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Bluprins® as Alternative Bud Break Promoter for 'Maxi Gala' and 'Fuji Suprema' Apple Trees

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Authors' contributions

This work was carried out in collaboration between all authors. Author CLF performed the statistical analysis and wrote the first draft of the manuscript. Author JLP designed the study and wrote the protocol. Authors AAS and MSDM managed the analyses of the study. Authors GCG and ESD managed the literature searches and the translation of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The effect of Bluprins[®] at different concentrations combined with calcium nitrate and ammonium nitrate on phenology, bud break induction and fruit production of 'Maxi Gala 'and' Fuji Suprema' apple tree cultivars was evaluated in mild winter conditions.

Study Design: The experiment was designed in randomised block and replicated five times.

Place and Duration of Study: The experiment was carried out in the municipality of Caçador, Brazil, during the growing seasons of 2013/2014, 2014/2015, 2015/2016 and 2016/2017.

Methodology: The study considered 'Maxi Gala' and 'Fuji Suprema' apple trees. The treatments evaluated were as follows: 1. Control (untreated); 2. Mineral oil 3.5% + hydrogen cyanamide 0.35%; 3. Bluprins[®] 3.0% + calcium nitrate 3.0%; 4. Bluprins[®] 5.0% + calcium nitrate 3.0%;

5. Bluprins[®] 3.0% + calcium nitrate 5.0%; 6. Bluprins[®] 5.0% + calcium nitrate 5.0%; 7. Bluprins[®] 3.0% + calcium nitrate 3.0% + ammonium nitrate 3.0%; 8. Bluprins[®] 5.0% + calcium nitrate 4.0% + ammonium nitrate 4.0%. Phenology, axillary and terminal bud break, fruit set, fruit production and average fruit weight were evaluated. The phenological stage of green tip (C-C3) and the beginning of bud break and flowering were anticipated by the application of Bluprins® and hydrogen cyanamide in compared to the control. The axillary and terminal bud break was increased by the application of bud break promoters for both cultivars considering the four growing seasons studied. The average fruit weight did not show significant differences between treatments in the growing seasons of 2013/2014 and 2015/2016. Bluprins®, in combination with calcium nitrate and ammonium nitrate, proved effective in inducing bud break of 'Maxi Gala' and 'Fuji Suprema' apple tree cultivars under mild winter conditions. The phenomenon is less effective with mineral oil in combination with hydrogen cyanamide. Bluprins® in combination with calcium nitrate and ammonium nitrate anticipates the bud break and flowering period and reduces the flowering period for 'Maxi Gala' and 'Fuji Suprema' apple tree cultivars in compared to plants without application of bud break promoters. Bluprins® does not affect considerably the fruit set and fruit production of 'Maxi Gala' and 'Fuji Suprema' apple tree cultivars.

Keywords: Malus domestica Borkh.; dormancy; mild winter chilling; bud break induction.

1. INTRODUCTION

The apple-growing (*Malus domestica* Borkh.) has a high production volume, estimated at 91.4 million tons of harvested fruit in 2016, which classifies apple as one of the most produced fruits in the world [1]. Apple production is of great importance in Southern Brazil. In this region, more than 4 thousand fruit growers harvested 1.05 million tons of apples in that year, representing a financial turnover of R\$ 6 billion and generating 195 thousand of jobs in marketing sector [2].

Apple trees present suspension of vegetative growth in winter, called dormancy. This mechanism allows it to survive periods of low temperatures [3]. To overcome dormancy, plants have to satisfy their chilling requirements to initiate spring bud break, shoot meristematic extension growth and anthesis [4]. Chilling requirements vary depending on the cultivar [5]. In Brazil, 90% of apple orchards are composed by 'Gala' and 'Fuji' cultivars and their spontaneous mutations [6]. These cultivars present chilling requirements of around 600 to 800 hours of temperatures below 7.2°C to overcome dormancy [7]. The insufficient chilling accumulation in a specified cultivar results mainly in the reduction of bud break and uneven flowering [8]. These dysfunctions have economic consequences due to the impact on fruit production and quality, may compromise management orchard techniques such as chemical thinning and influence the next harvest by reducing the production due to the lower formation of reproductive structures in the plants [9,10].

In some subtropical climate countries such as Brazil, a few regions present favourable conditions to overcome apple tree dormancy [11,6]. In these areas, the solution for the cultivation has been the selection of cultivars with low chilling requirement combined with the application of bud break promoters and cultural practices to break bud dormancy. These proceedings provide adequate bud break and flowering [12,13].

The desirable characteristics of chemical substances have good efficiency for the bud break induction, low cost and minimum toxicity to plants and environment [14]. Despite the existence of a large number of effective substances for bud break induction, only a few are used commercially. The high cost of application and high toxicity of the compounds are the primary limiting factors [15]. Hydrogen cvanamide combined with mineral oil is the most effective compound for bud break induction and is extensively used in the cultivation of apple trees and other temperate climate fruit trees for more than 20 years in Brazil [16,17]. However, the toxicity of hydrogen cyanamide is diversifying the standard recommendation to break dormancy of apple trees by alternative substances which is less harmful to the agrochemical applicator and environment [18].

Organic nitrogen compounds have shown potential for commercial use. The combination of

these inducers with calcium nitrate has proved promising for bud break induction of apple trees [19,10]. Several studies carried out in different countries have shown promising results of Bluprins® bio-stimulant on bud break induction and flowering uniformity for trees of table grapes, cherries and kiwifruit. Preliminarily, positive results were also obtained for apple and peach trees [20]. Bluprins® is a concentrated gel formulation for breaking bud dormancy of temperate fruit trees containing polysaccharides, amino acids, nitrogen and organic carbon [21].

