 1	20 ± 1
Flordaking	100 ± 0	100 ± 0	93 ± 5	93 ± 4	64 ± 3	48 ± 3	33 ± 2	30 ± 2	28 ± 1
Maycrest			100 ± 0	100 ± 0	100 ± 0	100 ± 0	92 ± 5	80 ± 4	66 ± 3
Spring Belle			100 ± 0	100 ± 0	100 ± 0	41 ± 3	0 ± 0	0 ± 0	0 ± 0
Armking	100 ± 0	100 ± 0	100 ± 0	92 ± 0	71 ± 0	32 ± 0	0 ± 0	0 ± 0	0 ± 0
Baby Gold					100 ± 0	66 ± 1	33 ± 2	18 ± 1	0 ± 0
September Free	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	74 ± 2	0 ± 0	0 ± 0	0 ± 0

^a Values expressed as percentage of blooming that occurred before leafing.

buds, reaching the stability when shoots were chilled for 600 h in 4 and 6 days for leaf and flower buds, respectively (Fig. 1a).

Although ‘Earlygrande’ (Fig. 1b) had a similar chilling requirement to ‘San Pedro 16–33’ (Fig. 1a), the evolution of MTB values with chilling accumulation was different. The MTB value for non-chilling shoots was double in ‘Earlygrande’ and was at least 50% higher for all chilling treatments. The MTB value of vegetative buds was higher than that of flower buds for non-chilled and shoots chilled for 100 h, whereas it

was lower after 200 h of chilling. Besides, the MTB value was stabilized in shoots chilled for 400 h compared with 500 h in ‘San Pedro 16–33’ (Fig. 1a and b). The point where the MTB for vegetative buds equals the MTB value for floral buds is defined as the point of critical chilling accumulation (PCCHA), which can be seen in Fig. 1b.

In ‘Maycrest’, MTB was not measurable for non-chilling or shoots chilled for 100 h since no leafing or blooming was observed after 45 days at 20 °C (Fig. 1c; Tables 1 and 2). The MTB

Table 4

Effect of chilling accumulation at 3.0 ± 0.1 °C on the mean time to budbreak (MTB) of vegetative (V) and floral buds (F) from excised shoot of different cultivars of peach and nectarine (*Prunus persica* L. Batsch) forced at constant temperature (20 °C) for 45 days

Cultivar	Bud	Chilling accumulation at 3.0 ± 0.1 °C ^a								
		0	100	200	300	400	500	600	700	800
Flordaprince	V	35 ± 1.0	32 ± 1.2	16 ± 0.8	10 ± 0.3	8 ± 0.5	7 ± 0.4	7 ± 0.4	7 ± 0.3	7 ± 0.4
	F	31 ± 0.8	29 ± 0.9	15 ± 0.5	12 ± 0.4	10 ± 0.4	9 ± 0.5	9 ± 0.2	9 ± 0.3	9 ± 0.2
Flordastar	V		29 ± 0.3	13 ± 0.3	9 ± 0.3	7 ± 0.3	6 ± 0.2	6 ± 0.2	6 ± 0.3	6 ± 0.2
	F	27 ± 0.7	25 ± 0.4	14 ± 0.6	11 ± 0.7	10 ± 0.4	9 ± 0.4	9 ± 0.5	9 ± 0.4	9 ± 0.4
Tropic Snow	V	25 ± 0.6	14 ± 0.5	8 ± 0.3	6 ± 0.3	5 ± 0.2	5 ± 0.2	5 ± 0.3	5 ± 0.3	5 ± 0.2
	F	20 ± 0.6	14 ± 0.4	11 ± 0.5	9 ± 0.4	8 ± 0.3	8 ± 0.2	8 ± 0.3	7 ± 0.2	7 ± 0.1
Sun Red	V	34 ± 0.8	28 ± 0.7	24 ± 1.7	19 ± 0.5	16 ± 0.6	15 ± 0.5	13 ± 0.5	12 ± 0.4	10 ± 0.4
	F	27 ± 1.0	25 ± 0.2	22 ± 1.2	19 ± 0.4	18 ± 0.4	16 ± 0.2	15 ± 0.2	13 ± 0.4	12 ± 0.3
Hermosillo	V		28 ± 0.1	14 ± 0.3	8 ± 0.2	7 ± 0.3	7 ± 0.7	7 ± 0.2	7 ± 0.3	7 ± 0.2
	F	27 ± 1.3	20 ± 1.7	12 ± 0.3	9 ± 0.5	9 ± 0.2	9 ± 0.7	9 ± 0.5	9 ± 0.3	9 ± 0.2
Flordaking	V		41 ± 1.0	27 ± 0.7	17 ± 0.7	11 ± 0.7	9 ± 0.2	8 ± 0.2	8 ± 0.3	8 ± 0.1
	F	30 ± 0.7	26 ± 0.5	19 ± 1.0	14 ± 0.2	10 ± 0.3	10 ± 0.4	10 ± 0.2	10 ± 0.2	10 ± 0.1
Spring Belle	V					25 ± 0.7	21 ± 0.6	18 ± 0.5	15 ± 0.4	13 ± 0.3
	F			30 ± 0.3	27 ± 0.2	22 ± 0.2	19 ± 0.3	18 ± 0.7	16 ± 0.4	15 ± 0.3
Armking	V				21 ± 0.8	17 ± 0.7	15 ± 0.5	13 ± 0.4	9 ± 0.3	9 ± 0.3
	F	24 ± 0.3	22 ± 0.2	19 ± 1.0	18 ± 0.3	16 ± 0.2	12 ± 0.2	12 ± 0.3	15 ± 0.4	15 ± 0.2
Baby Gold	V					28 ± 0.4	25 ± 0.4	22 ± 1.7	18 ± 1.1	15 ± 0.7
	F					22 ± 0.6	21 ± 0.4	19 ± 1.1	18 ± 0.3	16 ± 0.2
September Free	V				30 ± 0.5	27 ± 0.5	22 ± 0.6	19 ± 0.4	10 ± 0.4	9 ± 0.2
	F	31 ± 0.7	30 ± 0.4	27 ± 0.3	22 ± 0.7	21 ± 0.8	21 ± 0.4	18 ± 0.7	18 ± 0.4	16 ± 0.3

