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Bud burst induction in BRS-Carmem vine minicuttings under controlled environment

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Abstract

The search for new products and alternative techniques to artificially overcome dormancy of temperate climate plants has increased. Thus, the objective of this work was to evaluate the efficiency of the application of vegetable oil, mineral oil, garlic extract, and hydrogenated cyanamide on the bud burst of vines of the BRS-Carmem cultivar under controlled environment (biochemical oxygen demand). A randomized experimental design in a 2×6 factorial arrangement was used, with four replications and nine minicuttings per plot. The first factor consisted of exposure and not exposure of minicuttings to 400 hours of cold temperatures <7.2 °C, and the second factor consisted of 6 treatments: control, mineral oil (MO), vegetable oil (VO), MO+VO, hydrogenated cyanamide, and garlic extract. The percentage of swollen buds and sprouted buds were evaluated at 20 days after the application and the average times for bud swelling and bud sprouting and the bud swelling and bud sprouting speed indexes were calculated. Minicuttings that were not exposed to cold showed the best results for bud swelling and sprouting when using MO, followed by hydrogenated cyanamide. Minicuttings that were exposed to cold showed the best results of bud swelling and sprouting when using the combination of oils (MO+VO). Therefore, the best results for the induction of bud burst of BRS-Carmem vines are achieved when the minicuttings are not exposed to cold and the application of the combination oils (MO+VO) is used.

Keywords: alternative products, dormancy, hydrogenated cyanamide

Introduction

Vine (*Vitis* sp.) is a fruit plant of temperate climate whose fruits (grapes) are known by their antioxidant properties; the peel of fine grapes present large concentration of anthocyanins, which have positive affect on the prevention of several human diseases (Xia et al., 2010; Zhou & Raffoul, 2012; Fahmi et al., 2013)

The world grape production in 2018 was 77.8 million Mg, in an area of 7.4 million ha, (OIV, 2019). Brazil is the twenty-second world grape producing country, with 1.592.242 Mg in an area of 73.742 ha; table grape production stands out in the north region of the state of Paraná, which presents an area of 4.170 hectares and an annual production of 54.000 Mg (Beling, 2019).

BRS-Carmem is a hybrid vine cultivar originated from the crossing between the Muscat Belly O and BRS-Rúbea cultivars that was developed to be adapted to hot climate regions (Camargo et al, 2011). Fruits of BRS-Carmem vines are intended mainly to produce juices, since they present high sugar content and intense purple color, essential attributes to a quality juice, which can be consumed pure or used to compose blends with juices of fruits of other cultivars (Camargo et al., 2008).

Vines grown in tropical and subtropical regions need artificial induction of bud burst (Hawerroth et al., 2013). The plant dormancy is one of the main factors that affect the plant production; insufficient cold periods result in plants with irregular germination and development. Therefore, the use of products to overcome the dormancy is essential; hydrogenated cyanamide is the main active ingredient used for this function (Carvalho et al., 2016a). However, the use of this product is becoming limited in the world because it is classified as highly toxic (Settimi et al., 2005); thus, searching for alternative products that provide similar effect is essential (Carvalho et al., 2016a).

Studies have shown the efficiency of alternative

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products to overcome dormancy of fruit trees, such as garlic extract for vines, and vegetable oils combined with mineral oil for apple trees (Botelho et al., 2002; Marchi et al., 2017). Mineral oils, such as dinitro-ortho-cresol, thiourea, potassium nitrate, calcium nitrate, gibberellic acid, and kinetin, are efficient to overcome dormancy of many fruit tree species. These compounds can partially substitute the need for cold treatment and stimulate an early and uniform bud burst (Botelho et al., 2002; Botelho et al., 2009; Marchi et al., 2017).

Thus, the objective of the present work was to evaluate the efficiency of the application of vegetable oil, mineral oil, garlic extract, and hydrogenated cyanamide on the bud burst of BRS-Carmem vines under controlled environment (biochemical oxygen demand).

