Chilling requirement of four peach cultivars estimated by changes in flower bud weights[1]

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ABSTRACT

The adaptation of temperate fruit crops is a challenge being increased by the global warming. Chilling requirement is a key factor for adaptation. The objective of this study was to estimate the chilling requirement of peach cultivars BRS Bonão, Esmeralda, Granada and Eragil, using the Tabuenca test. Chilling accumulation was computed using four different chilling hour (d+ 7.2 ºC and d+ 11 ºC) models; and chill units using the Low Chill model and the Taiwan model. The fresh bud weight and bud water contents were also evaluated. The Tabuenca test (based on differences in bud´s dry weight) showed a fairly good efficiency for estimating the end of dormancy in peach. However, under mild winter conditions, it is better to use fresh bud weights. Either one of three chilling accumulation computation models (temperature d+ 7.2 °C, d+ 11°C, or Taiwan model) is suitable to classify comparatively different cultivars, but none is accurate enough to conclude on the adaptation of a given cultivar to a specific site. Using hours of temperatures d+ 11 ºC: 'BRS Bonão' needed around 180 hours for dormancy release; 'Esmeralda' around 250 hours; 'Granada' between 300 and 400 hours, and 'Eragil' more than 500 hours.

Key words: *Prunus persica* (L.) Batsch; Tabuenca test; mild winter; dormancy; low chill cultivars.

INTRODUCTION

Climatic patterns have undergone changes on a global scale, greatly affecting meteorological, environmental, biological, economic, and social processes (IPCC, 2013).

Perennial species of temperate climate are vulnerable to changes in temperature, since their development is largely dependent on this factor (Walthall *et al.*, 2012). Bud dormancy has been studied for years, aiming to understand the aspects involved in dormancy induction, maintenance and suppression (Hauagge & Cummins, 1991).

Peach (*Prunus persica* L. Batsch) is one of the temperate climate fruit species that has experienced the greatest expansion and is now found in subtropical and high altitude tropical regions. For this reason, and in view of the global warming, the development of low chilling cultivars is one of the priorities of most breeding programs.

Cold accumulation is the main responsible factor for dormancy release of deciduous fruit species. Thus, whenever these species are grown in regions with insufficient chilling accumulation, they do not adapt well and show symptoms as deficient leafing; strong apical dominance with consequent inhibition of lateral shoots; development of long terminal branches and uneven flowering which drastically affect production (Marodin *et al.*, 1992). Although the dormancy can be overcome using chemical substances, vegetative growth, yield and fruit quality are generally lower than those from adapted cultivars (Donadio, 2007). However, under field conditions it is practically impossible to estimate the exact chilling accumulation required for dormancy release of a specific cultivar, since other factors such as solar radiation, temperature fluctuations, among others, may not be controlled (Dennis, 2003).

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Much research has been carried out for estimating chilling requirement of stone fruit cultivars. Different mathematical models have been used, differing as to the relative efficiency of the various temperature ranges, among which the Utah model (Richardson *et al.*, 1974), Dynamic model (Fishman *et al.*, 1987), Taiwan model (Ou & Chen, 2000), and Low Chill model (Gilreath & Buchanan, 1981). There are also protocols called phenological models, which are generally used in combination with these models. Therefore, in addition to temperature data, phenological models also use plant phenology data over the years. Some researchers base this calculation on the beginning of leafing dates (10% bud burst), others base on the full flowering dates (50% opened flowers), and also others estimate chilling requirement by comparing to known cultivars.

Another approach used by several researchers are the biological methods (Herter *et al.*, 2001; Carvalho *et al.*, 2010; Malagi *et al.*, 2015). There are also variations among biological methods, such as the use of a whole plant or just a part of it, as isolated bud cuttings (Pouget, 1963), detached branches (Weinberger, 1950), and/or buds as in the Tabuenca protocol (Tabuenca, 1964). The Tabuenca test is an old biological method, still widely used, as it allows to establish when the chilling requirement has been satisfied. It has already been successfully applied in apricot (Tabuenca, 1964; Legave *et al*., 2010; Andreini *et al.*, 2014), peach and pear (Tabuenca, 1964), plum (Tabuenca, 1967) and apple (Malagi *et al.*, 2015).

The objective of the study was to estimate the chilling requirement of peach cultivars BRS Bonão, Esmeralda, Granada and Eragil, using the Tabuenca test.

