



# **Metamitron and Different Plant Growth Regulators Combinations in the Chemical Thinning of 'Eva' Apple Trees**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author CG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GAA and MBM managed the analyses of the study. Authors SBA, AB and AVS managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aims:** The objective of this study was to evaluate the effect of the application of Metamitron and the combinations of NAA + BA and Promalin + BA on the fruit thinning of the Eva cultivar apple tree in southern Brazil.

**Study Design:** The experimental design used was a randomized complete block design, with four replications, with two plants per treatment in each block, following a one-step scheme with 10 treatments for the thinning factor.

**Place and Duration of Study:** The experiment was carried out in the agricultural year of 2013/2014 in a commercial orchard located in the municipality of Morro Redondo, RS, Brazil (Lat. 31° 40' 60" S and Long. 52° 34' 50" W).

**Methodology:** Spraying of Metamitron was carried out at doses of 300, 350 and 400 mg L<sup>-1</sup> and

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combinations of 5 mg L<sup>-1</sup> of NAA + BA at doses 50, 75 and 100 mg L<sup>-1</sup> and 0.5 ml L<sup>-1</sup> of Promalin® + BA at doses of 50, 75 and 100 mg L<sup>-1</sup>. Only the doses of Promalin® were applied in full bloom, the other sprayings were carried out in fruiting with 5-8 mm in diameter. The variables analyzed were: number of fruits per trunk section area, number of fruits per plant, yield per plant, mean fruit mass, pulp firmness, fruit shape and diameter, fruit length, length/diameter ratio and soluble solids.

**Results:** A reduction in harvest load by Metamitron was only observed with the 300 mg L<sup>-1</sup> dose. The combination of NAA 5 mg L<sup>-1</sup> + BA 75 mg L<sup>-1</sup> showed the highest fruit reduction, with a low fruit density per cm<sup>-2</sup> and the highest average fruit mass. For the combinations of Promalin® + BA, as the increment of the dose of BA increased, there was a reduction of the number of fruits and consequently an increase in their size.

**Conclusion:** It was concluded that the Metamitron at the dose of 300 mg L<sup>-1</sup> was able to cause thinning when applied in the fruit formation phase (5-8 mm). And the combinations of NAA 5 mg L<sup>-1</sup> + BA 75 mg L<sup>-1</sup> and Promalin® 0.5 ml L<sup>-1</sup> + BA 100 mg L<sup>-1</sup> were able to reduce harvest load and increase the fruit size.

**Keywords:** *Malus domestica* (Borkh.); inhibitor of photosystem II; plant growth regulators; concentration; fruit set; fruit production.

## 1. INTRODUCTION

The apple tree crop usually presents an excessive load of flowers or fruits, more than the plant can support, causing a reduction in the quality of the fruits, due to competition for photoassimilates and nutrients. [1] reported that fruit species generally produce more flowers than necessary and that if all the flowers that appeared in flowering were to establish fruit development, the plant would not have the photosynthetic or structural integrity to mature those fruits correctly. Moreover, they also stated that fruit species have a self-regulating mechanism, capable of causing thinning of weak-structured, small-seeded and poorly-formed fruits, but that in general this mechanism is not sufficient to guarantee a commercial quality and size fruit.

Thus, fruit or flower thinning is necessary for commercial production of apples, improving fruit size and quality, leaving the plant with an ideal harvest load and increasing the flowering return [2].

The chemical thinning is a viable alternative to pomiculture through the use of different products, allowing the increase of the quality of the fruits and reducing the cost and time of labor, in relation to the manual thinning.

Thinning substances can be divided according to their mode of action; caustic or hormonal. Among the substances with hormonal action Naphthalene Acetic Acid (NAA), Carbaryl, Ethephon and Benzyladenine (BA) can be

mentioned [3]. These substances were shown to be preferred for use in chemical thinning in relation to caustic substances due to their greater selectivity in the thinning, induction in the abscission of flowers and fruits with less growth capacity, besides not inducing morphological damages to the remaining fruits.