Material and methods

The experiment was conducted from April 4 to 24, 2017, in the Laboratory of Plant Physiology and Nutrition of the Federal University of Paraná (UFPR), Palotina, PR, Brazil. The experiment was conducted using a completely randomized design, in a 2×6 factorial arrangement, with 12 treatments and four replications; each sample plot consisted of nine minicuttings, resulting in 432 experimental units. The first factor consisted of exposure and not exposure of minicuttings to 400 hours of cold temperatures <7.2 °C (Peruzzo et al., 2014), and the second factor consisted of 6 treatments: control, mineral oil (MO), vegetable oil (VO), MO+VO, hydrogenated cyanamide (H_2CN_2), and garlic extract (GE). A third factor (time) was considered for some variables analyzed up to 20 days after the implementation of the experiment.

Plants of the BRS-Carmem vine cultivar grafted on rootstocks of the IAC-766-Campinas cultivar were evaluated at a teaching orchard of the UFPR/Palotina (24°18'S, 53°55'W, and 310 m of altitude). The climate of the region is Cfa, subtropical, according to the classification of Köppen, with mean air temperature in the coldest month lower than 18 °C (mesothermal), mean temperature in the hottest month above 22 °C, hot summer, little frequent frosts in the coldest period, between late May and early September, and total annual rainfall depths between 1600 and 2000 mm (Caviglione et al., 2001).

After the pruning for production of the BRS-Carmem vines, 44 cuttings with 10 buds each were collected from the mid part of the branches. Half of the cuttings were maintained in a controlled environment (biochemical oxygen demand) under temperatures below 7.2 °C for 400 hours (Peruzzo et al., 2014). The remaining cuttings were cut in minicuttings with one bud each and approximately 5 cm length; they were maintained in a container with water to avoid dehydration until the application of the treatments.

The minicuttings were placed in germination boxes with phenolic foam and maintained in a controlled environment at temperature of 25 °C, without photoperiod. The foams were moistened daily with potable water during the storage period.

The garlic (*Allium sativum* L.) extract was prepared with a concentration of 20% of garlic (Kubota et al., 2000); 200 g of garlic was peeled, the cloves were crushed in a blender, and 1 L of potable water was added. The ground garlic was pressed and filtered simultaneously in a cotton cloth and the extract was stored for 24 hours in a container. The other treatments consisted of applications of commercial products: mineral oil (Nimbus®), vegetable oil (Neem Oil®), and hydrogenated cyanamide (Dormex®). The concentrations used were 4% of the commercial product, i.e., 40 mL per liter of water.

The treatments were applied by immersing the minicuttings in the solutions of the respective treatments for 30 seconds, place them in the phenolic foam, and subjected to 400 hours of cold. Then, the same procedure was done for the other cuttings.

The percentage of swollen buds and sprouted buds in the cuttings after 20 days of implementation of the experiment was evaluated. Based on the results of the variables over time, the mean times for bud swelling (MTBS) and sprouting (MTS) and the bud swelling (BSSI) and sprouting (SSI) speed indexes were calculated based on adapted formulas of mean time for emergence (Labouriau & Giorgio, 1983) and emergence speed index (Maguire, 1962):

$$\text{SSI or BSSI} = \sum \left(\frac{n_i}{t_i}\right)$$

$$\text{MTS or MTBS} = \frac{\sum n_i t_i}{\sum n_i}$$

where n_i is the number swollen buds at time *i*; t_i is the time after the implementation of experiment; SSI and BSSI are dimensionless; and MTS and MTBS are given in days.

