Phenology, reproductive biology and growing degree days of the grapevine 'Isabel' (*Vitis labrusca*, Vitaceae) cultivated in northeastern Brazil

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Abstract

Phenology and reproductive biology of cultivated species are important for the comprehension of the requirements for fruit and seed production and the management of pollinators. This study aimed to characterise the phenology, reproductive biology and growing degree days of the grapevine 'Isabel' (*Vitis labrusca*) in northeastern Brazil during January 2011 (P1), Augst 2011 (P2), April 2012 (P3) and August 2012 (P4). We recorded the duration (days) of the phenological stages, pruning (P), woolly bud (W), budburst (B), inflorescence development (ID), flowering (F), ripening (R) and harvest (H). We analysed the floral biology, the sexual system and the breeding system. We measured the growing degree days (GDD) required to reach the subperiods P-B, B-F and F-H. The periods P1, P2, P3 and P4 lasted for 116, 125, 117 and 130 days, respectively. The number of days of harvest were similar in the same dry (P1 and P3) and rainy (P2 and P4) periods. All the periods that we recorded were shorter than those observed in other regions of Brazil, which may be attributable to the mean temperature and carbohydrate metabolism. The flowers are green, hermaphroditic, with an odour of mignonette, low pollen viability and autogamous. The base temperature of 10°C was considered the most adequate for the subperiods as has been documented for other grape varieties in Brazil. Thus, temperature was also the most adequate for the cycles, presenting a smaller standard deviation (0.119, 0.147, 0.156 and 0.153 to P1, P2, P3 and P4, respectively) when compared to a base temperature of 12°C (0.122, 0.158, 0.165 and 0.160 to P1, P2, P3 and P4, respectively). The higher and the lower observed GDD were 1972.17 and 1870.05, respectively, both above the values recorded in other parts of Brazil for same variety. The phonological results, including knowledge of growing degree days, are important to the planning of cultures at the study site and in other regions that have similar climatic conditions and make it possible to pre-determine the harvest.

Keywords: breeding system, heat unit, grape, phenology, GDD.

Fenologia, biologia reprodutiva e graus-dias da videira "Isabel" (*Vitis labrusca*, Vitaceae) cultivada no nordeste do Brasil

Resumo

Fenologia e biologia reprodutiva de espécies cultivadas são importantes para a compreensão dos requerimentos para a produção de frutos e sementes, bem como para o manejo de polinizadores. O presente estudo objetivou caracterizar a fenologia, a biologia reprodutiva e a exigência térmica (graus-dias) da videira "Isabel" (*Vitis labrusca*) no nordeste do Brasil, durante janeiro/2011 (P1), Agosto/2011 (P2), Abril/2012 (P3) e Agosto/2012 (P4). Analisamos a duração (dias) dos estádios fenológicos poda (PO), gema-algodão (GA), brotamento (BR), aparecimento da inflorescência (AI), florescimento (FL), início da maturação (IM) e colheita (CO). Analisamos a biologia floral, o sistema sexual e o sistema reprodutivo. As exigências térmicas foram obtidas em termos de graus-dia (GD) necessários para atingir os subperíodos PO-BR, BR-FL e FL-CO. A duração do ciclo foi de 116, 125, 117 e 130 dias para as épocas P1, P2, P3 e P4 respectivamente, sendo o número de dias de colheita semelhantes no período seco (P1 e P3) e chuvoso (P2 e P4). Todos os períodos analisados foram mais curtos do que aqueles observados em outras regiões do Brasil, o que pode ser atribuído à temperatura média e ao metabolismo de carboidratos. As flores são verdes, hermafroditas, com odor almiscarado, baixa viabilidade polínica e autógama. A temperatura base de 10ºC foi considerada a mais adequada para os subperíodos e tem sido documentada para outras variedades de uva no Brasil; portanto, a temperatura foi também

a mais adequada para os ciclos, apresentando um desvio padrão menor (0,119; 0,147; 0,156 e 0,153 para P1, P2, P3 e P4, respectivamente), comparado com uma temperatura base de 12ºC (0,122; 0,158; 0,165 e 0,160 para P1, P2, P3 e P4, respectivamente). O maior é o menor GDD observado foram 1972,17 e 1870,05, respectivamente. Os resultados fenológicos, incluindo o conhecimento dos graus dias, são importantes para o planejamento de culturas no local e em outras regiões com condições climáticas semelhantes, tornando possível pré-determinar a colheita.

