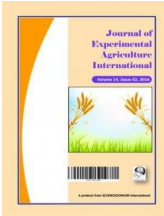

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Influence of Post-Flowering Chemical Thinning on Development and Fruit Quality of 'Fuji Suprema' and 'Maxigala' Apple Trees

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Authors' contributions

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

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ABSTRACT

Aims: Evaluate possible alterations in the development, anatomy and quality of fruits from 'Maxi Gala' and 'Fuji Suprema' apple plants treated with Metamitrom (MM) and Benzyladenine (BA) alone or in combination, applied at different stages of fruit development in the Midwestern region of the state of Santa Catarina.
Study Design: The experiment was arranged in a randomized block design with five replicates.
Place and duration of Study: The experiment was carried out in the municipality of Caçador-SC, Brazil, during the growing season of 2016/2017.
Methodology: Two products, BA (with hormonal action) and MM (photosynthesis inhibitor) were applied individually or in a tank mix in post-flowering periods (in fruits of 5-10 mm and 15-20 mm in equatorial diameter), which were compared with plants with no thinning and manual thinning of both cultivars. Treatments were as follows: Control (no thinning) MM, BA, MM + BA (in fruits of 5-10 mm

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in diameter); MM, BA, MM + BA, manual thinning (in fruits of 15-20 mm in diameter). The phenological stage of F2 (Full flowering (FF) was observed on September 28, 2016 for both cultivars. Evaluations of the anatomical characteristics of fruits at the time of harvest did not show any alteration in cell size (mean cell area - μm^2) and cell density (number of cells per fruit section area); the mean fruit mass was higher in MM 350 mg L^{-1} (5-10 mm), MM 350 mg L^{-1} + BA 40 mg L^{-1} (5-10 mm), and MM 350 mg L^{-1} (15-20mm) treatments. In the 'Fuji Suprema' cultivar, except for control treatments, MM 350 mg L^{-1} (5-10mm) and BA 80 mg L^{-1} (5-10mm), a significant reduction in production was observed (kg $plant^{-1}$ and $fruit^{-1}$). While in 'MaxiGala', all the thinning treatments reduced yield per plant. The cultural thinning practice, fruit load management, adapting the number of fruits according to plant size is the most important factor that producers are able to influence. Thinning reduces fruit load in the plant, which allows remaining fruits the possibility of greater growth and size gain. The combination of MM + BA is efficient in the thinning of apple fruits, especially when applied at the beginning of fruit development (5-10 mm in diameter). The application of chemical thinners, such as MM and BA, may influence the early stages of fruit development.

Keywords: Histology; productive capacity; cell density.

1. INTRODUCTION

In Brazil, the main cultivars produced belong to the groups Gala, Fuji and their colorful clones [1], whose plantations are located and restricted to regions with the highest winter cold.

The apple is included as the second most produced fruit in the world, whose production was estimated at 83.1 million tons, being surpassed only by the banana. Worldwide, China has a prominent position in production, reaching 49.8% of the total production, with around 41.4 million tons. According to Faostat [2], in Brazil production in 2017 was around 1.3 million tons. The states of Santa Catarina and Rio Grande do Sul account for 96% of the Brazilian production of apples. Where the apple tree culture involves more than 2,300 Brazilian producers, being 1,627 in Santa Catarina, 700 in Rio Grande do Sul, and 100 in Paraná [3]. The outflow of apple production is divided by internal natura consumption, representing 67.6%, external raw consumption only 7% of the total commercialized, and industrialization represents 25.4% of this total [4].

In apple trees, the fruit thinning practice is necessary to balance vegetation / production, maintain regular production, avoid alternating production and increase fruit size and quality, as well as fruit distribution into classes of higher commercial value [5]. Plant load adjustment presents the best results when carried out in the early stages of fruit development [6]; however, the cost is high due to the great need of manual labor in a short period of time. In this way, chemical thinning is a cheaper alternative to producers when properly applied [7].

The mode of action of the product can determine the chemical thinning efficiency [8]. For example, MM is a photosynthesis inhibitor and its thinning effect is dose dependent [9,10,11] and apple cultivars respond differently to the product [12]; thus, dose adjustment and application time should be specific for each cultivar. In addition, it should be considered that the MM efficacy as thinner can be variable in different years and within the same year [13], and due to environmental conditions such as low luminosity and mainly increase in night temperature after application, fruit fall may be intensified [14].

Products with hormonal action, such as BA have the ability to stimulate fruit growth and vegetative activity, influence cell division, increasing competition for assimilates, reducing the energy available for fruit development, and reducing the rate of net CO2 assimilation, which results in increased fruit fall [15]. Cytokinins increase fruit size in apple trees, even in the absence of thinning due to the promotion of cell division in apple tissues [16].

The final fruit size is determined by the coordinated progression of cell division and cell expansion during fruit growth and development [17]. The cell division and elongation period is limited by the expression of specific genes associated with the synthesis of enzymes involved in such physiological processes [18,19]. The genetic factor will determine the final fruit size, but can be influenced by the nutritional state of plants, cultural treatments and environmental factors [20]. The possible anatomical and histological interferences caused in fruits by the application of chemical products for load adjustment are still little known.

The aim of the present study was to identify possible alterations in development, anatomy and quality of fruits from 'MaxiGala' and 'Fuji Suprema' apple trees treated with MM and BA alone or in combination, applied at different stages of fruit development in the Midwestern region of the state of Santa Catarina.