The data of bud sprouting and swelling over time were analyzed by a mixed linear model with three factors, considering the cold, the treatments, and the time as fixed effects, and the experimental unit as a random effect. The date that presented the first occurrence of the variable was considered in the model as the initial time. When the results of the interaction or isolate effect over time were significant, they were fitted to logistic models of three or four parameters to obtain a sigmoidal curve, using the equation:

$$f(x) = c \; \frac{d - c}{1+e^{b(\log(x)-\log(e))}}$$

where *f(x)* is the response of the parameter; *x* is the time; *e* is the time for the response at the mid-point between *d* and *c*; *d* is the higher limit; *c* is the lower limit; and *b* is the relative slope.

The data of the mean times for sprouting and bud swelling and sprouting and bud swelling speed indexes were subjected to analysis of variance ($p<0.05$) and the means were compared by the Tukey's test ($p<0.05$). The data were tested for normality of errors and homogeneity of variances to validate the model. All analyses were carried out using the *R* program (*R* Core Team, 2020).

Results and Discussion

The effect of the interaction between the treatments on the mean time for bud swelling and bud swelling speed index was not significant, but the isolate effect of the factors (treatment and exposure to cold) was significant for these variables (Figure 1). The effect of the interaction between the factors (treatment and exposure to cold) was significant for bud sprouting speed index (Figure 2), but the mean time for bud sprouting presented no significant differences.

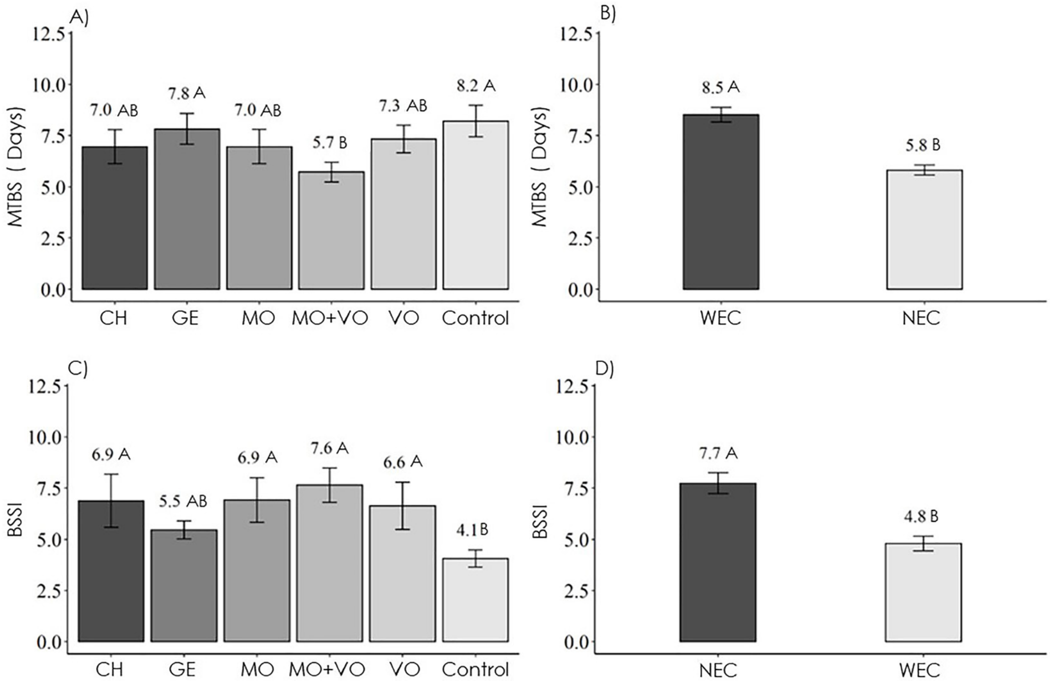

Figure 1. Mean time for bud swelling (MTBS) (A) and bud swelling speed index (BSSI) (C) in minicuttings of vines of the BRS-Carmem cultivar subjected to 6 treatments (hydrogenated cyanamide - HC, garlic extract - GE, mineral oil - MO, vegetable oil - VO, MO+VO, and Control), with (WEC) and without (NEC) exposure to 400 hours of cold (B and D). *Means followed by the same letter in the columns are not different by the Tukey's test ($p>0.05$). Bars indicate the standard error of the mean.

