



Different Sources and Concentrations of 6-BA in Chemical Thinning of Post-flowering in Apple Trees

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Authors' contributions

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

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ABSTRACT

Aims: Evaluate the efficiency of sources and dosages of 6-benzyladenine in the chemical thinning of the apple tree 'Fuji Suprema', as well as possible histological changes in the fruits produced, in the Midwestern region of the state of Santa Catarina.

Study Design: The experiment was arranged in a randomized block design and replicated six replications.

Place and Duration of Study: The experiment was carried out in the municipality of Caçador, Brazil (latitude 26°46 'S, longitude 51° W, altitude 960 meters), during the growing season of 2016/2017.

Methodology: Two commercial products based on 6-BA (Benzyladenine), Exilis[®] and Maxcel[®], both with 2% BA in their composition, were tested and applied individually in post-flowering period (in fruits of 5-10 mm), which were compared with plants with no thinning and manual thinning. Treatments were as follows: Control (no thinning); manual thinning; Exilis[®] (1.5, 3.0, 4.5 and 6.0 L

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ha⁻¹) and Maxcel® 4.5 L ha⁻¹), using a spray volume equivalent to 1000 L ha⁻¹. The fruit set and the number of fruit per cm² branch, was reduced in treated plants, regardless of the concentration of BA or formulation used. The productivity ranged from 38 to 56.5 t ha⁻¹ in the treatments of Exilis®, 60.5 t ha⁻¹ in manual scaling and 24.5 t ha⁻¹ in the Maxcel® 4.5 L ha⁻¹, characterizing excessive thinning. BA is efficient in fruit thinning in 'Fuji Suprema' apple trees, being the reduction of fruiting, and increase of fruit size, proportional to the applied concentration. There may be differentiated efficiency of the product by its formulation, even though they have concentrations of active ingredient equivalent. Exilis® was efficient in thinning of apple "Fuji Supreme", when applied to fruit 5 to 10 mm in diameter reduced the need for manual thinning, without causing toxicity. BA can promote the increase in the cell density of fruits produced in proportion to the applied concentration.

Keywords: Histology; productive capacity; cell density.

1. INTRODUCTION

Fruit plants, such as apple trees, generally produce more flowers than are necessary for a commercial crop. In years with abundant flowering and favorable environmental conditions for pollination, fruit set can be excessive, so that there is excess fruit on the plant, making it difficult to obtain large fruits and consequently of high commercial value. If all the flowers are fertilized and develop, the plant may lack photosynthetic resources or structural integrity for adequate fruit ripening [1]. In these conditions, thinning is a necessary and indispensable practice to obtain fruits of better quality (higher caliber and uniform color) and greater market value, as well as to avoid the alternation of production [2,3,4].

The use of chemical products aimed at fruit thinning is a technique consolidated in the apple tree culture, mainly due to the practicality of application, as it allows an adequate adjustment of the fruit load when applied correctly and at the right moment of the development (stage) of the fruits [5]. As the time of application of the chemical thinners, the formulation, the mode of action of these products, as well as the worked cultivar can determine the efficacy of chemical thinning [6,7,8].

Among the chemical thinners for the culture of the apple tree, stand out: ANA (Acetic Naphthalene Acetic), Carbaryl, Ethephon, Metamitron and BA (Benzyladenine). The results of the application of BA in the thinning can vary, according to the concentration, temperature, humidity, luminosity, volume of syrup applied and stage of fruit development in moment of application [9,10,11]. The knowledge of temperature and light in the three to four days after the application of chemical thinners has made the thinning process more predictable and reliable [12].

BA is a compound of the cytokinins group, which has the ability to stimulate fruit growth and vegetative activity, influences cell division, thus increasing competition for assimilates, reducing the energy available for fruit development, reducing the rate of net CO₂ assimilation, which results in an increased fruit fall [13]. Cytokinins increase fruit size in apples, even in the absence of thinning, due to the promotion of cell division in apple tissue [14,10]. According to Yuan and Greene [15], BA is considered a good product because it has low toxicological profile and imitates the biological action of the cytokinin that is synthesized in plants.