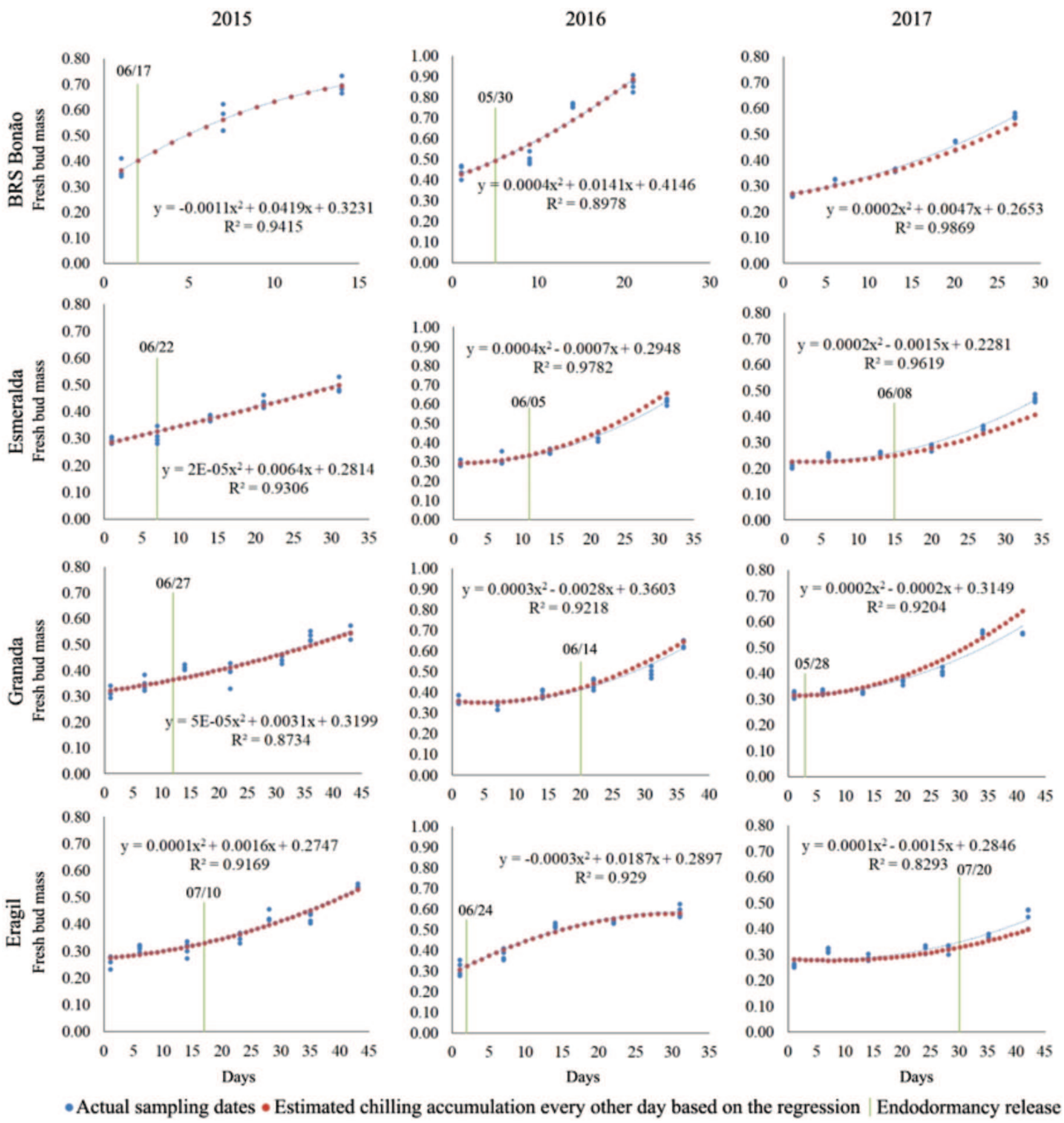

Figure 1: Regression curves for four peach cultivars in 2015, 2016, 2017 seasons, considering the fresh mass of flower buds. The date of dormancy break corresponds to the first significant increase of the bud weight, using the MSD test ($p \leq 0.05$).