In this context, the study aimed to test the efficiency of Bluprins® combined with calcium nitrate and ammonium nitrate as an alternative to hydrogen cyanamide in relation to bud break induction in 'Maxi Gala' and 'Fuji Suprema' apple tree cultivars.

2. MATERIALS AND METHODS

The experiment was carried out in an experimental orchard located in the municipality of Caçador, Santa Catarina State, Brazil (26°50'S, 50°58'O, 941 m a.s.l). Eight-year-old plants of 'Maxi Gala' and 'Fuji Suprema' apple trees were grafted on rootstock Marubakaido and M-9 as interstock. The orchard density was about 2,500 plants ha⁻¹ and the plants were trained to a central leader system. Orchard management practices were applied according to the recommendations for apple production system [22].

The experiment was carried out in a randomised complete block design following a 4 x 8 factorial structure (four growing seasons and eight bud break promoters) with five replications of a single tree. The growing seasons evaluated were 2013/2014. 2014/2015. 2015/2016 2016/2017, and the bud breakers evaluated were: 1. Control (untreated); 2. Mineral oil 3.5% + hydrogen cyanamide 0.35%; 3. Bluprins[®] 3.0% + calcium nitrate [Ca(NO₃)₂] 3.0%; 4. Bluprins[®] 5.0% + calcium nitrate 3.0%; 5. Bluprins® 3.0% + calcium nitrate 5.0%; 6. Bluprins® 5.0% + calcium nitrate 5.0%; 7. Bluprins® 3.0% + calcium nitrate 3.0% + ammonium nitrate [NH₄(NO₃)] 3.0%; 8. Bluprins[®] 5.0% + calcium nitrate 4.0% + ammonium nitrate 4.0%. The bio-stimulant used is the commercial product Bluprins[®], composed of water, ammonium nitrate, sugar cane molasses, amino acids, citric acid, sodium hydroxide, supplying 4% ammoniacal N, 4% nitric N, 0.7% organic N and 5.5% organic C. The commercial product Dormex®, has 52% of active

ingredient, used as a source of hydrogen cvanamide. The commercial product Assist® was used as a source of mineral oil (75.6%). Compounds were applied with a motorised backpack sprayer. Application time was performed on 06/09/2013, 03/09/2014, 26/08/2015 and 25/08/2016 for the growing seasons of 2013/2014, 2014/2015, 2015/2016 and 2016/2017, respectively. During the winter period, the growing seasons of 2013/2014, 2014/2015, 2015/2016 and 2016/2017 presented 544, 271, 189 and 612 hours of temperature below 7.2°C and 940, 884, 746 and 1,200 of chilling units (Modified North Carolina model [23]), respectively (Fig. 1).

The phenology, axillary and terminal bud break and fruit set were evaluated. The evaluation of flowering phenology consisted of determining the dates of occurrence of the green tip stage (C-C3), start of flowering, full bloom and end of flowering [22,18]. The C-C3 stage was considered when 50% of the dormant buds presented "green tip" (beginning of sprouting). The begining of flowering was considered when the plants had 5% of the flowers in opening stage, full bloom more than 80% and the termination of flowering was defined when the last flowers were open. The axillary bud break was obtained by counting both burst and dormant buds in five one-vear-old shoots previously selected, located in the middle third of the plant. A scaffold branch was selected to estimate the percentage of terminal bud break. These data were collected at 30 and 60 days after dormancy breaking (DADB). The fruit set was obtained as a percentage in 100 flower cluster in the same scaffold branch used to estimate terminal bud break. Fruit production per tree and average fruit weight was also measured and reported.

The data were statistically evaluated by using analysis of variance (ANOVA). Percentage data were transformed by the formula arcsine [(x + 1) / 100] 1/2 before being subjected to ANOVA. Treatment means were compared by using the Scott-Knott test at 5% probability level. The statistical analysis was performed by the Sisvar program version 5.6 [24].

3. RESULTS AND DISCUSSION

For both 'Maxi Gala' and 'Fuji Suprema' cultivars, the treatments resulted in a significant difference (p <0.05) for most of the evaluated variables. For 'Fuji Suprema' cultivar, there was a significant

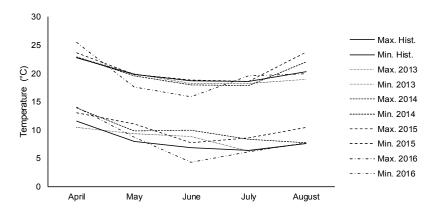


Fig. 1. Historical minimum (Min.) and maximum (Max.) average temperatures (°C) and minimum and maximum temperatures (°C) of the growing seasons of 2013/2014, 2014/2015, 2015/2016 and 2016/2017 during the dormancy period of apple trees. Caçador, SC

interaction between growing seasons and bud breaker promoters considering axillary and terminal bud break. However, for 'Maxi Gala' cultivar, there was no significant interaction between growing seasons and bud breaker promoters for any of the evaluated variables (data not shown).

For 'Maxi Gala' apple trees, the phenological stage C-C3 was different between the control treatment and the other treatments. The application of mineral oil in combination with hydrogen cyanamide advanced this stage 19 days, 21 days, 24 days and 23 days compared to the control treatment plant in the growing seasons of 2013/2014, 2014/2015, 2015/2016 and 2016/2017, respectively. The treatments containing Bluprins® also advanced this stage from 5 to 15 days in 2013/2014, from 13 to 19 days in 2014/2015, from 5 to 7 days in 2015/2016 and from 14 to 17 days in comparison to the control treatment in 2016/2017 (Table 1).

For 'Fuji Suprema' apple trees, there was not a defined period that characterised the C-C3 stage for the control treatment, In the 2013/2014 season; considering the treatments, this stage occurred practically at the same date. In 2014/2015 growing season, the treatments containing Blueprins® advanced the C-C3 stage from 1 to 8 days in relation to the control treatment and delayed this stage from 2 to 5 days in relation to mineral oil + hydroxygen cyanamide application. In 2015/2016 and 2016/2017 growing seasons, all the treatments advanced this stage from 2 to 8 days and from 4 to 10 days, respectively, in relation to the control treatment (Table 2).

