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Biological method of single-node cuttings for budburst in five fig cultivars subjected to artificial chilling

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ABSTRACT. Fig orchard expansion requires knowledge of the thermal requirements of genotypes available in different climatic regions. The budburst of fig cultivars was assessed by biological single-node cutting and exposure to artificial chilling. Cuttings from five cultivars were collected during two crop seasons, subsequently packaged in a horizontal position in a cold chamber (8 ± 0.5°C), and artificially chilled for 0, 40, 80, 120, and 160h. Cuttings were preserved under controlled conditions (23 ± 1°C, RH 85%, and 16h photoperiod). The variables evaluated were the budding velocity, average time and final rate of sprouting, vigorous shoot rate, and average time to leaf opening. The single-node cutting test allowed the assessment of dormancy, which is influenced by the accumulation of chilling in each crop season. The average time and final budding rate varied depending on the temperature requirements for budding. The cultivars were classified on an increasing scale according to their chilling requirements as follows: Pingo de Mel < Roxo de Valinhos < Brown Turkey < White Genova < Troyano. The traditional cultivar in Brazil is Roxo de Valinhos, but all of the cultivars studied had the potential to diversify the fig orchards in this region.

Keywords: *Ficus carica* L.; chilling requirements; dormancy; phenology.

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Introduction

Dormancy breaking of fruit trees in temperate climates directly influences the production of these species, as the chill hours in these areas are not sufficient to satisfy the needs of the trees (Abou-Zaid & Badawy, 2018).

Adaptation and growth of bud phenology have been studied in temperate climate species, which require a defined number of chilling hours to break dormancy, and consequently, to begin budding, flowering, and fruiting (El Yaacoubi et al., 2016). Souza, Silva, Leonel, and Escobedo (2009) studied the thermal requirements of cv. Roxo de Valinhos in Botucatu, São Paulo State, Brazil, and reported the minimum (8°C) and maximum (36°C) basal temperatures over five years of evaluation.

The dormancy period of fig trees is short because they require a mild winter (Oukabli & Mekaoui, 2012). Vossen and Silver (2000) reported that this species requires 0-150 chilling hours to overcome dormancy depending on cultivar. Chilling accumulation varies by genotype because each cultivar has distinct thermal requirements (Souza et al., 2021). Although the fig tree is grown in regions with mild winters, the development of buds can be unpredictable, which hinders production (Flaishman, Rover, & Stover, 2008).

The selection and evaluation of cultivars in terms of chilling requirements, yield, and fruit quality characteristics are important for diversification, especially in Brazil, where production is characterized by the use of a single fig cultivar, Roxo de Valinhos (Ferraz et al., 2021). The introduction of new genotypes is strategic for loading distribution and orchard crop management, as it results in a better seasonality of fruit for consumer markets. Knowledge of the thermal requirements for growth and development of temperate fruit trees is fundamental for achieving optimal yields (Mba, Guimaraes, & Ghosh, 2012; Tazzo, Fagherazzi, Lerin, Kretzschmar, & Rufato, 2015).

Mba et al. (2012) described the need to adapt crop improvement strategies to climate changes. Different cultivars of productive fig trees can be recommended in non-traditional regions, allowing for commercialization in the off-season.

The single-node cutting biological test assesses the phenological stages and temperature ranges from bud dormancy to budding of temperate fruit trees (Champagnat, 1989). This method uses stem cuttings with a single bud, which eliminates the effects of correlative inhibition and allows the bud to display its full potential (Champagnat, 1989). The average sprouting time is an indicator of dormancy depth, allowing for the practical analysis of the variables associated with sprouting and their relationship to physiological processes (Santos et al., 2020).

This test is widely used in tropical and subtropical regions worldwide to analyze the dormancy of temperate fruits, such as the peach (*Prunus persica*) (Bonhomme, Rageau, Richard, Erez, & Gendraud, 1999), pear (*Pyrus* sp.) (Bianchi, Arruda, Casagrande, & Herter, 2000), and apple (*Malus domestica*) (Carvalho & Silva, 2010). However, there are no reports on fig trees; therefore, research exploring the thermal requirements of fig cultivars is required for agroclimatic zoning of the crop.