The final size of the fruits is associated with the density and size of the cells present in the fruit, and these factors are determined by the genetic expression, which delimits the duration of the phases of cell division and elongation during fruit growth and development [16,17,18]. The genetic factor will predetermine the final size of the fruits, but may be influenced by the nutritional state of the plants, cultural traits and environmental factors [19].

Advances in the understanding of the physiological responses of plants and factors involved in the development of fruits, aim to improve technologies for adjusting loads to acceptable levels, with less dependence on manual thinning [20]. Information about possible anatomical and histological interferences caused in fruits by the application of chemicals for load adjustment, or even manual thinning, is still limited. The objective of the present study was to evaluate the efficiency of sources and dosages of 6-BA in the chemical thinning of 'Fuji Suprema' apple tree in the Midwest region of the state of Santa Catarina, as well as possible histological changes in the fruits produced.

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 26°46 'S, longitude 51° W, altitude 960 meters), in the 2016/17 season. Apple trees of "Fuji Suprema" cultivar, with Marubakaido / M-9 rootstock and planting density of 2,500 ha⁻¹ plants were used.

The 'Fuji Suprema' apple tree is a mutation of the 'Fuji' group, with high effective fruiting and fixation of 3 to 5 fruits per inflorescence. It is managed plants in the central leader in driving system, according to the recommendations of the apple production system [21], and management practices in the integrated system of apple production [22].

The experimental design was randomized blocks with seven treatments and six replicates. The treatments were: Control (No thinning); MT - Manual thinning; Exilis[®] (1.5, 3.0, 4.5 and 6.0 L ha⁻¹, values equivalent to the concentrations of BA 30, 60, 90 and 120 mg L⁻¹) and Maxcel[®] 4.5 L ha⁻¹ (BA 90 mg L⁻¹). The products used were: Exilis[®] SL (20g / L 6-BA), toxicological class I; Maxcel[®] SC (20 g / L 6-BA) toxicological class II. The treatment of manual scaling was performed using scissors, following the criterion of two fruits in terminal buds of brindila (long branches) and one fruit in spur, according to CP - productive capacity of the plants, 5 to 6 fruits per cm² of trunk section area [21]. The application of the treatments with chemical thinners was performed with a motorized costal spray, containing a tip with three D-S spray nozzles, using a volume of syrup equivalent to 1000 L ha⁻¹, up to the point of dripping.

The application of the treatments was performed on October 13, 2016, eighteen days after PF - full bloom, which occurred on September 25,

2016, when fruits were with an equatorial diameter between 5 and 10 mm. In the first days after the application of the treatments a significant increase in the maximum, minimum and average temperatures, and precipitation of 4.8mm was registered in the first day after the application (Table 1). The fruits were harvested at 166 days after PF, on March 10, 2017.

The variables evaluated were: The fruit set (%), determined in two branches per plant, was evaluated in the number of flower bunches during the PF and the number of fruits, 30 days after. In these same branches were counted the number of floral clusters with fruits, resulting in percentage of floral clusters with fruits, and number of fruits per inflorescence. At harvest (March 13, 2017): 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 (%); number of seeds per fruit; pulp firmness (lbpol⁻²) and soluble solids (° Brix) according to Scolari et al. [23].

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 (ToupView[®]).

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 and, or regression analysis at 5% probability. Statistical analyses were performed by the Sisvar software, version 5.6 [24].