The bud break was also advanced in both cultivars by the application of bud break promoters. However, there were differences between mineral oil + hydrogen cvanamide and the Bluprins® treatments. The begining, full bloom and termination of flowering were advanced in relation to the control treatment for 'Maxi Gala' and 'Fuji Suprema' cultivars in the four growing seasons, and the Bluprins® treatments showed a tendency to delay these phenological stages a few days in relation to treatment mineral oil + hydrogen cyanamide (Tables 1 and 2).

The growing seasons of 2014/2015 and 2015/2016 represented lower chilling accumulation and more extended flowering period than that of the growing seasons of 2013/2014 and 2016/2017. According to Kozmá et al. [25], duration of the flowering period is influenced by environmental conditions, being longer under low chilling accumulation during the winter season.

In 2013/2014 growing season, the flowering period was prolonged in the control treatment in compared to the other treatments. For 'Maxi Gala', the flowering period comprised of 14 days in the control trees, whereas the other treatments varied between 11 to 15 days. For 'Fuji Suprema', the flowering period comprised of 15 days in the control trees, and varied between 6 to 16 days in the other treatments. In 2014/2015, 2015/2016 and 2016/2017 growing seasons, the other treatments resulted in a shortened flowering period in compared to the control treatment only for 'Fuji Suprema', varying from 8 to 12 days, and from 10 to 17 days, respectively in 2015/2016 and 2016/2017, while the control

trees had a flowering period of 32 and 18 days, respectively. For 'Maxi Gala', the treatments presented this period equal to the control treatment or longer in the 2014/2015 and 2016/2017 growing seasons (Tables 1 and 2).

Petri and Leite [7] endorsed that prolonged flowering periods may difficult for some cultural practices such as thinning and disease control,

due to the occurrence of different phenological stages within the same plant. The duration of the flowering period can evaluate the efficiency of bud break promoters, and the most effective treatments are those with shorter flowering period and more uniform flowering, ripening and harvesting. It is revealed that Bluprins[®] acts in the advance and shortening of the flowering period of apple trees under mild winter conditions.

Table 1. Phenological stages of 'Maxi Gala' apple trees under the influence of compounds for bud break in four growing seasons (Date/Month). Caçador, SC, 2018

Treatments	C-C3	Bud break	Flowering		
			Start	Full bloom	End
	2013/201	4			
1. Control	10/10	14/10	14/10	21/10	28/10
2. MO 3,5% + HC 0,35%	21/09	06/10	08/10	12/10	19/10
3. B 3% + Ca(NO ₃) ₂ 3%	05/10	08/10	08/10	20/10	23/10
4. B 5% + Ca(NO ₃) ₂ 3%	25/09	08/10	08/10	18/10	23/10
5. B 3% + Ca(NO ₃) ₂ 5%	05/10	08/10	08/10	20/10	23/10
6. B 5% + Ca(NO ₃) ₂ 5%	25/09	06/10	08/10	12/10	23/10
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	25/09	06/10	08/10	18/10	25/10
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	05/10	12/10	12/10	21/10	26/10
	2014/201	5			
1. Control	12/10	12/10	12/10	24/10	28/10
2. MO 3,5% + HC 0,35%	21/09	25/09	28/09	03/10	14/10
3. B 3% + Ca(NO ₃) ₂ 3%	29/09	02/10	04/10	12/10	20/10
4. B 5% + Ca(NO ₃) ₂ 3%	26/09	29/09	29/09	12/10	18/10
5. B 3% + Ca(NO ₃) ₂ 5%	26/09	29/09	30/09	12/10	21/10
6. B 5% + Ca(NO ₃) ₂ 5%	23/09	25/09	30/09	08/10	18/10
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	28/09	01/10	01/10	12/10	21/10
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	29/09	03/10	04/10	12/10	21/10
	2015/201	6			
1. Control	10/10	-	10/10	28/10	05/11
2. MO 3,5% + HC 0,35%	16/09	21/09	21/09	24/09	30/09
3. B 3% + Ca(NO ₃) ₂ 3%	23/09	25/09	27/09	08/10	26/10
4. B 5% + Ca(NO ₃) ₂ 3%	23/09	25/09	25/09	01/10	04/10
5. B 3% + Ca(NO ₃) ₂ 5%	21/09	22/09	25/09	28/09	04/10
6. B 5% + Ca(NO ₃) ₂ 5%	21/09	25/09	26/09	11/10	26/10
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	21/09	24/09	25/09	30/09	09/10
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	21/09	22/09	25/09	28/09	04/10
	2016/201	7			
1. Control	05/10	08/10	08/10	16/10	20/10
2. MO 3,5% + HC 0,35%	12/09	17/09	21/09	30/09	06/10
3. B 3% + Ca(NO ₃) ₂ 3%	21/09	30/09	30/09	08/10	10/10
4. B 5% + Ca(NO ₃) ₂ 3%	19/09	30/09	30/09	06/10	10/10
5. B 3% + Ca(NO ₃) ₂ 5%	21/09	30/09	30/09	06/10	10/10
6. B 5% + Ca(NO ₃) ₂ 5%	21/09	30/09	30/09	05/10	15/10
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	18/09	22/09	30/09	06/10	10/10
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	18/09	20/09	25/09	21/10	08/10

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins®

Table 2. Phenological stages of 'Fuji Suprema' apple trees under the influence of compounds for bud break in four growing seasons (Date/Month). Caçador, SC, 2018