This study aimed to evaluate the biological method of single-node cutting for budburst in fig cultivars subjected to artificial chilling.

Material and methods

Experimental area characterization

A replicated trial was conducted during two crop seasons (2016/2017 and 2017/2018) on an experimental orchard of the São Paulo State University School of Agriculture, Botucatu, São Paulo State, Brazil, located at 22°51′55″ S, 48°26′22″ W, and 810 m a.s.l. The climate of the area is classified as Cfa, or warm temperate (mesothermic), according to the Köppen-Geiger system, with an average air temperature of 19.3ºC and annual precipitation of 1,344 mm (Cunha & Martins, 2009).

The number of hours with temperatures below 8ºC (Souza et al., 2009) was recorded from January to December in 2017 and 2018. From winter pruning (July) to fig cutting collection, 19.5 chill hours (CH) ≤ 8ºC were accumulated in 2017 and 2.7 CH ≤ 8ºC in 2018 (Citadin, Raseira, Herter, & Silveira, 2002; adapted). The air temperature was obtained using a Vaisala set (thermo-hygrometer HMP45C + multi-plate shield RM Young model 41002) installed at a height of 2 m and connected to a Micrologger CR23X with average data output every ten minutes.

The fig trees were four years old in 2017 and were planted at a 3 m spacing between rows and 2 m between trees, with management according to the recommendations for commercial fig orchards.

The experiment was undertaken using woody fig cuttings of the cultivars Roxo de Valinhos, Brown Turkey, Pingo de Mel, Troyano, and White Genova obtained from the branches remaining after winter pruning. 'Roxo de Valinhos' is the standard for Brazilian growers with fruits intended for fresh consumption (*in natura*) or the food industry (green figs); 'Brown Turkey' is commonly produced in the United States (California); 'Pingo de Mel', is also called Kadota or Dottado (in Italy it is referred to as Adriatic or Verdone), Mission (in California/US) and Fraga or Sepe (in Spain); Troyano is an Italian cultivar that is commonly grown in the United States; and 'White Genova' has a Italian background and was selected from the Campinas Agronomic Institute (IAC) (Ferraz et al., 2021).

Experimental design

The experimental design was completely randomized with four replicates, in a split-plot arrangement (5 cultivars × 5 artificial chilling times). The replicates consisted of ten cuttings per experimental plot. The data from the two crop cycles were evaluated separately.

Sampling, preparation, and storage of cuttings

Two hundred and fifty cuttings of each cultivar were standardized at a length of 20 cm and an average diameter of 7 mm. Each cut contained approximately five buds. The cuts were made at an angle at each end, one centimeter above the first bud and one centimeter below the last bud. Cuttings of each cultivar were separated into five lots wrapped in moistened paper. The lots were packed in a horizontal position and subjected to artificial chilling in a cold chamber (8 ± 0.5°C) for 0, 40, 80, 120, and 160 CH.

Biological test of single-node cuttings

After exposure to artificial chilling, the cuttings were standardized and cut to an average size of 7 cm, each containing one dormant bud, using the biological method of single-node cutting (Carvalho & Silva, 2010).

The cuttings were stored in white polyethylene trays with phenolic foam humidified with deionized water and preserved under controlled conditions of 23 ± 1°C, relative humidity of 85%, and photoperiod of 16h. Parafilm® tape was placed at the extremities of the cuttings to prevent dehydration.

Phenological characterization

Daily evaluations over 40 days identified the stages of green tip (GT), elongated bud (EB), and opened bud (OB). The GT stage was characterized by changes in bud color, whereby the upper end of the bud turned greenish-yellow (Figure 1[2]). The EB stage was represented by the elongation of unopened leaves (Figure 1[3]), and the GT stage by the expansion of open leaves (Figure 1[4]) (Carvalho, Biasi, Zanette, Santos, & Pereira, 2010). Photographs were taken 24 days after placing the cuttings under controlled conditions in the treatment where there was no submission to artificial chilling (control).