^a MTB (days).

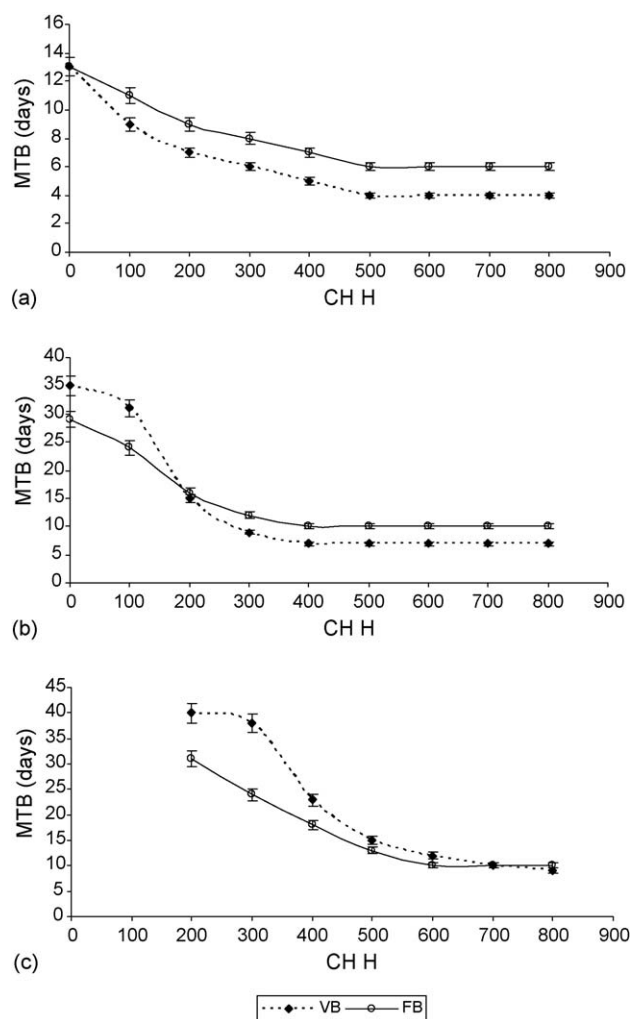


Fig. 1. Effect of chilling accumulation (CH H) at 3.0 ± 0.1 °C on the mean time to budbreak (MTB) of vegetative (VB) and flower buds (FB) from excised shoots of peach and nectarine (*Prunus persica* L. Batsch), cv. 'San Pedro 16–33' (a), 'Earlygrande' (b) and 'Maycrest' (c), forced at constant temperature (20.0 °C) for 45 days. MTB standard error for each sample is represented by the vertical bar.