MATERIAL AND METHODS

The experiment was conducted in commercial orchards located in Pelotas, RS, for three consecutive years (2015, 2016 and 2017). Adult plants of four peach cultivars, BRS Bonão, Esmeralda, Granada and Eragil, were used. The first three cultivars were developed by Embrapa Temperate Agriculture, and 'Eragil' is a cultivar selected by a grower in Santa Catarina state.

The plants of cvs. BRS Bonão and Esmeralda were located in Colonia Cristal, the 5th District of Pelotas (31°34'45.001"S; 52°28'42.895"W), those of 'Granada' in Colonia São Manuel, the 8th District of Pelotas (31°29 '26.020"S; 52°32'8.268"W) and the ones of 'Eragil', in Colonia Santa Eulália, the 5th District of Pelotas (31°33'30.917"S; 52°32' 22.549"W). These cultivars were chosen for their different chilling requirements. 'BRS Bonão' has the lowest chilling requirement (less than 200 hours ≤ 7.2 ºC), 'Esmeralda' and 'Granada', medium (250 and 400 hours ≤ 7.2 ºC, respectively), and 'Eragil', high chilling requirement (over 500 hours d" 7.2 ºC) (Franzon & Raseira, 2014).

The accumulation of chilling hours was calculated using temperatures ≤ 7.2 °C (Weinberger, 1950) and temperatures ≤ 11 °C (Chavarria *et al.*, 2000). Chill units (or cold units) were calculated for each collection date using Low Chill (Gilreath & Buchanan, 1981) and Taiwan (Ou & Chen, 2000) models. These models were chosen based on previous work in which they seemed to be the most suitable for the Pelotas area (Milech *et al.* 2018a, 2018b).

At the beginning of the experiment (2015), four uniform plants per cultivar were marked (each plant was a

