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4 **Phenology of apple cultivars with different**
5 **chilling requirements**
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10 **ABSTRACT**
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Under mild climate conditions where chilling requirements are not met, the different phenological stages tend to extend and vary according to the year and chill intensity. Knowledge on the phenological stages becomes important for the management of some cultural practices such as fruit thinning and phytosanitary treatments. The phenology of six apple cultivars with different chilling requirements was studied for a period of 20 years. The method employed made it possible to analyze the evolution of the different phenological stages, allowing the chronological determination of their development. The results suggest adaptability of the different cultivars in the same region, highlighting the influence of environmental factors. The duration of the different phenological stages varied according to the chilling requirements of each cultivar, being directly influenced by climatic factors.

12
13 *Keywords: Malus domestica, climate variability, phenological stages, chilling requirement.*
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16 **1. INTRODUCTION**
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18 Under mild climate conditions where chill needs are not met, phenological stages tend to
19 extend and vary according to the year and chill intensity. Fruit production is directly
20 influenced by flowering time, which is a highly heritable trait greatly affected by how a
21 genotype perceives its environment [1, 2, 3]. The evolution of the phenological stages of
22 apple, during the vegetative phase, beginning of bud breaking, full flowering, fruit
23 development and maturation, as well as yield and production quality, can be observed
24 visually and are influenced by climatic conditions [4]. Apple flowers are largely self-
25 incompatible, so cultivars that flower in a synchronized manner are required to ensure
26 adequate cross-pollination [5]. Commercial apple cultivars have a flowering cycle that
27 extends from a week to a month depending on the cultivar and environmental conditions.
28 Knowledge on the phenological stages becomes important for the management of some
29 cultural practices such as fruit thinning, phytosanitary treatments and nutrition. Among the
30 factors that influence apple phenology, the ones which stand out are chill needs of the
31 cultivar, degree days and rootstock. These phenological changes have been widely studied
32 and classified according to their development from dormancy to fruiting [6, 7, 8]. Despite the
33 long history of phenological observations, the term “phenology” appeared in 1949 [9]. In
34 regions with a mild climate where the chill needs of the cultivar are not met to exit dormancy,
35 there may be a series of anomalies in the phenological stages and development of apple
36 [10, 11, 12, 13]. The study of phenology is based on observations and measurements of a
37 number of plant organs in a given period[14]. This is an essential element in assessing the
38 adaptability of a species to certain environmental conditions. Phenology has been
39 associated with environmental factors such as precipitation, temperature, and photoperiod
40 [15].

41 The climate of the southern region of Brazil is distinct from the **climate** of the temperate
42 regions in the center of origin of the main apple species (Caucasus and eastern China) and
43 also distinct from those in the main areas where this fruit tree is cultivated [16]. When the
44 chilling requirements of **temperate fruit trees** are not fully met, they exhibit some anomalies
45 such as lower percentage of bud breaking and flowering, as well as a prolonged and uneven
46 flowering period [17]. Such uneven flowering causes plants to show several phenological
47 stages at the same time, hindering cultural practices, in addition to resulting in a lower and
48 low-quality production at the end of the production cycle [17]. Knowledge of phenology and
49 thermal requirements is a necessity in apple cultivation, as it allows the rationalization of
50 cultural practices and the estimation of probable harvest dates. Therefore, studies on the
51 phenological behavior of different cultivars in several cycles are necessary, since different
52 accumulations of chilling hours are observed in different years and may advance or delay
53 apple flowering, according to the chilling requirements of each cultivar. The occurrence of
54 frosts during flowering or effective fruiting can affect production, whereas late flowering is
55 less affected, so it is important to know the phenological stages of apple [18]. The amount of
56 accumulated chill is very important for the adaptation of temperate climate fruit trees, due to
57 the influence of chill on the exit from dormancy [19, 20]. After a long period of cold during the
58 winter, high temperatures in the early spring of the subsequent season promote final
59 development and flowering [21, 22]. However, these studies on the interaction between
60 environmental conditions and genotypes require a number of years of research, and some
61 cultural practices may also affect phenology. At the physiological level, harvest load, growth
62 dynamics, genotype, plant architecture, bearing habit (regular or biennial), and temperature
63 affect the time and rate of flower initiation in apple [23, 24, 25, 26, 27, 28, 29, 30]. High
64 temperatures during pre-flowering and flowering can shorten the flowering period [31]. The
65 phenological stages most observed in the field are the beginning of bud breaking, beginning
66 of full flowering and end of flowering, which are important to define phytosanitary treatments,
67 cultural practices such as thinning, and to predict early harvest. The objective of this study
68 was to evaluate the phenological development of six apple cultivars in a historical series
69 under the climatic conditions of Southern Brazil.

