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# Estimation of Chilling Accumulation for Blackberry (*Rubus* sp.) Using Mathematical Models

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### *Authors' contributions*

*This work was carried out in collaboration between all authors. Authors SC, and MCBR designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SC and RCF performed the statistical analysis and managed the literature searches. Author RCF, MCBR and SC interpreted the analyses of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

As the climate change takes place, the cultivation of temperate climate crops in subtropical areas has become a challenge. The success of fruit crops, such as blackberry, in certain areas depends basically on its chilling requirement and the chilling accumulation in those areas. Usually the models used to predict those accumulations presents widely variable results being necessary to test the models in the location where the species is cultivated. The objective of this work was to estimate the chilling requirement for bud break of blackberry cultivars Caingangue, BRS Cainguá, Guarani, Tupy, BRS Xavante, BRS Xingu, Brazos, Cherokee and Choctaw, using the models of Utah, Positive Utah, Low Chill, Taiwan, Chilling Hours ≤7.2°C, Chilling Hours ≤11°C, and Dynamic and both temperature and phenological data from 2010 to 2019. The results showed a high variability in cold accumulation for all studied cultivars in all tested models. None of the models performed perfectly to estimate chilling requirement however, the Taiwan and the Utah Positive models can be used to provide a rough estimate of this requirement. On the other hand, Utah and Low Chill models are clearly not suitable for estimating blackberry chilling requirement. The estimated

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chilling requirement showed that all the studied cultivars are well adapted to the climatic conditions of the southern Rio Grande do Sul, which is emphasized by their good productivity in most of the years.

*Keywords: Rubus sp.; adaptation; chill hours; dormancy; bud break.*

## 1. INTRODUCTION

The genus *Rubus* L., blackberry belonging to the Rosaceae family, is a plant of temperate climate. The plants typically have a perennial root system and crown and biennial canes. The plant has life expectancy between 15 and 50 years, depending on the management and cultivation conditions. Some differential characters at species level include life form, leaves shape, flower sex, production potential and chilling requirement. In different species of *Rubus*, chilling requirement is a genotype-dependent characteristic and varies from 200 to 900 h at temperatures between 0 and 7.2°C [1].

Due its adaptation, the blackberry is widely cultivated around the world. The main world producers are Europe, North and Central America [2], among them, Mexico is the number one producer in the world with an average cultivated area between 2018 and 2020 of more than 12000 ha [3]. There is a lack of data regarding the situation of the crop in Brazil, however, until the year 2014 there were estimated to be around 528 ha cultivated [4]. As it is an expanding crop, with the availability of new cultivars with lower chilling requirements and better fruit quality, as well as the development of new cultivation and management techniques in non-traditional areas, it is believed that this area, currently, is about 1200 ha.

In Brazil, blackberry is grown mainly in the South and Southeast regions, due to the climatic characteristics of these regions. Under these conditions, plants go into vegetative rest as days get shorter and temperatures lower. The blackberry requires a stop in growth, bud dormancy and chilling accumulation enough to overcome dormancy [1], for proper leafing and flowering.

Studies show that due to ongoing climate changes, the chilling accumulation has being decreased in many areas of the world, and that there is a tendency for this phenomenon to continue [5]. This temperature changes affect the perennial plants that depend on the winter cold to satisfactorily completion of their annual cycles, especially in the tropics and subtropics [6].

Particularly for temperate fruit species, these changes can cause substantial problems, leading many growers to adapt and rethink their choices and preferences regarding cultivars or species for cultivation, according to the chilling distribution [7], or even, in some cases, shifting the production area to a less vulnerable for the species [8].

Unfortunately, there are several gaps in knowledge that limit the ability of researchers and growers to project the impacts of climate change on crop phenology [9,10]. Among these gaps, are the lack of knowledge about the dormancy phase in which the accumulation of cold and heat is effective, the lack of visible indicators (morphological changes) of this accumulation, and the lack of accurate and accessible models for estimating both cold and heat accumulation in species and cultivars, in addition to their effect on the different stages of plant development [11].