The most recent alternative in pomiculture has been the inhibitors of photosystem II such as the Metamitron, which is capable of causing thinning when applied on fruits of 10 to 12 mm in diameter [4,5,6,7]. Metamitron disrupts the photosynthesis process 7 to 10 days after application, reducing electron rates by up to 60% [8]. According to recent studies conducted, the Metamitron herbicide of the chemical triazinone group has a positive effect on the activity of apple fruit thinning due to its direct action in the inhibition of photosynthesis [5,6,9].

The objective of this study was to evaluate the effect of the application of Metamitron and the combinations of NAA + BA and Promalin + BA on the fruit thinning of the Eva cultivar apple tree in southern Brazil.

## 2. MATERIALS AND METHODS

The experiment was carried out in the agricultural year of 2013/2014 in a commercial orchard located in the municipality of Morro Redondo, RS, Brazil (Lat. 31° 40' 60" S and Long. 52° 34' 50" W). Seven-year-old "Eva" apple plants grafted on a Marubakaido rootstock were used and conducted in the central leader system with a density of 2,500 ha<sup>-1</sup> plants.

The experimental design used was a randomized complete block design, with four replications, with two plants per treatment in each block, following a one-step scheme with 10 treatments for the thinning factor.

The treatments used were:

- T1 - Manual Thinning (30 days after full bloom);
- T2 - Metamitron 300 mg L<sup>-1</sup>;
- T3 - Metamitron 350 mg L<sup>-1</sup>;
- T4 - Metamitron 400 mg L<sup>-1</sup>;
- T5 - NAA 5 mg L<sup>-1</sup> + BA 50 mg L<sup>-1</sup>;
- T6 - NAA 5 mg L<sup>-1</sup> + BA 75 mg L<sup>-1</sup>;
- T7 - NAA 5 mg L<sup>-1</sup> + BA 100 mg L<sup>-1</sup>;
- T8 - Promalin® 0.5 ml L<sup>-1</sup> + BA 50 mg L<sup>-1</sup>;
- T9 - Promalin® 0.5 ml L<sup>-1</sup> + BA 75 mg L<sup>-1</sup>;
- T10 - Promalin® 0.5 ml L<sup>-1</sup> + BA 100 mg L<sup>-1</sup>.

The source of Metamitron was the herbicide Goltix® (70% active ingredient - ai), the commercial products Technical NAA (9.5% ai) and MaxCel® (2% ai) were used for naphthalene acetic acid (NAA) and 6-benzyladenine (BA), respectively. Promalin®, a phytohormone composed of gibberellins GA4 + 7 (1.8%) and cytokinin 6-benzyladenine (1.8%) (Valent Biosciences) was also used. In all treatments 0.2% Assist® mineral oil was added. The applications were performed by a sprinkler with costal spray, using a mean solution volume of corresponding to 1000 L ha<sup>-1</sup>. Promalin® was applied in full bloom (PF), Metamitron, NAA and BA were applied when the larger fruits reached 5 to 8 mm in diameter.

The variables analyzed were: number of fruits per trunk section area (cm<sup>2</sup>), trunk diameter at 20 cm from the ground, number of fruits per plant, production per plant (kg), mean fruit weight (g), pulp firmness (N), fruit shape through its diameter (mm), fruit length (mm), length and diameter ratio and soluble solids (°Brix).

The results were submitted to analysis of variance, and a comparison of means was made through the Tukey test, with a 5% probability of error.