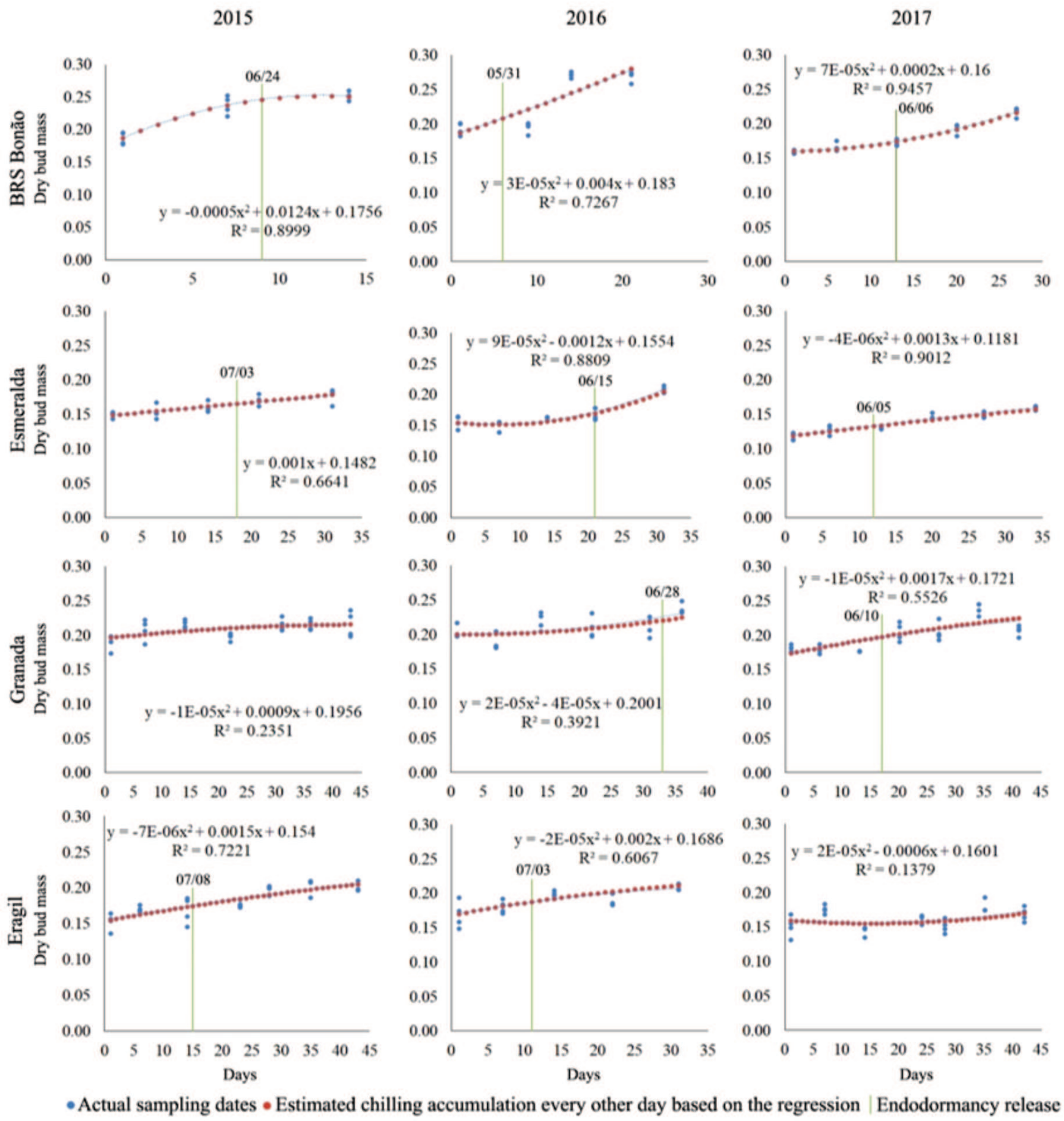

Figure 2: Regression curves for four peach cultivars in 2015, 2016, 2017 seasons, considering the dry mass of flower buds. The date of dormancy break corresponds to the first significant increase of the bud weight, using the MSD test ($p \leq 0.05$).

replication) for the three-year shoot collections. Sampling of five 30 cm long shoots per plant started near 50 hours of temperature ≤ 7.2 °C had been accumulated, in each collection site. These collections continued weekly, during the months of May, June, July and August, until beginning of blooming (anthesis of 10% flower buds) occurred in the orchard. Shoots were randomly collected in different orientation of the plant at the medium height of the canopy. So, in each collection date, 20 one-year old shoots per cultivar were cut and immediately taken to the laboratory and placed in vials containing 150 mL of 3% aqueous sucrose solution. They were kept for seven days in a germination chamber (Fitotron®), at a temperature of 21±1 ºC and a photoperiod of 12 hours. The sucrose solution was changed every two days.

The temperature data on the field were recorded from May 1st until the end of the winter by data loggers (Novus, Logchart II version 2.62), installed near each orchard.

Immediately after branch collection, 20 flower buds were removed from each sample, which constituted the experimental unit, totalizing 80 buds per cultivar. The bracts and pedicel were removed from the buds, and then each sample was weighed on an analytical scale to obtain their fresh mass. After, the buds were taken to drying oven at ± 70 °C, until constant mass was obtained (0.05% variation, ± 3 days), and then, they were weighed again to obtain their dry mass. The collections were carried out until a significant increase in the mass was observed, which it was defined as the date of the end of endodormancy.

Originally, the Tabuenca test (Tabuenca, 1964) is based on the changes in dry mass of the flower buds. However, in the present study, the bud fresh masses were also measured.

The experimental design was a completely randomized, with four replications of 20 buds per plot. First, the regression equations were calculated for each cultivar, considering the average masses obtained on each collection date. Subsequently, the daily average of the bud mass was calculated using regressions, and these were compared two by two by the minimum significant difference (MSD) to estimate the first significant increase of the bud mass.

For this established date, calculations were made for CH accumulation ≤ 7.2 °C (Weinberger, 1950), CH ≤ 11 °C (Chavarria *et al.*, 2000), and CU according to Taiwan (Ou & Chen, 2000), and the Low Chill model (Gilreath & Buchanan, 1981). Using the averages and standard error, comparisons were made among the chilling requirements of the cultivars (considering the dates obtained by the Tabuenca method and the chilling accumulation by the four models). A comparison was also made among years, within the same model. Additionally, for these established dates, the water content (%) of the buds was calculated.