The results found for MTBS (Figure 1A) showed that the treatment with MO+VO resulted in bud swelling within approximately 6 days, with significant difference from the other treatments. The minicuttings in the control an those treated with hydrogenated cyanamide presented later bud swelling, 8.2 and 7.0 days, on average, respectively. Minicuttings that were not subjected to cold (Figure 1B - NEC) presented a shorter time for bud swelling (5.8 days) than those subjected to cold (8.5 days) (Figure 1B - WEC).

Vine plants grown without a sufficient cold time may present serious physiological disturbances, such as deficient and uneven sprouting, and low yield (Hawerroth et al., 2013), which have probably occurred in the present work.

The BSSI found for the control treatment presented a slower sprouting, followed by the treatment with garlic extract, and the other treatments (Figure 1C). Minicuttings that were not exposed to cold (Figure D - NEC) showed high bud swelling speed index than those that were not subjected to cold (Figure 1D - WEC). The SSI of the treatments subjected to cold were, in general, slower than those that were not subjected to cold (Figure 2), and no significant difference in speed index was found between the treatments with cold.

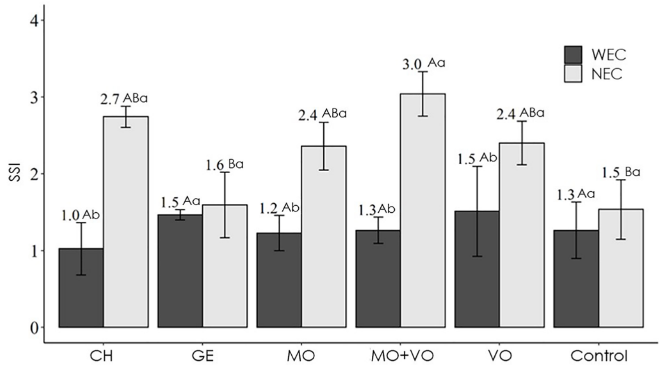

Figure 2. Sprouting speed index (SSI) in minicuttings of vines of the BRS-Carmem cultivar subjected to 6 treatments (hydrogenated cyanamide - HC, garlic extract - GE, mineral oil - MO, vegetable oil - VO, MO+VO, and Control), with (WEC) and without (NEC) exposure to 400 hours of cold. *Means followed by the same uppercase letter in the columns of the same color, or lowercase letters in the columns of different color, are not different by the Tukey's test (p>0.05). Bars indicate the standard error of the mean.

Among the treatments without cold, the control and the garlic extract presented less satisfactory results for bud sprouting and bud swelling, but with no significant differences from the other treatments, except MO+VO. The treatments that presented the highest bud swelling speed indexes, i.e., fast emergence of buds, resulted in higher stability and sprouting percentages. MO+VO was the best treatment for the mean times and speed indexes.

The interaction between the factors (exposure to cold, treatments, and time) was significant for the bud sprouting and bud swelling (Table 1). Thus, the variables were tested and subjected to logistic regression of three or four parameters (sigmoidal model), considering the exposure to cold and the treatments as qualitative variables, and the time as a quantitative variable. The values of the coefficients of the model are presented in Table 2.

Table 1. P-value of the F test of the mixed linear model for the parameters bud swelling and bud sprouting of vines of the BRS-Carmem cultivar subjected to the different treatments, with and without exposure to cold, over time after application.

Source of variation	Bud swelling (%)	Bud sprouting (%)
Treatments (A)	0.0078	0.2648
Cold (B)	<0.0001	0.0621
Time (C)	<0.0001	<0.0001
A × B	0.0870	0.0497
A × C	<0.0001	0.1814
B × C	<0.0001	0.0195
A × B × C	<0.0001	0.0321

Table 2. Parameters of the logistic model for bud swelling and bud sprouting of minicuttings of vines of the BRS-Carmem cultivar subjected to 6 treatments (hydrogenated cyanamide - HC, garlic extract - GE, mineral oil - MO, vegetable oil - VO, MO+VO, and Control), with (WEC) and without (NEC) exposure to 400 hours of cold, over time after application.