Table 1. Meteorological data observed three days before and three days after the application of the different treatments for load adjustment (thinning). Caçador, SC, Brazil, 2019

Date	Temperature (°C)			Precipitation (mm)
	Maximum	Mmnimum	Average	
1) 10/10/16	23,4	6,8	15,2	19,9
2) 11/10/16	18,6	11,2	13,8	13,2
3) 12/10/16	18,6	11,0	14,9	0,0
4) 13/10/16	22,4	13,2	17,0	0,0
5) 14/10/16	26,0	11,0	18,2	4,8
6) 15/10/16	22,0	16,5	19,1	0,0
7) 16/10/16	31,2	16,4	23,4	0,0

3. RESULTS AND DISCUSSION

When the effective fruiting and the number of fruits per cm^{-2} branch were observed, after the application of the treatments with chemical thinners, a significant reduction of these parameters in the treated plants was observed, independently of the concentration or formulation of BA used (Table 2). The mean number of fruits per inflorescence did not present significant differences.

This situation can be alarming, because in some cases, when considering the CP-productive capacity of apple trees, which according to Sezerino [21] would be around five fruits per cm^{-2} , the reduction in fruiting caused by the raleantes in the crop studied, may have been very severe, leading to a large reduction in orchard productivity. Considering a stand of 2,500 plants ha^{-1} , the treatments of Exilis[®] presented a productivity ranging from 38 to 56.5 t ha^{-1} , with manual thinning of 60.5 t ha^{-1} and Maxcel[®] treatment 4.5 L ha^{-1} of 24.5 t ha^{-1} , which is much below the production capacity, showing excessive thinning. In addition to the applied concentration, the time and the residual period of the product can help in the understanding and justify the results observed here. According to Ambrožič Turk and Stopar [25], BA can be active as a raleante in a broader period of phenological phases, from the end of flowering to fruits with 20 mm in diameter, not only in fruit between 10 mm, indicated by many reports, and the persistence of the raleante effect of BA can extend for up to 18 days after application. For an efficient chemical thinning with BA, the dose of the product must be taken into account, together with the climatic conditions and the cultivar [8].

Plant production (kg plant^{-1}) was significantly reduced by the application of Exilis[®] 3.0 L ha^{-1} and Maxcel[®] 4.5 L ha^{-1} treatments (Table 3). But when we analyzed the number of fruits per plant and the productive efficiency (kg cm^{-2}) all treatments with chemical thinners differed significantly from the control treatment and manual thinning, however, Maxcel[®] 4.5 L ha^{-1} was the treatment that caused the highest production efficiency. The mean mass of the fruits presented differences between the treatments, characterized by the significant increase in the mass of the fruits from plants submitted to the treatments of chemical thinning. Regardless of the concentration of the commercial Exilis[®] product applied, the average fruit masses were higher than the treatments of

manual thinning and Control, but lower than the treatment of Maxcel[®] 4.5 L ha^{-1} , which can be attributed to a reduction productivity caused by this treatment. It should be noted that the average mass of the fruits harvested in the plants of the treatment Maxcel[®] 4.5 L ha^{-1} increased 54,8% in relation to the Control.

Similar behavior was observed in fruit classification, where the largest size class (65mm), which presented the highest commercial value, all treatments of Exilis[®] differed significantly from the control and manual thinning treatments, increasing the percentage of fruits of larger caliber and reducing however, the Maxcel[®] 4.5 L ha^{-1} was superior to all treatments tested. Szot et al. [26] obtained excellent results with the application of BA 300mg L^{-1} (Maxcel[®] 7.5 L ha^{-1}) and BA 300mg L^{-1} + Extract of algae, characterized by a very uniform crop formed by only two fruit size classes, 75-85 mm and above 85 mm in diameter, such results would be excellent at the producer level, as it would eliminate the need for manual thinning and increase profitability. Petri et al. [9] working with concentrations of 80 and 120 mg L^{-1} of BA obtained large fruit proportions (<135) greater than 75% and when associated BA 80 mg L^{-1} + Carbaryl 1000 mg L^{-1} the results were superior to 80% of the fruits in categories of greater commercial value.