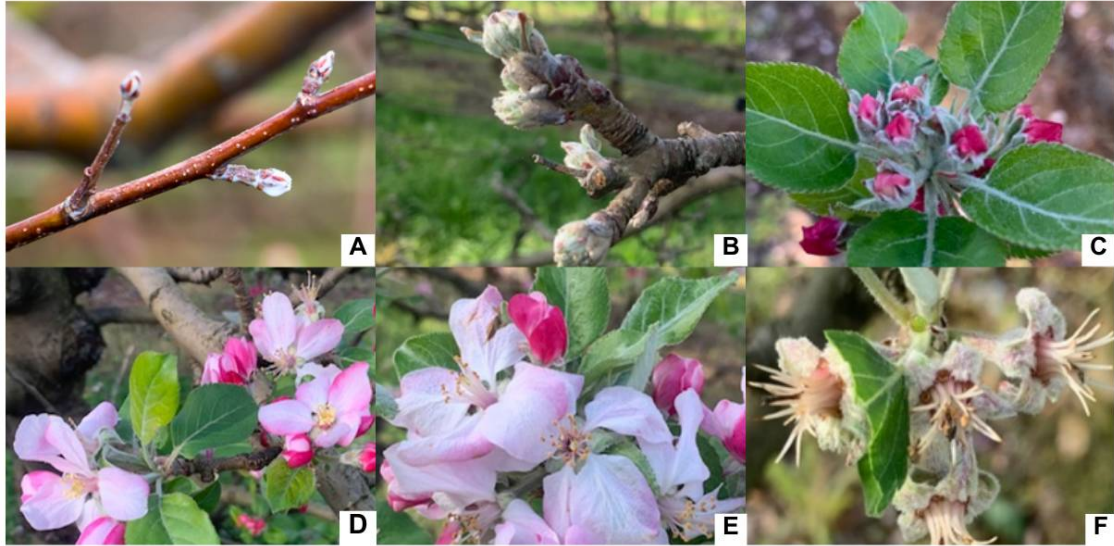
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71 **2. MATERIAL AND METHODS**

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73 The experiment was carried out in an experimental orchard located in the municipality of
74 Caçador, SC, Brazil (26°46'S latitude, 51° W longitude, 960 m altitude), belonging to the
75 EPAGRI Experimental Station – Caçador, SC. The climate of this region is characterized as
76 temperate, constantly humid, with mild summers, classified as Cfb according to Köppen's
77 classification. The soil of the orchard under study is classified as a Nitossolo Bruno distrófico
78 (Ultisol). The average annual rainfall is 1,670 mm and the average relative humidity is 78%
79 (Ciram/Epagri). The average chilling accumulation during the autumn-winter period,
80 according to the modified North Carolina model [32] is 1,058 chilling units on average.