value for flower buds declined up to 600 h of chilling while the MTB for vegetative buds did not reach its minimum value in our experiment (Fig. 1c). The MTB value of 'Flordaprince', 'Flordastar', 'Tropic Snow', 'Hermosillo', 'Flordaking' and 'Armking', showed particular changes with chilling but with the same pattern of evolution observed for 'Earlygrande' (Table 4; Fig. 1b) reaching stable values between the range of chilling treatments used in this experiment, whereas 'Sun Red', 'Baby Gold', 'September Free' and 'Spring Belle', did not reach a stable MTB value, as occurred in 'Maycrest' (Table 4; Fig. 1c).

A comparison between the suggested chilling requirements and the range of chilling accumulation needed to reach a percentage of budbreak higher than 50% for both floral and vegetative buds is shown in Table 5.

4. Discussion

The increase in budbreak and endodormancy release caused by chilling observed in this work is well known (Erez et al.,

Table 5

Range of chilling accumulation at which different peach cultivars showed a percentage of floral and vegetative budbreak higher than 50%

Cultivar	CH H ^a	Range of chilling accumulation with budbreak >50% ^b
Flordaprince	150 ^c	200–800
Flordastar	250 ^c	200–300
Earlygrande	260 ^c	200–800
Tropic Snow	250 ^c	200–800
Sun Red	250 ^{d,f}	0–300
San Pedro 16–33	297 ^c	100–800
Hermosillo	350 ^c	200–800
Flordaking	450 ^c	300–800
Maycrest	600 ^c	600–800
Spring Belle	650 ^g	600–800
Armking	600 ^{e,f}	500–700
Baby Gold	850 ^{d,f,h}	+800
September Free	850 ^f	400–800

^a Chilling hours: chilling requirement of the cultivar.

^b Measured as the percentage of excised shoots that reached the green tip stage.

^c Valentini (2002).

^d Barbosa et al. (1997).

^e Powel (1998).

^f Okie (1998).

^g Adjusted according to its behaviour in Valencia, Spain.

^h Agustí (2004).

1979; Faust et al., 1997; Fuchigami and Wisniewsky, 1997; Dennis, 2003). However, we noted that some low chilling cultivars had a relatively high budbreak ($\approx 50\%$) and a low MTB value without exposure to low temperature, mainly in flower buds as compared to vegetative buds. Under insufficient chilling conditions, buds open but do not develop further, and flowers fail to set fruits (Dennis, 2003). The results using the cutting test may consider these limitations to predict field behaviour.

Excessive chilling diminished the percentage of flower budbreak (see Table 2) in low chilling cultivars (<400 h). These symptoms were also observed in shoots of low chilling requirement cultivars from the Southern Brazil breeding program, which were chilled for 800 h and the effect was attributed to a physiological injury of buds and shoots caused by longer exposure to low temperatures (Citadin et al., 2001). These symptoms may be observed in cultivars of high chilling requirement at higher chilling accumulation treatments not considered in this trial.

Except in cv. 'San Pedro 16–33', the MTB value of the vegetative buds for non-chilled shoots was higher compared with that of floral buds. The intensity of rest was therefore higher in vegetative than in flower buds at the beginning of the rest, explaining why the percentage of floral budbreak was higher compared with leaf buds at low chilling accumulation treatments (see Tables 1 and 2), why less chilling was generally necessary to reach maximum budbreak in flower buds, and why blooming occurred earlier than leafing in low chilling treatments (see Tables 3 and 4 and Fig. 1). Additionally, the occurrence of blooming after leafing in non-chilled shoots of 'San Pedro 16–33' (see Table 3) could be explained by the MTB value of the flower and leaf buds being equal in non-chilled

shoots (see Fig. 1a). This is an additional reason for using the method of measuring the time required to reach a specific stage of bud opening versus the percentage of budbreak within a fixed time interval for measuring the response to chilling (Dennis, 2003). Furthermore, we observed that chilling caused a continuous decline in the intensity of rest, whereas no increase in the depth of dormancy was observed during the early phases of the rest, as observed in other trials (Arias and Crabbé, 1975; Walser et al., 1981).