Palavras-chave: sistema reprodutivo, exigência térmica, uva, fenologia, graus-dias.

1. Introduction

The phenology and the growing degree days (i.e, thermic time, heat unit, Pedro-Júnior and Sentelhas, 2003) of the grape are important parameters for the efficient maintenance of this plant (Murakami et al., 2002). The growing degree days is an expression of the amount of energy that a cultivate plant species needs to satisfactorily complete its production cycle; it constitutes the accumulated difference between the mean environment temperature and the base-temperature (the value below which the plants cannot develop; Pedro-Júnior and Sentelhas, 2003). The growing degree days is the most used parameter in tropical viticulture because of its easy application and reliability (Sentelhas, 1998). When phenological and climatic data are combined, it is possible to comprehend the relationship between the duration of plant developmental phases and seasonal variations. Additionally, it is possible to understand how a specific plant species interact with the different climatic regions (Terra, 1993; Leão and Pereira, 2000).Therefore, phenological analysis and degree-days measurement may, be used as tools to evaluate the climatic potential of a region for the development of a crop species (Pedro-Júnior et al., 1993) and contribute for the knowledge about the periods of harvest, improving agricultural practices (Boliani and Pereira, 1996).

The NE region of Brazil has several areas where grape varieties are grown (Instituto FNP Consultoria e Comércio, 2009); for example, the 'Isabel' variety (*Vitis labrusca* L.) is cultivated in more than 400 ha in a region called 'agreste'. The region is characterised by a high level of precipitation in the winter and a milder climate throughout the year (Tavares and Lima, 2009). Grapes are produced in two annual harvests and have certain features that result from the accentuated declivity and familiar systems of production; the grapes are mostly used for *in natura* fruit market and also for the production of juice and table wine, with exporting prospects (Tavares and Lima, 2009). This local tendency to expand Isabel cultivation follows a global trend resulting from the relative rusticity of this vine; it easily adapts to edaphic-climatic conditions and has a high productivity and longevity, which is a consequence of its resistance to the main diseases of grapevines (Grigoletti Junior and Sônego, 1993).

Despite the socioeconomic relevance of viticulture at the local and regional scales, and despite the interest in production enhancement, there are no data on phenology, reproductive biology or growing degree days for this variety (Tavares and Lima, 2009). Moreover, there are few studies concerning grape phenology in Brazil and worldwide. The reproductive strategies of the genus *Vitis* are variable. Species may be dioecious, poligamodioecious or hermaphroditic (Dorsey, 1912). Flowers seem to be spontaneously self-pollinated, but there are differences regarding the importance of pollen vectors (Free, 1970; McGregor, 1976; Mullins et al., 1992).

The objectives of this study were to investigate the phenology, the growing degree days (GDD) and the reproductive biology of the grape *Vitis labrusca* cultivar 'Isabel' at distinct pruning times using as a model plantations located in the climatic conditions of the city of São Vicente Férrer, Pernambuco state, Brazil.

2. Material and Methods

2.1. Study area

The study was conducted in three areas in the rural zone of the municipality of São Vicente Férrer, PE, Brazil (07°35'06.7"S 035°31'11.5"W). The altitude is 570 m, and the climate is As' (warm and humid, with the rainy season occurring in the autumn and winter *sensu* Köppen, Beltrão and Macêdo, 1995). The annual precipitation and temperature means are 1.103 mm and 24°C, respectively. The rainy season begins in Jan-Feb (precipitation is higher than 100 mm) and ends in Sep-Oct (Leão and Borges, 2009). The soil is orthic red-yellow podzolic (Brasil, 1981). In the study areas the grapevines were planted in 1997, spaced at 1.5 m x 1 m; the trellis/training system used was a single-curtain cordon system, which consisted of a horizontal canopy. The plants were watered in periods of eight days.