2. MATERIALS AND METHODS

The study was conducted in an experimental orchard in the Midwestern region of Santa Catarina, in the municipality of Caçador (latitude 26º46 'S, longitude 51º W, altitude 960 meters), in the 2016/17 season. Apple trees of "Fuji Suprema" and "Maxi Gala" cultivars, with Marubakaido / M-9 rootstock and planting density of 2,500 ha^{-1} plants were used, both conducted in the central-leader system.

The experiment was conducted in a randomized blocks experimental design consisting of 8 treatments with five replicates, the experimental unit consisting of one plant, totaling 80 plants, 40 plants per cultivar. Two products, BA (with hormonal action) and MM (photosynthesis inhibitor) were applied individually or in a tank mix in post-flowering periods (in fruits of 5-10 mm and 15-20 mm in equatorial diameter), which were compared with plants with no thinning and manual thinning of both cultivars. Treatments were as follows: T1-Control (no thinning); T2-MM, T3-BA, T4-MM + BA (in fruits of 5-10 mm in diameter on October 14, 2016); T5-MM, T6-BA, T7-MM + BA, T8-manual thinning (in fruits of 15-20mm in diameter on October 28,2016). The phenological stage of F2 (FF) was observed on September 28, 2016 for both cultivars.

The concentrations adopted were: BA 80 mg L^{-1} for 'Fuji Suprema' and 40mg L^{-1} for 'Maxi Gala'; MM 350 mg L^{-1} for both cultivars. Commercial product MaxCel® (2% BA) was used as source of 6-benzyladenine (BA), and as source of Metamitron, commercial product Goltix® 700 WG was used. Products were applied with a motorized costal spray (20L) with tip containing three fan-type D-S nozzles, with average flow rate of 1000 L Ha^{-1}. Manual thinning adopted the criterion of two fruits in twigs and one fruit in spur, and in the chemical thinning, no manual thinning complement was performed.

The variables evaluated were: fruit growth rate; production (kg $plant^{-1}$ and fruits $plant^{-1}$); productive efficiency (kg cm^{-2} and fruits cm^{-2}); average fresh fruit mass (g); classification of fruits by size classes (%); fruit color (%); number of seeds per fruit; pulp firmness (lb $inch^{-2}$) and soluble solids (° Brix) according to Scolaro et al. [21]; density and cell area; and mineral content of fruits [22].

The fruit growth rate was determined by the weekly measurement of ten fruits per plant (duly identified). The equatorial measure of the fruits was taken with digital pachymeter, always in the same position (region of the fruit marked with permanent writing pen). The gain in diameter at each reading was divided by the number of days referring to the period between the readings.

For the anatomical analyses of fruits, two fruits per plant were collected during the cycle, only of terminal twig buds. After collection, samples were fixed in FAA solution (Formalin / acetic acid / ethyl alcohol 1: 1: 8); fractionated and processed; included in historesin; submitted to microtomy, confection and staining of microscopy slides; capture and analysis of images in specific software.

Fruit collections were performed according to the following scheme:

1. First collection: Fruits T1, T2, T3 and T4 (20 days after full flowering (DAFF) October 18, 2016 fruits with 5-10 mm in diameter (4 days after first application).
2. Second collection: Fruits T1, T2, T3, T4, T5, T6, T7 and T8. (33 DAFF) October 31, 2016 fruits with 15-20 mm in diameter (3 days after second application).
3. Third collection: Fruits T1, T2, T3, T4, T5, T6, T7 and T8. Harvest (149 DAFF 'Fuji Suprema'); (133 DAFF 'Maxi Gala').

Fruit growth rate was determined by the marking of five fruits per plant, only previously identified twig fruits, and the equatorial diameter of fruits was weekly measured with the aid of a pachymeter.

Statistical analysis of data was performed through analysis of variance, and variables whose results revealed significance ($P<0.05$) were submitted to comparison of means by the Scott-Knott test at 5% probability. Statistical analyses were performed by the Sisvar software, version 5.6 [23].

3. RESULTS AND DISCUSSION

The fruit growth rate (mm/day) was quite variable among cultivars, and the load adjustment treatments showed a certain influence on fruit development (Fig. 1). In the 'Fuji Suprema' cultivar, ununiform fruit growth was observed among plants during the cycle; however, MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10mm) treatment showed fruits with the largest diameters at the time of harvest, while the other treatments did not differ from each other or from control (without thinning). For 'Maxi Gala' cultivar, the fruit growth rate was also quite variable among treatments during the evaluation period; however, at the time of harvest, it was observed that fruits from plants treated with MM 350 mg L^{-1} (5-10 mm) showed greater diameter gain compared to the other treatments, which results were even higher than those observed in manual thinning treatment. This interference in fruit development was also observed by Rosa et al., [4], who reported an increase in the growth rate of 'Royal Gala', 'Cripps Pink' and 'Red Delicious' fruits treated with MM 165mg L^{-1} at 8-12 mm in diameter, and the increase in final fruit size was higher than plants treated with BA 150 mg L^{-1}.

In 'Fuji Suprema' cultivar, except for control, MM 350 mg L^{-1} (5-10 mm) and BA 80 mg L^{-1} (5-10 mm) treatments, significant production reduction was observed (kg $plant^{-1}$ and fruits $plant^{-1}$). Among treatments evaluated, the combination of MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10 mm) provided the highest fruit mass (g $fruit^{-1}$), followed by MM 350 mg L^{-1} (5-10 mm), MM 350mg L^{-1} (15-20 mm) and MM 350 mg L^{-1} + BA 80mg L^{-1} (15-20 mm) treatments. With the exception of BA 80 mg L^{-1} (5-10 mm), all plants that received load adjustment treatments were superior to control treatment (without thinning), that is, they showed fruit mass gain (Table 1).