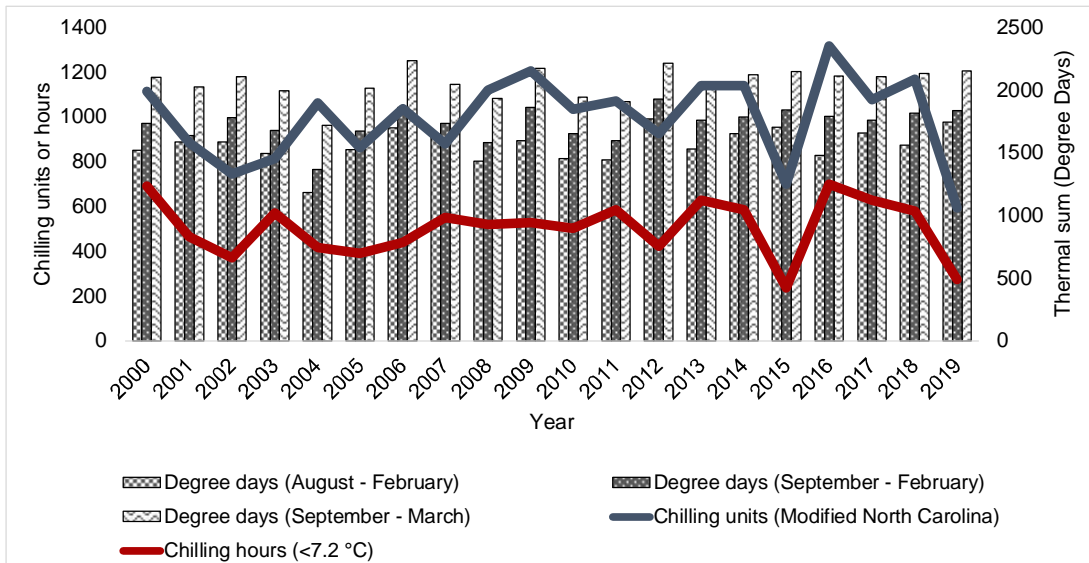
81 The phenology of six apple cultivars with different chilling requirements was evaluated for a
82 period of 19 years during the cycles from 2000 to 2019. The method to determine the
83 evolution of the different phenological stages was visual, allowing the chronological
84 determination of their development (Figure 1). To determine the phenology, the evaluation
85 consisted of determining the dates of occurrence of the following stages: green tip (C-C3);
86 beginning of bud breaking; beginning, full and end of flowering; and beginning and end of
87 harvest for each cultivar. The beginning of bud breaking was considered when 50% of the
88 reproductive buds had 1.3 cm of green tip; beginning of flowering was considered when the
89 plants had 10% of open flowers; full flowering was considered when more than 70% of
90 flowers were open; and end of flowering was determined when 90% of the flowers had their
91 petals falling.



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Figure 1 – Phenological stages of apple. A: Silver tip (B); B: Green tip (C-C3); C: Pink; D: Beginning of flowering; E: Full flowering; F: Petal fall.

96 Data of chilling units according to the Modified North Carolina model [32], chilling hours
97 below 7.2 °C, during the period of phenological evaluations, and degree days with base
98 temperature of 10 °C were collected (Figure 2). The cultivars studied were ‘Condesa’ (low
99 chilling requirement), ‘Imperatriz’, ‘Baronesa’ and ‘Fred Hough’ (medium chilling
100 requirement) and ‘Gala’ and ‘Fuji Suprema’ (high chilling requirement). The orchard used
101 has a planting density of 2,500 plants per hectare, all cultivars are grafted on the M-9
102 rootstock and trained to the central leader system. Orchard management practices followed
103 the technical recommendations of the production system [33]. To overcome dormancy, the
104 dormancy breaking treatment was performed with a mixture of mineral oil and hydrogenated
105 cyanamide in all seasons studied. Phenological development was evaluated twice a week
106 from the C-C3 stage until the end of flowering and the beginning and end of fruit harvest.
107 The cultivars were studied separately and were not considered causes of variation. The
108 results compare the adaptive capacity of different cultivars in the same region and the
109 influence of environmental factors.
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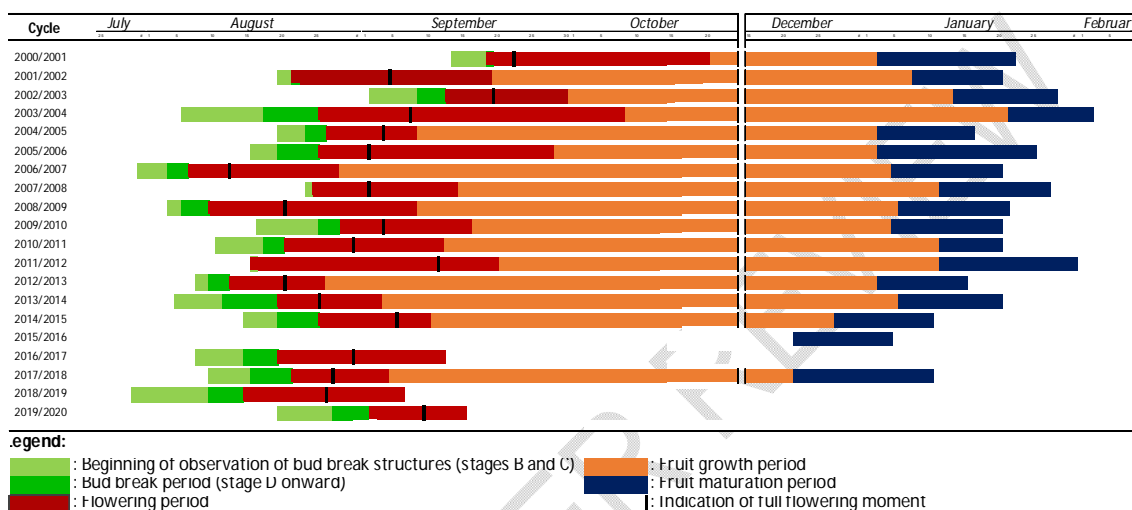
Figure 2 – Chilling units according to the modified North Carolina model, chilling hours below 7.2 °C and degree days in different periods in the years 2000 to 2019. Caçador, SC, Brazil, 2023.