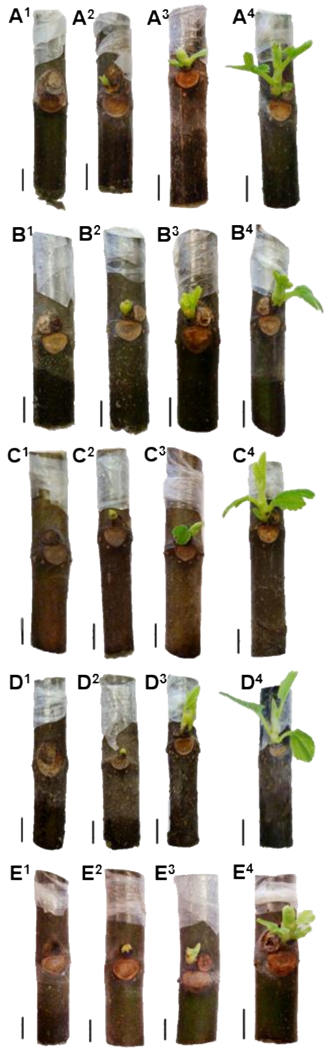

Figure 1. Cuttings of fig tree (*Ficus carica* L.) cultivars: Roxo de Valinhos (A), Brown Turkey (B), Pingo de Mel (C), Troyano (D), and White Genova (E). Botucatu, São Paulo State, Brazil. [1]Dormant bud, [2]Green tip, [3]Elongated bud, and [4]Opened bud. Bar = 1 cm.

The following variables were evaluated according to phenological stage (Carvalho et al., 2010):

i) Velocity of budding (VB) (buds day^{-1}): budding time [$\text{VB} = \sum (\text{n}i\ \text{t}i^{-1})$, where: ni = number of buds that reached the GT stage at the time "i," and ti = time after test onset ($i = 1 \rightarrow 40$)];

ii) Average time of sprouting (ATS) (days $^{-1}$): average number of days between the onset of the experiment and the GT stage;

iii) Final sprouting rate (FSR) (%): percentage of cuttings with buds in the GT stage;

iv) Vigorous shoot rate (RVS) (%): percentage of cuttings with buds at the GT stage that progressed to OB [RVS = (% of cuttings at OB) × (100 ÷ FSR)]; and

v) Average time to leaf opening (ATOL) (days^{-1}): number of days between the GT and OB stages.

Statistical analysis

Before performing the analysis of variance, the data were subjected to a normality test. When significant, the Scott-Knott test was used for comparison of means, while data referring to artificial chilling hours were analyzed through regression at 0.1, 1.0, and 5.0% probability. Logarithmic transformation (x) was applied to the day and percentage data, and they were expressed as $\sqrt{(x + 0.5)}$, where x is the value of each variable. All analyses used the system for analysis of variance software (Sisvar, version 5.6) (Ferreira, 2019).

Results

There was a significant interaction among the cultivars and chilling hours for ATS, FSR, and RVS in 2017. In 2018, a significant interaction was observed for ATS. The remaining variables presented isolated effects (Table 1).

Table 1. F-value, degrees of freedom (DF), coefficient of variation (CV), and means of the velocity of budding (VB), average time of sprouting (ATS), final sprouting rate (FSR), vigorous shoot rate (RVS), and average time to leaf opening (ATOL) in the crop cycles of 2017 and 2018. Botucatu, São Paulo State, Brazil.