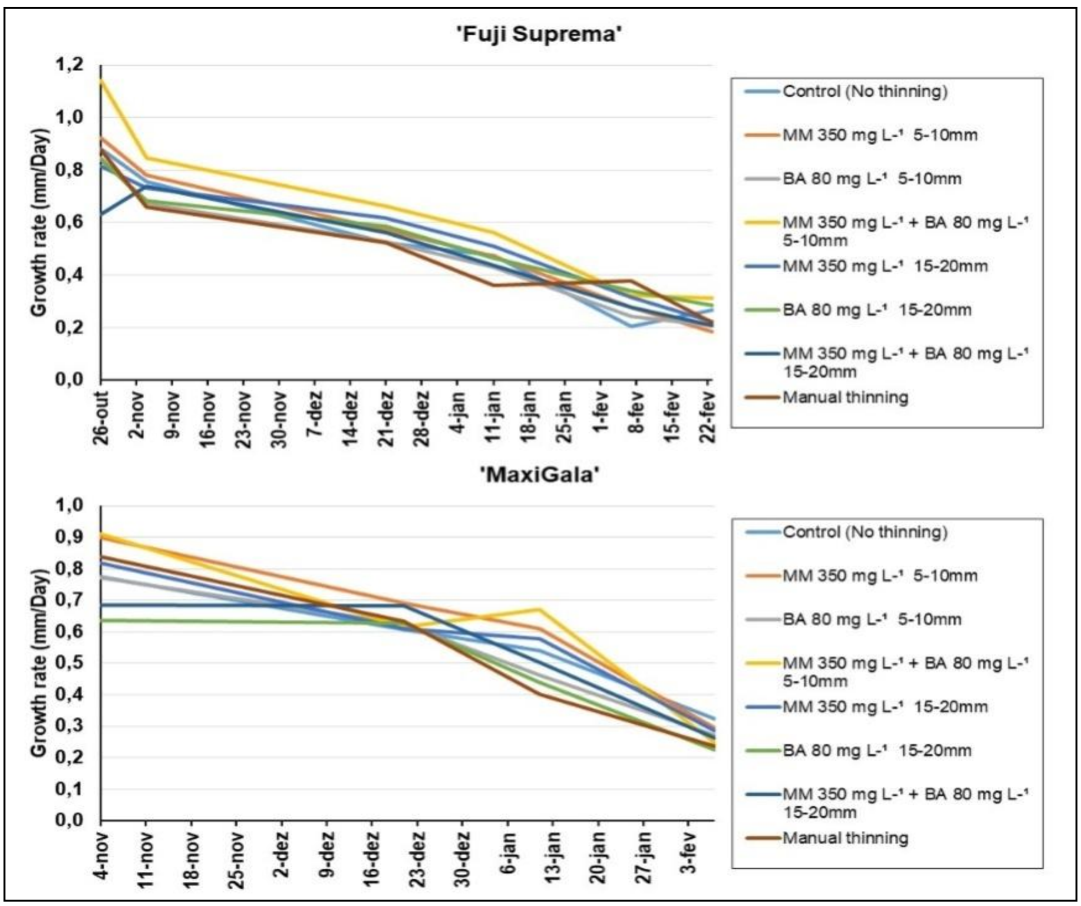

Fig. 1. Fruit growth rate (mm/day) of 'Fuji Suprema' and 'Maxi Gala' apple trees submitted to different load adjustment treatments. 2016/2017 agricultural years with BA and MM application, in the 2016/2017 season, Caçador-SC, Brazil, 2019

MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10 mm); MM 350 mg L^{-1} (15-20 mm); BA 80 mg L^{-1} (15-20 mm); MM 350 mg L^{-1} + BA 80 mg L^{-1} (15-20 mm) and manual thinning treatments reduced the productive efficiency of plants (kg cm^{-2} and fruits cm^{-2}), being efficient in adjusting fruit load. The fruit size was improved in some treatments (Table 2), especially MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10 mm), which resulted in higher allocation of fruits into the greatest size category (<135) and significantly reduced the percentage of small fruits (> 180). Plants with no thinning, BA 80 mg L^{-1} (5-10 mm), BA 80 mg L^{-1} (15-20 mm) and manual thinning showed the highest percentages of small fruits and low percentage of large-sized fruits, when compared to other treatments. However, the percentage of fruits produced and allocated into the intermediate size category was similar in all treatments.

The physicochemical characteristics of fruits were improved in MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10 mm); MM 350 mg L^{-1} (15-20 mm); BA 80 mg L^{-1} (15-20 mm); MM 350 mg L^{-1} + BA 80 mg L^{-1} (15-20 mm) and manual thinning treatments, in which the highest levels of soluble sugars (°Brix) were observed at the time of harvest compared to the other treatments. On the other hand, pulp firmness was not affected by treatments at the time of harvest.