Historically, mathematical models were proposed to estimate the chilling requirement and to study the appropriate conditions for each species and cultivars and explain the progression of dormancy, which become important as the cultivation of temperate climate species expanded beyond the traditional zones. However, choosing a suitable model to be applied in a particular region implies comparing several models, over several years, for a particular cultivar(s) of each species.

Three mathematical models are most frequently used to quantify the chilling accumulation to overcome dormancy: the Chill Hours (CH) model [12], which considers as one CH each hour of temperature ≤7.2°C; the Utah Model, which establishes different relative values of Chill Units (CU) according to the temperature range [13], and the Dynamic model, which uses Cold Portions (CP), and proposes the formation of an intermediate product produced by low temperature that can be reversed if temperatures rise. However, once the intermediate product reaches a certain level, the CP are fixed and no longer affected by the action of high temperatures [14]. However, these models were developed for stone fruits and apple trees.

In addition to these, there are models developed for regions with mild winter conditions, such as the Low Chill [15] and Taiwan [16] models, among others, equally developed for peach trees and both are expressed in CU.

There are also modified versions of the models, which were adapted to better meet the temperature conditions of the places where they were studied, such as the Chill Hours ≤11°C model, which is an adaptation, for warmer places, of the Weinberger model, in which for CH accumulation, hours of temperature equal to or less than 11°C are considered [17], and the Utah Positive model, adapted from the Utah model, which disregards the negative effects of high temperatures, so that temperatures above 16°C do not contribute negatively to the accumulation of CH [18].

As there are no mathematical models developed or indicated to estimate the chilling requirement for bud break in blackberry, in Brazil, the application of models already established for other species can be used as an alternative. Therefore, the objective of the present work was to estimate the chilling requirement of 10 blackberry cultivars using some of the available mathematical models.

## 2. MATERIAL AND METHODS

The phenological data of the 10 blackberry cultivars used in this work were obtained from the records kept by the blackberry breeding program of Embrapa Clima Temperado in Pelotas/RS, Brazil. The hourly temperature data for the years 2010 to 2019 were obtained at the Embrapa meteorological station, at the same location, and were used to calculate the chilling accumulation according to each model.

According to the Köppen climate classification [19], the region's climate is subtropical, humid, with no dry period. The annual precipitation average is 1366.9 mm, with the highest precipitation rate in February and the lowest in October, 188.2 and 74.7 mm, respectively.

The chilling requirement for vegetative bud breaking was calculated from June 1st of each year, until the beginning of leafing (10% of the buds at a green tip stage) of each cultivar. The date of June 1st was chosen because it is the month in which, in most years, the first temperature accumulations below 7.2°C were recorded and the plants had dropped their leaves.

The cultivars used in the study were: Caingangue, BRS Cainguá, Guarani, Tupy, BRS Xavante and BRS Xingu, from the blackberry breeding program of Embrapa Clima Temperado; ‘Brazos’, from Texas A & M University; ‘Cherokee’, ‘Comanche’ and ‘Choctaw’, from the University of Arkansas.

Chilling requirements were estimated in Chill Hours (CH) according to the models: hours of temperature ≤7.2°C [12] and hours ≤11°C [17], in Chill Units (CU) according to the models of Utah [13], Low Chill [15], Taiwan [16], Utah Positive [18] and in chilling portions, Dynamic [14].

The data for the chilling requirements of the cultivars for each model were submitted to analysis of variance, and to the Scott & Knott mean grouping test, using the years as replications. To perform the analysis it was used the SISVAR statistical software [20].

## 3. RESULTS AND DISCUSSION

No statistically significant differences between cultivars regarding the chilling accumulation for vegetative bud break within each model, in the 10 years studied were observed (Table 1), also there was a very large variation in this accumulation between years for the same cultivar.

As well as occurred with the cultivars, all the models presented a high variability. The highest coefficient of variation (CV) were observed for Utah, Low Chill and ≤7.2ºC models respectively. ‘Dynamic’ and the ≤11ºC were very similar whereas Taiwan and ‘Positive Utah’ models presented the smallest variation among all tested models with CV of 21.9 and 26.8%, respectively. But in general, except for ‘Utah’ and ‘Low Chill’, all the others were similar. However, in the present study, the CV can be considered high for all the tested models.