Variation source	DF	VB (buds day^{-1})	ATS[1] (days^{-1})	FSR[1] (%)	RVS[1] (%)	ATOL[1] (days^{-1})
2017						
Cultivar (C)	4	5.65**	1.37^{NS}	6.64**	0.79^{NS}	1.11^{NS}
Hours (H)	4	30.79**	5.93**	10.94**	8.72**	3.36**
C*H	16	1.20^{NS}	1.77**	1.93**	2.31**	1.66^{NS}
CV. 1		42.29	13.76	23.25	36.00	32.20
CV. 2		39.83	15.62	20.28	37.95	31.52
Mean		0.31	4.02	6.97	6.68	2.63
2018						
Variation source	DF	VB (buds day^{-1})	ATS[1](days^{-1})	FSR[1] (%)	RVS[1] (%)	ATOL[1] (days^{-1})
Cultivar (C)	4	8.23**	2.19^{NS}	5.25**	3.53**	8.07**
Hours (H)	4	8.89**	40.22**	4.99**	3.93**	3.65**
C*H	16	1.62^{NS}	1.93**	0.82^{NS}	0.69^{NS}	1.28^{NS}
CV. 1		36.25	8.68	17.77	27.63	15.66
CV. 2		35.98	6.99	17.84	30.37	23.48
Mean		0.26	4.07	8.01	7.97	2.78

[1]Data transformed to square root (x + 0.5). **significant at 1% probability; ^{NS}Not significant. CV.: coefficient of variation. VB = velocity of budding; ATS = average time of sprouting; FSR = final sprouting rate; RVS = vigorous shoot rate; ATOL = average time to leaf opening.

Higher averages occurred in both cycles in relation to the increase in artificial chilling hours for ATS. The cultivars Roxo de Valinhos, Brown Turkey, Troyano, and White Genova displayed linear increases in ATS in 2017, while Pingo de Mel displayed a reduction in ATS until 49.3 CH (Figure 2a).

In the 2018 season, cv. Roxo de Valinhos presented a quadratic effect for ATS, increasing up to 123.8 CH and subsequently decreasing. The other cultivars showed linear mean increases in ATS (Figure 2b). Based on these results, during the second crop cycle cv. Roxo de Valinhos was the only cultivar to reach this stage.

ATS showed significant differences during the 2017 crop season when the cultivars were subjected to 120 CH. The average ATS of 'Pingo de Mel' was lower than those of the other cultivars (Figure 2a). During the 2018 cycle, the only difference among cultivars occurred when they were exposed to 40 CH. 'Roxo de Valinhos' showed the longest ATS (Figure 2b).

The hours of artificial chilling above the accumulation of chilling in the field (19.5 CH) generally promoted a reduction in the average FSR in 2017, negatively affecting sprouting (Figure 2c).

For the cv. Brown Turkey, the chilling of the 2017 season (19.5 CH) that was added to the hours of artificial chilling increased the dormancy of the buds of this cultivar. The ATS increased linearly (Figure 2a), whereas the FSR decreased (Figure 2c).

The cultivars Roxo de Valinhos and Troyano presented quadratic increases in FSR with up to 42 and 73 CH, respectively, followed by a decrease in rate (Figure 2c). The exposure of cuttings to artificial cold may have reduced bud vitality.

The cultivars Roxo de Valinhos and Troyano showed increases in linear ATS and quadratic FSR with up to 42 and 73 CH, respectively, in 2017, which highlights that Roxo de Valinhos requires less chill accumulation than Troyano during dormancy, and consequently overcomes this stage more quickly (Figure 2a and c).