Table 1. Production and average fruit mass (AFM) of 'Fuji Suprema' fruits submitted to different load adjustment treatments (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	Production / plant		AFM (g)	Productive efficiency	
	kg	fruits		kg cm^2	fruits (cm^2)
T1-Control	20.5 a	161.0 a	127.0 d	1.33 a	10.41 a
T2-MM*	18.7 a	116.6 a	165.0 b	1.38 a	8.70 a
T3-BA*	23.6 a	175.2 a	134.1 d	1.88 a	13.96 a
T4-MM + BA*	12.5 b	63.0 b	197.9 a	0.82 b	4.15 b
T5-MM**	14.9 b	87.2 b	172.0 b	0.88 b	5.28 b
T6-BA**	11.1 b	74.6 b	147.5 c	0.66 b	4.46 b
T7-MM + BA**	9.9 b	62.8 b	160.4 b	0.57 b	3.62 b
T8-Manual thinning**	9.5 b	63.6 b	153.5 c	0.60 b	3.99 b
Mean	15.1	100.5	157.2	1.02	6.82
VC (%)	39.4	40.5	8.4	49.0	50.9

**Applied on fruits with 5 to 10 mm diameter; **Applied on fruits with 15 to 20 mm diameter. Means followed by the same letter in the column do not differ from one another by the Scott-Knott test (P=.05)*

Table 2. Fruit classification by size, ° Brix and pulp firmness, of 'Fuji Suprema' fruits submitted to different load adjustment treatments (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	Size			Brix°	Pulp firmness (Lb)
	>180	140-150	<135		
T1-Control	41.5 a	40.8^{ns}	17.7 c	7.5 b	17.2 ^{ns}
T2-MM*	13.5 c	35.9	50.7 b	6.5 b	16.6
T3-BA*	32.4 a	45.7	21.9 c	5.1 c	18.0
T4-MM + BA*	10.3 c	25.7	64.0 a	9.6 a	18.3
T5-MM**	17.4 b	37.2	45.4 b	9.4 a	17.1
T6-BA**	35.6 a	32.5	31.9 c	9.9 a	18.1
T7-MM + BA**	22.4 b	32.1	45.5 b	8.9 a	20.1
T8-Manual thinning**	29.8 a	32.4	37.8 b	10.9 a	17.9
Mean	25.4	35.3	39.3	8.5	17.9
VC (%)	16.7	15.2	19.8	16.4	8.0

**Applied on fruits with 5 to 10 mm diameter; **Applied on fruits with 15 to 20 mm diameter. Means followed by the same letter in the column do not differ from one another by the Scott-Knott test (P=.05).*
** ns: not significant (P>.05)*

The visual characteristics of harvested fruits were improved in MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10 mm); MM 350 mg L^{-1} (15-20 mm); BA 80 mg L^{-1} (15-20 mm); MM 350 mg L^{-1} + BA 80 mg L^{-1} (15-20 mm) treatments, where significant reductions in the percentage of fruits with red coloration below 50% were observed, as well as increase of the percentage of colored fruits, that is, with more than 80% of colored epidermis (Table 3).

In ‘MaxiGala’ cultivar, all thinning treatments reduced the average number of fruits per plant in comparison to plants without thinning and, consequently, production per plant also suffered reduction. However, the average fruit mass did not change among treatments (Table 4). Rosa et al., [4] emphasize the greater effectiveness to promote fruit fall of MM compared to 6-BA, when applied in fruits with diameter between 8 and 12 mm in 'Royal Gala', 'Cripps Pink' and 'Red Delicious' cultivars. It is important to point out that the studied harvest season, 2016/17, was a very atypical period, in which excessive natural fruit fall in 'Gala' apple trees was observed in most of producing regions, with the fall of fruits with diameter greater than 20 mm. There are many hypotheses to try to explain such phenomenon such as the nutritional status of plants and climatic factors. This excessive natural fall made it very difficult to implant and evaluate experiments with chemical thinners. Thinning treatments significantly reduced the productive efficiency (kg cm^{-2} and fruits cm^{-2}) of treated plants in comparison to plants without thinning, and in some cases promoting excessive thinning MM 350 mg L^{-1} (5-10 mm), MM 350 mg L^{-1} + BA 40 mg L^{-1} (5-10 mm) and (15-20 mm) treatments provided higher fruit distribution into higher size categories compared to the other treatments. No significant difference among treatments for pulp firmness was observed;

Table 3. Red coloration of 'Fuji Suprema' fruits submitted to different load adjustment treatments (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	Red coloration of fruits (%)		
	<50	50-80	>80
T1-Control	37.6 a	48.3 ^{ns}	14.1 b
T2-MM*	25.7 a	48.4	25.9 b
T3-BA*	28.4 a	49.1	22.5 b
T4-MM + BA*	13.4 b	29.6	57.0 a
T5-MM**	20.3 b	33.1	46.7 a
T6-BA**	16.7 b	37.7	45.6 a
T7-MM + BA**	16.2 b	28.6	55.2 a
T8-Manual thinning**	16.6 b	36.4	47.0 a
Mean	21.9	38.9	39.2
VC (%)	25.5	30.6	27.4

**Applied on fruits with 5 to 10 mm diameter; **Applied on fruits with 15 to 20 mm diameter. Means followed by the same letter do not differ from each other by the Scott-Knott test (P=.05). ns: not significant (P>.05)*