**Table 1. Average chilling accumulation over 10 years, for vegetative bud breaking in blackberry cultivars according to the models of chill hours ≤7.2°C and ≤11°C, the models of chill units of Utah, Low Chill, Taiwan, Utah Positive and the model of chill portions, Dynamic**

| **Cultivar** | **Chilling accumulation models** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **≤7.2°C** | **CV$^1$** | **≤11°C** | **CV$^1$** | **Utah** | **CV$^1$** | **Low Chill** | **CV$^1$** | **Taiwan** | **CV$^1$** | **Utah Positve** | **CV$^1$** | **Dynamic** | **CV$^1$** |
| Brazos | 176.8$^{ns}$ | 36.6 | 612.9$^{ns}$ | 31.8 | 126.6$^{ns}$ | 213.6 | 347.2$^{ns}$ | 65.7 | 761.1$^{ns}$ | 29.0 | 619.9$^{ns}$ | 31.5 | 29.9$^{ns}$ | 36.1 |
| Caingangue | 179.6 | 35.3 | 624.1 | 30.5 | 69.2 | 585.3 | 297.0 | 103.9 | 773.3 | 25.8 | 627.2 | 28.8 | 30.0 | 31.2 |
| BRS Cainguá | 179.3 | 35.2 | 624.7 | 29.5 | 134.8 | 224.3 | 341.2 | 76.8 | 755.1 | 20.2 | 623.3 | 27.1 | 29.6 | 25.0 |
| Cherokee | 183.3 | 34.6 | 644.5 | 29.9 | 91.8 | 344.7 | 322.1 | 86.8 | 797.6 | 22.4 | 648.8 | 28.1 | 32.0 | 24.7 |
| Choctow | 180.0 | 35.3 | 635.3 | 30.0 | 118.1 | 282.3 | 333.7 | 84.5 | 774.6 | 21.1 | 634.8 | 27.8 | 30.3 | 30.5 |
| Comanche | 177.4 | 31.1 | 620.3 | 29.0 | 152.2 | 189.1 | 352.1 | 72.7 | 751.4 | 20.9 | 622.8 | 27.1 | 28.8 | 30.0 |
| Guarani | 172.7 | 34.0 | 595.1 | 29.7 | 154.6 | 186.7 | 343.2 | 71.9 | 715.9 | 20.5 | 595.6 | 26.7 | 26.8 | 32.6 |
| Tupy | 181.8 | 34.5 | 637.5 | 28.0 | 88.4 | 409.4 | 315.4 | 98.5 | 785.0 | 19.0 | 640.4 | 25.8 | 31.1 | 25.9 |
| BRS Xavante | 177.3 | 36.2 | 612.0 | 30.3 | 126.8 | 239.1 | 334.1 | 74.5 | 747.1 | 24.4 | 613.5 | 28.7 | 29.3 | 33.1 |
| BRS Xingu | 183.2 | 34.6 | 648.1 | 29.5 | 133.8 | 221.7 | 350.6 | 76.8 | 789.0 | 21.8 | 648.6 | 27.9 | 31.1 | 29.3 |
| **CV (%)$^2$** | **33.2** | | **28.6** | | **255.1** | | **77.4** | | **21.9** | | **26.8** | | **28.8** | |

*$^{ns}$non-significant, $^1$Coeficient of variation of each cultivar for each model, $^2$ Coefficient of variation for the model*

The high CVs found in this study are consequence of the more expressive variation in chilling accumulation between years than between the cultivars within each model, with differences greater than 3.5 times between years being observed, as in the case of 2014 and 2010 (CH ≤7.2ºC, data non shown). Similarly, in a study evaluating the chilling accumulation in peach trees in the same region, in Pelotas, RS, large variations among years were found for the same cultivar [21].

