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Garlic extract and hydrogen cyanamide on 'Tupy' blackberry bud sprouting, flowering and harvest

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Abstract

Considering the reduction of synthetic compounds used in sustainable fruit production system, this work aimed to evaluate and compare the effects of applying garlic extract and hydrogen cyanamide ($C_{22}H_{19}Cl_2NO_3$) in the bud sprouting, flowering and harvesting of blackberry. The research was conducted during the season of 2011 and 2012. The experiment design was randomized in split plots, consisting of 12 blocks. Each plot corresponded to the six treatments with hydrogen cyanamide, garlic extract and control (0%); and subplots were formed from the two crop seasons. 'Tupy' blackberry were 2 and 3 years-old, trained to 1.2m high espalier-type trellis with 4 lateral canes retained in a T configuration, with a spacing of 0.6 m between plants and 4.0 m between rows. The plants were pruned in August and the treatments applied immediately afterwards: 4% hydrogen cyanamide (standard product) and garlic extract in doses of 4.0%, 8.0%, 12.0% and 16.0%, and control. Both products were applied only once; and separately by using knapsack sprayer in the early morning hours. The hydrogen cyanamide 4% performed best, bringing forward more uniform bud sprouting and fruit harvesting. The intervals between: pruning and bud sprouting and pruning and harvesting were 14.5 and 87.5 days, respectively. The evaluated garlic extract concentrations showed similar values of production compared with the other treatments, except at 16% in 2011, being the highest concentration used on cultivated blackberry at São Manuel, São Paulo state, Brazil.

Keywords: bud break; climate adaptation; dormancy; plant growth regulator; *Rubus spp*.
Abbreviations: SB_ Sprouted buds; GE_ Garlic extract; HC_ Hydrogen cyanamide; NFP_ Number of fruit per plant; PR_ Production; Y_ Yield

Introduction

In Brazil, commercial cultivation of blackberries (*Rubus* spp) has been improved since 1970, when the first cultivar was introduced from the United States; and by using imported materials. Therefore, Brazilian cultivars have been acknowledged (i.e. 'Tupy' cultivar, which has been more dominant in Brazil ever since). In the state of Rio Grande do Sul, one can find the largest production of blackberries in Brazil due to cold winter. However, there are other areas that favor this kind of production (Campagnolo and Pio, 2012).
The production of these fruits can be an interesting alternative to small farmers, as these crops are highly productive in small areas; and consumer demand has increased in recent years. Blackberries have gained more consumers due to its high content of bioactive compounds, such as antioxidants (e.g. anthocyanins, polyphenols and flavonoids) (Hassimoto et al., 2008).

São Paulo state has presented an increasing demand (Attílio et al, 2009); thus cropping is also interesting from an economic point of view. In addition to that blackberry (*Rubus* spp.) is one of the most promising of the fruit species, especially with regards to increasing production in relation to demand and supply relationship (Jacques and Zambiazi, 2011).

São Paulo state presents subtropical climates. Therefore, species from temperate climates when cultivated in tropical regions, the number of hours of chilling during the winter is insufficient; the breaking of dormancy tends to be reduced and/or uneven. In this way, plants may present a longer delay between bud sprouting period; lower percentage of buds and flowers, reflecting on lower productivity; non-uniform fruit production; and fruit quality may be affected (Gao et al., 2002). There are some techniques that support the cultivation time, such as defoliation; pruning; irrigation, and plant growth regulators (i.e. natural or chemical products), which are considered as an alternative that enable the production in tropical areas.

Regarding to the chemical products (Settimi et al., 2005), one of the most popular is hydrogen cyanamide (HC) that induces sprouting in temperate fruits; providing a uniform bud; and allowing early harvest (Sagredo et al., 2005), but its efficacy faces the challenge to reduce synthetic and toxic substances. There was a preference in sustainable production systems instead of the chemical braking of dormancy, as one can observe through certain studies in Oceania (Mcartney and Walker, 2004) and Brazil (Botelho et al., 2007 a and 2007 b).
By searching for alternatives to breaking of dormancy in temperate fruits, Kubota and Miyamuki (1992) studied the application of garlic paste in greenhouses. The authors based their hypothesis on the use of this product and the presence of active substances [i.e. sulphur and allyl group (H_2CHCH_2), mainly diallyl disulfide, which is the most abundant sulphate in garlic], possibly acted by using the same mechanism as