MTB value of leaf buds decreases faster than floral buds with chilling and consequently, the MTB value of leaf buds was lower than flower buds for high chilling accumulation treatment (see Fig. 1 and Table 4). Thus, each cultivar showed a point of chilling accumulation at which the MTB value was equal for both floral and leaf buds. When chilling accumulation was lower than the point of critical chilling accumulation (PCCHA) blooming tended to occur first, whereas when chilling accumulation was higher than PCCHA, leafing tended to occur earlier. Plum showed the same response to chilling as that described for peach, but not apricot, in which the MTB value of leaf buds was always higher than flower buds in the three cultivars studied (data not shown). This evidence, together with the decrease in flower budbreak and the increase in the proportion of shoots leafing before blooming with the increase of chilling exposure, is in accordance with the conclusion of Citadin et al. (2001), who observed that prolonged chilling enhances leafing more than blooming in low chill requirement cultivars, and that after years of higher chilling accumulation, leafing can occur before blooming. In fact, in cv. 'San Pedro 16–33' leafing occurs before or together with blooming under the field cultivation conditions of the central-east area of Santa Fe Province (Argentina) (unpublished data) because the depth of rest of leaf buds was always equal or lower (but not higher) than flower buds (see Fig. 1a). Similar observations were made in the area of Valencia Spain, in the 'Springcrest' and 'Zincal 5' cultivars, when winter chilling was higher than the average year (>600 h of chilling).

Citadin et al. (2001) observed differences in the heat requirement between flower and vegetative buds in peach cultivars with low chilling requirements. This observation also occurred in our experiment, since when the MTB reached a stable low value and longer exposure at low temperature did not produce further MTB decrease, we therefore considered that the end of rest had occurred (Balandier et al., 1993), and that the number of days to blooming or leafing was the time necessary to cover the heat requirement. Consequently, in all the peach cultivars studied, the heat requirement for leafing was lower than for blooming when sufficient chilling was accumulated (see Fig. 1 and Table 4).

It is interesting to note that different varieties with similar chilling requirements showed different responses to chilling; thus, the cutting test measuring the response of vegetative and floral buds gives a great deal of information about the characterisation of the cultivar compared with the sole and traditional data of chilling requirement. For example, 'Flordastar' and 'Flordaprince', or 'September Free' and 'Baby Gold' had similar chilling requirement but 'Florda-

prince' and 'September Free' showed a higher percentage of budbreak in a wide range of chilling accumulation treatments, compared with 'Flordastar' and 'Baby Gold', respectively (Table 5). On the other hand, 'Maycrest' needed more chilling to reach a higher percentage of budbreak compared with its chilling requirement (Table 5).

It is known that flower buds require less chilling than leaf buds (Tabuenca, 1965). However, both flower and leaf buds reached the end point of endodormancy with the same chilling requirement in at least four of the cultivars studied (see Fig. 1 and Table 4) indicating that their chilling requirements are the same. This is more in accordance with the work of Guerriero et al. (1986), who proposed that vegetative buds would have a lower chilling requirement than floral buds. The fact that their depth of dormancy was different could possibly cause some confusion. To avoid it, the figure that represents the MTB evolution of leaf and floral bud with chilling, clarifies the concepts of intensity of rest measured as MTB, chilling requirement, CHH required for a constant MTB and heat requirement to sprout (constant MTB multiplied by daily heat accumulation).

Finally, the method of excised shoots is at present used to study the evolution of endodormancy in leaf buds, extracting twigs at different times during winter (Balandier et al., 1993; Dennis, 2003). Also, recent works have used this method to simulate artificial chilling under controlled conditions (Citadin et al., 2001; Dennis, 2003). Our results confirm the utility of the method and suggest that rest of both leaf and flower buds, and not that of leaf buds only, may be studied to clarify the physiological process of dormancy.

The main conclusions of this work are that some cultivars showed a high blooming and leafing without exposure to chilling; chilling enhances leafing and blooming but the percentage of increment was higher in leaf buds; excessive chilling caused a reduction of the percentage budbreak in flower but not in leaf buds; generally, flower buds generally have a lower intensity of rest; the intensity of rest declines more slowly in flower than in leaf buds with chilling; flower buds had higher heat requirements than leaf buds when the chilling requirement had been covered. Consequently, each peach cultivar studied had a point of critical chilling accumulation below which blooming tended to occur earlier and above which leafing tended to occur first.

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