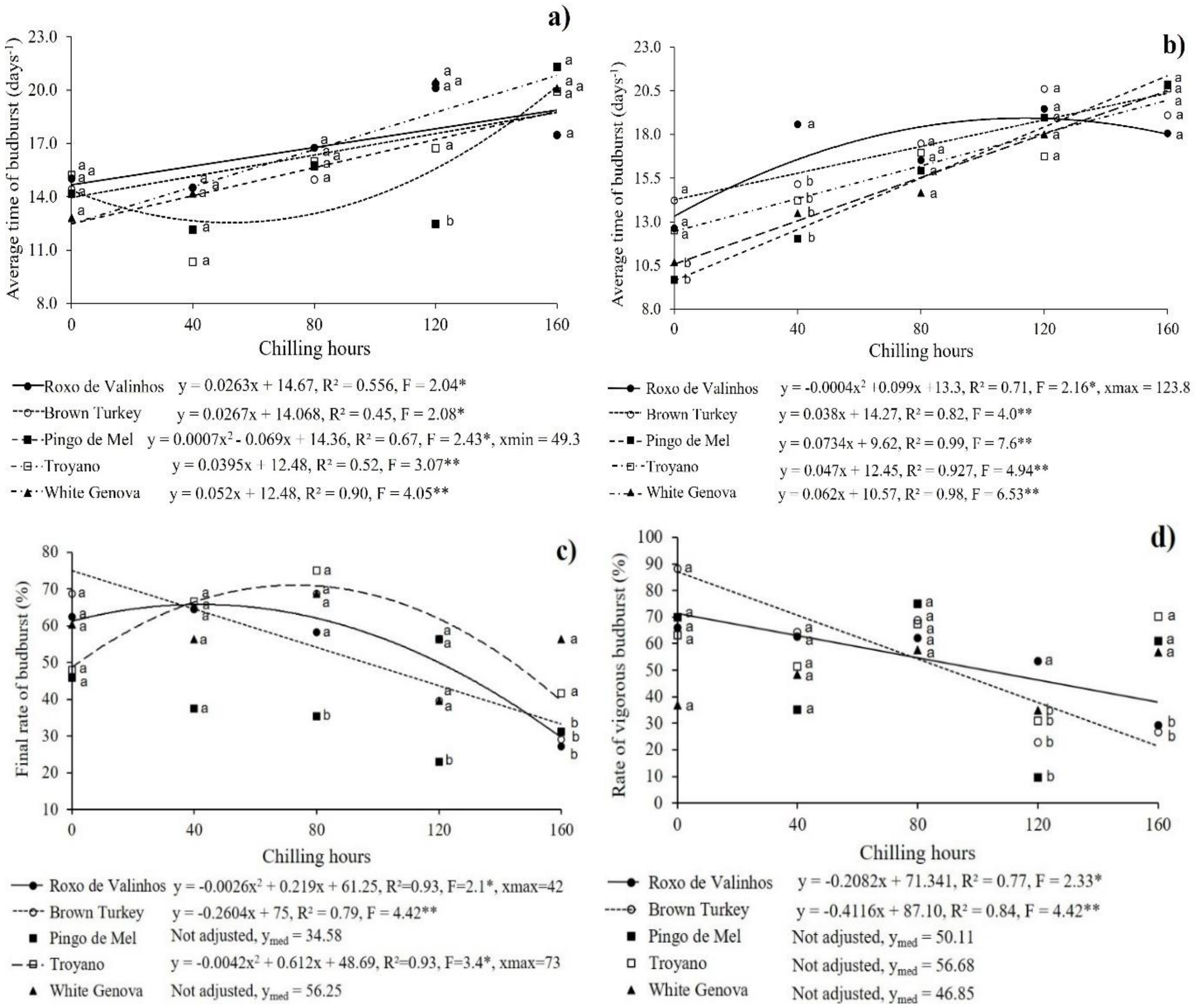

Figure 2. Average time of sprouting (ATS) in 2017 (a) and 2018 (b), final sprouting rate (FSR) in 2017 (c), and vigorous shoot rate (RVS) in 2017 (d). The same letter among the fig cultivars indicates no difference by the Scott-Knott test at 1% probability. Botucatu, São Paulo State, Brazil.

The cultivars Roxo de Valinhos and Brown Turkey showed linear reductions in the RVS, as well as an increase in the accumulation of artificial chilling (Figure 2d). The cultivars differed when the cuttings were exposed to 120 and 160 artificial CH (Figure 2d). 'Brown Turkey' had the lowest RVS at both CH durations. This observation reinforces that the accumulation of natural cold by the cultivar in 2017 and supplementary chilling intensified its bud dormancy.