3. RESULTS AND DISCUSSION

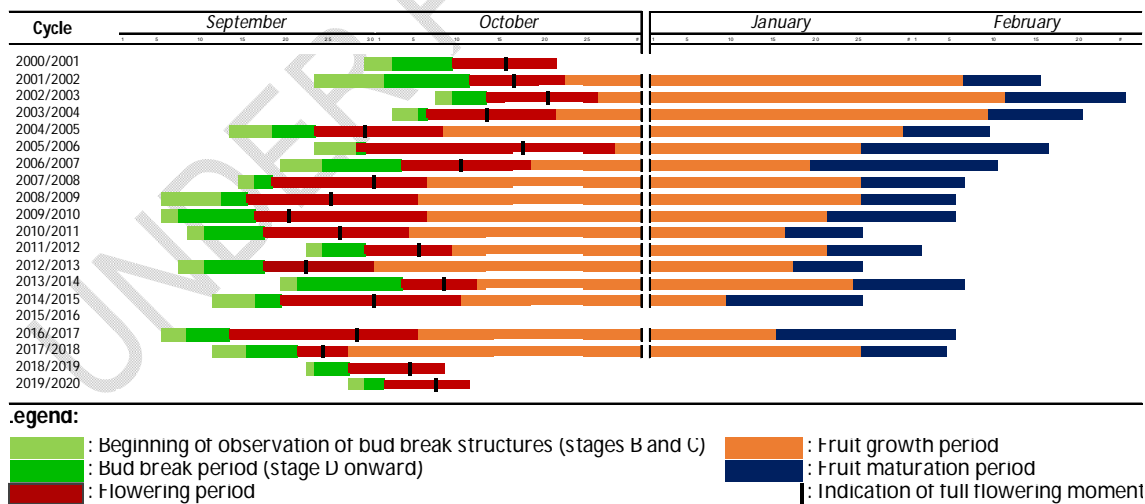
The different phenological stages varied according to the chilling requirements of the cultivar. Within the same cultivar, the phenological stages tend to advance in years with greater chill intensity (Figures 3 to 8 and Tables 1 and 2). The cv. ‘Condesa’, the one with the lowest chilling requirement, showed a variation in the beginning of bud breaking and flowering concentrated in August, but with great variability according to the year. In turn, the cultivars ‘Imperatriz’, ‘Fred Hough’ and ‘Baronesa’, with medium chilling requirement, concentrated their flowering in September and the first half of October, and the cultivars with highest chilling requirement, ‘Gala’ and ‘Fuji Suprema’, concentrated their flowering in October. These results demonstrate that the cultivars with lower chilling requirements have their bud breaking and flowering earlier than those with medium and high chilling requirement, showing that the chilling requirement of the cultivar influences the times for the beginning of bud breaking and flowering. Within the same cultivar, there were variations in the different phenological stages according to the years, which also had great variability in terms of the amount of chill, showing that the variation of the phenological stages is linked to climatic factors. The greater the number of chilling units during dormancy, the shorter the time and the need for thermal units for the sprouting of apple buds [34]. According to the climatic conditions observed, the average air temperature recorded from dormancy break to bud breaking is influenced by temperature, and the increase in temperature in the period following the dormancy breaking treatment accelerates bud breaking [35]. Full flowering in cv. ‘Gala’ was very variable according to the year, with a difference of up to 34 days among the sequences of the years (Figure 6). This also occurred in the other cultivars, regardless of the chilling requirement, which indicates that climatic variables influence the flowering date. The number of days from the beginning to the end of flowering also showed great variability between the years, regardless of the cultivar, with no evident correlation with the chilling units or chilling hours (Table 1). However, there are several factors that affect apple phenology, including the rootstock, training system, arching of the branches, time of application of bud break inducers, cultivar, environmental conditions and type of fruiting structure [4]. It is worth pointing out that there are differences in the phenological behavior of apple trees when cultivated under conditions that do not meet their chill needs. Under these