Treatments	C-C3	Bud break			
			Start	Flowering Full bloom	End
	4				
1. Control	-	07/10	07/10	15/10	22/10
2. MO 3,5% + HC 0,35%	25/09	04/10	05/10	08/10	16/10
3. B 3% + Ca(NO ₃) ₂ 3%	25/09	06/10	06/10	15/10	22/10
4. B 5% + Ca(NO ₃) ₂ 3%	25/09	03/10	05/10	08/10	15/10
5. B 3% + Ca(NO ₃) ₂ 5%	25/09	04/10	05/10	08/10	16/10
6. B 5% + Ca(NO ₃) ₂ 5%	25/09	04/10	05/10	08/10	16/10
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	25/09	03/10	06/10	08/10	12/10
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	23/09	04/10	03/10	07/10	15/10
	2014/201				
1. Control	01/10	03/10	10/10	20/10	28/10
2. MO 3,5% + HC 0,35%	25/09	27/09	30/09	04/10	08/10
3. B 3% + Ca(NO ₃) ₂ 3%	30/09	02/10	06/10	10/10	18/10
4. B 5% + Ca(NO ₃) ₂ 3%	28/09	30/09	01/10	08/10	12/10
5. B 3% + Ca(NO ₃) ₂ 5%	28/09	01/10	03/10	10/10	18/10
6. B 5% + Ca(NO ₃) ₂ 5%	29/09	30/09	03/10	10/10	15/10
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	23/09	29/09	28/09	03/10	18/10
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	27/09	30/09	03/10	10/10	18/10
	2015/201				
1. Control	25/09	25/09	26/09	-	28/10
2. MO 3,5% + HC 0,35%	17/09	20/09	18/09	25/09	30/09
3. B 3% + Ca(NO ₃) ₂ 3%	23/09	23/09	22/09	27/09	30/09
4. B 5% + Ca(NO ₃) ₂ 3%	20/09	22/09	21/09	26/09	30/09
5. B 3% + Ca(NO ₃) ₂ 5%	19/09	21/09	21/09	26/09	30/09
6. B 5% + Ca(NO ₃) ₂ 5%	17/09	21/09	18/09	26/09	30/09
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	17/09	21/09	19/09	26/09	30/09
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	19/09	21/09	21/09	26/09	30/09
	2016/201				
1. Control	24/09	03/10	26/09	06/10	14/10
2. MO 3,5% + HC 0,35%	14/09	23/09	20/09	30/09	07/10
3. B 3% + Ca(NO ₃) ₂ 3%	20/09	26/09	26/09	04/10	12/10
4. B 5% + $Ca(NO_3)_2$ 3%	18/09	23/09	25/09	30/09	07/10
5. B 3% + Ca(NO ₃) ₂ 5%	14/09	25/09	25/09	01/10	05/10
6. B 5% + $Ca(NO_3)_2$ 5%	14/09	24/09	20/09	30/09	05/10
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	14/09	20/09	20/09	30/09	04/10
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	15/09	25/09	25/09	01/10	07/10

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins®

The bud break promoters maximized the axillary bud break at 30 and 60 DADB for 'Maxi Gala' and 'Fuji Suprema' apple trees in the four growing seasons (Tables 3 and 4). According to Petri [26], the rate of axillary bud break is the variable that better expresses the effectiveness of bud break promoters, and can be used as indicative of cultivar adaptation to local environmental conditions. However, the efficiency of bud break promoters depends, in addition to the cultivar, on the vigour of the plant, time of application and concentration of the bud break promoter.

For 'Maxi Gala' cultivar, the treatment mineral oil + hydrogen cyanamide showed higher axillary bud break in relation to the other treatments at 30 and 60 DADB in the four growing seasons. The treatments Bluprins® $3\% + \text{Ca}(\text{NO}_3)_2 \ 3\%$ and Bluprins® $5\% + \text{Ca}(\text{NO}_3)_2 \ 3\%$ differed from the other treatments in the 2013/2014 growing season. In 2014/2015 and 2016/2017, there were no significant differences between Bluprins® treatments and the control treatment. In 2015/2016, the treatment Bluprins® $3\% + \text{Ca}(\text{NO}_3)_2 \ 5\%$ provided higher axillary bud break in relation to the control treatment and other Bluprins® treatments (Table 3).

For 'Fuji Suprema' apple trees at 30 and 60 DADB, all treatments showed higher axillary bud break compared to the control treatment in the 2013/2014 growing season, without differences among them. Pasa et al. [10] reported that a nutritive solution containing calcium nitrate and mineral oil showed similar kind of effects to hydrogen cyanamide on axillary bud break of this cultivar. In 2014/2015, except for the treatment Bluprins[®] 3% + Ca(NO₃)₂ 3%, all treatments presented higher axillary bud break compared to the control treatment, for this cultivar. The treatment mineral oil + hydrogen cyanamide presented the highest axillary bud break. followed by Bluprins® 5% + Ca(NO₃)₂ 3%. In 2015/2016, the treatment Bluprins $Ca(NO_3)_2$ 4% + NH_4 (NO_3) 4% was superior to the other Bluprins® treatments at 30 DADB. At 60 DADB, this treatment and Bluprins® 3% + $Ca(NO_3)_2$ 3% + NH_4 (NO_3) 3% treatment were superior to the other Bluprins® treatments. However, both treatments were lower than the treatment of mineral oil + hydrogen cyanamide at 30 and 60 DADB. In 2016/2017, the treatment Bluprins[®] 5% + Ca(NO₃)₂ 4% + NH₄ (NO₃) 4% was superior to the other Bluprins® treatments at 30 and 60 DADB, and did not differ from the treatment mineral oil + hydrogen cyanamide (Table 4).