The analysis of the isolated effect of each factor confirmed that the VB was affected by both cultivar and chilling time in both crop cycles (Table 1). In 2017, the chill in the field was 19.5 CH, and the cultivar Pingo de Mel had the lowest VB (0.22 buds day^{-1}). In 2018, with the accumulation of 2.7 CH, cv. Roxo de Valinhos had the lowest VB (0.16 buds day^{-1}) (Table 2). These results highlight the fact that these cultivars showed the greatest dormancy under their respective conditions.

Table 2. Mean velocity of budding (VB), vigorous shoot rate (RVS), final sprouting rate (FSR), and average time to leaf opening (ATOL) in fig cultivars. Botucatu, São Paulo State, Brazil.

Fig cultivar	VB[1] (buds day^{-1})	VB[2] (buds day^{-1})	RVS[2] (%)	FSR[2] (%)	ATOL[2] (days^{-1})
Roxo de Valinhos	0.30 a**	0.16 c**	64.92 b*	48.33 b*	7.75 b**
Brown Turkey	0.31 a	0.29 a	79.50 a	75.83 a	9.23 a
Pingo de Mel	0.22 b	0.29 a	54.75 b	67.50 a	6.75 b
Troyano	0.37 a	0.23 b	65.67 b	68.33 a	6.20 b
White Genova	0.33 a	0.31 a	82.93 a	71.65 a	8.60 a
Standard error	0.0235	0.0208	6.3511	5.0640	0.5141

[1]Data corresponding to the 2017 year. [2]Data corresponding to the 2018 year. The same letter indicates no differences by the Scott-Knott test at **1% and *5% probability.

The relationship between VB and chilling hours showed that VB was linearly reduced in both crop cycles. Longer chilling times related to slower sprouting (Figure 3a and b). The decrease in VB observed in fig buds may be associated with the lower chilling requirements of the genotypes.