proposed by Pinto et al. (2007), in breaking of dormancy in temperate fruits, i.e. through oxidative stress; through accumulating H_2O_2 and thus with the possibility for promising results in flowering plants. Kubota et al. (2000) stated that by applying garlic paste or oil causes buds on vines to break dormancy. In Brazil, Botelho (2007 a) also evaluated using garlic extract (GE) on apple trees and in table grapes (2007 b). Also, Maia et al. (2013) evaluated the effect of a hydrolate obtained from *Gallesia integrifolia* in the bus sprouting, yield and enzyme activity of peroxidase and catalase on grapevines, concluding that *Gallesia* hydrolate in the doses between 100 and 150 mL L-1 can be an alternative to break dormancy of grapevine 'Benitaka' in tropical regions for sustainable viticulture.

GE is a natural product that is an alternative for breaking of dormancy in temperate fruits, especially in production systems that limit the use of agrochemicals. However, the studies on this product are inconclusive; and further researches are needed to clarify how these compounds work and their effects in different regions that occurs the cultivation.

There are several reports on using GE to induce breaking of dormancy in blackberries in the cultivation conditions in the subtropical climate of the São Paulo state (Dias et al., 2011 and Segantini et al., 2011), in which the authors highlighted the need for further studies that enable this product recommendation. In recent years, the population's interest in consuming blackberries and raspberries has increased due to their high antioxidant content (Kuskoski et al., 2006; Hassimoto et al., 2008). At the same time, interest has grown in production systems that are organic or by using minimal synthetic chemical products. Bearing in mind that there are no reports available on the thermal needs in terms of chilling hours needed to cultivate blackberries, and little is known of the need to use chemical products to breaking of dormancy in subtropical climates, therefore the aim of this study is to evaluate the effects of applying GE and HC in the bud sprouting, yield and crop cycle of the blackberry in the São Manuel region, São Paulo state, Brazil.

Results and Discussion

Number of chilling hours below 7.2 and 13ºC

In 2011, in the city of Sâo Manuel, the number of chilling hours below 7.2ºC and 13ºC were 51.91 and 347.83 hours, respectively. In 2012, the number of chilling hours below 7.2 ºC was 767.5 (Table 2). These numbers are below those reported by Silveira et al.(2007); therefore it is necessary about 215 chilling hours below 7.2ºC for breaking of dormancy from "Tupy" blackberries. The data in Table 1 reflect that the number of chilling hours < 7.2ºC was higher in 2011 (51.91 hours), but in 2012 there was more chilling hours < 13ºC (767.50 hours) compared with 2011 (347.83 hours). The higher number of chilling hours (< 13ºC) in the 2012 cycle, compared with 2011; thus it encouraged early bud sprouting and harvest (Table 3), allowing it to be inferred that the buds naturally broke of dormancy, i.e. irrespective of the used compound.

However, it will be necessary further studies to define basal temperatures and thermal time according to the phenological stages of cultivation; it is also important to consider that there is no data available on the number of chilling hours needed for the 'Tupy' blackberry to breaking of dormancy for the studied area. Although, it is known that the number of chilling hours needed to breaking of dormancy may vary between cultivars within same species. Thus, genetic improvements aim to select cultivars to better adapt into different locations; this fact will push back frontiers of fruit trees species, whether there is an interest in establishing plantations and in the consuming market, as it is the case of São Manuel, São Paulo state, Brazil.

Phenological cycle

Regarding to the charcteristics of the phenological cycle, there was no significant difference between the years of 2011 and 2012. In this way, mean was calculated for this period. Therefore, GE did not influence on the blackberry season of 2011-2012 (Table 3). By using GE, the season varied between 124 days (GE 8, 12 and 16%) and 127.5 days (GE 4%).