Phenological data were obtained in an area of three ha, and data on reproductive biology were collected in this area and also in two other areas of approximately one ha and two ha (approx. 1km far from each other).

2.2. Phenological data

Phenological observations were performed in the period between fruit pruning and harvest [i.e., 29 January to 25 May 2011 (P1), 24 August to 29 December 2011 (P2), 24 April to 25 August 2012 (P3) and 13 August 2012 to 23 December 2012 (P4)]. The harvest P1 and P3 occurred during the rainy season, and P2 and P4 occurred in the dry season. After pruning, the plants received the growth regulator 3% Dormex®. We used a random experimental design, and each plant (n = 20 individuals) was considered a sample unit (Boliani and Pereira, 1996; Leão and Pereira, 2001). We analysed the following seven phenological stages: pruning (P), woolly bud (W), budburst (B), inflorescence development (ID), flowering (F), ripening

(R) and harvest (H) (Pedro-Júnior et al., 1990; Baillod and Baggiolini, 1993). We also recorded the duration (days) of the following periods: pruning to woolly bud (P-W), woolly bud to budburst (W-B), budburst to inflorescence development (B-ID), inflorescence development to flowering (ID-F), flowering to ripening (F-R), and ripening to harvest (R-H) (Boliani, 1994; Guerreiro, 1997). These data were recorded weekly for a randomly selected branch from each individual. The phase change was defined as the time at which 50% of the branches from each individual exhibited a particular stage.

2.3. Floral biology

We estimated the means of (1) open flowers per day in each inflorescence until senescence of all inflorescences for selected individuals (2) flowers per inflorescence and (3) inflorescences per individual. These data were collected from 30 randomly selected individuals in the same area of phonological observations.

Floral buds in the pre-anthesis stage were monitored daily until flower senescence to determine flower longevity (n = 30 buds from 10 inflorescences and 10 individuals). Fresh flowers were collected (30 flowers from 10 individuals), preserved in a 70% alcohol solution and observed under a stereomicroscope to determine floral morphology and to obtain morphometric data (i.e., floral diameter, gynoecium and androecium heights) using a digital calliper (error: 0.01).

The mean number of pollen grains produced per anther was estimated using 10 anthers of 10 different buds from 10 individuals. Pollen viability was evaluated with the acethocarmine technique (Dafni et al., 2005) for all anthers of 30 buds collected from 30 individuals. We analysed the first 200 pollen grains on each glass slide. These buds were also used to estimate ovule production.

The period of stigmatic receptivity was verified using the potassium permanganate method (Dafni et al., 2005) in two-hour intervals of during the day (from 6 h 00 min to 16 h 00 min), using flowers that were bagged since the bud stage (n = 10 individuals and n = 35 flowers). The presence of odour emission was checked by closing 15 flowers (seven individuals) in a capped glass vials for 30 minutes and inhaling the odour (Dafni et al., 2005).

2.4. Sexual and breeding system

The sexual system was determined after observation for the presence of anthers with pollen and pistils containing ovules (30 floral buds were preserved in 70% alcohol, from 10 individuals; three buds per individual, collected from different inflorescences, were analysed). The pollen viability was tested using the acetho-carmine technique (for 10 inflorescences) as described above.

The breeding system was determined by controlled hand pollinations of all flowers in 10 inflorescences for each treatment, previously isolated with paper bags at pre-anthesis stage. The treatments performed were as follows. (1) For cross-pollination, flowers received a mixture of pollen collected from two or more individuals. Flowers that were used as pollen-donors were collected and maintained in the field in plastic gerbox boxes containing a 2% agar solution. (2) For apomixis, buds were emasculated before anther dehiscence. (3) For wind pollination, emasculated flower buds were placed in mesh bags that allowed the entrance of pollen grains (carried by the wind) but did not allow contact with pollinators. (4) For hand self-pollination, flowers received their own pollen or received pollen from other flowers from the same individual. (5) For spontaneous self-pollination, inflorescences were bagged after the exclusion of previously open flowers. (6) For natural pollination, flowers were only marked on the pedicel.