	Treatment	Bud swelling (%)				Bud sprouting (%)		
		b	*c*	*d*	*E*	*B*	*d*	*E*
WEC	Control	-7.4897	2.3022	45.1949	9.9052	-6.8230	49.5830	15.6524
	MO	-1.7041		77.9465	12.9525	-9.8295	48.6043	15.6382
	VO	-1.7014		74.4174	12.9151	-9.7621	49.9524	14.9675
	MO+VO	-1.7246		55.2933	4.9088	-9.6482	56.1058	16.3085
	HC	-6.4101	2.9238	41.4798	8.3884	-9.2173	28.9196	13.8464
	GE	-1.0395		113.8498	26.5058	-5.2959	66.8849	16.8750
NEC	Control	-3.7807		37.8765	5.7471	-9.5359	39.1669	15.2239
	MO	-3.0475		61.2462	4.4543	-8.0463	47.5196	13.7806
	VO	-3.7094		62.8795	5.0420	-10.8941	51.5771	14.4397
	MO+VO	-3.7974		56.2380	4.4969	11.5103	60.6370	13.7800
	HC	-3.3502		58.3069	3.6013	4.3402	104.8867	18.3789
	GE	-2.5218		46.0315	5.7544	5.9645	59.4349	18.0012

e = time for the response at the mid-point between *d* and *c*; *d* = higher limit; *c* = lower limit; *b* = relative slope

Little difference between the treatments was found for bud swelling after 10 and 20 days of evaluation when the treatments were subjected to cold (Figure 3A), except for the hydrogenated cyanamide, which had the lowest values due to a possible phytotoxic effect; moreover, it is known that the success in sprouting using hydrogenated cyanamide depends on the concentration and environmental temperature (Maia & Camargo, 2007). However, treatments with hydrogenated cyanamide without exposure to cold (Figure 3B) presented the highest results, followed by MO+VO, whereas the control and the garlic extract presented the lowest. This result was also found for the sprouting variable (Figure 3C and 3D).