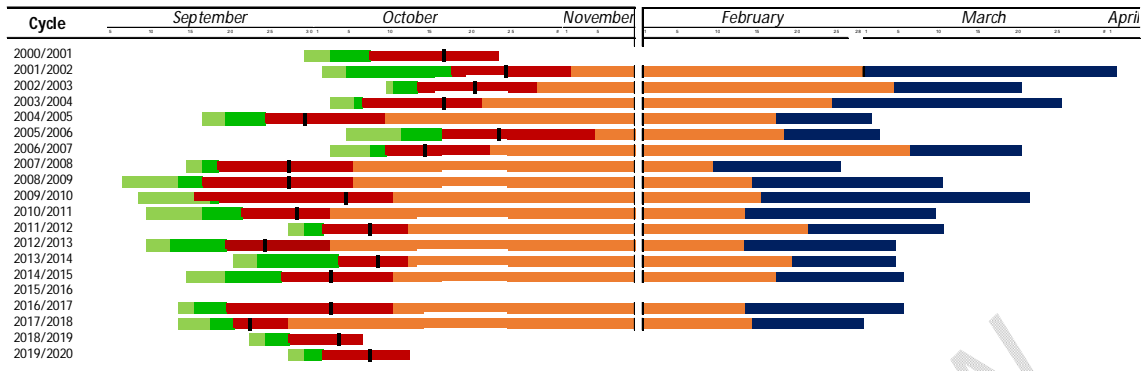
147 conditions, chemical treatment needs to be applied to induce bud breaking and flowering,
 148 and the post-treatment temperatures influence the beginning of bud breaking and flowering.
 149 The increase in temperature during the days following the dormancy breaking treatment
 150 accelerates bud breaking, while lower temperatures prolong the period and consequently
 151 delay flowering [35]. This may explain why there was no correlation between the date of the
 152 phenological stages and chilling units or hours for the different cultivars studied. The year
 153 2000/2001, one of those with the highest chilling units and hours (Figure 2), was among the
 154 years with the longest delay in the beginning of bud breaking for the cultivars studied
 155 (Figures 3 to 8).

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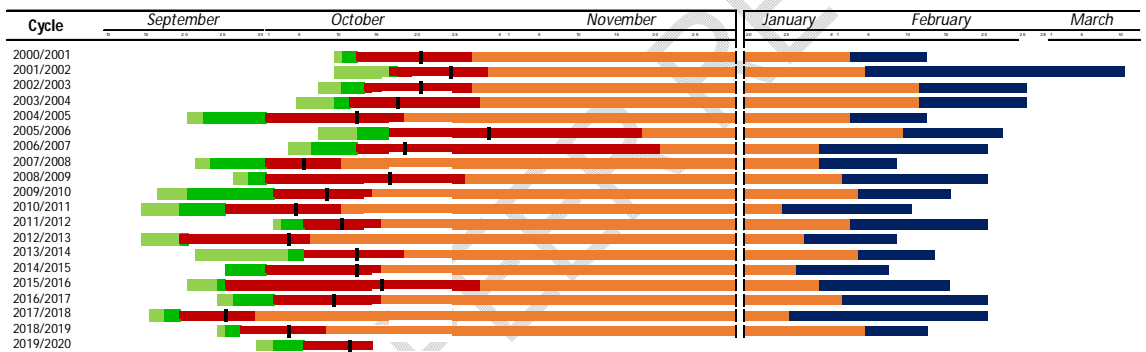


legend:

■ : Beginning of observation of bud break structures (stages B and C) ■ : Fruit growth period
■ : Bud break period (stage D onward) ■ : Fruit maturation period
■ : Flowering period | : Indication of full flowering moment

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Figure 5 – Date and duration of the main phenological events that occurred from stage C (Green tip) until the fruit harvest of the apple cultivar 'Fred Hough'. Caçador, SC, Brazil, 2023.