Marchioretto et al. [27], reported the occurrence of changes in the physical-chemical characteristics of 'Fuji Suprema' apple fruits treated with BA at 60 mg L^{-1} compared to untreated plants, characterized by an increase in soluble solids concentration and firmness of pulp, as was observed in the present study, in which Exilis[®] 3.0 L ha^{-1} , Exilis[®] 4.5 L ha^{-1} and Maxcel[®] 4.5 L ha^{-1} were superior to the other treatments of thinning and plants without control.

When analyzing the characteristics of the fruits harvested, the plants treated with Maxcel[®] 4,5 L ha^{-1} presented the highest values for the variables average fresh mass, height and diameter of the fruits, surpassing all other treatments (Table 4). These results can be related to the great reduction of the effective fructification caused by this treatment, as well as the type of structure that gave origin to these fruits, which in this case were only fruits from brindilas, structures with greater productive capacity and that produce fruits of better quality [28]. Although differences were found in the number of seeds present in the fruits, this did not

cause a change in the shape of the fruits harvested. Although there was no difference in cell size (μm^2), fruits from plants submitted to manual thinning, Exilis[®] 3.0 L ha⁻¹ and Maxcel[®] 4.5 L ha⁻¹ had cell density of cells per fruit section area increased by 18.5%, 21.1% and 30.1%, respectively, compared to fruits of plants with no thinning (Control). Several studies corroborate the results observed in the present study, such as Marchioretto et al. [27], who

observed an increase in the mean fresh mass, in the height and diameter of the fruits of 'Fuji Suprema' apple trees treated with BA at 60 mg L⁻¹. Szot et al. [26] report the fruit yield of 'Jonagold Red Prince' apples with a mean mass of 68.3% higher than the no-rare plants, with the application of BA 300mg L⁻¹ (Maxcel[®] 7.5L ha⁻¹), with fruits with a mean mass of 304.6 g and 181.0 g, respectively.

Table 2. Fruit set (%), mean number of fruits per inflorescence and number of fruits per cm² of branch cross section, Fuji Suprema cultivar, submitted to different treatments for load adjustment (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	Fruit set (%)	n° fruits cm ⁻² branch	Fruits per inflorescence
Control (no thinning)	109.0 a	6.9 a	2.5 ^{ns}
Manual thinning	119.9 a	6.2 a	2.2
Exilis [®] 1.5 L ha ⁻¹	59.5 b	2.9 b	1.8
Exilis [®] 3.0 L ha ⁻¹	58.3 b	3.3 b	1.8
Exilis [®] 4.5 L ha ⁻¹	76.0 b	3.7 b	2.0
Exilis [®] 6.0 L ha ⁻¹	66.6 b	2.6 b	1.7
Maxcel [®] 4.5 L ha ⁻¹	25.9 b	1.4 b	1.2
Average	73,6	3,9	1,9
VC (%)	33,7	39,3	32,4

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 ($P>.05$).

Table 3. Production (kg plant⁻¹ and fruits plant⁻¹), MFM (average fresh mass - g fruits⁻¹), productive efficiency and firmness pulp of fruit 'Fuji Suprema' apple tree, submitted to different treatments for load adjustment (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	Produção / Planta		MFM (g)	Productive Efficiency	
	kg	frutos		kg cm ²	frutos cm ²
Control (no thinning)	25.2 a	191.5 a	131.4 c	1.3 a	10.0 a
Manual thinning	24.2 a	177.7 a	137.2 c	1.4 a	10.5 a
Exilis [®] 1.5 L ha ⁻¹	22.6 a	142.8 b	158.4 b	1.0 b	6.1 b
Exilis [®] 3.0 L ha ⁻¹	15.2 b	92.8 c	163.5 b	1.0 b	6.1 b
Exilis [®] 4.5 L ha ⁻¹	20.8 a	124.0 c	170.2 b	0.9 b	5.5 b
Exilis [®] 6.0 L ha ⁻¹	20.5 a	118.3 c	173.6 b	1.0 b	6.1 b
Maxcel [®] 4.5 L ha ⁻¹	9.8 b	47.5 d	203.4 a	0.4 c	1.8 c
Average	19,8	127,8	162,5	1,0	6,6
VC (%)	24,0	24,0	10,2	25,4	27,8
Treatments	Caliber			Brix°	Firmness of Pulp (Lib)
	>65 mm	55-65 mm	<55 mm		
Control (no thinning)	29.7 d	48.8 a	21.4 a	11.3 b	16.4 b
Manual thinning	44.5 c	39.2 b	16.2 a	11.8 b	16.5 b
Exilis [®] 1.5 L ha ⁻¹	60.2 b	28.0 c	11.8 b	11.3 b	16.3 b
Exilis [®] 3.0 L ha ⁻¹	59.0 b	31.3 b	9.6 b	12.1 a	18.0 a
Exilis [®] 4.5 L ha ⁻¹	67.4 b	23.3 c	9.3 b	12.3 a	16.0 b
Exilis [®] 6.0 L ha ⁻¹	67.7 b	25.3 c	7.0 b	12.0 b	16.6 b
Maxcel [®] 4.5 L ha ⁻¹	83.4 a	10.2 d	6.4 b	13.1 a	18.4 a
Average	58,8 b	29,5	11,7	12,0	16,9
VC (%)	11,3	17,0	28,3	7,8	8,8