To eliminate the possibility of spontaneous self-pollination prior to anthesis (Beach, 1892), in treatments 1, 2 and 3, all floral buds of each inflorescence were emasculated. After treatments 1, 2, 3, 4 and 5, the flowers were bagged until senescence. All treated and marked flowers were monitored until the total development of fruits.

A chi-square test was performed to compare the fruit sets after controlled hand pollination treatments, using BioEstat software (Ayres et al., 2003). The self-incompatibility index (ISI) was calculated as the ratio between the fruit set after hand self-pollination and cross-pollination (*sensu* Bullock, 1985).

2.5. Growing degree days

The growing degree days were characterised by the sum of the 'heat units' for each calendar day of the growing season from pruning until harvest, considering four production cycles (two in the wet and two in the dry season). These data were also collected during the subperiods pruning (P) to budburst (B), budburst (B) to flowering (F), and flowering (F) to harvest (H). Meteorological data were obtained at the Meteorological Station of Surubim (7°49'48" S 35°46' 48"W).

The following Equations 1 and 2 were used (Villa-Nova et al., 1972):

$$GDD = \frac{(T_m - T_b) + (T_M - T_b)}{2} \quad \text{for } T_m > T_b \tag{1}$$

$$GDD = \frac{(T_M - T_b)^2}{2(T_M - T_m)} \quad \text{for } T_m < T_b \tag{2}$$

and GDD = 0, for $T_M < T_b$, where GDD = growing degree days; T_M = maximum daily temperature (°C); T_m = minimum daily temperature (°C); and T_b = base temperature (°C). The growing degree days were calculated for two base temperatures (10 and 12°C), to establish the lower standard deviation (in days) according the following Equation 3 (Arnold, 1959):

$$Sd = \frac{Sdd}{X_t - t_b} \tag{3}$$

where Sd = standard deviation (days); Sdd = standard deviation (growing degree days); x_t = air mean temperature during the considered period (°C); and t_b = base temperature (°C).

3. Results

3.1. Phenology

The phenological pattern observed was similar for the rainy (P1 and P3) and the dry (P2 and P4) seasons. The total duration (days) was shorter in the rainy than in the dry season (116 and 117 days, respectively); we observed a difference of 8 days longer in P2 (125) and 13 days longer in P4 (130, as shown in Table 1). Flowering occurred 33 days after pruning, and the harvest occurred 87 days after this period.

3.2. Floral biology

Each individual produced a mean of 20 ± 10 inflorescences, and each inflorescence had a mean of 9 ± 6 open flowers daily (97 ± 34 flowers in total). The anthesis lasted one day and began approximately at 6 h 30 min (see Figure 1a, b); at 7 h 30 min the flowers were completely opened

Table 1. Mean number of days between each phenological stage of the vine 'Isabel' (*Vitis labrusca*) in three pruning periods in São Vicente Férrer, PE, Brazil.

Phenological stages	Pruning / Phenological period (days)			
	Pruning 1 29/01/2011	**Pruning 2 24/08/2011**	**Pruning 3 24/04/2012**	**Pruning 4 13/08/2012**
P –W	6	7	8	7
W – B	6±2.0	7	5	5
B – ID	8±3.4	5	7	7
ID – F	14	22	12	15
F – R	71	69	71	70
R - H	11	15	14	27
Total period (P – H)	116	125	117	130
Harvest	25/05/2011	29/12/2011	21/08/2012	23/12/2012

P-W: pruning to woolly bud; W-B: woolly bud to budburst; B-ID: budburst to inflorescence development; ID-F: inflorescence development to flowering; F-R: flowering to ripening; R-H: ripening to harvest and P-H: pruning to harvest. Standard deviation was zero, except to W-B and B-IB in the Pruning 1.