Table 4. Production, average fruit mass-g (AFM) of 'MaxiGala' fruits submitted to different load adjustment treatments (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	Production / plant		AFM (g)	Productive efficiency	
	kg	fruits		kg cm^2	fruits cm^2
T1-Control	9.7 a	58.8 a	165.7^{ns}	0.65 a	3.98 a
T2-MM*	1.8 c	8.4 c	212.0	0.12 c	0.59 c
T3-BA*	5.5 b	34.2 b	162.2	0.44 b	2.76 b
T4-MM + BA*	1.4 c	7.6 c	188.4	0.09 c	0.47 c
T5-MM**	3.5 c	15.8 c	246.8	0.19 c	0.84 c
T6-BA**	1.2 c	7.4 c	154.9	0.07 c	0.42 c
T7-MM + BA**	1.8 c	11.2 c	178.5	0.09 c	0.57 c
T8-Manual thinning**	1.5 c	9.6 c	153.6	0.11 c	0.72 c
Mean	3.3	19.1	182.8	0.22	1.29
VC (%)	51.9	56.6	33.1	66.6	71.1

**Applied on fruits with 5 to 10 mm diameter; **Applied on fruits with 15 to 20 mm diameter. Means followed by the same letter do not differ from each other by the Scott-Knott test (P=.05). ns: not significant (P>.05)*

however, the concentration of soluble sugars (°Brix) was higher in plants that received some form of thinning in comparison to control plants (without thinning) (Table 5).

When analyzing fruit growth and development, significant reduction in the average fruit mass was observed in 'Fuji Suprema' cultivar at four days after application of treatments: MM 350 mg L^{-1} (5-10 mm); BA 80 mg L^{-1} (5-10 mm); and MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10 mm); resulting from the initial action of thinners (Table 6). Fruits from the control treatment (without thinning) showed average fruit mass 0.27 g above fruits from treatments with chemical thinners, characterizing a direct interference of thinners on fruit development, a result that is in agreement with that reported by Gabardo et al. [11], in which the use of Metamitron did not change fruit growth.

Fruit height variable shows that the initial action of isolated BA used in fruit of 5-10 mm did not significantly alter fruit morphology in comparison to untreated plants (without thinning). However, significant reduction in the mean height of fruits treated with MM was observed, alone or in association with BA (Table 6). However, there was no expressiveness for the A / D ratio, which indicates that the fruit shape was not affected by treatments.

Differences were observed in the average number of seeds, cell area and number of cells per section (Table 6). In fruits from BA treatment, due to the cell division action of the product, an inverse relationship between cell area and cell density was observed. This difference, for this cultivar, also relates the number of cells per section with the average number of seeds, which shows that the initial action of BA altered the morphological characteristic of fruits. Carminatti [24] obtained results similar to those of this work; however, the effect of the product varied according to the application period, and at 17 days after petal fall (DAPF) the effect was less expressive.

At 33 DAPF, three days after the application of treatments in fruits of 15-20 mm and thirteen days after the application of treatments in fruits of 5-10 mm in diameter, no difference was observed for the following variables: average fruit mass and fruit diameter and height. However, the A / D ratio presented alterations in BA 80 mg L^{-1} (5-10 mm) and MM 350 mg L^{-1} + BA 80 mg L^{-1} (15-20 mm) treatments, with reduction in values compared to the other treatments, which characterizes the production of flattened fruits, an undesired characteristic for the consumer market.

For variables average number of seeds, cell density and number of cells per section, no difference was observed; however, it was observed that, for cell area, manual thinning and treatments with BA applied alone, showed cell size increase (Table 6), an effect described by Byers [14] as a stimulant in cell division, resulting from cytokinin mimicking.

The analysis of fruits collected at the time of harvest presented changes for average mass, in which MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10 mm); MM 350 mg L^{-1} (15-20 mm); BA 80 mg L^{-1} (15-20 mm) treatments produced fruits with the

Table 5. Fruit classification by size, ° Brix and pulp firmness, of 'MaxiGala' fruits submitted to different load adjustment treatments (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	Size			Brix°	Pulp firmness (Lb)
	>180	140-150	<135		
T1-Control	10.2ns	49.9 a	39.9 b	9.3 b	18.2 ns
T2-MM*	2.2	12.1 b	85.7 a	12.3 a	20.0
T3-BA*	13.8	47.1 a	39.1 b	12.4 a	19.4
T4-MM + BA*	5.5	18.0 b	76.6 a	13.3 a	19.1
T5-MM**	6.1	39.9 a	54.0 b	12.4 a	17.5
T6-BA**	11.8	43.6 a	44.6 b	12.5 a	18.8
T7-MM + BA**	3.5	29.5 b	67.0 a	12.1 a	18.2
T8-Manual thinning**	15.7	41.7 a	42.6 b	12.3 a	21.7
Mean	8.6	35.2	56.2	12.1	19.1
VC (%)	92.1	47.5	34.0	9.9	12.4