RESULTS AND DISCUSSION

The Tabuenca method, followed by the regression calculations and MSD of the fresh and dry mass of the buds, between two consecutive days, allowed to estimate the date of the dormancy break for each cultivar (Figures 1 and 2). Tabuenca (1964 and 1967) used only the flower bud dry mass, however, his work was carried out in Zaragoza, Spain, where winters are well defined and

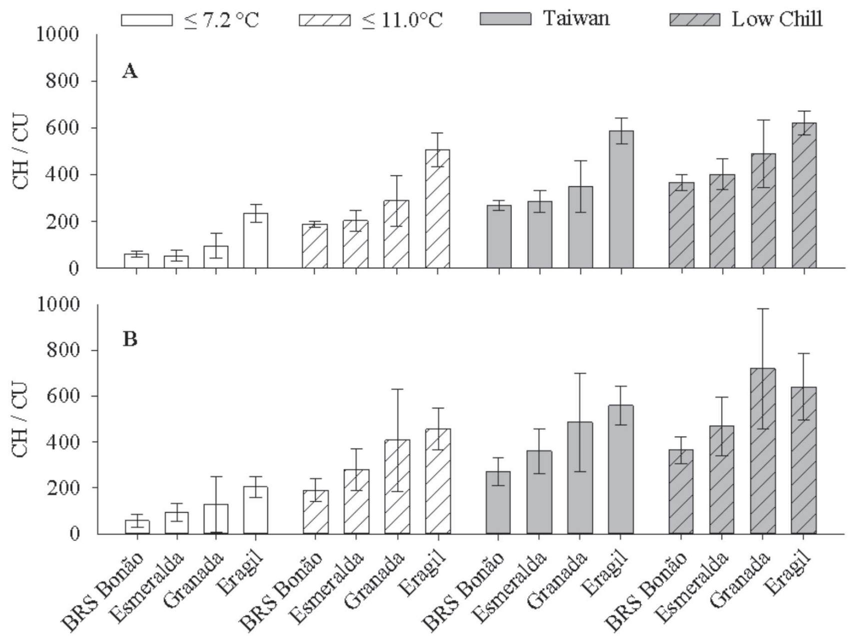

Figure 3: Chilling requirement of four peach cultivars estimated by four models using bud fresh weight (A) or dry bud weight (B). Vertical bars represent the standard deviations of the mean; CH= Chill hours; CU= Chill units.

Table 1: Chilling accumulation until the dormancy breaking of each cultivar in chill hours (CH) ≤ 7.2 °C and ≤ 1°C and in chill units (CU) according to Taiwan and Low Chill models

Year	Model	Fresh weight				Dry weight			
		‘BRS Bonão’	‘Esmeralda’	‘Granada’	‘Eragil’	‘BRS Bonão’	‘Esmeralda’	‘Granada’	‘Eragil’
2015	≤ 7.2 °C	75	96	98	163	112	120	ne	158
	≤ 11 °C	198	244	304	369	272	300	ne	355
	Taiwan	284	344.5	391	482.5	372.5	434	ne	463.5
	Low Chill	326.5	399.5	507.5	512	432	510.5	ne	488
2016	≤ 7.2 °C	48	55	185	250	48	146	250	250
	≤ 11 °C	174	247	465	521	177	412	613	532
	Taiwan	242	308	504.5	574.5	249	463	686.5	632
	Low Chill	390.5	499.5	708.5	676.5	402.5	650.5	964.5	772
2017	≤ 7.2 °C	ne	13	6	294	13	13	9	ne
	≤ 11 °C	ne	114	88	619	110	108	182	ne
	Taiwan	ne	189.5	139.5	673	172,5	164	267	ne
	Low Chill	ne	277.5	219.5	632	244	224.5	451.5	ne

ne = not estimate. There was not a significant mass increase from beginning of sample collection until the last collection date, then the dormancy break date was not calculated.

cultivars have high chilling requirement, which is not the case of Southern Brazil. In the present study, the differences among genotypes were better observed when using the flower bud fresh mass. The same comparative order was maintained, regardless of use CH or CU, except for a slight difference (between ‘Granada’ and ‘Eragil’) in the Low Chill model, when using the dry mass (Figure 3).

The comparison among genotypes using means and respective standard deviation (Figure 3), confirmed the classification of ‘BRS Bonão’ as the one with the lowest chilling requirement but it did not significantly differ from ‘Esmeralda’ and ‘Granada’, differing from ‘Eragil’, which it has the highest need in chilling accumulation among them.