The HC 4% showed: greater uniformity; and early phenological stages of 'Tupy' blackberry, i.e. low cycle from pruning to harvesting in both seasons. When HC 4% was used, bud sprouting and harvesting were 30.5 and 36.5 days earlier, respectively; compared with the control and GE treatment (Table 3). Such results show that HC is efficient at bringing forward bud sprouting in blackberries and making them more uniform. Vergara et al. (2012) explained that HC is efficient because of respiratory stress that leads to breaking of dormancy. The authors found that after applying HC, the expression of genes associated to the fermentative process increased.

In a warm winter region, while evaluating buds from four different grapes, Corrales-Maldonado et al.(2010), quantified the percentage of sprouted bud after applying GE compounds, compared with HC and control. Both garlic compounds (3% v/v) and HC (5% v/v) promoted an early bud sprouting in 4 cultivars; hastened sprouting of bud by 19-28 days, compared to the control. Also, the plants that received the treatments reached 50% sprouted bud from 9 to 25 days before control.

The efficacy of applying HC 4% to bring forward more uniform bud sprouting in 'Tupy' blackberries has also been observed by Attílio et al. (2009), in Selvíria, MS, Brazil. This area is characterized by a very warm climate. The authors found that a cycle lasted 105 days from pruning to harvesting; the harvest peak was in mid-October, with earlier production compared to colder regions. The singular effect of HC in making buds more uniform and earlier bud sprouting favored in establishing the cultivation of blackberry in warm areas. Therefore, better price was obtained since there were favorable conditions for harvesting compared to colder areas (Ceasa Campinas, 2013).

Sprouted buds, number of fruits, production and yield

The effect of GE concentrations varied within two seasons, with significant interaction between percentages of SB (Table 4). The linear and quadratic regression models were adjusted for the years of 2011 and 2012 respectively (Fig 1). According to Nascimento et al. (2009), the effect of plant growth regulators can change according to some factors, such as used dose; application time; cultivar; and temperature. In this study, there was no difference within the mentioned factors in the season of 2011-2012, but temperature had. Therefore, the differences in the results for SB; NFP; PR and Y could be related to the number of chilling hours below 13°C, i.e. 582.58 hours (2011) and 1259.75 hours (2012).

In 2011, a higher percentage of SB (34%) was at 16% GE; i.e. an increase of 203% SB compared with control. In 2012, the maximum point of the function was obtained with an estimated dose of 6% GE; this led to 53% of sprouted buds

Table 1. Soil analysis of experimental area of blackberry 'Tupy', São Manuel, São Paulo state, 2011 and 2012.

2011							
Amostra (cm)	pH CaCl2	M.O g dm^{-3}	P mg dm^{-3}	H + Al	K	Ca	Mg
				-------------mmolc dm-3-------------			
0 – 20	5.5	8.0	10.0	11.0	2.0	10	3.0
20 – 40	5.3	6.0	5.0	10.0	1.6	10	4.0
	B	Cu	Fe	Mn	Zn		
	---------------------------- mg dm-3 -------------------------						
0 – 20	0.20	0.5	22	8.3	0.7		
20 -40	0.16	0.4	19	7.9	0.5		
2012							
Amostra (cm)	pH CaCl2	M.O g dm^{-3}	P mg dm^{-3}	H + Al	K	Ca	Mg
				-------------mmolc dm^{-3}-------------			
0 – 20	5.4	7.0	9.0	12.0	1.8	20	3.5
20 – 40	5.2	8.5	6.0	11.0	1.4	16	4.0
	B	Cu	Fe	Mn	Zn		
	---------------------------- mg dm^{-3} -------------------------						
0 – 20	0.20	0.6	20	8.0	0.6		
20 -40	0.15	0.5	22	6.5	0.6		

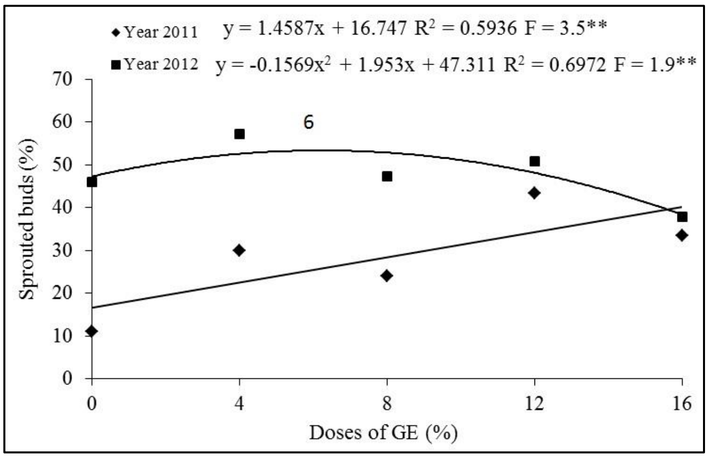

Fig 1. Percentage of sprouted buds (SB) of blackberry plants subject to doses of garlic extract (GE). São Manuel, São Paulo state, 2011 and 2012.

