



et al., 2002), biochemical methods (Bartolini et al., 2004), detached twigs (Egea et al., 2003) and changes in carbohydrate composition (Marquat et al., 1999). However, determination of the thermal requirements for breaking dormancy under laboratory conditions based on one year data must be carefully considered because chill and heat accumulation were shown to be interdependent processes (Alonso et al., 2005). Besides, these procedures are costly and time-consuming when analyzing a large number of genotypes (Leida et al., 2012). Thus, statistical models that rely on the analysis of historical blooming dates have been developed to estimate chilling and heat requirements for flowering (Alonso et al., 2005; Luedeling et al., 2009b). In order to quantify chill and heat, researchers have proposed several models that convert temperature records into a metric of coldness or heat, respectively. Due to the lack of information of the biochemical and physiological mechanisms controlling dormancy, almost all models have been developed empirically or statistically to fit the responses of tree species to local weather conditions (Fan et al., 2010). In peach (*Prunus persica* (L.) Batsch), few reports dealt with the estimation of chill and heat requirements. The first estimates of chill requirements were quantified in 18 varieties through the variation of dry weight in the flower buds method (Tabuenca, 1964). Citadin et al. (2001) investigated differences in heat requirement for blooming in 7 peach accessions using artificially chilled excised shoots and potted trees. Later, Pawasut et al. (2004) determined chilling and heat requirements of 11 ornamental peach genotypes by the classical method of bud breaking, when flower buds are able to sprout by placing branches in warm conditions during the progress of dormancy. The same procedure was employed by Razavi et al., (2011) to study the requirements for breaking dormancy and flowering in 5 peach genotypes.

The aims of this study were to estimate the requirements for flower bud break of 63 nectarine and 118 peach accessions grown in Argentina and compare the accuracy of four chilling models and two statistical methods. Moreover, we took advantage of these estimates to find a relationship between the effects of chilling and heat accumulation on blooming date.

## 2. Materials and methods

### 2.1. Data collection

Observations of nectarine and peach phenology have been recorded at the Estaci3n Experimental Agropecuaria INTA, San Pedro, Argentina (31°41'12"S–60°47'32"W) in 2003 and from 2005 to 2012. In order to estimate the requirement for floral bud break we considered the date of anthesis for 50% of the flowers (F50) of 63 nectarine and 118 peach genotypes for at least four years. Genotypes were arranged in a completely randomized design with three replications, each of them consisted of three trees planted at 5 × 4 m row spacing. Daily and hourly air temperatures have been recorded at the meteorological station placed in the experimental field since 1965. We extracted daily and hourly mean temperatures between 1 May 2003 and 1 October 2011 from the complete dataset.

### 2.2. Chilling and heat models

To calculate the requirements for flower bud break we used four chilling models that require hourly temperatures as input data. The Chilling Hours model, also known as the Weinberger model, calculates the accumulated 'chill hours' (CH) as the number of hours when the temperature is between 0 and 7.2 °C (Weinberger, 1950). Although it was first developed for peaches in United States, nowadays is widely used to describe accession requirements since it is easy to understand and calculate (Okie, 1998). The second model tested was the Utah model which quantifies the degree of

accumulated chilling in 'chill units' (CU). It assigns different weights according to different ranges of temperatures and accounts for negative influence of high temperatures during dormancy period (Richardson et al., 1974). As in milder climates weather patterns usually result in heat and chill units on the same day, unlimited negation may be a problem. Therefore, Utah model has been adapted by limiting or ignoring the chill negation. One of the modified versions is the Positive Utah model and has performed well in warm regions such as South Africa. It consists on removing the negative contributions of warm temperatures from the original equation. Each temperature hour is treated independently and is allocated a 'positive chill unit' (PCU), then they are summed over (Linsley-Noakes and Allan, 1994). The last chilling model tested was the Dynamic which is the only one that explains experimental evidence from controlled temperature studies in Israel. It has been also used in South Africa (Erez et al., 1990; Fishman et al., 1987a, 1987b), Spain (Ruiz et al., 2007) and Chile (P3rez et al., 2008). It postulates that winter chill accumulates in a two-step process. During the first step, cold temperatures lead to the formation of a precursor for the dormancy breaking factor. Then, once a certain quantity has accumulated, the precursor can be transformed into Chill Portion (CP) by a process that requires relatively warm temperatures. On the other hand, the accumulation of heat was calculated as 'growing degree hours' (GDH) (Richardson et al., 1975). Then, the minimum accumulation of GDH per hour is 0 °C GDH and the maximum amount 20.5 °C GDH.