The cultivar BRS Bonão, in the year 2015, had a significant increase of the bud mass, on June 17. On this date, the accumulated chilling hours of temperature d” 7.2 ºC and d” 11 ºC were 75 CH and 198 CH, respectively. If models of chill units are used, it would correspond to 284 CU and 326.5 CU for Taiwan and Low Chill models, respectively (Table 1). In 2016, the date of significant increase in fresh flower bud mass occurred on May, 30. This was possibly related to the fact that in the year of 2016, temperatures in May were lower than in 2015 and 2017, and, consequently, the CU accumulation in this period was practically double (Table 2).

May temperatures are of great influence, mainly for low chill cultivars, as they can determine if these cultivars will or not go into dormancy. Dormancy entry and depth are strongly correlated with cold winter temperatures (Malagi *et al.*, 2015). On May 30 the chilling accumulations were 48 CH ≤ 7.2 °C, 174 CH ≤ 11 °C, and 242 CU, by the Taiwan model and 390.5 CU by the Low Chill model. Cold unit accumulation at the beginning of May possibly supplied enough chill and since it was followed by warm temperatures at the end of the same month, the increase in the bud weight was stimulated. These results can be explained by the very low chilling requirement of ‘BRS Bonão’.

Low temperatures have a double function on dormancy mechanisms of deciduous fruit trees. They induce the entry and exit of dormancy, in order to allow leafing and flowering development (Putti, 2001). The transition between the endodormancy and ecodormancy phases is not well defined, but it is assumed that this transition may become shorter due to global warming, causing earlier flowering (Aguilera *et al.*, 2014).

In 2017 there was not a significant difference in the ‘BRS Bonão’ flower bud fresh mass, due to the fact that temperatures remained high in the fall, which probably led this cultivar not going into deep dormancy (Figure 1).

‘Esmeralda’ had the first significant increase in the flower bud fresh mass on June 22, in 2015 (Figure 1). By then, there was an accumulation of 96 CH (≤ 7.2 °C), 244 CH (≤ 11 °C), and 344 CU, by the Taiwan model and 399.5 CU by the Low Chill model (Table 1). In 2016, 'Esmeralda' showed similar dynamics as the previous year, for models of hours below 11 °C and the Taiwan model, with the significant increase occurred on June 5, with an accumulation of 247 CH (≤ 11 °C) and 308 CU by the Taiwan model. The difference in dates was probably due to the cold that occurred in May of 2016, and thus necessary chill accumulation for this cultivar occurred earlier (Table 2). In 2017, the significant increase in the bud fresh mass was observed on June 8, with chilling accumulation well below the previous years (Table 1). 'Esmeralda' is considered a medium chilling requirement cultivar, however, in May 2017, temperatures did not drop much (the daily average was between 16 °C and 17 °C). So, it is very likely that the cultivar did not go into endodormancy that year, thus responding to the higher temperatures. Temperate species, when grown in areas of mild climate, such as Southern Brazil, rarely meet their chilling requirements during winter (Hawerroth *et al.*, 2010). Thus, it is believed that over time there will be some level of adaptation of the cultivars to the climate, with increasingly warm temperatures.

Considering the changes in the bud weight of 'Granada', there was not the same trend in the three years of the experiment (Figure 1). According to the literature, 'Granada' is also considered as medium chill cultivar, requiring between 250 and 400 hours of temperature ≤ 7.2 °C. However, as the hourly temperatures and monthly accumulations in the winter months were very variable from one year to the next, it was not possible to estimate conclusively the chilling requirement of this cultivar by any of the models for chill accumulation computation. Analyzing the temperature and chilling accumulation data for the winter months, in the years in which the experiments were carried out (2015, 2016 and 2017) it was possible to observe large fluctuations in hourly temperatures and a large difference in the onset of cold among the years (Table 2).

The established dates, based on the MSD, both for fresh (Figure 1) and dry mass of buds (Figure 2), were different as well as the chilling accumulation. In 2015, MSD for bud fresh mass of 'Granada' occurred on June 27 which corresponded to 98 CH (d" 7.2 °C) and 304 CH (d"11 °C), and for the Taiwan and Low Chill models, 391 and 507.5 CU, respectively. Dormancy entry and depth are strongly correlated with cold winter temperatures. The quality and regularity of the cold during dormancy are extremely important for the development of the peach tree (Gonçalves, 2014). In other words, the effects of cold can be assessed in terms of duration (quantitative aspect) and intensity (qualitative aspect). Under warm winter conditions, as Southern Brazil, the three classic phases of dormancy (paradormancy, endodormancy and ecodormancy) are difficult to differentiate, where the endodormancy, if it does occur, is mild and short. The results for 'Granada' (Table 1) in 2016 were 185 CH (≤ 7.2 °C), 465 CH (≤ 11 °C), 504.5 CU (Taiwan model) and 708.5 CU (Low Chill model), on June 14. As already referred, the regularity with which the cold occurs is of great importance. Temperature fluctuations increase the need for chilling hours to satisfy the plant's requirements (Erez & Lavee, 1971). It is important that enough chilling occurs during the winter (especially in the beginning) to satisfactorily overcome the dormancy (Champagnat, 1973). When this