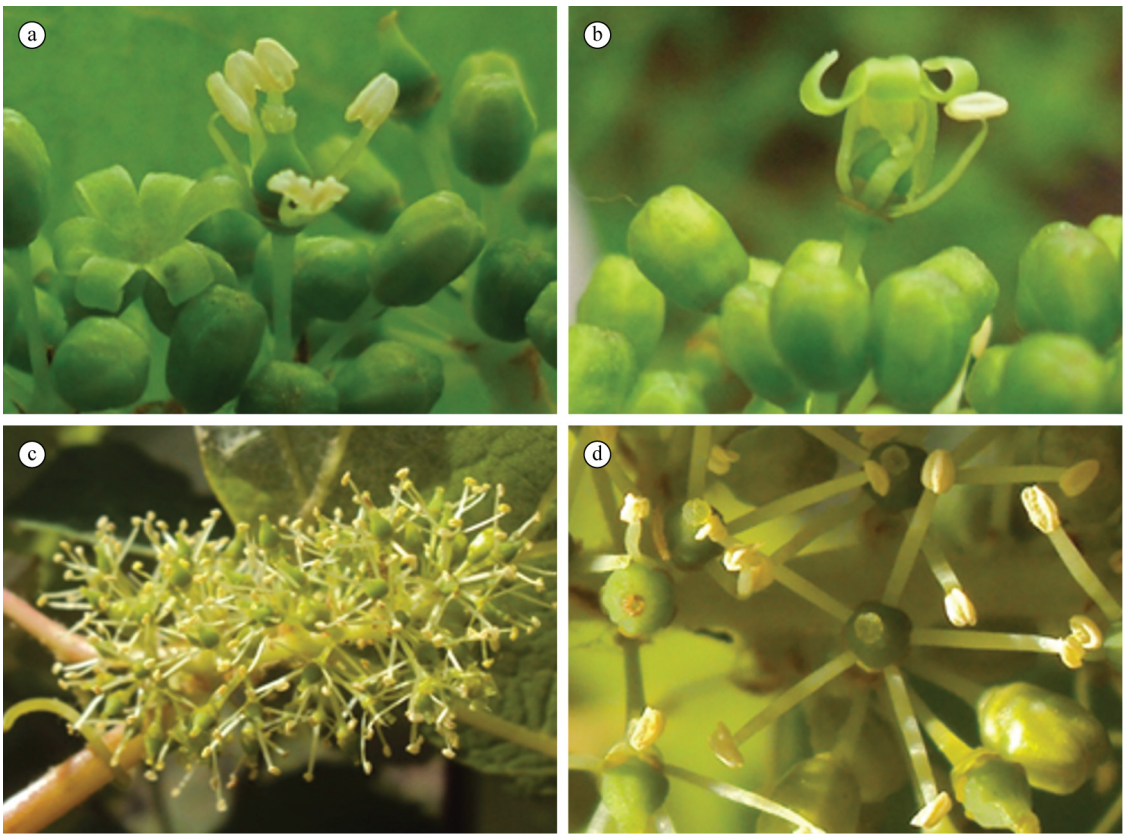

Figure 1. *Vitis labrusca*; (a) Initial anthesis; (b) Flower shedding the calyptras; (c) Opened flowers; (d) Inflorescence.

(see Figure 1b, c). In the pre-anthesis stage, the anthers contacted the stigma, and some of them had already released pollen, thus allowing self-pollination to occur. In the afternoon, the anthers became dry and the filaments were reflexed.