### 2.3. Estimation of chilling and heat requirements for flower bud break

Two different procedures were carried on to estimate the chilling requirements (CR) and heat requirements (HR) for flower bud break. The methodology developed by Alonso et al. (2005) is based on the temperature effects on blooming dates according to the dormancy stage in almond. We defined a temperature matrix by the number of days from 1 May (day 1) to 1 Oct (day 154) and years (2005–2011) and the average of the mean daily temperature measured during a 5 days period was introduced in each cell. The matrix of blooming dates was defined by two axes: years and genotypes. Then, the vector of each day was correlated to the vector of blooming dates of each accession by calculating the Pearson coefficients between both vectors and the endodormancy breaking date was considered to be given by the first significant negative coefficient. After that, chill was computed yearly until the transition date and heat from that day to F50. The average chill and heat accumulation were considered to be the CR and HR, respectively. The other method was proposed by Luedeling et al. (2009) to explain walnut phenology. It assumes that endodormancy ends when the chilling requirements are fulfilled, whereas ectodormancy length is determined by the necessary time to complete the heat requirements. Therefore, we plotted the accumulated chill since 1 May against the heat that remains to be accumulated to flower bud break and obtained a yearly curve per genotype. Since an approximation of the theoretical intersection point was acquired, we were able to estimate CR and HR for floral bud break for each accession.

### 2.4. Validation of modelled requirements

In an attempt to confirm whether chilling and heat requirements were properly estimated, F50 of 51 genotypes were predicted based on hourly temperatures recorded in 2003 and modelled requirements. Then, expected dates were compared to the ones registered in 2003 blooming season. We used two indicators of prediction accuracy to verify if the statistical methods and chilling models were useful to estimate the requirements for flower bud break. To begin with, we calculated the differences between theoretical and

**Table 1**  
Chill accumulation in seven years according to Chilling Hours, Utah, Positive Utah and Dynamic models.

	15-May	31-May	15-Jun	30-Jun	15-Jul	31-Jul	15-Aug	31-Aug
Chilling hours model								
2005	13.0	70.0	26.0	55.0	50.0	143.0	68.0	62.0
2006	20.0	75.0	61.0	25.0	24.0	62.0	94.0	78.0
2007	78.0	101.0	125.0	141.0	199.0	133.0	157.0	148.0
2008	74.0	34.0	94.0	110.0	17.0	31.0	79.0	71.0
2009	28.0	55.0	125.0	106.0	104.0	152.0	85.0	50.0
2010	55.0	29.0	43.0	55.0	99.0	155.0	156.0	48.0
2011	32.0	22.0	60.0	88.0	93.0	115.0	75.0	107
Mean	42.9	55.1	76.3	82.9	83.7	113.0	102.0	80.6
Standard deviation	26.2	28.7	39.1	40.0	62.1	48.2	38.1	35.8
cv (%)	61.0	52.1	51.3	48.2	74.2	42.6	37.3	44.4
Utah model								
2005	21.0	50.0	53.0	118.0	64.5	184.0	105.5	102.0
2006	14.5	84.0	74.0	98.0	46.5	73.0	132.0	54.5
2007	118.0	192.5	187.5	221.0	222.0	185.0	195.0	216.0
2008	31.5	46.0	108.0	198.5	0.0	127.0	101.5	26.5
2009	38.5	80.5	175.0	122.5	116.0	187.0	66.5	0.0
2010	29.0	28.0	125.5	140.0	113.0	243.0	220	28.5
2011	1.0	98.5	153.0	148.5	109.5	194.0	50.5	181.5
Mean	36.2	82.8	125.1	149.5	95.9	170.4	124.4	87.0
Standard deviation	38.1	54.3	50.4	44.7	70.0	54.6	63.1	83.2
cv (%)	105.2	65.6	40.3	29.9	73.0	32.0	50.7	95.6
Positive Utah model								
2005	70.0	141.0	73.0	142.5	116.0	231.5	129.0	156.5
2006	108.0	125.0	126.5	131.5	85.5	115.5	162.5	138.5
2007	125.5	217.5	216.5	222.0	228.0	212.5	225.5	241.0
2008	139.5	63.0	150.0	217.5	77.0	145.5	154.0	134.0
2009	85.5	113.0	198.0	169.5	164.5	197.0	155.0	95.0
2010	83.5	91.0	151.5	156.5	121.0	251.5	225.5	99.0
2011	83.5	126.0	176.0	159.5	144.5	222.5	117.5	200.0
Mean	99.4	125.2	155.9	171.3	133.8	196.6	167.0	152.0
Standard deviation	25.6	48.2	47.7	35.3	51.6	48.9	43.0	52.9
cv (%)	25.7	38.5	30.6	20.6	38.6	24.9	25.7	34.8
Dynamic model								
2005	3.0	5.7	4.0	7.3	6.0	8.4	7.2	7.5
2006	3.0	6.0	7.2	6.4	4.0	4.6	8.3	6.7
2007	6.1	10.6	9.0	10.4	10.6	10.0	9.6	10.7
2008	3.4	2.6	7.7	10.3	4.0	7.2	7.9	6.3
2009	1.8	4.9	8.3	8.0	8.7	10.2	5.4	4.5
2010	1.7	4.0	7.9	8.6	5.3	11.3	10.3	5.0
2011	3.0	7.0	8.9	8.2	7.4	10.9	5.2	11.1
Mean	3.1	5.8	7.6	8.5	6.6	8.9	7.7	7.4
Standard deviation	1.5	2.5	1.7	1.5	2.5	2.4	1.9	2.6
cv (%)	46.2	43.9	22.4	17.4	37.3	26.9	25.3	35.1