(Fig. 1); i.e. an increase of 155% SB compared with control. Such results are interesting, as they show the importance of the number of chilling hours measured in both cultivation seasons. In 2011, there were fewer chilling hours compared to 2012; and thus better results were obtained with the higher dose of GE (16%). In 2012, there was partial breaking bud dormancy, because the number of chilling hours with GE (6%) was sufficient to enable a higher percentage of SB. These explanations corroborates with the one found by Clark and Moore (2005), which suggested that phenological alterations of the blackberry in different crop seasons are commons, mainly because of the low temperatures during the dormancy.

There was a significant effect in the treatment of 2011 by comparing the results from SB percentages obtained with GE and HC; thus the highest value for SB was of 82.2% by applying 4% HC, differing significantly from the other treatments. For GE, similar values were obtained for all doses evaluated (Table 4).

In 2012, there was no difference between the treatments evaluated, which is attributed to the higher number of chilling hours < 13º C (1259.75 hours), i.e. buds broke dormancy occurred naturally. The efficiency in supplying of chilling requirements may be evident in sprouted buds with control (0% GE) during both seasons. In 2012, the highest number of chilling hours enhanced naturally budburst. Inadequate winter chilling can modify the budburst pattern, leading to poor bud sprouting, delayed foliation and protracted bloom, as the major consequences (Sagredo et al., 2005).

There was a significant interaction between the doses of GE and crop seasons for the NFP, PR and Y for the blackberry. Examining the interaction for the concentrations of GE and the HC (Tables 4 and 5), it can be seen that, in 2011, higher mean values were obtained for yield and for NFP when HC 4% was applied (2.3 kg $plant^{-1}$ and 352 fruit per $plant^{-1}$), differing only from treatment with 16% GE, which obtained the lowest values, with the PR of 1.1 kg $plant^{-1}$ and 170 fruit $plant^{-1}$, respectively.

In 2012, no differences were found between the treatments for yield or number of fruit per $plant^{-1}$; which can be attributed to the higher number of chilling hours in this year, enabling the buds to break dormancy. According to Pérez and Lira (2005), by applying HC and higher number of chilling hours can inhibit catalase activity, which is the main enzyme responsible for breaking down hydrogen peroxide (H_2O_2) in buds of fruiting trees from temperate climates. Inhibiting the action of catalase enables mitochondrial respiration; thus waking the plant from dormancy. Moreover, genes related to biosynthesizing vegetable hormones such as gibberellins and cytokinins may well be activated, as when during bud sprouting processes of cell division, starch breakdown and growth regulated by these hormones occur. In 2011, the NFP and PR with 4% HC and 0, 4, 8 and 12% GE; and 0, 4, 8, 12 and 16% in 2012 are greater when compared with the results

Table 2. Number of chilling hours below 7.2 and 13ºC, in São Manuel, São Paulo state, 2011 and 2012.

Months	n° of hours < 7.2 °C		n° of hours < 13 °C	
	2011	2012	2011	2012
January	0.00	15.3	0.00	302.7
February	0.00	0.00	0.00	48.7
March	0.00	0.00	0.00	9.2
April	0.00	0.00	0.00	67.4
May	0.00	7.20	29.58	108.40
June	39.58	0.00	156.58	267.98
July	0.00	0.00	60.42	309.15
August	12.33	0.00	101.25	81.97
September	0.00	8.60	32.17	40.09
October	0.00	0.00	147.08	22.77
November	11.08	0.00	55.50	1.39
December	0.00	0.00	0.00	0.00
Total	62.99	31.08	582.58	1259.75

Data collected at experimental farm São Manuel, São Paulo State, 2011 and 2012.