Although some of the cultivars used in the present study, such as Comanche and Cherokee are said to have medium and medium-high chilling requirement respectively [22], under the studied conditions, they were not different from the others, such as cultivars BRS Xavante [23], Caingangue [24], Brazos [25], Tupy and BRS Xingu [26] and BRS Cainguá [27], which have an estimated chilling requirement between 200 and 300 hours (≤7.2 ºC). And almost for all the tested models a very small difference in chilling accumulation to bud break between the cultivars was noticed.

For the present work, the amount of chilling accumulated until leafing was calculated for each cultivar in each year, using all the seven models. However, only the Taiwan and Utah Positive models will be presented (Table 2), as they were the models with smaller CV, which means that they are more stable, so the most suitable.

**Table 2. Chill accumulation to vegetative bud break, calculated by the model of Taiwan and Utah Positive from 2010 to 2019 for the blackberry cultivars Brazos (Brz), Caingangue (Cai), BRS Cainguá (Cga), Cherokee (Che), Choctaw (Cho), Comanche (Com), Guarani (Gua), Tupy (Tup), BRS Xavante (Xav) e BRS Xingu (Xin), Embrapa Clima Temperado, Pelotas-RS, Brazil**

| | **Chill Units calculated by the Taiwan model** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Brz** | **Cai** | **Cga** | **Che** | **Cho** | **Com** | **Gua** | **Tup** | **Xav** | **Xin** |
| 2019 | 1035.0 | 1141.5 | 957.5 | 988.5 | 1015.5 | 806.0 | 864.5 | 969.0 | 1035.0 | 969.0 |
| 2018 | 976.0 | 976.0 | 936.0 | 976.0 | 980.0 | 986.0 | 986.0 | 986.0 | 976.0 | 986.0 |
| 2017 | 490.5 | 556.0 | 564.0 | 556.0 | 564.0 | 556.0 | 551.0 | 597.5 | 522.0 | 551.0 |
| 2016 | 624.5 | 656.5 | 702.0 | 690.0 | 834.0 | 656.5 | 656.5 | 702.0 | 656.5 | 809.0 |
| 2015 | 490.0 | 527.0 | 522.5 | 535.0 | 535.0 | 514.5 | 514.5 | 578.0 | 522.5 | 503.0 |
| 2014 | 575.0 | 600.5 | 630.0 | 719.5 | 630.0 | 690.0 | 618.0 | 690.0 | 575.0 | 690.0 |
| 2013 | 735.5 | 824.5 | 840.0 | 875.0 | 840.0 | 840.0 | 824.5 | 840.0 | 785.5 | 854.0 |
| 2012 | 703.0 | 710.0 | 710.0 | 723.5 | 710.0 | 709.0 | 703.0 | 723.5 | 709.5 | 723.5 |
| 2011 | 1049.5 | 915.0 | 815.0 | 1038.0 | 847.0 | 966.5 | 669.0 | 938.0 | 886.0 | 979.0 |
| 2010 | 932.0 | 825.5 | 873.5 | 874.5 | 789.5 | 789.5 | 772.0 | 825.5 | 803.0 | 825.5 |
| **Average** | **761.1** | **773.3** | **755.1** | **797.6** | **774.5** | **751.4** | **715.9** | **785.0** | **747.1** | **789.0** |
| **SD (±)[1]** | **209.2** | **189.1** | **144.8** | **169.8** | **155.0** | **149.2** | **139.0** | **141.4** | **173.1** | **163.0** |
| **CV (%)[2]** | **27.5** | **24.5** | **19.2** | **21.3** | **20.0** | **19.8** | **19.4** | **18.0** | **23.2** | **20.7** |
| | **Chill Units calculated by the Utah Positive model** | | | | | | | | | |
| | **Brz** | **Cai** | **Cga** | **Che** | **Cho** | **Com** | **Gua** | **Tup** | **Xav** | **Xin** |
| 2019 | 782.0 | 775.0 | 778.5 | 809.0 | 664.0 | 699.5 | 764.5 | 770.5 | 782.0 | 770.5 |
| 2018 | 811.0 | 811.0 | 812.5 | 811.0 | 812.5 | 812.5 | 796.0 | 812.5 | 811.0 | 812.5 |
| 2017 | 350.0 | 402.5 | 407.0 | 402.5 | 402.5 | 402.0 | 407.0 | 414.5 | 375.0 | 402.0 |
| 2016 | 600.5 | 657.5 | 773.5 | 624.5 | 624.5 | 624.5 | 668.5 | 668.5 | 624.5 | 751.0 |
| 2015 | 343.0 | 351.0 | 351.0 | 346.0 | 346.0 | 346.0 | 346.0 | 375.5 | 346.0 | 346.0 |
| 2014 | 410.0 | 483.5 | 428.5 | 417.0 | 478.5 | 418.5 | 428.5 | 478.5 | 410.0 | 478.5 |
| 2013 | 665.0 | 803.5 | 782.0 | 773.0 | 782.0 | 773.0 | 782.0 | 782.0 | 728.5 | 785.5 |
| 2012 | 603.0 | 604.5 | 604.5 | 604.5 | 604.5 | 603.0 | 604.5 | 604.5 | 604.5 | 604.5 |
| 2011 | 873.5 | 869.5 | 726.5 | 792.0 | 829.5 | 601.0 | 706.0 | 804.5 | 766.5 | 843.0 |
| 2010 | 761.0 | 729.5 | 683.5 | 692.5 | 683.5 | 675.5 | 729.5 | 692.5 | 686.5 | 692.5 |
| **Average** | **619.9** | **648.8** | **634.8** | **627.2** | **622.8** | **595.6** | **623.3** | **640.4** | **613.5** | **648.6** |
| **SD (±)[1]** | **195.3** | **182.6** | **176.3** | **180.7** | **168.6** | **159.1** | **169.0** | **165.2** | **176.2** | **180.7** |
| **CV (%)[2]** | **31.5** | **28.1** | **27.8** | **28.8** | **27.1** | **26.7** | **27.1** | **25.8** | **28.7** | **27.9** |