actual F50 and a modified version of Shapiro–Wilk normality test was applied (Mahibbur and Govindarajulu, 1997). Then, differences between groups were evaluated by paired *t*-test for normally distributed data or Wilcoxon signed rank test for skewed data. We also performed a classical linear regression between the estimated and actual F50 verifying if the residuals were normally distributed. Nevertheless, if the assumption of normality was violated, robust linear regression was achieved. Finally, we tested if the slope and the origin of the regression equation were equal to 1 and 0, respectively. Analyses were performed with R Development Core Team (2013), SAS Institute Inc. (2011) and InfoStat (Di Rienzo et al., 2013).

### 3. Results and discussion

#### 3.1. Chilling accumulation in field conditions

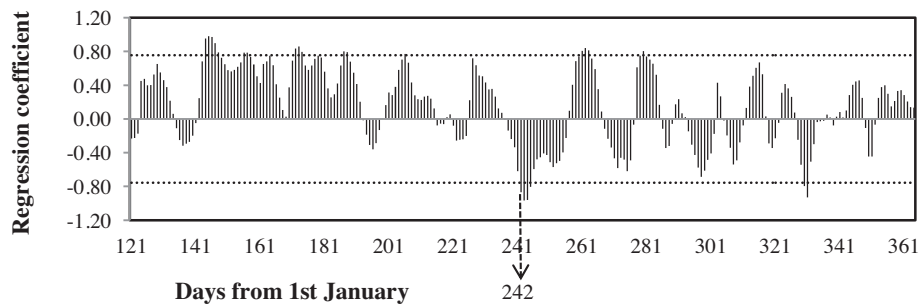
Chilling accumulations were calculated by the four models from 1 May to 31 August at the experimental station during 7 consecutive years and are presented in Table 1. In our field conditions, the average chilling accumulation during May is generally rather low while the most efficient month is July. These results were consistent with all chilling models. The coefficients of variation ( $cv_s$ ) allow a comparison among models. The most homogeneous results along with years were obtained with the Positive Utah model and the

Dynamic model. On the other hand, the variability achieved with the Utah model was very high as the  $cv_s$  for the chilling accumulation of each fortnight were approximately twice those obtained with the modified version and the Dynamic model. Considering the  $cv_s$  of each range of time obtained with the Dynamic and Utah models, the first fortnight of May and the last fortnight of June had the highest and lowest variation among years, respectively. When chill was quantified according to the Chilling Hours Model, the first fortnight of July and August had the highest and lowest variation among years, respectively. However, those obtained with the Positive Utah model were homogeneously distributed in all periods of time.