**Applied on fruits with 5 to 10 mm diameter; **Applied on fruits with 15 to 20 mm diameter. Means followed by the same letter do not differ from each other by the Scott-Knott test (P=.05). ns: not significant (P>.05)*

highest average mass, and the other treatments did not differ fromcontrol. Results consistent with those found in two years of research by Gabardo et al. [11], working with 'Fuji Suprema' cultivar and testing two MM doses (350 and 700 mg L^{-1}) applied isolated or in combination with BA (0.8 mgL^{-1}), observed higher allocation of fruits in the highest size categories and significant reduction of small size fruits (less than 100 g). For fruit diameter, an increase in MM 350 mg L^{-1} + BA 80 mg L-1 (5-10 mm); MM 350 mg L^{-1} (15-20 mm); BA 80 mg L^{-1} (15-20 mm); MM 350 mg L^{-1} + BA 80 mg L^{-1} (15-20 mm) treatments was observed. For height, an increase in MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10 mm); MM 350 mg L^{-1} (15-20 mm) treatments was observed. However, it did not present for A / D ratio, sustaining the shape of the fruits. The evaluations of the anatomical characteristics of fruits at the time of harvest did not show any alteration in cell size (average cell area - μm^2) and cell density (number of cells - cell mm^{-2}) among treatments, although the average mass of sampled fruits for the cell count was higher in MM 350 mg L^{-1} (5-10 mm), MM 350 mg L^{-1} + BA 40 mg L^{-1} (5-10 mm), and MM 350 mg L^{-1} (15-20 mm) treatments. Fruit shape did not change among treatments, and no significant difference in the number of seeds per fruit was observed, which may influence fruit development (receptacle).

Table 6. Average fruit mass (g) AFM, diameter (mm), height (mm) and height/diameter ratio (A/D), Average number of seeds (ANS) and cell area (µm²), of apple fruits from different lead adjustment treatments, 'Fuji Suprema' cultivar, at 24, 33 and 149 DAFF in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	AFM (g)	Diameter (mm)	Height	A/D	ANS	Cell area µm²
24DAFF. Four days after the application of treatments						
T1-Control	0.88 a	10.38^{ns}	12.9 a	1.24^{ns}	5.67 a	24924,52 a
T2-MM*	0.68 b	8.99	10.7 b	1.19	3.50 b	24789,19 a
T3-BA*	0.58 b	9.33	11.7 a	1.26	6.17 a	17522,78 b
T4-MM + BA*	0.57 b	8.65	10.4 b	1.20	3.50 b	21771,40 a
Mean	0.7	9.3	11.4	1.2	4.7	22251,9
VC (%)	24.0	11.9	12.6	7.2	30.8	18,4
33 DAFF. Three days after the application of treatments in 15-20 mm fruits						
T1-Control	4.02^{ns}	18.69^{ns}	20.10^{ns}	1.08a	4.79^{ns}	-
T2-MM*	4.42	19.42	21.30	1.10a	4.00	61028,78 b
T3-BA*	3.85	19.05	19.81	1.04b	5.67	65315,36 a
T4-MM + BA*	4.05	18.77	21.79	1.16a	4.33	56089,02 b
T5-MM**	3.78	18.41	20.29	1.10a	5.33	57960,28 b
T6-BA**	3.80	18.01	19.31	1.07a	3.67	68749,34 a
T7-MM + BA**	4.12	19.35	19.89	1.03b	6.00	62538,70 b
T8-Manual thinning**	4.37	19.01	20.90	1.10a	4.17	73403,19 a
Mean	4.05	18.8	20.4	1.1	4.7	63583,5
VC (%)	14.9	9.6	20.4	5.9	33.9	14,2
149 DAFF. Fruit harvest						
T1-Control	176.2 b	73.8 b	64.95 b	0.88 ^{ns}	5.8 ^{ns}	130231,7^{ns}
T2-MM*	217.9 b	77.9 b	67.43 b	0.87	4.83	139670,6
T3-BA*	215.3 b	79.3 b	68.22 b	0.86	7.17	147628,4
T4-MM + BA*	254.67 a	81.9 a	75.75 a	0.93	4.00	143414,9
T5-MM**	255.2 a	83.5 a	75.21 a	0.90	3.83	156436,5
T6-BA**	232.9a	82.3 a	69.46 b	0.85	3.67	149956,6
T7-MM + BA**	222.9 b	82.3 a	67.05 b	0.81	4.33	159987,4
T8-Manual thinning**	198.6 b	79.3 b	65.21 b	0.82	5.83	150590,5
Mean	221.7	80.0	69.16	0.87	4.94	147239,6
VC (%)	14.0	5.3	7.2	7.3	41.9	11,8

**Applied on fruits with 5 to 10 mm diameter; **Applied on fruits with 15 to 20 mm diameter. Means followed by the same letter do not differ from each other by the Scott-Knott test (P=.05). ns: not significant (P>.05)*

In the 'MaxiGala' cultivar, when analyzing fruits collected four days after the application of treatments in the 5-10 mm phase, it was observed that plants treated with MM suffered a reduction in the average fruit mass. Basak [25] obtained satisfactory results in their experiment with the use of MM in single and repeated applications, both at concentration of 350 mg L^{-1}, in which the final fruit diameter was elevated with MM, while those treated with BA presented higher average fruit mass. For fruit diameter and height, treatments with MM were inferior to treatment with BA and Control (No thinning) (Table 5). Petri et al. [26], reported that the use of BA or MM alone provided fruits with lower mass in relation to their combined use.

Treatment with isolated BA increased cell area in comparison to the other treatments; in turn, treatments that included MM did not differ and were inferior to Control (without thinning). At cell density level, BA showed the lowest values in cells per mm² and treatment with isolated MM provided the highest cell density. When comparing the cell density and area results, as well as the average number of cells per section, it was observed that cell development is not exclusively influenced by the number of seeds, since there was a variation in the average number of seeds among fruits from different treatments, and the highest cell density is not directly related to the high number of seeds (Table 7).