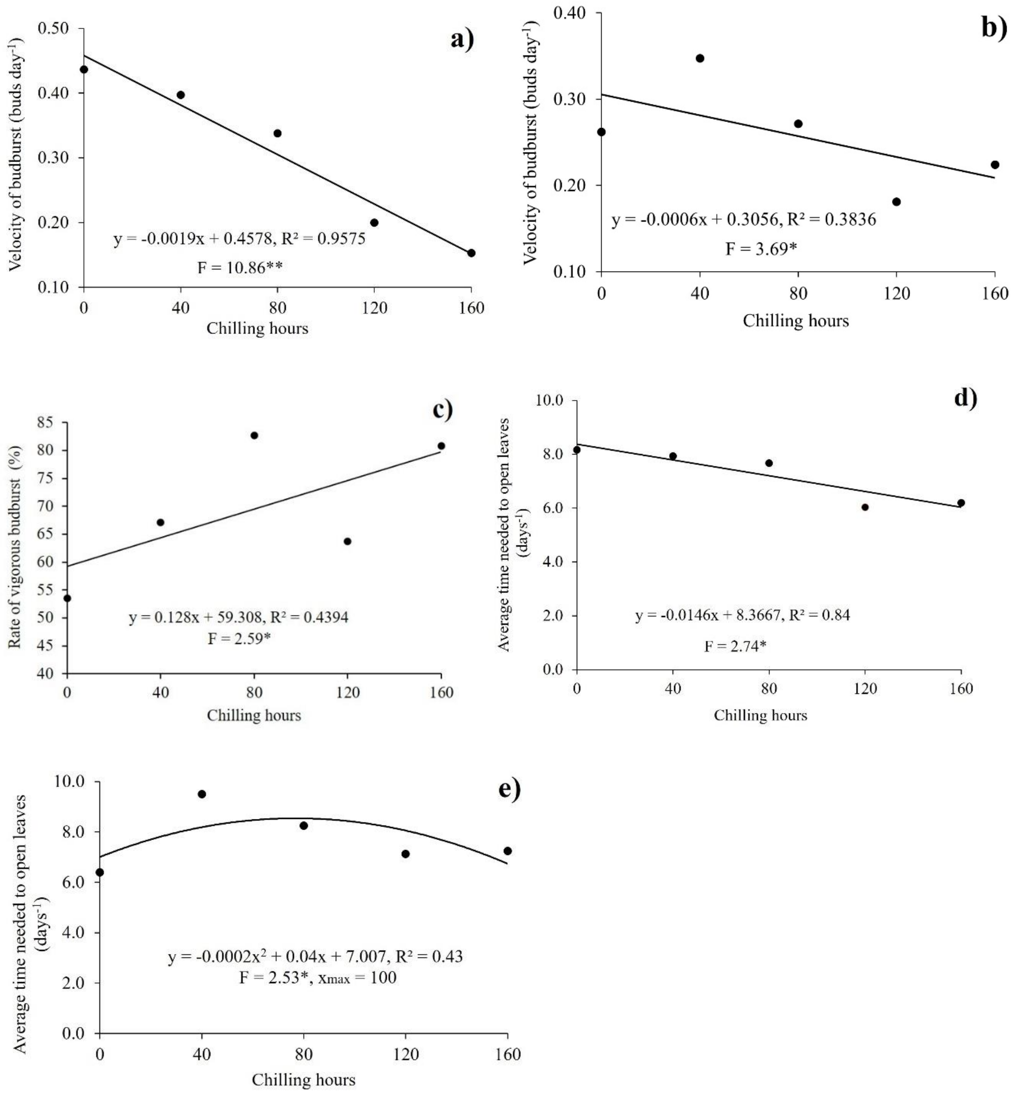

Figure 3. Velocity of budding (VB) in 2017 (a) and 2018 (b), vigorous shoot rate (RVS) in 2018 (c), average time to leaf opening (ATOL) in 2017 (d), and 2018 (e). Botucatu, São Paulo State, Brazil.

Only the isolated cultivars and CHs affected the FSR and RVS of the fig cuttings in 2018 (Table 1). The ATOL highlighted the isolated effect of CH in both cycles and cultivar in 2018 (Table 1). 'Brown Turkey' and 'White Genova' had higher mean values of ATOL and RVS in the 2018 season (Table 2). 'Roxo de Valinhos' showed the lowest FSR, and the other cultivars showed no difference for this variable (Table 2).

The RVS means were adjusted for linear regression in the 2018 season (Figure 3c). As for ATOL, in the 2017 season, the increase in chilling time promoted a linear decrease in the mean (Figure 3d), while in the 2018 cycle, there was a quadratic increase in ATOL up to 100 CH (Figure 3e).

Discussion

The variation in the ATS between cultivars depends on genetics and temperature (Ímrak, Küden, Küden, Sarier, & Çimen, 2016). Along with the effective temperatures to accumulate chilling, higher temperatures are also required, as they intensify the metabolic activity of the buds and stimulate sprouting (Abou-Zaid & Badawy, 2018). Leonel and Tecchio (2010) observed increased initial sprouting and vegetative growth in fig trees that were pruned during longer days with higher temperatures, due to the greater amount of photosynthesis, which resulted in increased fruit production.

The modification of the ATS of a single cultivar over the two years depended on the quantity, quality, and duration of accumulated chilling hours, which varied for each cycle. This observation corroborates the findings of Santos et al. (2020), who reported that changes in the accumulation of chilling hours, mainly due to insufficient chilling, can cause uneven and less intense sprouting. According to Carvalho and Biasi (2012), a reduction in the average sprouting time affects dormancy breaking.

Budbreak variation can also depend on the thermal amplitude in the present study, as the region has a temperate warm climate, without well-defined climatic seasons, and is sometimes subject to sudden and adverse weather changes that compromise crop loading. The interaction between the cultivar and its environment is important for temperate fruit yields. Knowledge of the adaptability and stability of the trees in a specific region or different years increases productivity (Citadin et al., 2014).

Anzanello, Fialho, and Santos (2018) reported that after dormancy induction by low temperature accumulation and minimum achievement of budbreak, FSR increased as the chill needs were met, and dormancy breaking occurred in grape vines (*Vitis vinifera* L.). According to Carvalho and Biasi (2012), the dormancy intensity of the bud is directly proportional to the ATS and inversely proportional to the FSR.