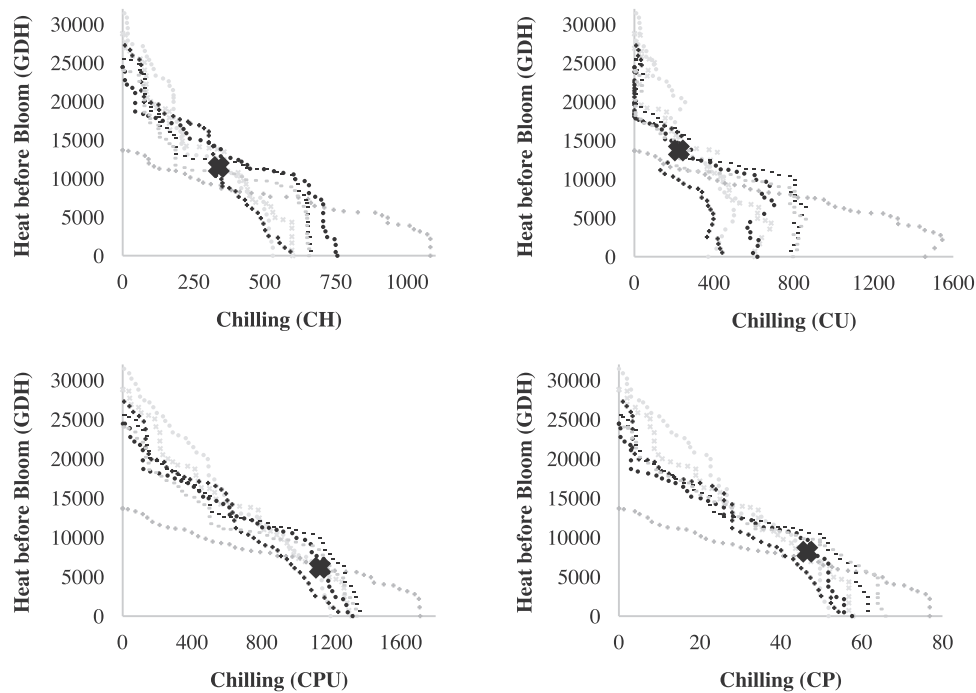
#### 3.2. Chilling and heat requirements for breaking dormancy

Chilling and heat requirements for breaking dormancy and flowering were estimated according to the models proposed by Alonso and Luedeling. Fig. 1 shows an example of the estimation of the endodormancy breaking date according to the Alonso model. On the other hand, Fig. 2 depicts an example of chilling and heat requirements estimation by the Luedeling model.

Some of the estimated requirements are in Table 2. The first accession to break endodormancy depends on the model used to estimate the CR. Among the cultivars shown in the table, in the climatic conditions of San Pedro, genotype Lara (98 CH, 103 CU, 225



**Fig. 1.** Establishment of endodormancy breaking of 'María Delizia' cultivar by analysing the evolution of the correlation coefficients between the average temperatures in a 5 days period and the date of full bloom during the 2005–2011 seasons. The arrow indicates the first negatively significant correlation coefficient obtained, and thus the endodormancy breaking date.



**Fig. 2.** Sets of curves of 'María Delizia' cultivar for all chilling models during seven seasons (◻ 2005, ● 2006, + 2007, + 2008, ● 2009, - 2010, ■ 2011). Heat units remaining until budbreak, as a function of accumulated chilling during the winter season. Under the assumption that the chilling and heat requirements are fulfilled sequentially, all seasonal observations of remaining heat vs chilling should pass through a common intersection point. An approximation of the theoretical point was acquired and is indicated by an 'X'.