The small flowers (diameter of 8.0 ± 1.2 mm, range from 3.3 to 9.4) opened randomly within the inflorescence (Figure 1c, d). The calyx is pale green, inconspicuous and forms an arc at the base of the flower. The corolla is green and pentamerous; the petals form a cap (called calyptra) and break away from the base but remain fastened to each other at the top. When the petals dropped, the sexual elements became exposed (Figure 1b). The androecium has five erect stamens (4 ± 0.4 mm in length, range from 3.4 to 5.23), bearing yellow anthers with longitudinal dehiscence. Each anther produced 2.260 ± 286 pollen grains, and pollen viability was 36 ± 8%. The gynoecium (2 ± 0.3 mm in length, range from 1.55 to 2.7) has a large stigma (that was receptive in all periods tested, including the pre-anthesis stage), a short style and a superior, bilocular, ovary with two ovules per locule. Five yellow nectaries alternated with the stamens were observed at the base of the flowers (Figure 1c); it was impossible to collect nectar because of its very low volume.

Flowers produced a delightfully fragrant aroma, recalling the odour of mignonette early in the morning and waned during the day.

3.3. Sexual and breeding system

The flowers are hermaphroditic with erect stamens and a functional gynoecium. No temporal separation between male and female phase was observed.

Most of the 2.636 flowers that were used to test the breeding system withered after the manipulations (as shown in Table 2), consequently, low fruit production was observed for all treatments. Fruit set after natural pollination (37%) was higher than that after pollinations treatments, except for hand self-pollination (43%; $\chi^2 = 1.464$, $P > 0.05$). Only the clusters from the control treatment and spontaneous self-pollination produced well developed and commercially viable grapes. The others showed little fruit bunches of unequal sizes and had uncoordinated colour changes. All treatments set one or two seeds, and the ISI was 6.14 (species with values above 0.25 are considered self-compatible, *sensu* Bullock, 1985).

3.4. Growing degree days

Data on growing degree days are presented in Table 3. The base temperature (Tb) that was more adequate for the subperiods and for the cycle was 10°C, which had a lower standard deviation when compared to the Tb of 12°C. The growing degree days necessary for the complete cycle (pruning to harvest) were 1870.05 GD (P1) [summer], 1895.80 GD (P2) [spring], 1642.05 GD (P3) [winter] and 1972.17 GD (P4) [spring] (as shown in Table 3).

Table 2. Values for flowers used and fruits produced in the treatments of the reproductive system of *Vitis labrusca* cv. 'Isabel' in São Vicente Férrer, PE, Brazil.

Treatments	% fruits (flowers)
Hand self-pollination	43 (242)
Cross-pollination	7* (58)
Apomixy	16* (100)
Wind pollination	11* (918)
Spontaneous self-pollination	28* (418)
Natural pollination	37 (900)

* Significance difference from the control (χ^2, $P < 0.05$).

4. Discussion

4.1. Phenology

The observed cycles (between 116 and 130 days), even when the pruning time was considered, were shorter than those recorded in regions of mild climate, such as Serra Gaúcha, RS (164 days since the pruning, Camargo, 2006) and Maringá, PR (99 days after flowering, Roberto et al., 2004; Sato, 2007). Longer periods were also observed in SE Brazil, such as in Campina Verde, MG (141 days, Maia et al., 2002; Hernandes et al., 2010) and in the south of MG (177 days, Regina et al., 2003). Similarly, longer periods were recorded in areas of NE Brazil with a semiarid climate, such as in the Vale do São Francisco, BA (94 days after flowering, Lima et al., 2004).

The differences between our results and those from other studies may be a result of the mean temperature and carbohydrate metabolism. The mean temperatures at the study site are higher (24°C) than those found in the SE and S regions of Brazil, resulting in faster vegetative development and, consequently, shorter periods to complete the entire cycle. Additionally, the shorter cycle duration may be related to the fact that vegetative development and the duration of the grapevine cycle are influenced by the production and accumulation of carbohydrates resulting from photosynthesis, which is directly dependent on climatic conditions.

Generally, the grapevine photosynthetic rates are almost nil at temperatures ≤ 10°C; the peaks of carbohydrate production and accumulation occur at temperatures between 25 and 35°C, and the photosynthetic rate declines when the temperature is higher than 40°C (Mullins et al., 1992). However, in Brazil, it is possible to find plants whose carbohydrate production is relatively high at 40°C (Pommer et al., 2003). Thus, it is reasonable to suggest that the relationship between temperature and photosynthesis caused the shorter cycle and high vegetative growth of plants cultivated in areas that had higher mean temperatures, such as the study site and Vale do São Francisco, as opposed to observations made in areas that had lower temperatures (SE and S Brazil).