*[1] Standard deviation, [2] Coefficient of variation*

The chilling accumulation necessary for bud break in blackberries (10% of buds on the green tip stage), estimated by the Taiwan model was in a range of 715.9 ('Guarani') to 797.6 ('Cherokee'), and for the Utah Positive model, the lowest chilling accumulation was 595.6 ('Comanche') while the highest was 648.8 ('Caingangue'), being the difference even smaller than those observed in the Taiwan model. As the bud break dates were observed only under field conditions, it is possible that the studied cultivars need even less chilling accumulation than those observed on this study to overcome dormancy, since the paradormancy effect was not eliminated and also the heat necessity was not considered.

To more precisely determine the chilling requirement to overcome dormancy, it is necessary to carry out tests using biological methods, which use detached branches or entire plants, kept at controlled temperatures and subsequently subjected to ideal growth conditions [28]. In the present work, biological methods were not used but it is believed that, as it occurred in peach trees submitted to forcing conditions, leafing occurred earlier or, with lower chilling accumulation than observed in the same cultivars under field conditions [21], this also may be observed for the blackberry.

The Utah and Low Chill models are widely used and effective for the CU calculation, however, for the blackberry, in the climatic conditions of Pelotas, these were the models that showed the greatest variation in the CU accumulation, with 255.1% and 77.4% (Table 1), respectively, being clearly unsuitable for the crop in this region.

As mentioned before, the variations observed for Taiwan and Positive Utah models were quite high for being applied accurately for estimation of chilling requirement for blackberry bud break. Thus, it may be interesting to compare the chilling accumulation estimated by the models (Table 2), with the obtained production in each year (Table 3), which can give a good idea of the minimum chilling amount necessary to obtain a satisfactory productivity.

In this case, it is important to observe the fruit production of the cultivars, which can be considered as an indicator of their adaptation to the growing conditions, since the insufficient chill accumulation causes several disorders in temperate fruit trees, such as erratic sprouting, prolonged flowering period, low fruit set and, consequently, low productivity [29,30]. The cultivars in this study, in general, had similar production (Table 3), which suggests that the chilling requirement for all of them is in fact very similar.