Hawerroth et al. [27] reported lower axillary bud break in 'Imperial Gala' than in 'Fuji Suprema' cultivar, corroborating the higher difficulty in inducing bud break in 'Gala' cultivars. The low axillary bud break, similar to that verified in this work, has already been endorsed by Leite et al. [28], conclude that temperate fruit trees cultivated in subtropical climate conditions, where the chilling requirement is not satisfied, represent low bud break levels associated with high bud break and flowering heterogeneity along the branches.

For 'Maxi Gala' apple trees, considering terminal bud break, the treatment mineral oil + hydrogen cyanamide showed higher values than that of other treatments at 30 and 60 DADB in the 2013/2014 and 2015/2016 growing seasons. This treatment and treatment of Bluprins® 3% + Ca (NO₃)₂ 3%, achieved higher terminal bud break than the other treatments at 30 DADB in 2014/2015 and at 60 DADB in 2016/2017. The Bluprins® treatments were superior to the control at 30 DADB in 2013/2014, 2014/2015 and 2015/2016. In 2016/2017, all the Bluprins® treatments were superior to the control at 30 and 60 DADB, and at 30 DADB, they did not differ from the treatment mineral oil + hydrogen cyanamide (Table 5). Marchi et al. [29] reported that even terminal buds, required low stimulus to break the dormancy, [30] only showed a high bud break by the application of mineral oil + hydrogen cyanamide. However, Pasa et al. [10] endorsed no significant differences between plants treated with hydrogen cvanamide, a nutrient solution containing calcium nitrate and control (untreated) plants, considering terminal bud break.

Table 3. Axillary bud break (%) of 'Maxi Gala' apple trees under the influence of compounds for bud break during four growing seasons. Caçador, SC, 2018

Treatments	Axillary bud break (%)									
	2013/2	014	2014/2	015	2015/2	2015/2016		017		
	30	60	30	60	30	60	30	60		
	DADB	DADB	DADB	DADB	DADB	DADB	DADB	DADB		
1. Control	1.1 c	3.8 c	1.2 b	4.9 b	0.9 c	1.8 c	0.0 b	1.0 b		
2. MO 3,5% + HC 0,35%	41.5 a	43.8 a	20.9 a	26.0 a	23.0 a	25.7 a	13.5 a	24.4 a		
3. B 3% + Ca(NO ₃) ₂ 3%	22.0 b	23.7 b	2.5 b	9.5 b	2.2 c	4.6 c	0.9 b	2.0 b		
4. B 5% + Ca(NO ₃) ₂ 3%	22.4 b	25.3 b	3.9 b	7.4 b	1.8 c	1.8 c	3.1 b	3.8 b		
5. B 3% + Ca(NO ₃) ₂ 5%	9.2 c	12.5 c	3.8 b	10.0 b	8.5 b	9.8 b	5.7 b	7.7 b		
6. B 5% + Ca(NO ₃) ₂ 5%	16.6 c	19.6 c	6.7 b	10.2 b	3.1 c	4.7 c	3.5 b	5.3 b		
7. B 3%+Ca(NO ₃) ₂ 3%	11.8 c	16.5 c	3.8 b	5.9 b	1.6 c	3.5 c	3.5 b	7.6 b		
+NH ₄ (NO ₃) 3%										
8. B 5%+Ca(NO ₃) ₂ 4%	8.2 c	9.8 c	5.1 b	10.7 b	2.2 c	3.1 c	3.5 b	4.9 b		
+NH ₄ (NO ₃) 4%										
CV (%)	72.2	61.4	55.8	33.2	66.2	57.6	69.0	43.0		

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins[®]. DADB: Days after dormancy breaking; CV: coefficient of variation. Means followed by same letter do not differ by Scott-Knott test at 5% probability

Table 4. Axillary bud break (%) of 'Fuji Suprema' apple trees under the influence of compounds for bud break during four growing seasons. Caçador, SC, 2018

Treatments	Axillary bud break (%)								
	2013/2014		2014/20	15	2015/20	2015/2016		17	
	30	60	30	60	30	60	30	60	
	DADB	DADB	DADB	DADB	DADB	DADB	DADB	DADB	
1. Control	3.3 b	12.6 b	0.0 d	5.2 d	0.0 d	4.4 c	15.2 c	22.6 c	
2. MO 3,5% + HC 0,35%	53.1 a	60.2 a	48.0 a	51.9 a	50.1 a	50.1 a	65.5 a	81.0 a	
3. B 3% + Ca(NO ₃) ₂ 3%	39.3 a	43.0 a	2.6 d	7.5 d	2.9 d	5.7 c	20.2 c	25.8 c	
4. B 5% + Ca(NO ₃) ₂ 3%	52.7 a	59.4 a	22.3 b	30.3 b	5.1 c	7.3 c	32.3 b	48.4 b	
5. B 3% + Ca(NO ₃) ₂ 5%	48.0 a	58.5 a	14.4 c	20.3 c	1.6 d	7.2 c	41.2 b	45.9 b	
6. B 5% + Ca(NO ₃) ₂ 5%	56.1 a	62.3 a	11.3 c	14.2 c	0.6 d	4.6 c	33.4 b	37.2 b	
7. B 3%+Ca(NO ₃) ₂ 3%	72.4 a	73.8 a	13.7 c	20.0 c	7.9 c	11.0 b	25.2 c	29.2 c	
+NH ₄ (NO ₃) 3%									
8. B 5%+Ca(NO ₃) ₂ 4%	55.6 a	71.3 a	11.4 c	14.9 c	18.8 b	19.9 b	67.3 a	70.9 a	
+NH ₄ (NO ₃) 4%									
CV (%)	27.8	27.9	47.2	31.0	49.0	35.4	25.5	21.5	

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins[®]. DADB: Days after dormancy breaking; CV: coefficient of variation. Means followed by same letter do not differ by Scott-Knott test at 5% probability