CPU, 9 CP) is the first to break endodormancy, considering the estimates obtained by Alonso model, and accessions Late Dwarf (71 CH, 5 CU) and Flordaglo (46 PCU, 1 CP) are the first to break endodormancy according to the requirements estimated by the Luedeling model. On the other hand, the lowest heat requirement estimated by Alonso model corresponds to GaLa (1348 GDH), whereas the lowest values obtained by the Luedeling approach belong to genotypes María Anna (5853 GDH, 4275 GDH, 4617 GDH) and Early Giant (3960 GDH), considering the Chilling Hours, Utah, Dynamic and Positive Utah models, respectively. The requirements showed a huge variability not only among accessions but also among models. So as to conclude which approach is the best for our germplasm collection, a validation of the modelled requirements must be performed.

### 3.3. Validation

Predicted blooming dates were compared with the observed ones during 2003 season. The analysis revealed significant differences between expected and actual F50 when we considered the

chilling requirements in CU, PCU and CP and heat requirements in GDH obtained by the Alonso model. The same results were achieved when we calculated the blooming dates based on the chilling requirements in CU and CP and heat requirements in GDH obtained by the Luedeling model. In contrast, we did not obtain significant differences between registered and modelled F50 when we considered the requirements estimated by the procedure proposed by Luedeling with Chilling Hours and Positive Utah models (Table 3). Chilling requirements estimated by the Alonso model in CH were not included in the analysis since we were able to predict the F50 of only 15% of the accessions tested. As the requirements were overestimated, it seemed that winter cold of 2003 season had not been enough for flower bud to release endodormancy in 85% of the genotypes studied. However, as all accessions had flowered in that season we conclude that those requirements were not accurately estimated.

Additionally, we evaluated the models through a classical or robust linear regression between estimated and actual F50 if the distribution of regression residuals was normal or not, respectively. After testing if the slope and the origin of the regression equation

**Table 2**  
Average blooming dates and requirements for breaking dormancy estimated by Alonso and Luedeling models.

Cultivar	Mean F50 <sup>a</sup>	Alonso					Luedeling							
		CR (CH)	(CU)	(PCU)	(CP)	HR CH/CU/PCU (GDH)	CR (CH)	(CU)	(PCU)	(CP)	HR CH (GDH)	CU (GDH)	PCU (GDH)	CP (GDH)
Nectarine														
Caldessi 2000	254	633	745	1179	54	2640	316	210	732	33	9002	11004	8294	8291
Early Giant	256	636	776	1201	56	2796	342	249	1111	46	8677	10260	3960	5772
Firebrite	253	646	757	1206	55	1794	308	198	735	34	7498	9436	6933	7054
Lara	218	98	103	225	9	10242	93	47	219	9	9338	8699	8973	8809
María Anna	257	631	774	1194	55	3393	426	392	1005	46	5853	4275	4489	4617
María Lucía	251	667	816	1240	57	15131	413	244	1112	47	6777	9933	3964	4901
May Glo	214	101	108	227	9	9039	98	84	275	12	8242	7367	7413	7296
Roseprincess	254	646	757	1206	55	2077	313	207	741	33	9000	11201	8248	8288
Sunraycer	212	131	144	275	11	8728	90	45	226	10	9086	8852	8642	8396
Peach														
Babygold 5	268	642	786	1219	56	5464	498	364	1186	53	8505	10797	5148	5481
Fireprince	256	631	774	1194	55	2938	341	226	863	36	8488	10783	7133	8007
Flordaglo	201	244	269	538	24	1830	79	11	46	1	8394	12588	12973	13571
Gala	247	631	774	1194	55	1348	306	206	858	30	7415	9433	5075	7399
Guglielmina	266	642	786	1219	56	5115	488	381	1137	51	8543	10737	6136	6509
Late Dwarf	249	331	409	668	30	8978	71	5	148	6	16493	17784	16396	16018
María Delizia	266	631	774	1194	55	5576	338	223	1135	47	11504	13723	6148	8187
María Marta	257	644	752	1196	55	2829	327	212	771	34	9252	11406	8429	8581
Rich Lady	240	325	404	657	30	6985	73	6	161	11	14086	15116	14137	12034
Rosa del West	269	644	752	1196	55	6211	434	378	1145	51	9879	11360	6106	6572

<sup>a</sup> Days after 1 January.