The FSR decrease began at different levels for ‘Roxo de Valinhos’ and 'Troyano,' which may have been associated with the greater difficulty in advancing to the green tip stage when exposed to prolonged low temperatures. It is likely that the reduction in FSR observed after a specific accumulation of chilling was related to the lack of sufficiently high temperatures for sprouting. Figs respond to higher temperatures at the end of winter with an increase in respiratory activity due to the reduction of inhibitors and increase in growth promoters (Souza et al., 2021).

Ferraz et al. (2020) assessed the phenology of fig cultivars and concluded that Roxo de Valinhos reached the budding stage more quickly than Troyano, which is classified as having a longer harvest cycle.

Dormancy is directly proportional to the ATS and inversely proportional to the RVS (Carvalho & Biasi, 2012). This study confirms that the sprouting of 'Roxo de Valinhos' buds was negatively affected by the hours of artificial chilling, as a reduction in the sprouting rate occurred as the CHs increased. Adaptation of the ability to sprout and grow is one of the main advantages of a specific cultivar in areas with low chilling accumulation.

According to Carvalho and Biasi (2012), dormancy intensity is inversely proportional to the VB, and along with ATS, this variable most influenced dormancy intensity (Carvalho & Silva, 2010).

The VB values of fig cultivars in both crop cycles were reduced as the cultivars were exposed to less chilling accumulation in the field (2018 season), particularly for the Roxo de Valinhos and Troyano cultivars. Chilling accumulation was necessary to break dormancy in these cultivars. VB was higher in the 2018 season for cv. Pingo de Mel indicating that this cultivar does not require a prolonged cold temperature, as it presented a higher VB with less chilling. Cold accumulation had an inhibitory effect on sprouting performance.

In the cultivars Roxo de Valinhos and Brown Turkey, VB was reduced because of the lower accumulation of cold in the field. The use of sprouting inducers can be promising for obtaining more vigorous and uniform buds, especially in crop cycles with low chilling conditions. Uneven sprouting and flowering occur because of insufficient accumulation of cold hours. This explains the lower VB in the 2018 season and reinforces the need to use inducers with these cultivars.

This observation suggests that the biological test of single-node cuttings was efficient, especially for 'Brown Turkey' and 'White Genova', as the shoots advanced to the green tip stage. In addition, the FSR results were not obtained only from the cuttings performed, indicating that they had previous reserves for the sprouting process.

In addition to having the lowest FSR in 2018, cv. Roxo de Valinhos presented the lowest VB in the same crop cycle, thereby demonstrating the lowest sprouting of the cultivars. VB fluctuations may be due to the direct effect of low temperature on sprouting. If the necessary chilling is not provided, sprouting slows, causing a reduction in the FSR (Alves, Biasi, & Mio, 2016).

El Yaacoubi et al. (2016) reported that ATOL reduction is associated with dormancy breaking. The results obtained in 2017 showed that all artificial CHs were efficient in promoting sprouting because the ATOLs were lower than those of cuttings that were not subjected to artificial chilling. Dormancy breaking appeared only when the cuttings were subjected to more than 100 CH in 2018. According to Carvalho et al. (2010), ATOL is an efficient variable for assessing bud developmental capacity.

The present study showed that fig cultivars responded differently to the application of artificial chilling hours. Gariglio, Rossia, Mendow, Reig, and Augusti (2006) stated that the biological test of single-node cuttings assessed the chilling requirements of each cultivar. That report confirms the relevance of the present study, as undetermined thermal needs causes inadequate selection of cultivars, which can reduce tree performance in tropical or subtropical regions (Ruiz, Campoy, & Egea, 2007).

Conclusion

The biological test of single-node cuttings allowed for the evaluation of the withdrawal from vegetative rest, which was influenced by the accumulation of chilling that occurred in each crop season. On an increasing scale of chilling hours required for sprouting, the cultivars were classified as follows: Pingo de Mel < Roxo de Valinhos < Brown Turkey < White Genova < Troyano.

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