Analyzing fruits at the time of harvest, it was observed that only 350 mg L^{-1} (5-10 mm); MM 350 mg L^{-1} + BA 40 mg L^{-1} (5-10 mm); and MM 350 mg L^{-1} (15-20 mm) treatments influenced the average fruit mass, increasing it in relation to the other treatments. However, this may be related to the higher allocation of fruits into higher size categories, such as the case of MM 350 mg L^{-1} + BA 40 mg L^{-1} (5-10 mm) treatment, which generally showed significant reduction of the number of fruits per plant and consequently higher average fruit mass. McArtney and Obermiller [10] found similar results when using MM concentration of 350 mg L^{-1} and observed the product efficiency as thinner, as well as the better distribution of fruits into categories of greater commercial value.

There was an increase in the average fruit mass as a function of MM treatment, but BA use did not show superiority in relation to Control (without thinning). These results are not in

Table 7. Average fruit mass (g) AFM, diameter (mm), height (mm) and height/diameter ratio (A/D), Average number of seeds (ANS), cell area (µm²), cell density (cel./mm²) and Average number of cells per fruit section area (ANCFSA) of apple fruits from different lead adjustment treatments, 'Maxi Gala' cultivar, fruit diameter of 5-10 mm in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	AFM	Diameter (mm)	Height	A/D	NMS	Cell area µm²	ANCFSA
24 DAF. Four days after the application of treatments							
T1-Control	0.32 b	6.59 a	7.22 a	1.11^{ns}	4.0 b	16837.3 b	2042.8^{ns}
T2-MM*	0.22 c	5.27 b	5.58 b	1.06	4.8 b	13477.0 c	1653.4
T3-BA*	0.45 a	7.75 a	8.29 a	1.07	6.7 a	20906.4 a	2307.1
T4-MM + BA*	0.18 c	5.46 b	6.09 b	1.12	6.0 a	14855.1 c	1611.5
Mean	0.29	6.27	6.79	1.09	5.4	16518.9	1903.7
VC (%)	16.9	16.2	13.8	6.1	27.5	12.2	27.7
133 DAFF. Fruit harvest							
T1-Control	167.1 b	72.2^{ns}	65.1^{ns}	0.90^{ns}	6.5^{ns}	108346.8^{ns}	39942.9^{ns}
T2-MM*	201.0 a	73.6	69.2	0.94	7.5	106094.5	42395.9
T3-BA*	180.6 b	72.9	65.6	0.90	6.7	111793.5	39047.7
T4-MM + BA*	194.5 a	74.5	69.9	0.94	8.0	113821.1	39915.1
T5-MM**	184.6 a	74.3	69.4	0.93	6.7	119946.0	41265.7
T6-BA**	174.2 b	73.3	66.7	0.91	6.8	112446.1	39796.1
T7-MM + BA**	173.7 b	71.2	65.9	0.93	7.8	109986.9	38787.2
T8-Manual thinning**	162.3 b	70.2	61.9	0.88	7.0	110064.0	37400.9
Mean	179.7	72.8	66.7	0.92	7.1	111562.4	39818.9
VC (%)	11.4	3.5	6.6	6.7	27.5	10.4	11.9

**Applied on fruits with 5 to 10 mm diameter; **Applied on fruits with 15 to 20 mm diameter. Means followed by the same letter do not differ from each other by the Scott-Knott test (P=.05). ns: not significant (P>.05)*

agreement with those found in the study by Cline et al. [27], in which the use of BA as thinner was efficient, generating an increase in fruit size, and results still contradict the hypothesis developed by the authors, since Table 7 shows that there was no significant change in size or number of cells as a function of treatments.

In Table 8, it is possible to observe variation in the mineral contents present in fruits as a function of the applied treatment and, consequently, the relationships among these minerals also changed. Ca is an essential nutrient for the post-harvest maintenance of fruits, and in 'Fuji Suprema' fruits, Ca had increased concentration in MM 350 mg L^{-1} + BA 80 mg L^{-1} (10-15 mm) and manual thinning treatments in comparison to the other treatments. In 'Maxi Gala' cultivar, the highest Ca concentrations were observed in BA 40 mg L^{-1} and MM 350 mg L^{-1} + BA 40 mg L^{-1} (5-10 mm) treatments. K was very influenced by thinning treatments, with high variation in contents found in both cultivars. Higher nitrogen (N) concentrations were observed in fruits from plants without treatment and plants treated with MM 350 mg L^{-1} (5mm); BA 40 mg L^{-1} (5-10 mm); and MM 350 mg L^{-1} + BA 40 mg L^{-1} (5-10 mm). All thinning treatments reduced P content in fruit pulp compared to untreated plants of 'Maxi Gala' cultivar, but in 'Fuji Suprema' cultivar, P concentration was higher in MM 350 mg L^{-1} and MM 350 mg L^{-1} + BA 80 mg L^{-1} (5-10 mm) treatments, while the Mg contents were higher in 350 mg L^{-1} (10-15 mm), MM 350 mg L^{-1} + BA 40 mg L^{-1} (10-15mm) and manual thinning (10-15mm) treatments. In 'Maxi Gala' cultivar, the N/Ca ratio was higher in fruits harvested from control plants (No thinning) and in MM 350 mg L^{-1} (5 mm) treatment. The other nutritional ratios (K/Ca and K + Mg/Ca) showed great variation among treatments in both cultivars.