**Table 3**  
Results of *t* and Wilcoxon tests of differences between predicted and actual blooming dates.

	<i>t</i> -test <i>t</i> value	Wilcoxon Z value
Alonso–Utah model		−4.70***
Alonso–Positive Utah model		−5.79***
Alonso–Dynamic model		5.88***
Luedeling–Chilling Hours model		−0.99 <sup>ns</sup>
Luedeling–Utah model		6.03***
Luedeling–Positive Utah model	−1.58 <sup>ns</sup>	
Luedeling–Dynamic model	−2.13*	

<sup>ns</sup>, \*, \*\*\*: non-significant or significant at  $P < 0.05$  or  $P < 0.001$ , respectively.

**Table 4**  
Results of the test for the null hypothesis that slope (*m*) and origin (*h*) of the regression equation were equal to 1 and 0, respectively. Values obtained for the slope (*m*), origin (*h*) and coefficient of determination ( $R^2$ ) of each linear regression.

	<i>F</i> value	<i>M</i>	<i>h</i>	$R^2$
Classical linear regression				
Luedeling–Chilling Hours model	2.17 <sup>ns</sup>	1.16	−40.28	0.79
Luedeling–Positive Utah model	2.60 <sup>ns</sup>	1.13	−32.95	0.80
Luedeling–Dynamic model	2.88 <sup>ns</sup>	1.12	−31.55	0.69
Robust linear regression				
Alonso–Utah model	244.7***	0.73	80.19	0.48
Alonso–Positive Utah model	158.8***	0.76	70.73	0.55
Luedeling–Utah model	17.3***	0.99	−0.79	0.64
Alonso–Dynamic model	298.4 <sup>ns</sup>	0.68	92.10	0.58

<sup>ns</sup>, \*\*\*: non-significant or significant at  $P < 0.001$ , respectively.

were 1 and 0, respectively, we conclude that the Luedeling model is better to estimate CR and HR for flower bud break of nectarine and peach genotypes than the Alonso approach when the Chilling Hours or Positive Utah models are applied in our field conditions. Moreover, when the Dynamic model is applied both methods seem to estimate the requirements correctly (Table 4). However, based on the coefficients of determination and the results shown in Table 3, it is recommendable to calculate the chilling accumulation in accordance with the Chilling Hours or Positive Utah models and estimate the requirements for flower bud break by the Luedeling method in our field conditions.

### 3.4. Chilling requirements, heat requirements and blooming dates

Once we recognized that requirements were accurately estimated by the Luedeling approach when we quantified chill in CH or PCU, we measured the strength of association between those requirements and average blooming dates. As the variables were not normally distributed, Spearman's rank correlation analysis was used. The analysis revealed that the correlations between chilling requirements and average F50 were 0.69\*\*\* and 0.73\*\*\*, when they were estimated in CH and PCU, respectively. On the other hand, correlations between heat requirements and average blooming dates were 0.08<sup>ns</sup> and −0.04<sup>ns</sup>, when they were estimated considering a data set based on chill accumulation in CH and PCU, respectively. Thus, only the associations between average F50 and chilling requirements for flower bud break are significantly positive. Consequently, accessions with low CR will flower earlier than genotypes with high CR. In agreement with our results, studies carried on in south-east Spain showed that flowering date of some almond accessions was influenced more by chilling than heat requirements (Egea et al., 2003) as well as differences in chilling requirements had stronger influence on blooming date of sweet cherry genotypes than do heat requirements (Albuquerque et al., 2008). In addition, our results suggest that in the climatic condition of San Pedro, chilling requirements seem to be more important than heat requirements to regulate blooming date in nectarine and peach accessions due to the late satisfaction of chilling, mostly in August.

## 4. Conclusions

The Luedeling method was reliable for estimating chilling and heat requirements for flower bud break from historical blooming dates of nectarine and peach trees grown in the Estación Experimental Agropecuaria INTA, San Pedro, Argentina. Our analysis clearly made the Chilling Hours and Positive Utah models stand out as the most accurate ways of quantifying winter chill in our field conditions. Moreover, our results suggest that chilling requirements have stronger influence on blooming date than do heat requirements of nectarine and peach genotypes. Knowledge of the requirements for flowering is important not only to select accessions according to the climatic conditions but also to choose parents



in breeding programs concerning blooming date. Additionally, this information would allow progress in research on control mechanisms for the activity-dormancy cycle in buds as well as on the impact of climate change on peach phenology.

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