Means followed by the same letter in the column do not differ from one another by the Scott-Knott test ($P= .05$)

Table 4. MFM (mean fresh mass - g fruits⁻¹), diameter and height (mm) and height / diameter ratio (A / D), mean number of seeds (NMS), cell area of cells per fruit section area (NCASF) of the fruits of brindilas, harvested from 'Fuji Suprema' apple trees, submitted to different treatments for load adjustment (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

Treatments	MFM (g)	DØ	Hei.	A/D	NMS	Area cell (µm ²)	NCASF
		mm					
Control (no thinning)	189.6 b	75.9 b	64.5 b	0.85 ^{ns}	7.5 a	132844.17 ^{ns}	34607.85 b
Manual thinning	179.9 b	78.4 b	64.6 b	0.82	5.8 b	118157.49	41010.51 a
Exilis [®] 1.5 L ha ⁻¹	205.4 b	80.1 b	65.8 b	0.82	6.4 b	131140.91	38815.61 b
Exilis [®] 3.0 L ha ⁻¹	223.3 b	80.8 b	67.2 b	0.83	7.8 a	123031.67	41928.62 a
Exilis [®] 4.5 L ha ⁻¹	217.0 b	78.6 b	68.6 b	0.87	5.9 b	128621.05	37735.46 b
Exilis [®] 6.0 L ha ⁻¹	201.9 b	76.7 b	66.2 b	0.86	6.3 b	128990.22	35836.01 b
Maxcel [®] 4.5 L ha ⁻¹	281.9 a	86.6 a	73.7 a	0.85	6.9 a	131740.23	45032.66 a
Average	214.1	79.6	67.2	0.85	6.7	127789.39	39280.96
VC (%)	16,6	5,7	6,6	6,1	15,2	8,8	11,5

Means followed by the same letter in the column do not differ from one another by the Scott-Knott test (P=0.05).

* ns: not significant (P>0.05)