For 'Fuji Suprema' apple trees, all the treatments showed higher terminal bud break compared to the control at 30 DADB in 2013/2014. At 60 DADB, the treatments Bluprins® 3% + Ca(NO₃)₂ 3% and Bluprins® 3% + Ca(NO₃)₂ 5% provided lower terminal bud break than the other Bluprins® treatments and the treatment mineral oil + hydrogen cyanamide was superior to the other treatments in 2014/2015 at 30 DADB, whereas the treatment Bluprins® 3% + Ca(NO₃)₂ 3% was superior to the control treatment and other Bluprins® treatments. At 60 DADB, the treatments mineral oil + hydrogen cyanamide,

Bluprins® $3\% + Ca(NO_3)_2$ 3% and Bluprins® $5\% + Ca(NO_3)_2$ 3% were superior to the other treatments. In 2015/2016, at 60 DADB, the treatments Bluprins® $3\% + Ca(NO_3)_2$ 5%, Bluprins® $5\% + Ca(NO_3)_2$ 3% and Bluprins® $5\% + Ca(NO_3)_2$ $4\% + NH_4(NO_3)$ 4% did not differ from the control, while the other treatments were superior to the control, not differing from the treatment mineral oil + hydrogen cyanamide. In 2016/2017, only the treatment Bluprins® $5\% + Ca(NO_3)_2$ 3% did not differ from the control treatment. The other treatments presented higher terminal bud break and did not differ from each other at 30 and 60 DADB (Table 6).

Table 5. Terminal bud break (%) of 'Maxi Gala' apple trees under the influence of compounds for bud break during four growing seasons. Caçador, SC, 2018

Treatments	Terminal bud break (%)								
	2013/20	14	2014/20	15	2015/20	16	2016/20	2016/2017	
	30	60	30	60	30	60	30	60	
	DADB	DADB	DADB	DADB	DADB	DADB	DADB	DADB	
1. Control	15.9 c	60.3 b	12.6 c	52.9 ^{ns}	8.2 c	35.6 b	26.7 b	45.7 c	
2. MO 3,5% + HC 0,35%	96.1 a	96.4 a	71.8 a	77.1	80.6 a	90.8 a	77.2 a	90.6 a	
3. B 3% + Ca(NO ₃) ₂ 3%	57.8 b	57.5 b	43.9 b	68.1	27.9 b	49.2 b	55.4 a	66.8 b	
4. B 5% + Ca(NO ₃) ₂ 3%	46.8 b	68.4 b	36.0 b	59.2	37.6 b	61.2 b	53.4 a	70.1 b	
5. B 3% + Ca(NO ₃) ₂ 5%	42.2 b	60.3 b	32.0 b	72.1	38.4 b	60.8 b	59.5 a	83.5 a	
6. B 5% + Ca(NO ₃) ₂ 5%	68.3 b	78.5 b	45.9 b	63.2	34.5 b	65.1 b	57.7 a	69.6 b	
7. B 3%+Ca(NO ₃) ₂ 3%	62.9 b	71.9 b	51.3 b	73.3	33.4 b	44.0 b	63.1 a	72.8 b	
+NH ₄ (NO ₃) 3%									
8. B 5%+Ca(NO ₃) ₂ 4%	51.0 b	64.8 b	37.3 b	71.7	29.6 b	56.7 b	65.4 a	74.2 b	
+NH ₄ (NO ₃) 4%									
CV (%)	24.8	17.9	29.8	18.3	26.1	21.9	24.2	15.6	

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins[®]. DADB: Days after dormancy breaking; CV: coefficient of variation. Means followed by same letter do not differ by Scott-Knott test at 5% probability. ns: not significant

Table 6. Terminal bud break (%) of 'Fuji Suprema' apple trees under the influence of compounds for bud break during four growing seasons. Caçador, SC, 2018

Treatments	Terminal bud break (%)							
	2013/2	014	2014/20	015	2015/20	016	2016/2017	
	30	60	30	60	30	60	30	60
	DADB	DADB	DADB	DADB	DADB	DADB	DADB	DADB
1. Control	46.7 c	80.2 b	24.3 c	80.3 b	30.9 ^{ns}	83.6 b	69.2 b	82.6 b
2. MO 3,5% + HC 0,35%	89.7 a	94.0 a	86.3 a	96.2 a	73.3	99.4 a	95.0 a	97.7 a
3. B 3% + Ca(NO ₃) ₂ 3%	68.5 b	86.2 b	59.2 b	87.5 a	52.5	98.3 a	78.5 b	91.3 b
4. B 5% + Ca(NO ₃) ₂ 3%	88.9 a	100 a	44.1 c	88.2 a	53.1	97.0 a	95.1 a	97.8 a
5. B 3% + Ca(NO ₃) ₂ 5%	74.5 b	87.9 b	44.9 c	77.8 b	48.4	93.6 b	95.3 a	95.8 a
6. B 5% + Ca(NO ₃) ₂ 5%	95.2 a	97.8 a	29.2 c	82.7 b	46.1	92.3 b	91.0 a	97.5 a
7. B 3%+Ca(NO ₃) ₂ 3%	96.3 a	99.6 a	37.8 c	70.0 b	63.2	95.6 a	92.6 a	98.2 a
+NH ₄ (NO ₃) 3%								
8. B 5%+Ca(NO ₃) ₂ 4%	90.7 a	99.4 a	36.0 c	81.2 b	59.9	80.3 b	96.2 a	99.3 a
+NH ₄ (NO ₃) 4%								
CV (%)	16.0	7.1	28.1	14.1	21.9	12.2	13.1	10.7

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins[®]. DADB: Days after dormancy breaking; CV: coefficient of variation. Means followed by same letter do not differ by Scott-Knott test at 5% probability. ^{ns}: not significant

For both 'Maxi Gala' and 'Fuji Suprema' apple trees, the high terminal bud break confirms the excellent efficiency of Bluprins[®] associated with calcium nitrate regardless of its concentration.