Table 3. Average of number of days between pruning and peak blackberry bud sprouting, flowering and harvest, according to application of hydrogen cyanamide (HC) and garlic extract (GE) of season 2011-2012. São Manuel, São Paulo state.

Compound	Number of days		
	Bud Sprouting	Flowering	Harvest
HC 4%	14.5 B	59.5 B	87.5 B
Control 0%	45.0 A	76.0 A	127.5 A
GE 4%	45.0 A	76.0 A	127.5 A
GE 8%	45.0 A	83.0 A	124.0 A
GE 12%	45.0 A	79.5 A	124.0 A
GE 16%	45.0 A	76.0 A	124.0 A
CV (%)	31.1	10.8	1.5

Data followed by the same letter are not different by the Tukey’s test (p≤0.05).

Table 4. Number of fruit per plant (NFP) and sprouted buds (SB) percentage in blackberry plants subject to doses of hydrogen cyanamide (HC) and garlic extract (GE). São Manuel, São Paulo state, 2011 and 2012.

Compound	NFP		SB percentage (%)	
	2011	2012	2011	2012
HC 4%	352 Aa	222 Ab	82.2 Aa	52.4 Ab
Control 0%	270 ABa	253Aa	11.1 Cb	46.0 Aa
GE 4%	231 ABa	303Aa	30.1 BCb	57.1 Aa
GE 8%	223 ABa	261Aa	24.0 BCb	47.4 Aa
GE 12%	303 Aa	234Aa	43.4 Ba	50.9 Aa
GE 16%	170 Bb	274Aa	33.6 Ba	37.9 Aa
CV 1 (%)	30.8		29.5	
CV 2 (%)	32.4		30.1	

Data followed by the same letter, upper case letter in columns and low case letters in rows, are not different by the Tukey’s test (p≤0.05).

Table 5. Production (PR) and yield (Y) for blackberry plants subject to doses of hydrogen cyanamide (HC) and garlic extract (GE). São Manuel, São Paulo state, 2011 and 2012.

Compound	PR (kg plant^{-1})		Y (ton ha^{-1})	
	2011	2012	2011	2012
HC 4%	2.3 Aa	1.4 Ab	9.5 Aa	6.0 Ab
GE 0%	1.8 ABa	1.6Aa	7.3 Aba	6.9 Aa
GE 4%	1.5ABa	2.0 Aa	6.2 Aba	8.2 Aa
GE 8%	1.4ABa	1.7Aa	6.0 Aba	7.1 Aa
GE 12%	2.0 ABa	1.5Aa	8.2 Aba	6.3 Aa
GE 16%	1.1 Bb	1.8 Aa	4.6 Bb	7.4Aa
CV 1 (%)	31.8		31.8	
CV 2 (%)	32.6		32.6	

Data followed by the same letter, upper case letter in columns and low case letters in rows, are not different by the Tukey’s test (p≤0.05).

obtained by Tadeu et al. (2015) for 'Tupy' blackberry without spraying of chemical or natural compounds to breaking dormancy. The plants had 196 fruits, with an average production of 1.187,1 g per plant^{-1}.

The application of GE leads to a number of questions, i.e. correct dosage and application time. According to Corrales-Maldonado et al. (2010) the mechanism that GE compounds use to induce bud sprouting, is still unknown, and then further researches should be directed to elucidate how buds sprouting.

The different results for the 2011 and 2012 crop seasons are attributed to climatic conditions that led to differences between the buds' state of dormancy by inducing SB. The most evident effects of HC was in bringing forward SB, and the subsequent decrease in the crop season, such as earlier harvesting.

There were significant differences in relation to the application of the HC 4% between the two evaluated years. In 2012, it was observed a reduction of the 130 fruits plant^{-1} compared with 2011 (Table 4), reflecting on yield reduction: 3.5 ton ha^{-1} (Table 5). Differences between the years proved favorable effects of the application dose in the years that had less number of chilling hours (i.e., < 7.2ºC and < 13ºC), which showed best sprouting, flowering and production. In 2012, the dose of 16% GE showed better results with an increase of 104 fruits plant^{-1} and 2.8 ton ha^{-1} in yield. In 2012, it was recorded 1259.75 hours of chilling < 13ºC, which is more than 2011, indicating more influence of low temperatures in plants production that also received the product concentration; therefore the control (2011) showed better results than control (2012)..