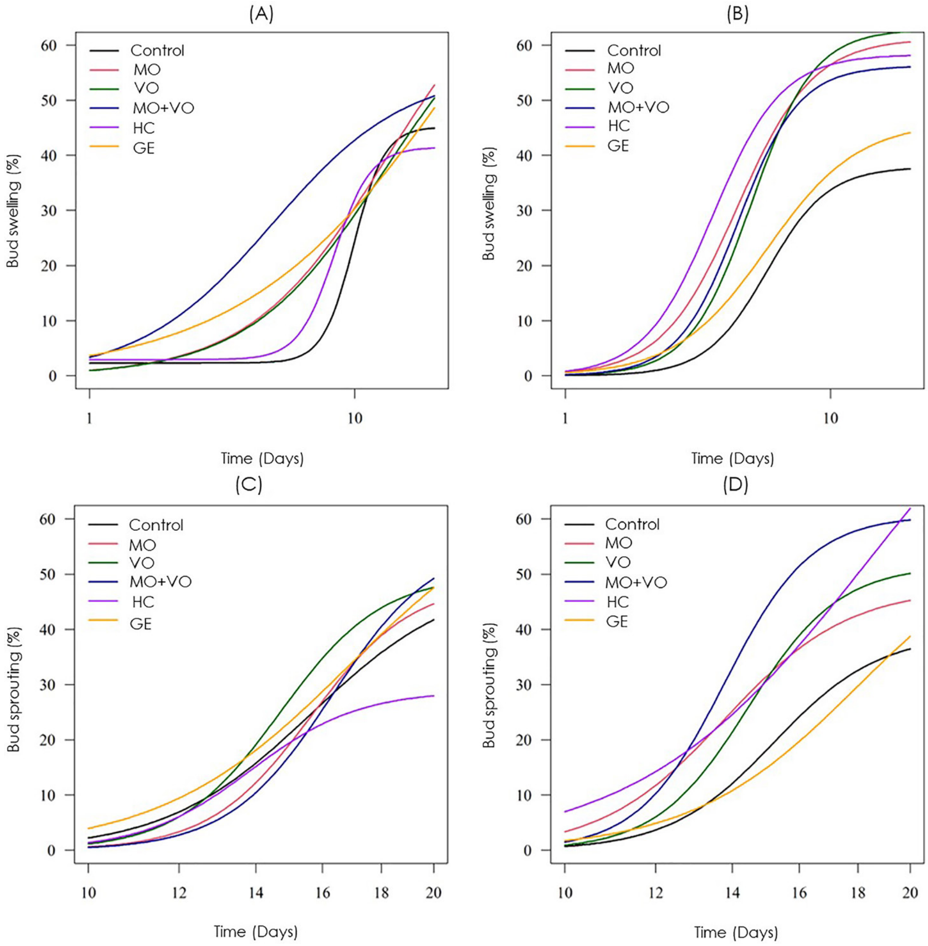

Figure 3. Bud swelling and bud sprouting in minicuttings of vines of the BRS-Carmem cultivar subjected to 6 treatments (hydrogenated cyanamide - HC, garlic extract - GE, mineral oil - MO, vegetable oil - VO, MO+VO, and Control), with (A and C) and without (B and D) exposure to 400 hours of cold.

Few studies with vine cuttings in controlled environment are found in the literature, thus, the results found were compared to field experiments. Carvalho et al. (2016b) found that the treatment with 20% garlic extract is not efficient for the sprouting of vines of the BRS-Rúbea and BRS-Cora cultivars, but lower percentages combined with mineral oil were efficient. Similar results were found in the present study, the treatment with 20% garlic extract presented less satisfactory results, which may be due to a phytotoxicity, whereas lower percentages resulted in increases in sprouting. El-Salhy et al. (2017) found similar results for 0.1% and 0.2% garlic extracts, which improved the sprouting and vigor of vines of the Flame Seedless cultivar.

Moreover, the treatment with hydrogenated cyanamide presented lower sprouting than the other treatments after 15 days when the cuttings were subjected to cold. This was also found for the bud swelling, since increases in the number of swollen buds were found only after 9 days.

The lower results found for hydrogenated cyanamide in the cuttings exposed to cold may be due to a genetic factor, since the BRS-Carmem cultivar was developed to be grown in hotter regions (Camargo et al., 2015). Therefore, the cold temperature that the minicuttings were subjected may have caused some disturbance to the buds, inhibiting their initial development.

In addition, vines of the BRS-Carmem cultivar usually present difficult to sprout, even with the use of hydrogenated cyanamide (Camargo et al., 2015); in the present study, it was indeed harmful to the minicuttings. According to Soltys et al. (2011), despite the efficiency of hydrogenated cyanamide, it may present a phytotoxic effect and cause disturbances to plants. When hydrogenated cyanamide is used inappropriately, it can inhibit the sprouting of buds, as described in the commercial product label. Thus, this harmful effect can be related to the bud need for a cold period of 400 hours, and the absence of effect of hydrogenated cyanamide was probably because it is recommended for crops that did not reached this climatic demand, or even by the toxic effect of the high rate applied (4%), since many studies with vine use rates of 1% to 5% (Werle et al., 2008; Leão et al., 2008); however, these rates vary according to the cultivar.

Peruzzo et al. (2014) found that *Vitis labrusca* plants of the Isabel, Concord, Bordô, and Niágara cultivars require accumulation of 70 to 250 hours of cold for maximum sprouting, whereas *Vitis vinífera* plants of the Chardonnay, Merlot, and Cabernet Sauvignon cultivars require 150, 300, and 400 hours of cold, respectively (Anzanello et al., 2021). Considering that *V. labrusca* cultivars present low cold demand and the hybrid used in the present study was adapted to subtropical and tropical regions, the use of 400 hours of cold was above the plant requirement.