For 'Maxi Gala' apple trees, considering fruit set, the treatments Bluprins® 3% + Ca(NO₃)₂ 3% and Bluprins[®] $3\% + Ca(NO_3)_2 \ 3\% + NH_4(NO_3) \ 3\%$ were superior to the other treatments in the 2015/2016 growing season; in 2013/2014, 2014/2015 and 2016/2017, the treatments showed no significant differences (Table 7). For 'Fuji Suprema' apple trees and fruit set, the treatments Bluprins[®] 5% + Ca(NO₃)₂ 3% and Bluprins[®] 3% + Ca(NO₃)₂ 3% + NH₄(NO₃) 3% did not differ from the control treatment and were significantly superior to the other treatments, in 2013/2014; in 2014/2015 and 2015/2016, the treatments showed no differences, and in 2016/2017, the control treatment showed a higher fruit set in relation to the other treatments (Table 8). El-Agamy et al. [31] endorsed a negative effect of the treatments with hydrogen cyanamide on the fruit set for Anna cultivar. Erez [14], Petri and Leite [7] argued the possibility of reduction in the fruit set when bud break are promoters applied due to synchronisation of the pollination between cultivars under conditions of insufficient chilling accumulation during the winter period. The fruit set may also be reduced due to weather conditions that affect the activity of pollinators and the pollen viability [32]. However, the high fruit set values obtained in some treatments

indicates that there were no problems related to pollination and that the concentration of the flowering period for the treatments with bud break promoters did not reduce the fruit set, even though the flowering period was more concentrated in the treatments with bud break promoters in comparison to the control treatment. According to Erez [14], the use of bud break promoters may resulted in a drastic reduction of the fruit set due to the nutritional competition established between vegetative and reproductive sinks. The Bluprins® treatments were equal or superior to the treatment of mineral oil + hydrogen cyanamide for fruit set of both cultivars in all the growing seasons.

For 'Maxi Gala' apple trees, considering the fruit production per tree, the treatments Bluprins® 5% + Ca(NO₃)₂ 3%, Bluprins® 3% + Ca(NO₃)₂ 3%+ NH₄(NO₃) 3% and Bluprins® 5% + Ca(NO₃)₂ 4%+ NH₄(NO₃) 4% resulted in higher values compared to the other treatments, in the 2013/2014 growing season. Apple trees treated with Bluprins® 3% + Ca(NO₃)₂ 3%+ NH₄ (NO₃) 3% produced 19.9 kg tree $^{-1}$ and the control treatment, 9.0 kg tree $^{-1}$, an increase of 121.1%. There were no significant differences between treatments in the 2014/2015 growing season (Table 9). The harvesting was not evaluated in 2015/2016. In 2016/2017 growing season, the treatments Bluprins® 3% + Ca(NO₃)₂ 3% + NH₄(NO₃) 3% and mineral oil + cyanamide hydrogen resulted in higher fruit production per tree than the other treatments.

Table 7. Fruit set (%) of 'Maxi Gala' apple trees under the influence of compounds for bud break during four growing seasons. Caçador, SC, 2018

Treatments	Fruit set (%)							
	2013/2014	2014/2015	2015/2016	2016/2017				
1. Control	16.8 ^{ns}	11.7 ^{ns}	12.8 b	2.2 ^{ns}				
2. MO 3,5% + HC 0,35%	21.8	9.5	6.9 b	1.0				
3. B 3% + Ca(NO ₃) ₂ 3%	23.4	10.4	86.7 a	37.5				
4. B 5% + Ca(NO ₃) ₂ 3%	30.2	13.4	43.3 b	23.3				
5. B 3% + Ca(NO ₃) ₂ 5%	17.7	4.8	25.7 b	4.7				
6. B 5% + Ca(NO ₃) ₂ 5%	20.9	29.6	3.9 b	1.5				
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	20.1	10.6	140.7 a	11.2				
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	28.0	15.6	40.0 b	10.4				
CV (%)	69.2	51.8	102.1	161.0				

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins[®]. CV: coefficient of variation. Means followed by same letter do not differ by Scott-Knott test at 5% probability. ^{ns}: not significant

Table 8. Fruit set (%) of 'Fuji Suprema' apple trees under the influence of compounds for bud break during four growing seasons. Caçador, SC, 2018

Treatments	Fruit Set (%)							
	2013/2014	2014/2015	2015/2016	2016/2017				
1. Control	380.0 a	242.0 ^{ns}	159.3 ^{ns}	359.4 a				
2. MO 3,5% + HC 0,35%	181.9 b	205.5	76.9	109.6 b				
3. B 3% + $Ca(NO_3)_2$ 3%	260.2 b	266.3	95.6	208.7 b				
4. B 5% + $Ca(NO_3)_2$ 3%	300.5 a	163.3	65.9	70.5 b				
5. B 3% + Ca(NO ₃) ₂ 5%	192.8 b	257.9	49.9	191.8 b				
6. B 5% + $Ca(NO_3)_2$ 5%	224.9 b	168.3	61.2	200.5 b				
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	304.3 a	164.7	37.3	148.6 b				
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	170.1 b	173.4	29.0	148.3 b				
CV (%)	34.5	39.9	57.1	32.1				

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins[®]; CV: coefficient of variation; ^{ns}: not significant at 5% probability

For 'Fuji Suprema' apple trees and fruit production per tree, the treatments Bluprins $^{\circ}$ 3% + Ca(NO₃)₂ 3% and Bluprins $^{\circ}$ 3% + Ca(NO₃)₂ 3%+ NH₄(NO₃) 3% resulted in lower fruit production per tree than that of other treatments and did not differ from the control treatment in the 2013/2014 growing season. In the 2014/2015 growing season, except for the treatment Bluprins $^{\circ}$ 3% + Ca(NO₃)₂ 3%+ NH₄(NO₃) 3%, the treatments resulted in lower production of fruit per tree in compared to the control treatment. In 2015/2016, the harvest was not evaluated and in 2016/2017, the treatments did not differ from each other (Table 10).