These results reinforce the idea that prior data on the phenological behaviour of grapevines facilitate better cultural practices and make it possible to predict probable

Table 3. Growing degree days (GD) calculated for a base temperature of 10 and 12°C and standard deviation (sd) in days. Data were collected in the following subperiods of four consecutive harvests of 'Isabel' grapes (*Vitis labrusca*), cultivated in São Vicent Férrer, PE.

Subperiods	Pruning 1				Pruning 2				Pruning 3				Pruning 4			
	10°C		12°C		10°C		12°C		10°C		12°C		10°C		12°C	
	GD	sd	GD	sd	GD	sd	GD	sd	GD	sd	GD	sd	GD	sd	GD	sd
P – B	240.75	0.024	212.75	0.028	198.35	0.058	146.55	0.069	223.45	0.059	195.45	0.068	160.05	0.051	144.15	0.062
B – F	330.50	0.033	290.50	0.033	362.70	0.051	215.80	0.051	291.80	0.044	266.95	0.044	317.05	0.028	236.05	0.028
F – HH	1298.8	0.060	1298.8	0.060	1334.75	0.037	1118.4	0.036	1126.8	0.051	939.65	0.051	1495.07	0.072	1329.45	0.069
P – H	1870.05	0.119	1636.05	0.122	1895.8	0.147	1480.75	0.158	1642.05	0.156	1402.05	0.165	1972.17	0.153	1709.65	0.160

P-B: pruning to budburst; B-F: budburst to flowering; F-H: flowering to harvest; P-H: pruning to harvest.

harvest periods (Abrahão and Nogueira, 1992). Due to the shorter cycle for this vine, it is possible to obtain two annual harvests if the appropriate production techniques are employed (Roberto et al., 2004).

4.2. Reproductive biology

The high ISI (6.14) indicates that the species does not depend on pollinators to produce fruit; the possibility of self-pollination during the pre-anthesis stage was also reported by Beach (1892 apud Mc Gregor, 1976). It is important to note that fruits resulting from self-pollination were adequate for commercialisation. Even with spontaneous self-pollination and non-dependence of pollinators, their presence may influence fruit features that are commercially important, such as smaller and sweeter pulp (Nunes et al., unpublished data).

The low flower:fruit ratio observed was a result of excessive flower loss, most likely because of physiological and/or pathological causes. In grapes, many flowers are not fecundated and abort (Marro, 1989). The physiological causes may be plant debility, inefficient pollination, low pollen viability and the absence of nutrients (Marro, 1989). Fecundation may occur until two days after pollination (Leão and Borges, 2009).

4.3. Growing degree days

The base temperature of 10°C was also recorded for other grape varieties in Brazil (Pedro-Junior et al., 1994; Nagata et al., 2000; Roberto et al., 2004; Santos et al., 2007; Neis et al., 2010; Ribeiro et al., 2010).

In NW and in N Paraná (S Brazil), we recorded 1238.2 and 1260.9 GD growing degree days, and cycles of 127 and 148 days, respectively, for the cultivar 'Isabel' (Roberto et al., 2004; Sato, 2007). These values are lower than those presented in this paper, even with a shorter cycle, indicating that the species needs more energy to complete the cycle in the study area. These results do not substantiate the generally accepted idea that the longer the cycle duration, the greater the need for energy accumulation to complete the cycle. However, it is important to note that the concept of GDD assumes that the relationship between crop development and temperature is linear. Therefore, other environmental conditions are not evaluated, and the GDD varies across regions (Pedro Júnior et al., 2006).

Our results related to phenology and the concept of growing degree days are important for planning cultures at the study site and in other regions that have similar climatic conditions and make it possible to predict the harvest. However, it is necessary to observe more production cycles to obtain a better characterisation of these data.

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