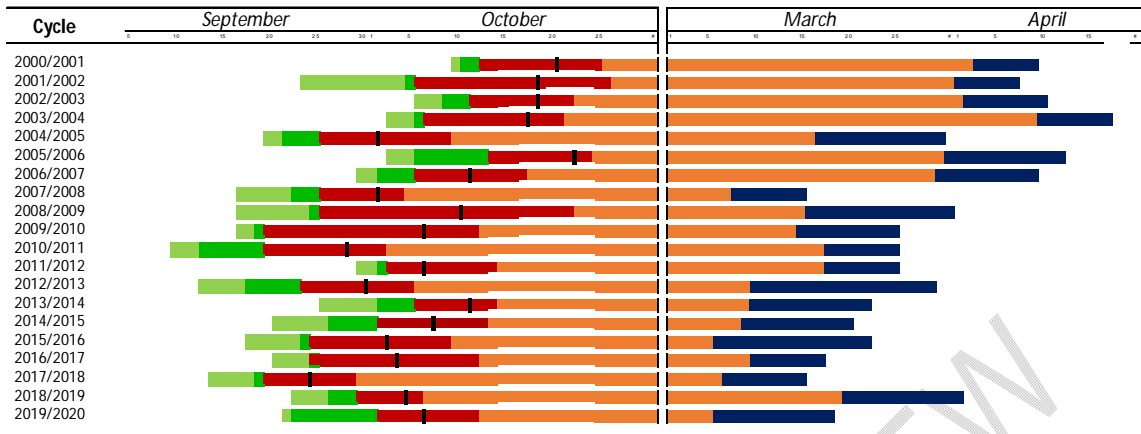


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■ : Beginning of observation of bud break structures (stages B and C) ■ : Fruit growth period
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Figure 6 – Date and duration of the main phenological events that occurred from stage C (Green tip) until the fruit harvest of the apple cultivar 'Gala'. Caçador, SC, Brazil, 2023.

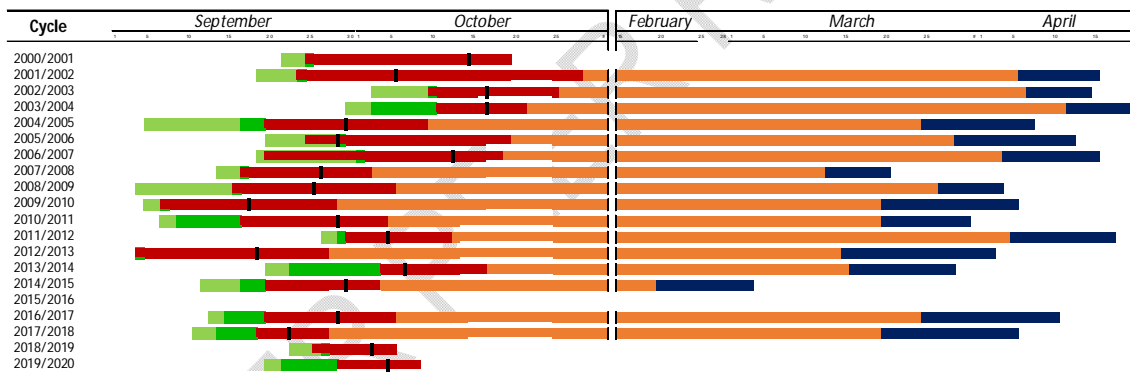


Legend:

■ : Beginning of observation of bud break structures (stages B and C) ■ : Fruit growth period
■ : Bud break period (stage D onward) ■ : Fruit maturation period
■ : Flowering period | : Indication of full flowering moment

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Figure 7 – Date and duration of the main phenological events that occurred from stage C (Green tip) until the fruit harvest of the apple cultivar ‘Fuji Suprema’. Caçador, SC, Brazil, 2023.



Legend:

■ : Beginning of observation of bud break structures (stages B and C) ■ : Fruit growth period
■ : Bud break period (stage D onward) ■ : Fruit maturation period
■ : Flowering period | : Indication of full flowering moment

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Figure 8 – Date and duration of the main phenological events that occurred from stage C (Green tip) until the fruit harvest of the apple cultivar ‘Baronesa’. Caçador, SC, Brazil, 2023.