### 3. RESULTS AND DISCUSSION

The fruits per plant variable stood out at a dose of Metamitron 400 mg L<sup>-1</sup> with a mean of 105.5 fruits, not differing from NAA 5 mg L<sup>-1</sup> + BA 100

mg L<sup>-1</sup> with 92 fruits, which shows that these treatments were not effective in reducing the harvest load. However, it was observed that the dose of Metamitron 300 mg L<sup>-1</sup> with 34.75 fruits and Promalin® 0.5 ml L<sup>-1</sup> + BA 100 mg L<sup>-1</sup> with 28 fruits were the treatments that presented the greatest reduction in harvest. When analyzing the treatments and comparing them with the manual thinning control, it was observed that of the Metamitron treatments, only Metamitron 300 mg L<sup>-1</sup> containing the lowest dosage of the product showed a reduction of fruits differing statistically from manual thinning (Table 1). The author obtained a reduction of effective fruiting and increase of average mass of fruits in the Gala Must cultivar with application of 350 mg L<sup>-1</sup> of Metamitron [6]. [8] reported, application of a photosystem II inhibitor, such as Metamitron, three weeks after flowering increases fruit sensitivity to abscission due to transient excess and stress of carbohydrates within the fruit caused by inhibition of electrons transport.

With the treatments sprayed with the NAA + BA mixture, the highest reduction occurred in NAA 5 mg L<sup>-1</sup> + BA 75 mg L<sup>-1</sup>, whereas those treated with a 0.5 ml L<sup>-1</sup> Promalin® dose during flowering and different BA doses during fruiting presented a progressive reduction in the harvest load as the dose increased, with the combinations Promalin® 0.5 ml L<sup>-1</sup> + BA 75 mg L<sup>-1</sup> and Promalin® 0.5 ml L<sup>-1</sup> + BA 100 mg L<sup>-1</sup> differing statistically from manual thinning (Table 1).

Manual thinning, Metamitron 400 mg L<sup>-1</sup> and NAA 5 mg L<sup>-1</sup> + BA 100 mg L<sup>-1</sup> were those that expressed the highest results in relation to the number of fruits by the cross-sectional area of the trunk, on the other hand, Metamitron 300 mg L<sup>-1</sup>, NAA 5 mg L<sup>-1</sup> + BA 75 mg L<sup>-1</sup> and Promalin® 0.5 ml L<sup>-1</sup> + BA 100 mg L<sup>-1</sup> were the lowest results found in the same way as those with the highest reduction in the number of fruits per plant (Table 1). The reduction, even when excessive, in the combination of Promalin® + BA was probably due to the excess cytokinin the plant received. Significant results of thinning with the application of NAA + BA in the Gala cultivar [10], however in the Golden Delicious cultivar the NAA + BA mixture reduced the number of fruits by the section of the trunk area, but did not differ from isolated doses of the plant growth regulators [11]. The effective action of the Promalin® + BA blend in the Fuji Suprema cultivar when applied on fruits with 20 mm diameter, but did not obtain effectiveness when applied in fruits of 5-8 mm [12], differing from the results found in this study.

**Table 1. Number of fruits per plant, fruits per trunk cross-sectional area and yield per plant, in different treatments on Eva cultivar**

Treatments	N <sup>o</sup> . of Fruits per plant	Fruit cm <sup>-2</sup> TCSA	Yield per plant (Kg plant <sup>-1</sup> )
T1 - Manual Thinning	86.25 ab	8.01 ab	9.25 a
T2 - Metamitron 300 mg L <sup>-1</sup>	34.75 cd	2.98 e	3.91 b
T3 - Metamitron 350 mg L <sup>-1</sup>	83.25 ab	7.52 a-c	8.55 a
T4 - Metamitron 400 mg L <sup>-1</sup>	105.5 a	9.45 a	9.87 a
T5 - NAA 5 mg L <sup>-1</sup> + BA 50 mg L <sup>-1</sup>	58.5 bc	5.84 b-d	5.28 b
T6 - NAA 5 mg L <sup>-1</sup> + BA 75 mg L <sup>-1</sup>	32.5 cd	2.81 e	3.6 b
T7 - NAA 5 mg L <sup>-1</sup> + BA 100 mg L <sup>-1</sup>	92 a	8.42 ab	9.36 a
T8 - Promalin <sup>®</sup> 0.5 ml L <sup>-1</sup> + BA 50 mg L <sup>-1</sup>	60 bc	5.0 c-e	5.64 b
T9 - Promalin <sup>®</sup> 0.5 ml L <sup>-1</sup> + BA 75 mg L <sup>-1</sup>	41 cd	3.96 de	4.35 b
T10 -Promalin <sup>®</sup> 0.5 ml L <sup>-1</sup> + BA 100 mg L <sup>-1</sup>	28 d	2.63 e	3.04 b
Mean	62.17	5.60	6.28
Coefficient of variance (%)	18.64	19.10	17.61

Means followed by the same letter do not differ from each other, by the Tukey test, at 5% probability. ( $p < 0.05$ ).