This was confirmed in the present study, since biochemical oxygen demand was used, which excludes the climatic effect throughout the experiment; therefore, 400 hours of cold was enough for minicuttings from plants of the BRS-Carmem cultivar to sprout, making the use of hydrogenated cyanamide unnecessary.

Vine plants have an endodormancy period that is overcome by the beginning of cold times ($T \leq 7.2$ °C), and each genotype has a specific cold demand to overcome this physiological state and start sprouting (Peruzzo et al., 2014). Therefore, the use of products to induce sprouting is needed in subtropical and tropical regions, and the most used product is hydrogenated cyanamide. It has an inhibitory effect on the catalase activity, triggering the oxidative stress process to overcome dormancy; it is supposedly related to transitory increases in H_2O_2 levels, which combined with the beginning of the signal transduction process that ends the endodormancy and starts the bud sprouting (Pérez & Lira, 2005; Pinto et al., 2007; Marchi et al., 2017).

According to Wurz et al. (2020), vine plants of the Niágara Branca cultivar showed better results under field conditions when using hydrogenated cyanamide, whereas the control treatment presented little sprouting. However, the cold time demand is dependent on the species and cultivars (Fioravanço & Santos, 2013), and the BRS-Carmem does not need a long time of cold.

Camili et al. (2010) evaluated vine plants of the Superior Seedless cultivar and found that the use of hydrogenated cyanamide results in 75.8% sprouted buds at 13 days after pruning. Similar results were found in the present study for cuttings without cold, which presented 60% of sprouted buds at 18 days when using hydrogenated cyanamide. Mohamed & Gouda (2017) also found the highest number of sprouted buds (67.66%) when using hydrogenated cyanamide at 5% of the commercial product in vine plants of the Superior Seedless cultivar. In addition, Rodrigues et al. (2019) found 70.26% of sprouting when using branch bending combined with hydrogenated cyanamide at 5% of the commercial product in vine plants of the Itália Muscat cultivar.

Several studies have shown the effects of hydrogenated cyanamide on bud sprouting of vines and on the anticipation of sprouting when compared to controls (Camili et al., 2010; Camargo et al., 2015; Rodrigues et al., 2019). In the present study, the minicuttings treated with oils and hydrogenated cyanamide without cold presented better performance than the control. The control presented 25% sprouting at 16 days, whereas the treatment with MO+VO resulted in 50% sprouting.

The number of buds obtained when applying MO+VO were similar to that found by Marchi et al. (2017) in an experiment with apple trees of the Fuji Suprema cultivar, with a higher percentage of buds for the treatment with MO+VO at 50 days after sprouting; despite the difference between the species evaluated, both are plants from temperate climate that present a dormancy period (Petri et al., 1996). Mineral oils affect the respiration process by causing anaerobic conditions in the buds (Erez et al., 1980). The results found show that vegetable oils probably have the same mechanism, considering the low correlation between sprouting and enzymatic activity.

The cold time combined with the alternative treatments or hydrogenated cyanamide had a negative effect, changing physiological aspects of the BRS-Carmem cultivar, making it difficult to plants overcome dormancy, resulting in a low development of buds. Therefore, despite the information on the action of different factors on the metabolism and endodormancy of these plants (Pio, 2014), no information about the interrelation between these factors is found; thus, these results can be different under field conditions due to environmental effects.

Conclusions

The best results for the induction of bud burst of BRS-Carmem vines, considering the bud swelling and sprouting of minicuttings, are achieved when the minicuttings are not exposed to cold and the combined application of mineral oil and vegetable oil is used. Hydrogenated cyanamide application is harmful when the cuttings are subjected to 400 hours of cold.

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