Table 2: Comparison of the monthly chill accumulation in May, June and July, in the years of 2015, 2016 and 2017, in one specific site (Colônia São Manuel, Pelotas, and the average mean temperature, the average maximum, and the average minimum temperature, for the same months and years for Pelotas

Year		Embrapa Weather Station[1]			Colônia São Manuel[2]	
		Average	Maximum	Minimum	≤ 7.2°C	≤ 11°C
2015	May	16.9	21.8	13.2	27	95
	June	14.3	19.4	9.9	71	211
	July	13.9	17.9	10.7	25	178
				Total	123	484
2016	May	13.6	17.5	10.8	67	223
	June	10.6	15.3	7.5	183	396
	July	12.6	17.0	9.0	119	273
				Total	369	892
2017	May	16.7	20.7	13.4	6	100
	June	14.9	20.0	10.9	73	226
	July	15.3	21.3	10.9	115	203
				Total	194	529

[1]Data of Embrapa Clima Temperado (31°40'49" S; 52°26'23" W; 57m); [2]Data of the Colônia São Manuel (31°29' 26"S 52° 32' 08"W; 223m).

occurs, the development of the floral primordium is then dependent on the heat accumulation which is also variable depending on the cultivar (Citadin *et al.*, 2001). The year 2017 was a very unusual and hot year, and when the minimum significant difference in the bud fresh mass occurred in ‘Granada’ (May 28), there was practically none or very little CH accumulated of temperatures ≤ 7.2 ºC or ≤ 11 ºC, and also very low CU accumulation (Table 2). Therefore, it is very likely that ‘Granada’ did not go into dormancy that year (Table 1).

Large temperature fluctuations in winter besides canceling the CH already accumulated induces the plants to early flowering, causing significant damage to production (Gonçalves, 2014). Moreover, the significant increase of the flower bud mass of some low cultivars and not in others may be an indication of a different heat requirement for blooming.

The results for ‘Eragil’ were very inconsistent. Observing the 2015 data, a significant increase in bud fresh mass occurred on July 10, with 163 CH (≤ 7.2 °C), 369 CH (≤ 11 °C), 482.5 CU for the Taiwan model and 512 CU for the Low Chill model. On the following year (2016), the date was advanced to June 24, and the chilling accumulations were already higher than the previous year, with values of 250 CH, 521 CH, 574.5 CU and 676.5 CU, for temperature ≤ 7.2 °C, ≤ 11 °C, Taiwan and Low Chill models, respectively. In 2017, ‘Eragil’ had a significant increase in bud fresh weight later than the previous two years (July 20). For this date, 294 CH (≤ 7.2 °C), 619 CH (≤ 11 °C), 673 CU (Taiwan) and 632 CU (Low Chill) were accumulated. The deeper the endodormancy, the greater the amount of CH needed to overcome it, which implies the crop failure of some cultivars of temperate climate when cultivated in subtropical or tropical environments (Erez, 2000). However, the biggest problem of adaptation of deciduous fruit crops to the region under study refers to the fact that large temperature fluctuations generally occur during the