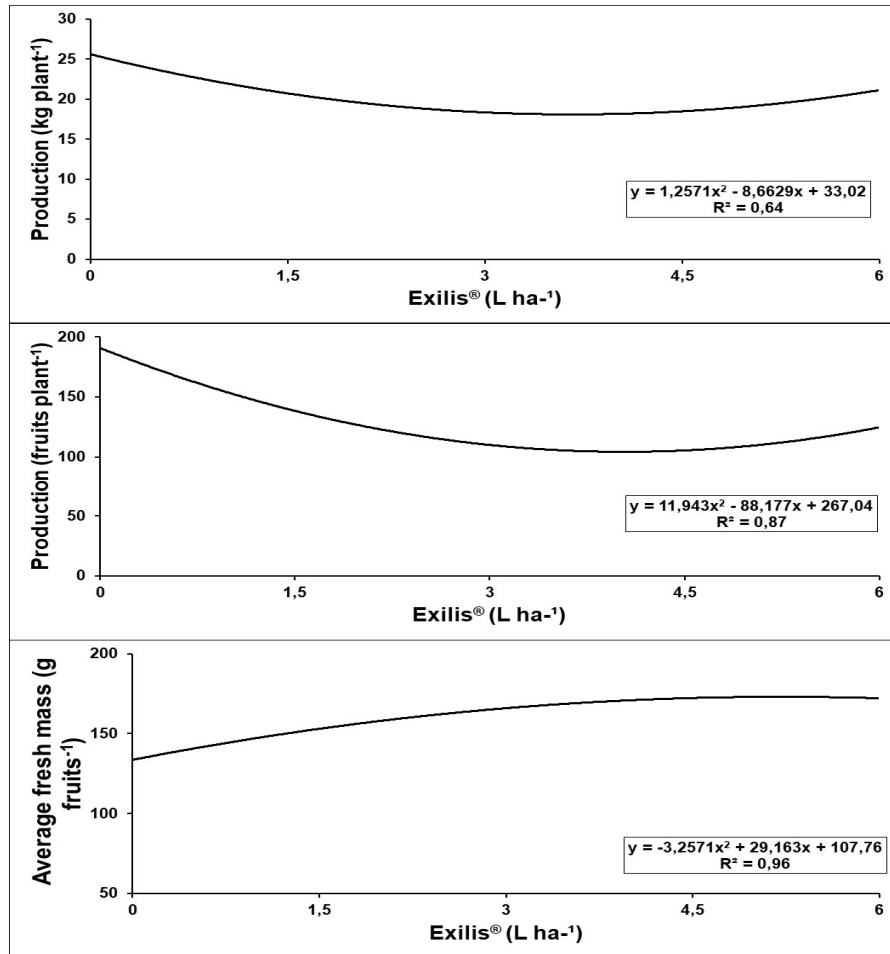


Fig. 1. Production trend lines (kg plant⁻¹ and fruits plant⁻¹) and average fresh mass of 'Fuji Suprema' apple trees, submitted to different treatments for load adjustment (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

When analyzing the concentrations of BA applied, more specifically the Exilis® formulation (1.5, 3.0, 4.5 and 6.0 L ha⁻¹, values equivalent to the concentrations of BA 30, 60, 90 and 120 mg L⁻¹), a quadratic response (kg plant⁻¹ and fruits plant⁻¹), with maximum reduction of production (kg plant⁻¹ and fruits plant⁻¹) in doses of 3.44 L ha⁻¹ (68.9 mgL⁻¹) and 3.69 L ha⁻¹ (73.8 mgL⁻¹), respectively (Fig. 1). However, for the average fresh fruit mass, the opposite behavior was observed, with a maximum increase in the mean fruit mass in the dose of Exilis 4,53 L ha⁻¹ (90.6 mgL⁻¹). Similar results are presented by other authors, such as Barreto et al. [8], which, working with increasing doses of BA for load adjustment in peach trees, reported behavior similar to those observed in the present study, characterized by an increase in the percentage of abscission and mass of fruits, in response to the increase of applied dosage, excess of fruits with doses of 400 mg L⁻¹. For 'Fuji Suprema' apple trees it has

already been proven in other studies that the highest concentrations of BA are more effective in the thinning, however, there is some reduction in its effectiveness when applied to fruits with a diameter greater than 10 mm in comparison to the application in fruits [9].

When analyzing only the characteristics of fruits harvested from thinned fruits, no changes were observed in fruit shape (height and diameter), but the average fresh fruit mass has a quadratic behavior proportional to the dose increase of Exilis® applied, reaching the maximum point yield at the dosage of 3.2 L ha⁻¹, which corresponds to 65.9 mg L⁻¹ BA. Cellular dimensions were not altered by increasing doses of Exilis® applied, however, cell density had a similar behavior to fruit mass, with a greater increase in the number of cells per section of the fruits with the dose of 3.04 L ha⁻¹ of Exilis®, corresponding to 60.8 mg L⁻¹ BA (Fig. 2).