According to Botelho et al. [33], the response of bud break promoters on fruit production is dependent on the climatic conditions during the fruit development period. Once the climatic conditions were adequate during this period in the growing seasons studied, there was no marked difference in the fruit production per tree by the use of bud break promoters, for both cultivars.

The average fruit weight did not showed significant differences between treatments in the 2013/2014 and 2016/2017 growing seasons for 'Maxi gala', and for 'Fuji Suprema' cultivar andinsignificant between treatments in 2013/2014, 2014/2015 and 2015/2016. For 'Maxi Gala' apple trees in the 2014/2015 growing season, the treatments Bluprins $^{\$}$ 5% + Ca(NO₃)₂ 3%, Bluprins $^{\$}$ 3% + Ca(NO₃)₂ 3%+ NH₄(NO₃) 3% and Bluprins $^{\$}$ 5% + Ca(NO₃)₂ 4%+ NH₄(NO₃) 4% did not differ from the control treatment and resulted in lower values than the other treatments (Tables 9 and 10).

It was observed that there were some differences between 'Maxi Gala' and 'Fuji Suprema' cultivars, such as the flowering period, lower in 'Maxi Gala', and the percentage of buds sprouted, higher in 'Fuji Suprema'. However, both cultivars presented similar kind of behaviour in response to climatic factors such as the anticipated beginning of bud break and flowering in the growing seasons with colder winter, and higher bud break by the application of bud break

Table 9. Fruit production per plant (FPP, kg) and average fruit weight (AFW, g) of 'Maxi Gala' apple trees under the influence of compounds for bud break during tree growing seasons.

Caçador, SC, 2018

Treatments	2013/2014		2014	2015	2016/2017	
	FPP (kg)	AFW (g)	FPP (kg)	AFW (g)	FPP (kg)	AFW (g)
1. Control	9.0 b	145.1 ^{ns}	8.0 ^{ns}	120.9 b	4.7 b	132.8 ^{ns}
2. MO 3,5%+HC 0,35%	3.9 b	137.4	13.1	138.0 a	8.4 a	139.7
3. B 3% + Ca(NO ₃) ₂ 3%	9.4 b	165.3	10.1	133.0 a	5.0 b	154.3
4. B 5% + Ca(NO ₃) ₂ 3%	13.9 a	141.3	13.6	118.6 b	4.9 b	148.3
5. B 3% + Ca(NO ₃) ₂ 5%	16.1 a	142.6	11.0	130.3 a	3.7 b	149.4
6. B 5% + Ca(NO ₃) ₂ 5%	9.6 b	149.6	14.6	130.1 a	6.8 b	151.8
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	19.9 a	140.6	15.2	121.4 b	10.5 a	150.1
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	16.6 a	140.0	12.8	119.2 b	6.0 b	140.9
CV (%)	33.2	11.2	39.8	8.1	52.1	12.4

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins®; CV: coefficient of variation. Means followed by same letter do not differ by Scott-Knott test at 5% probability. ns: not significant

Table 10. Fruit production per plant (FPP, kg) and average fruit weight (AFW, g) of 'Maxi Gala' apple trees under the influence of compounds for bud break during tree growing seasons.

Caçador, SC, 2018

Treatments	2013/2014		2014	2015	2016/2017*	
	FPP (kg)	AFW (g)	FPP (kg)	AFW (g)	FPP (kg)	AFW (g)
1. Control	25.9 b	123.6 ^{ns}	35.1 a	129.2 ^{ns}	19.6 ^{ns}	101.7 ^{ns}
2. MO 3,5%+HC 0,35%	35.6 a	113.9	19.7 b	120.5	11.8	118.2
3. B 3% + Ca(NO ₃) ₂ 3%	23.4 b	116.7	15.4 b	138.0	14.6	102.2
4. B 5% + Ca(NO ₃) ₂ 3%	34.5 a	120.3	23.0 b	124.8	15.4	111.1
5. B 3% + Ca(NO ₃) ₂ 5%	40.6 a	118.7	17.8 b	126.6	14.4	104.6
6. B 5% + Ca(NO ₃) ₂ 5%	42.7 a	113.6	19.6 b	125.5	17.9	102.5
7. B 3%+Ca(NO ₃) ₂ 3%+NH ₄ (NO ₃) 3%	14.2 b	125.9	37.3 a	122.7	11.8	114.8
8. B 5%+Ca(NO ₃) ₂ 4%+NH ₄ (NO ₃) 4%	38.0 a	111.3	13.3 b	122.1	15.2	112.0
CV (%)	31.9	118.0	22.7	126.2	33.1	12.2

MO: Mineral oil; HC: Hydrogen cyanamide; B: Bluprins®; CV: coefficient of variation. Means followed by same letter do not differ by Scott-Knott test at 5% probability. ns: not significant

promoters, especially mineral oil + hydrogen cyanamide.

4. CONCLUSION

Bluprins® in combination with calcium nitrate and ammonium nitrate proved effective in inducing bud break of 'Maxi Gala' and 'Fuji Suprema' apple tree cultivars under mild winter conditions, however, this is less effective than mineral oil + hydrogen cyanamide. Bluprins® in combination with calcium nitrate and ammonium nitrate anticipates the bud break and flowering period and reduces the flowering period for 'Maxi Gala' and 'Fuji Suprema' apple tree cultivars, in compared to plants without application of bud break promoters. Bluprins® does not affect the fruit set considerably and fruit production of 'Maxi Gala' and 'Fuji Suprema' apple tree cultivars.

COMPETING INTERESTS

Authors have declared competing interest for the company name "Bluprins®".

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