TCSA – trunk cross-sectional area

When analyzing the average mass of fruits, a direct relation can be established with fruits per cm<sup>2</sup> of cross-sectional area of the trunk, where the three treatments that expressed the lowest fruit density per plant were those that presented the highest average fruit mass (Metamitron 300 mg L<sup>-1</sup> with 113.16 grams, NAA 5 mg L<sup>-1</sup> + BA 75 mg L<sup>-1</sup> with 111.94 grams and Promalin<sup>®</sup> 0.5 ml L<sup>-1</sup> + BA 100 mg L<sup>-1</sup> with 109.89 grams) and consequently the same ones that obtained the smallest yields in kilos per plant (Table 2). The application of Metamitron or Metamitron + BA was efficient in fruit thinning in the Fuji Suprema, MaxiGala and Fred Hough apple cultivars, proving their potential to compose the chemical thinning program of apple trees in substitution of Carbaryl in the Brazilian climatic conditions [12]. In addition, the possibility of using Metamitron in the thinning of fruits with larger diameter was observed, due to the efficiency of Metamitron in fruit thinning from 5 mm to 20 mm in diameter in the Fred Hough apple cultivar [13].

In Table 2, when analyzing the treatments that had Metamitron in their formulation, it is observed that as the product dosage is increased, the average mass of fruits decreases, different from what happened with the treatments with BA (combined with NAA as well combined with Promalin<sup>®</sup>), the increase in BA dosage resulted in a progressive increase in mean fruit mass. The combination of different BA + NAA 5 mg L<sup>-1</sup> doses showed effective results in increasing fruit mass and a reduction in yield per plant in Kozara cultivar [14]. However, using the BA + NAA mixture provides dwarf fruits [15].

Soluble solids were not significantly affected by the treatments used, presenting an average of 12.51° Brix, different from the pulp firmness that presented statistical difference between the treatments, with NAA 5 mg L<sup>-1</sup> + BA 50 mg L<sup>-1</sup> with 81.37 N being the best result found and Promalin<sup>®</sup> 0.5 ml L<sup>-1</sup> + BA 50 mg L<sup>-1</sup> with 69.94 N the lowest result (Table 2).

The high pulp resistance in the NAA 5 mg L<sup>-1</sup> + BA 50 mg L<sup>-1</sup> is probably due to a paralysis of cell expansion, since this was the treatment that obtained the lowest mean mass and the lowest fruit diameter among the analyzed treatments (Tables 2 and 3). Good results in relation to fruit size and quality when NAA + BA was applied in Senabija, Pasinka and Kozara cultivars in Montenegro in Europe [14]. The combination of NAA and BA increased the size of 'Summerred' fruits [16]. BA is considered a good thinning agent because the substance has a low toxicological profile [17].

The fruit shape is evaluated by the diameter, length and the relation between them, and consumers generally take this into account, always looking for symmetrical fruits, that is, those that do not appear flat, that do not have deformations in some sides and are not too long. Table 3 presents the variables that evaluate the fruit shape. It is observed that in all treatments, the length was larger than the diameter, with the L/D ratio being always higher than 1.0. Thus it can be inferred that during the year of the experiment, the fruits of the Eva cultivar tended to be more elongated than round, denoting a possible problem of pollination and seed