In the comparison between production per plant and CU accumulation in the period between 2010 and 2015, it is observed that both in the years of 2015 (lower CU number), and 2011 (higher CU number), despite the difference in chilling accumulation, the productivity of the cultivars did not show large differences, and some were even better in 2015 than in 2011, as for example 'Guarani' and 'Choctow'. And except for 'Xavante' (the lowest productivity in 2015), probably because some biotic factors, and growth habit (with secondary branches only in the superior third of the cane), for the other cultivars the productivity was quite similar, reassuring that for some, the accumulation of a large amount of chilling is not necessary for floral differentiation [31], and that the tested blackberry cultivars are very well adapted to the climatic conditions of the study site.

In temperate climate plants, the phenological events that occur in spring (leafing and flowering) are usually coordinated by two temperature-dependent processes, the chilling accumulation to overcome the endodormancy and the heat accumulation necessary for leafing and flowering to occur. The temperature increase in the second phase can anticipate these events, however, when this increase occurs during the chilling accumulation phase, it can delay leafing and flowering due to the delay in the accumulation of the chilling hours necessary to overcome dormancy [32,33].

However, regardless of the model, cultivars with similar chilling accumulation over the years, in most cases, do not show similar production. This is because other factors are also important for the overcoming plant dormancy, such as water stress, thermal stress, pest attack, among others, which can act alone or together. Excessive rainfall, for example, can interfere with the pollination of flowers and fruit set, while thermal stress can compromise both pollen and ovule viability [34].

But in general, both in years of high and low chilling accumulation, the studied cultivars showed satisfactory production, demonstrating that they are well adapted to the climatic conditions of Pelotas, RS.

**Table 3. Production per plant (in grams) between 2010 and 2015, Embrapa Clima Temperado, Pelotas-RS, Brazil**

| **Cultivar** | **Production per plant (g)[1]** | | | | | |
|---|---|---|---|---|---|---|
| | **2015** | **2014** | **2013** | **2012** | **2011** | **2010** |
| Brazos | 1790 | 1923 | 2582 | 1289 | - | 1082 |
| Caingangue | 1720 | 2187 | 1706 | 1580 | 1765 | 1272 |
| BRS Cainguá | 2873 | 1717 | 1780 | 1520 | - | 965 |
| Cherokee | 1330 | 973 | 1650 | 1894 | 1986 | 2111 |
| Choctow | 2650 | 1367 | 1726 | 1798 | 1633 | 1221 |
| Comanche | 1340 | 1200 | 1501 | 2013 | 2185 | 1281 |
| Guarani | 1590 | 1060 | 1545 | 1035 | 1318 | 1652 |
| Tupy | 1980 | 2816 | 2226 | 2110 | 2181 | 1707 |
| BRS Xavante | 430 | 1278.3 | 1888 | 1415 | 1691 | 1227 |
| BRS Xingu | 3062 | 4468 | 1528 | 2394 | 3744 | 3496 |
| **Average** | **1876.5** | **1898.9** | **1813.2** | **1704.8** | **2062.9** | **1601.4** |

*[1] Plants without irrigation or trellis; maintained short by pruning.*

Regarding adaptation, the blackberry breeding program carried by the Embrapa Clima Temperado, focuses, among other characteristics, on the development of cultivars with low chilling requirement, in order to mitigate the effect of raising temperatures resulting from climate change, in addition, providing in due course, materials for cultivation in areas that have low chill accumulation. In this regard, we can mention the release of the Tupy cultivar, one of the most planted in warm temperate zones, with high fruit quality and good productivity [25,35], especially in Brazil and Mexico [35], plus other areas with mild winter. Therefore, the program seeks low chilling requirement, without neglecting fruit quality, disease resistance, among other important characteristics for the success of the crop.

## 4. CONCLUSION

The estimation of cold accumulation for cultivars by all applied models was highly variable.

The most suitable models to estimate the chilling requirement of the tested blackberry cultivars were the Taiwan and the Utah Positive models.

Utah and Low Chill models are not suitable for estimating blackberry chilling requirement.

All cultivars in the study presented good crop and adaptation with less than 800CU or less than 650 CU by the Taiwan and Utah Positive models, respectively.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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