183 The flowering period (beginning to end) varied according to the year and the cultivar, but
 184 there was no relationship between the chilling requirement of the cultivar and the number of
 185 days from the beginning to the end of flowering (Table 1). There was a great variability
 186 between the years in the flowering period, as seen in the ‘Gala’ cultivar, whose flowering
 187 period ranged from nine to 38 days, with an average of 17 days. Among the cultivars, the
 188 average of the different years ranged from 13.4 days to 21.6 days. A more concentrated
 189 flowering period becomes more advantageous due to the uniformity of maturation, less need
 190 for labor and greater efficiency of thinners; however, the probability that flowers will be
 191 pollinated in a longer flowering period becomes higher, especially under adverse conditions
 192 to pollination, impairing the work of bees [35].

193 **Table 1** – Number of days from the beginning of flowering to the end of flowering in several
 194 apple cultivars. Caçador, SC, Brazil, 2023.

Year	'Condesa'	'Imperatriz'	'Fred Hough'	'Baronesa'	'Gala'	'Fuji Suprema'
2000	22	-	15	25	14	22
2001	27	10	13	34	12	27
2002	17	12	14	15	13	17
2003	34	14	14	10	16	34
2004	11	14	14	19	17	11
2005	32	29	17	24	32	32
2006	21	14	12	28	38	21
2007	19	17	16	15	9	19
2008	28	19	18	19	25	28
2009	19	19	24	20	12	19
2010	19	16	10	17	14	19
2011	35	9	10	12	9	35
2012	12	12	12	23	16	12
2013	13	8	8	12	12	13
2014	14	20	13	13	19	14
2015	-	-	-	-	-	-
2016	22	21	20	15	13	22
2017	12	7	6	8	9	12
2018	21	10	8	9	10	21
2019	33	9	10	9	-	33
Mean	21.6	14.4	13.4	17.2	17.0	21.6

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 196 Considering the period from full flowering to the beginning of harvest, in days, there was a
 197 great variation between cultivars and within the same cultivar between years, which may be
 198 related to maximum and minimum daily temperatures (Table 2). Among the cultivars, those
 199 with the latest harvest were the ones with the longest cycle, namely 'Baronesa' and 'Fuji
 200 Suprema', with an average of 176.8 and 161.5 days, respectively. For the other cultivars, the
 201 average cycle duration was 114.6 days for 'Gala', 113.7 days for 'Imperatriz', 124.3 days for
 202 'Condesa' and 135.5 days for 'Fred Hough'. Variability was high within the different years, as
 203 in the case of 'Fuji Suprema', whose cycle ranged from 150 to 175 days, i.e., 25 days. The
 204 individual study of each period is extremely important, as it can provide direct evidence and
 205 specific information about which events, such as climate variations and changes, may
 206 influence [35]. There was a reduction of up to 25 days in the case of 'Fuji Suprema', but it
 207 was observed that the degree days also showed great variability between the years,
 208 influencing the cycle of the cultivars. Despite that, the index of degree days from full
 209 flowering is appropriate for possible calculations of the period from flowering to fruit maturity,
 210 which requires the monitoring of degree days during the cycle.

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 212 **Table 2** – Number of days from full flowering to the beginning of fruit maturation of several
 213 apple cultivars. Caçador, SC, Brazil, 2023.

Year	'Condesa'	'Imperatriz'	'Fred Hough'	'Baronesa'	'Gala'	'Fuji Suprema'
2000	116	114	131	171	116	165
2001	126	114	128	184	104	165
2002	117	115	136	173	115	166
2003	136	120	132	178	118	175
2004	122	124	142	177	114	167
2005	123	108	119	182	104	160
2006	115	102	144	174	105	170

2007	132	118	136	168	117	154
2008	138	123	141	183	109	157
2009	124	124	135	184	119	150
2010	134	113	139	173	113	171
2011	122	109	138	183	116	163
2012	136	118	143	178	117	162
2013	134	109	135	161	115	150
2014	114	102	139	178	107	153
2015	-	-	-	-	-	-
2016	113	110	135	178	116	158
2017	115	124	146	179	123	165
2018	124	104	139	174	125	167
2019	120	110	118	181	125	152
Mean	124.3	113.7	135.5	176.8	114.6	161.5

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216 4. CONCLUSION

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218 The different phenological stages vary with the year and the cultivar, being influenced by
219 climatic variables and cultural practices such as the budbreak induction treatment.

220 Cultivars with low chilling requirement show advance in the beginning of bud breaking and
221 flowering compared to cultivars with higher chilling requirement.

222 The number of days from the beginning of flowering to the end of flowering varied with the
223 years, but did not correlate with chilling units or chilling hours.

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UNDER PEER REVIEW