All load adjusting treatments promoted improvements in fruit quality, especially in relation to size gain without changes in fruit

Table 8. Mineral contents and nutritional ratios of 'Fuji Suprema' and 'MaxiGala' apple trees submitted to different load adjustment treatments (thinning) in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	Mineral contents (mg/Kg)					Nutritional ratios		
	N	P	K	Ca	Mg	N/Ca	K/Ca	K+Mg/Ca
'Fuji Suprema'								
T1-Control	371.0 a	162.7 b	961.7 c	38.7 c	53.0 b	9.7 a	24.7 b	26.3 b
T2-MM*	363.7 a	198.7 a	935.7 c	36.0 c	49.7 b	10.0 a	26.0 b	27.3 b
T3-BA*	291.0 c	169.0 b	1056.0 b	43.7 b	58.3 a	6.3 d	24.3 b	25.7 b
T4-MM + BA*	312.3 b	186.7 a	1093.0 b	44.7 b	54.3 b	7.0 c	24.3 b	25.7 b
T5-MM**	312.3 b	161.7 b	1349.3 a	41.7 b	60.3 a	7.7 b	32.7 a	34.3 a
T6-BA**	298.0 c	165.7 b	1194.3 b	43.7 b	49.7 b	7.0 c	28.3 a	29.3 b
T7-MM + BA**	265.3 d	159.7 b	1466.3 a	47.3 a	52.3 b	5.7 e	31.0 a	32.0 a
T8-Manual thinning**	295.3 c	136.3 c	1456.7 a	48.3 a	61.0 a	5.7 e	30.0 a	31.3 a
Mean	313.6	167.5	1189.1	43.0	54.8	7.4	27.7	29.0
VC (%)	3.9	9.0	9.5	6.7	6.2	6.6	11.0	10.3
'MaxiGala'								
T1-Control	344.0 a	387.7 a	1241.7 b	43.7 b	56.0 b	8.0 a	28.7 a	29.7 a
T2-MM*	379.3 a	321.0 b	965.0 d	43.7 b	51.0 b	9.0 a	22.3 b	23.3 b
T3-BA*	336.0 a	245.0 c	953.3 d	49.0 a	51.7 b	7.0 b	19.3 c	20.3 c
T4-MM + BA*	353.7 a	299.3 b	1114.3 c	52.7 a	56.7 b	6.7 b	21.3 c	22.3 c
T5-MM**	310.3 b	213.3 d	1231.0 b	44.0 b	70.7 a	7.0 b	28.0 a	29.7 a
T6-BA**	283.7 b	236.0 c	1036.7 c	44.0 b	61.3 b	6.3 b	24.3 b	25.3 b
T7-MM + BA**	316.0 b	263.3 c	1399.3 a	45.7 b	70.3 a	6.7 b	30.3 a	31.7 a
T8-Manual thinning**	312.7 b	330.7 b	1203.0 c	44.7 b	70.7 a	7.0 b	26.7 a	28.7 a
Mean	329.5	287.0	1143.0	45.9	61.0	7.2	25.1	26.4
VC (%)	8.8	8.2	7.9	6.3	12.0	11.7	9.6	9.0

**Applied on fruits with 5 to 10 mm diameter; **Applied on fruits with 15 to 20 mm diameter. Means followed by the same letter do not differ from each other by the Scott-Knott test (P=.05)* shape, which justifies the use of this tool. According to Argenta [4], apples are classified according to external characteristics, among them formation defects. Therefore, for better sector profitability, fruits classified into better categories are desired, so, the non-alteration of the natural fruit shape through the use of the product is a desirable situation.

Petri et al. [28] reported that BA has thinning effect on post-flowering 'Fuji Suprema' apple trees, obtaining results similar to manual thinning without the need for manual application after chemical thinning. In this study, higher average fruit mass, diameter and height results were observed in treatments in which the use of BA was associated with MM in fruits of 5-10 mm. Cytokinins, such as BA-Benzyladenine, have the ability to stimulate fruit growth and vegetative activity, influencing cell division, increasing competition for assimilates, reducing the energy available for fruit development, reducing the net CO_2 assimilation rate, resulting in increased fruit drop [15]. Cytokinins increase fruit size, even in the absence of thinning, due to the promotion of cell division in apple tissues [16].

The best benefits of fruit thinning are related to the time of its application, and the earlier it is performed, the better the results [29]. MM has stronger activity with applications in fruits with diameter between 10 and 13 mm [13], losing the thinning potential in fruits with diameter greater than 25 mm [11].

According to Harada et al. [30], in the first 35 to 50 days after fertilization, there is intense cell division in fruits formed. After this period, fruit growth becomes practically linear due to cellular expansion; first characterized by cell vacuolization, then by the rapid increase in the size of individual cells, and finally by the rapid development of intercellular spaces. Later fruit growth stages are largely associated with cell expansion. Differences in fruit size at harvest are results of the difference in the number of cells and not in cell size [10].

In the present study, it was found that the combination of increased cell division capacity and higher degree of cell growth are involved in the increase in fruit size, which is determined by the genetic load of each cultivar [30].

4. CONCLUSION

The cultural thinning practice to manage fruit load, balancing the number of fruits according to plant size is the most important factor that producers can influence, because thinning reduces fruit load in the plant, which allows the remaining fruits the possibility of greater growth and size gain.

The combination of MM + BA is efficient in the thinning of apple fruits, especially when applied at the beginning of fruit development (5-10 mm in diameter).

The application of chemical thinners, such as MM and BA, may influence fruit development.

The application of chemical thinners reduces the labor force for this practice in apple trees.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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