**Table 2. Average mass of fruits, total soluble solids and pulp firmness, in different treatments in the Eva cultivar**

Treatments	Average Mass of fruits (g)	SS (°Brix)	PF (N)
T1 - Manual Thinning	107.04 ab	12.55 ns	71.27 b
T2 - Metamitron 300 mg L <sup>-1</sup>	113.16 a	11.53	76.31 ab
T3 - Metamitron 350 mg L <sup>-1</sup>	97.63 ab	13	75.57 ab
T4 - Metamitron 400 mg L <sup>-1</sup>	93.82 ab	12.76	70.41 b
T5 - NAA 5 mg L <sup>-1</sup> + BA 50 mg L <sup>-1</sup>	88.02 b	12.7	81.37 a
T6 - NAA 5 mg L <sup>-1</sup> + BA 75 mg L <sup>-1</sup>	111.94 a	12.93	77.78 ab
T7 - NAA 5 mg L <sup>-1</sup> + BA 100 mg L <sup>-1</sup>	102.73 ab	12.33	70.19 b
T8 - Promalin® 0.5 ml L <sup>-1</sup> + BA 50 mg L <sup>-1</sup>	94.35 ab	12.1	69.94 b
T9 - Promalin® 0.5 ml L <sup>-1</sup> + BA 75 mg L <sup>-1</sup>	106.77 ab	12.16	73.62 ab
T10 - Promalin® 0.5 ml L <sup>-1</sup> + BA 100 mg L <sup>-1</sup>	109.89 a	12.26	74.09 ab
Mean	102.93	12.51	74.05
Coefficient of variance (%)	8.24	8.91	5.53

Means followed by the same letter do not differ from each other, by the Tukey test, at 5% probability. ( $p < 0.05$ ). ns – not significant. SS – Soluble Solids. PF – Pulp Firmness in N – Newton

**Table 3. Evaluation of the fruit shape through diameter, length and length/diameter ratio, in different treatments in the Eva cultivar**

Treatments	Diameter (mm)	Length (mm)	L/D
T1 - Manual Thinning	60.5 a	61.75 ab	1.02 ab
T2 - Metamitron 300 mg L <sup>-1</sup>	61.37 a	65.5 a	1.06 ab
T3 - Metamitron 350 mg L <sup>-1</sup>	59 ab	61.25 ab	1.03 ab
T4 - Metamitron 400 mg L <sup>-1</sup>	58.62 ab	59.5 b	1.01 b
T5 - NAA 5 mg L <sup>-1</sup> + BA 50 mg L <sup>-1</sup>	56.37 b	60.25 ab	1.06 ab
T6 - NAA 5 mg L <sup>-1</sup> + BA 75 mg L <sup>-1</sup>	59 ab	63.5 ab	1.07 ab
T7 - NAA 5 mg L <sup>-1</sup> + BA 100 mg L <sup>-1</sup>	58.87 ab	63.5 ab	1.07ab
T8 - Promalin® 0.5 ml L <sup>-1</sup> + BA 50 mg L <sup>-1</sup>	57.12 b	61.5 ab	1.07 ab
T9 - Promalin® 0.5 ml L <sup>-1</sup> + BA 75 mg L <sup>-1</sup>	59.37 ab	63.5 ab	1.06 ab
T10 - Promalin® 0.5 ml L <sup>-1</sup> + BA 100 mg L <sup>-1</sup>	59.37 ab	65.5 a	1.1 a
Mean	58.96	62.57	1.06
Coefficient of variance (%)	2.33	3.57	3.46

Means followed by the same letter do not differ from each other, by the Tukey test, at 5% probability

formation and also by varietal characteristics. The use of BA increases the size of the fruit by the thinning effect, besides promoting increase in fruit size because it is a compound of the cytokinins group, acting in the increase of cell division [12]. Because of this increase in cell division, BA can increase fruit size even in the absence of thinning [18].

#### 4. CONCLUSIONS

The use of a Metamitron dose of 300 mg L<sup>-1</sup> was able to cause thinning when applied during the fruit formation stage (5-8 mm).

The combinations of NAA 5 mg L<sup>-1</sup> + BA 75 mg L<sup>-1</sup> and Promalin® 0.5 ml L<sup>-1</sup> + BA 100 mg L<sup>-1</sup> were able to reduce harvest load and increase fruit size.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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