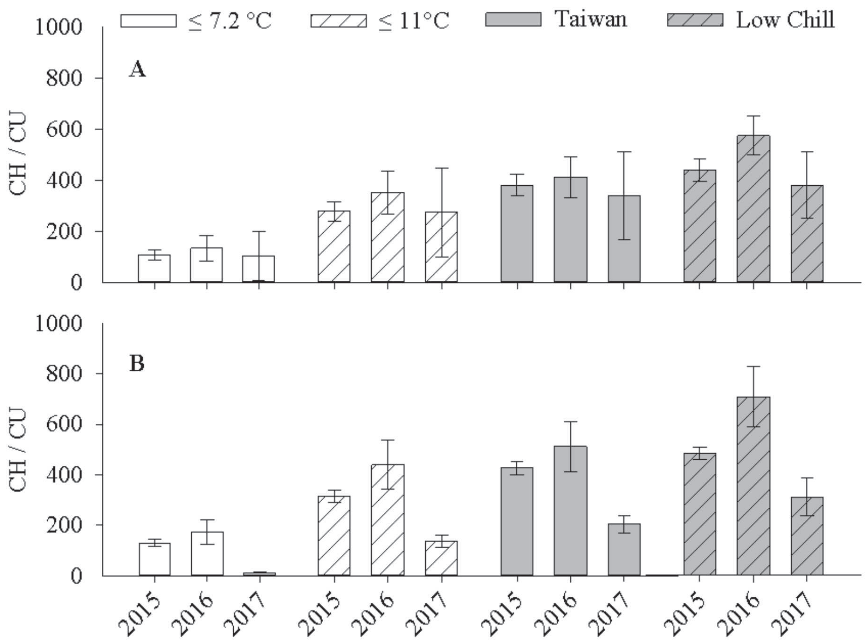

Figure 4: Comparison among years for average chilling accumulation, using four models and bud fresh weight (A) and dry bud weight (B), of four peach cultivars (BRS Bonão, Esmeralda, Granada and Eragil). Vertical bars represent the standard deviation of the mean; CH= Chilling hours; CU= Chill units.

Table 3: Water content (%) in flower buds of four peach cultivars at the estimated date of dormancy release

Cultivar	2015	2016	2017	CV (%)
BRS Bonão	48.31 B[1]	61.25 A	ne	5.54
Esmeralda	50.10 ns	53.06	49.47	4.93
Granada	46.26 B	52.57 A	45.45 B	4.41
Eragil	45.46 B	46.41 B	53.32 A	4.09

[1]Means followed by the same letters in the line (comparing years) do not differ by the Tukey test at 5% probability; ns = not significant by the F-test ($p \leq 0.05$); ne = not estimate; CV = coefficient of variation.

winter season. It is important to point out that it is not uncommon the occurrence of days when temperatures exceed 25 °C, even in coldest winter months.

The chilling requirement estimation for a certain cultivar should be the same over different years if the method and model adopted were sufficiently accurate. The Tabuenca model recommends using the flower bud dry weight, however the results obtained in our study were not consistent. For this reason, we decided to compare the results obtained using the bud fresh mass and their dry mass in the three studied years, in order to verify which is the most reliable for chilling requirement estimation. Observing the data set of four cultivars, for the three studied years, with the exception perhaps of the estimates made by the Low Chill model, there was no statistical difference between the years, considering the bud fresh weights. However, the same was not true for the dry weights (Figure 4). This makes sense considering that among the characteristics that undergo modification when a dormant cell becomes active, the cell turgor is one of the most noticed. And it is strictly linked to the percentage of water in the tissues.

The results for bud water content were stable, with approximately 50% for all cultivars, on the date of the estimated endodormancy break. The differences, even when statistically significant, were less than 10% (Table 3). The water content was associated with the plant dormancy state, in previous studies, in apple (Malagi *et al.*, 2015; Sachet, 2013), peach (Leite *et al.*, 2006; Bonhomme *et al.*, 1997), and pear trees (Marafon *et al.*, 2011; Simões *et al.*, 2014). The percentage of water in the flower buds probably reveals the end of endodormancy and could be an alternative method for estimating it.

CONCLUSIONS

Under the conditions of this work we conclude that:

'BRS Bonão' needs around 190 CH of temperature ≤ 11 °C for dormancy breaking; 'Esmeralda' needs close to 250 CH; 'Granada' needs between 300 and 400 CH; and 'Eragil' needs more than 500 CH.

Either one of three models (hours of temperature ≤ 7.2 °C, hours of temperature ≤ 11 °C, or chill units by the Taiwan model) is suitable to classify comparatively different cultivars, but none of them is accurate enough to conclude on the adaptation of a given cultivar to a specific site.

The Tabuenca test has a fairly good efficiency to estimate the end of a dormancy phase in peach.

Under warm winter conditions for the Tabuenca test, it is better to use the fresh bud weight than the dry weight.

Water content in the buds can be an alternative method to estimate the end of the dormancy period.

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