Materials and Methods

Location and soil classification

The experiment was conducted in the Sao Manuel Experimental Farm, Botucatu School of Agronomy, UNESP (22°44'28"S, 48°34'37"W; 740m m altitude). The mean annual rainfall of São Manuel is 1433 mm; and the mean annual temperature is 23°C. According to the Köppen classification, the climate in the region is mesothermic, *Cwa*, in other words, humid and subtropical, dry in the winter with a rainy season between November and April. The soil is classified as Dystrophic Red Latosol (Oxisoil), the soil chemical characteristics are shown in Table 1.

Estimating the number of chilling hours below 7.2 and 13ºC

Thermal data were obtained using a Vaisala assembly (HMP50 thermo hygrometer + multi-plate RM Young model 41002 cover) installed at 2m height and connected to a CR23X Micrologger scanning every 10 seconds, storing data every 5 minutes, provided by the Department of Natural Resources of the Botucatu School of Agronomy, UNESP (Table 1). The numbers of chilling hours below 7.2º C and 13 ° C were calculated; and the experiment took place over the crop seasons of 2011 and 2012.

Plant materials and experimental design

The same 'Tupy' blackberry plants, planted on the 23rd of June 2009 were used in the 2011 and 2012 crop season. Tupy' blackberry plants were 2 and 3 years-old trained to 1.2m high espalier-type trellis with 4 lateral canes retained in a T configuration, with a spacing of 0.6 m between plants and 4.0 m between rows and a density of 4166 plants per ha^{-1}.

The pruning was done on the 17th of August 2011 and 13th of August 2012, being selected 6 branches, with approximately 30 to 40 cm per plant. One day after the prunning, the treatments were applied: 4% HC, GE in doses of 4.0%, 8.0%, 12.0% and 16.0%, and untreated control, without spraying. The commercial product used was HC (Dormex®), containing 490 gl^{-1} H_2CN_2 and GE (Bioalho®), containing 70% GE. Both products were applied only once; and separately by using knapsack sprayer in the early morning hours. In average, each plant received 250 mL of the solution. The experiment design was randomized in split plots, consisting of 12 blocks and 10 usable plants per plots. Each plot corresponded to the six treatments with HC, GE and control (0%); and subplots were formed from the two crop seasons

Phenological cycles

The following variables were evaluated: number of days between pruning and peak bud sprouting; flowering and harvesting. To determine the occurrence of the phenological stages of the 'Tupy' blackberry each plant had one of their canes marked and the number of buds was counted previously to the treatments. After the treatments, the number of buds, sprouted buds, flowers and harvested fruits were counted twice a week in order to determine the peaks of each phenological stage. The number of days from pruning to peak of bud sprouting was determined when more than 50.0% of buds had sprouted, from pruning to peak of flowering when more than 50.0% of flowers were opened, from pruning to peak of harvest when more than 50.0% of fruit were harvested.

Sprouted Buds, number of fruits, production and yield

Percentage of sprouted buds was determined by the following equation: % bud sprouting = (nº total sprouted buds/ vegetative buds) x 100.

Production was determined by the product of the mean number of fruit produced by each plant per plot, totaling 120 plants per treatment and the respective mean weight (kg plant^{-1}) and the yield in, kg ha^{-1}, measured by considering density of planting of 4166 plants per ha^{-1}.

Statistical analysis

Results were subjected to variance analysis and means from treatment with GE and HC were compared by Tukey test, at 5% level of significance and doses of GE by using polynomial regression analysis. The software used for the analyses of variance was SISVAR (Ferreira, 2011).

Conclusion

When the weather conditions were satisfactory to naturally break bud dormancy, the hydrogen cyanamide and garlic extract did not affect the bud sprouting and yield. Hydrogen cyanamide 4.0% performed best, as it concentrated the season and promoted earlier harvest. Doses higher than 12% for garlic extract can decrease the bud sprouting and yield; therefore it is not recommend for 'Tupy' blackberry cultivar in São Manuel, São Paulo state.

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