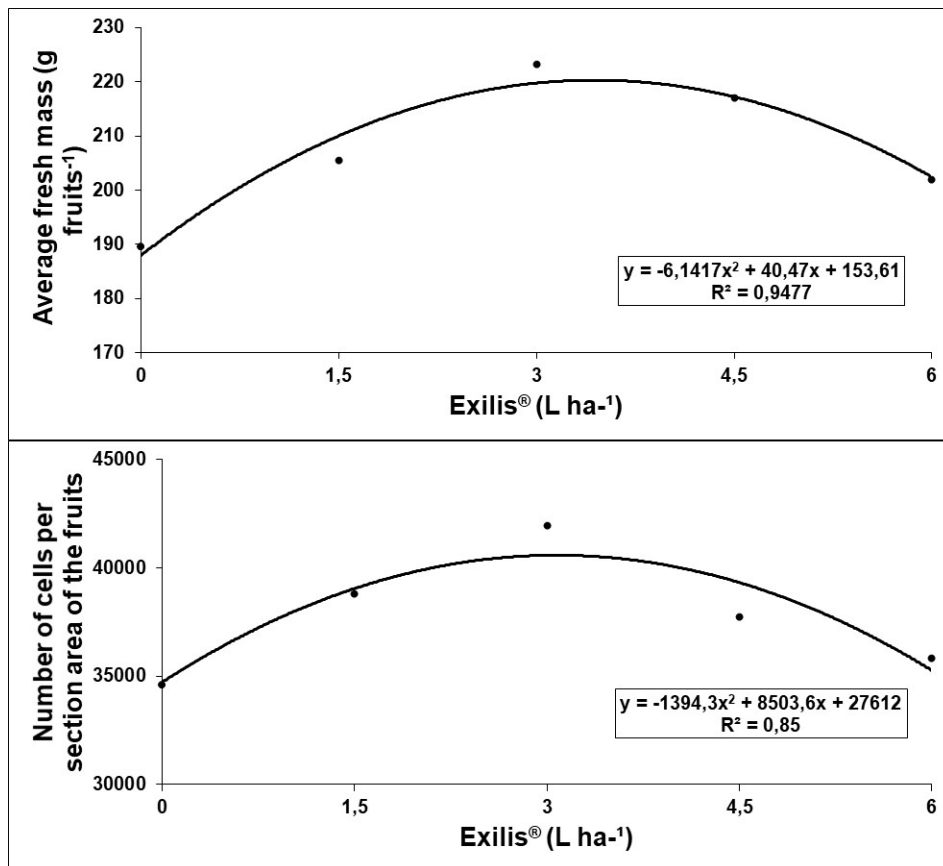


Fig. 2. Trend lines for average fresh mass (g fruit⁻¹) and Number of cells per section area of the fruits of brindilas, harvested from 'Fuji Suprema' apple trees, submitted to different treatments for load adjustment (thinning), in the 2016/2017 season, Caçador-SC, Brazil, 2019

4. CONCLUSION

The BA is efficient in adjusting fruit load in 'Fuji Suprema' apple trees, reducing fruiting, as well as improving fruit quality and caliber proportional to the applied concentration.

There is a differentiated response in the efficiency of the chemical thinners by their formulation, even though both have concentrations of active ingredient equivalent.

Exilis® was efficient in the thinning of the 'Fuji Suprema' apple tree, when applied in fruits with 5 to 10 mm of diameter increased the percentage of fruits of greater caliber that has greater commercial value. With higher gains in the mean fruit mass with the application of the 4.53 L ha⁻¹ dose (90.6 mgL⁻¹ BA)

BA can promote the increase in the cell density of fruits produced in proportion to the applied concentration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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