



Chemical thinning programs for vigorous ‘Maxi Gala’ apple trees under black anti-hail netting in Southern Brazil¹

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ABSTRACT

Hail netting increases natural fruitlet drop. Mixing lower concentrations of Plant Growth Regulators (PGRs) and spraying on more advanced fruitlets could promote adequate crop load management and improve fruit quality. The objective of this study was to evaluate thinning programs with low concentrations of PGRs sprayed at advanced fruitlet size looking for reducing crop load, enhancing fruit quality, and preserving yield. On 2020/21 and 2021/22, in an orchard covered with hail netting in Bom Jesus-RS, eight programs were tested on vigorous ‘Maxi Gala’: Benzyladenine (BA)+Gibberellic acid₄₊₇ (BA-GA) at full bloom (FB) and BA+Ethephon (ETH) at 15mm; BA-GA at FB and Naphthalene acetic acid+Carbaryl (CB) at 7 mm; BA-GA at FB and BA+CB at 15 mm; ETH+CB at 15 mm and Metamitron (MM)+ETH at 20 mm; BA-GA at FB and BA at 7 mm and MM at 20 mm; MM at 7 mm and MM at 20 mm; Manual Thinning Only; Untreated Control. The treatments containing CB reduced fruit set, although diminished yield unsustainably. All PGR programs improved fruit quality. In conclusion, BA-GA at FB and BA+ETH at 15 mm, and MM at 7 mm and MM at 20 mm reduce crop load, preserve yield and improve fruit quality.

Keywords: (CLM) crop load management; *Malus domestica* Borkh.; fruit set; semi-vigorous rootstock; fruit weight.

INTRODUCTION

The installation of anti-hail netting in apple orchards is an effective management to avoid damage to fruits and tree structure, besides assuring the profitability of the activity. On the productive aspect, hail netting improves the hydric status of apple trees, thus decreasing stresses during drought or high sunlight intensity, potentially improves leaf photosynthesis and both light and water use efficiency as seen in many semi-arid places (Kalcsits *et al.*, 2018; Mupambi *et al.*, 2018). Nevertheless, in unstressed orchards, the shading promoted by the netting reduces available solar radiation and stimulates the trees to drive energy towards shade avoidance strategies at the expense of fruit production,

eventually leading to higher fruitlet fall and reduced crop load (Amarante *et al.*, 2009; Bosco *et al.*, 2015).

Under netting, the less favorable microclimate for fruit development (Solomakhin & Blanke, 2010) leads to a higher fruitlet natural abscission rate (Brglez Sever *et al.*, 2021). In such conditions the use of Plant Growth Regulators (PGRs) for fruitlet thinning must be carefully assessed to avoid overthinning. On the other hand, the carbohydrate supply may be limiting to sustain a high crop load (Lakso & Robinson, 2015), and the effect of some PGRs used for chemical fruitlet thinning may enhance fruitlet drop in such condition, leading to overthinning (Clever, 2022).

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The combination of BA+GA₄₊₇ (Promalin®) is sprayed at full bloom (FB) due to its effect on increasing fruit elongation and also on increasing budbreak, improving leaf area, and promoting some fruitlet thinning due to the Benzyladenine (BA) (Greene *et al.*, 2016). Naphthalene Acetic Acid (NAA) is more effective when sprayed from FB up to fruitlets with 10 mm of diameter (Petri *et al.*, 2017; Rademacher, 2015). Also, this PGR acts on inhibiting photosynthesis, increasing respiration, and enlarging cells leading to higher consumption of reserves (Milić *et al.*, 2017; Untiedt & Blanke, 2001). In addition, Carbaryl is also a synthetic auxin that has similar properties of NAA, although it can be used from FB until fruitlets with 20 mm of diameter (Rademacher, 2015).

BA is sprayed on fruitlets ranging from 5 to 10 mm of diameter and has thinning effect when sprayed in the whole canopy, promoting intense cell multiplication, making less assimilates available to the fruitlets, thus triggering more abscission (Yuan & Greene, 2000). Ethephon when into the cell plasma is hydrolyzed, releasing ethylene; Also, this PGR is effective on a wide range of growth stages that could be from FB up to 20 mm of diameter. This PGR increases the respiration rate, increasing the consumption of reserves, besides acting directly on activating the abscission zone (Petri *et al.*, 2017; Untiedt & Blanke, 2001). The herbicide Metamitron (MM) is more effective in promoting fruitlet thinning when sprayed on fruitlets between 11.5 and 14 mm of diameter (Gonzalez *et al.*, 2019). This PGR temporarily impairs the photosynthesis due to the uncoupling of the photosystem II, which leads to a shortage of assimilates for the fruitlets (Eccher *et al.*, 2013; Gonzalez *et al.*, 2019).

The PGRs used as chemical thinners have a common mechanism of action for inducing fruitlet abscission, which is reducing the availability of assimilates to the fruitlets, then increasing the competition (Eccher *et al.*, 2013; Gonzalez *et al.*, 2019; Milić *et al.*, 2017). In addition to the setbacks caused by the netting, and the higher natural fruitlet abscission found on covered orchards, it could be reasonable to hypothesize that reducing the concentrations of the PGRs for fruitlet thinning sprayed at post blossom (Lakso & Robinson, 2015), or to mix different PGRs with different mechanisms of action (e.g., metamitron inhibits the assimilates production, and ethephon increases respiration and reserves depletion) looking for optimizing the thinning response (Elfving & Cline, 1993; Stover *et al.*, 2001; Cline *et al.*, 2019), as well as spray the PGRs at later

fruitlet development stages, as the susceptibility to fruit abscission tends to decrease (Stover *et al.*, 2001; Lordan *et al.*, 2020) could promote sufficient chemical thinning, improve fruit quality, without the risk of overthinning apple trees under hail netting, especially when using semi-vigorous rootstocks and consequently shaded inner canopies.

In this context, in Southern Brazil, farmers opt for doing manual thinning only or one PGR spray followed by manual thinning in the orchard before December Drop (June in the NH), however, this practice may correspond to a big share of the production costs (Lazzarotto, 2018), as chemical thinning may be unpredictable due to the reduced sunlight supply and large tree canopies with shadowed interior. Thus, the objective of this study was to evaluate different chemical thinning programs elaborated with the combinations of different mixtures of low doses of plant growth regulators at post-blossom that would be suitable to safely reduce crop load, enhance fruit quality, and maintain adequate cropping yield of vigorous apple trees grown under black anti-hail netting.

MATERIAL AND METHODS

The experiment was carried out in the growing seasons of 2020/21 and 2021/22 in a commercial apple orchard located in the municipality of Bom Jesus - RS, Brazil (28°38'45.3" S, 50°36'57.3" W, elevation of 1030 m above sea level). The orchard was planted in 2011 with the cultivar Maxi Gala grafted on the semi-vigorous rootstock Marubakaido with a 20 cm M9 interstock filter, planted at 4.25x1 meters in a density of 2353 trees per hectare, and 'Fuji Mishima' was the pollinizer.

The trees were trained as tall spindle, in which every November, tertiary branches were green pruned to improve light interception in the inner canopy, leaving only the horizontal scaffold branches. Also, the orchard had no irrigation system. The orchard was covered with black anti-hail netting (18% shading) with a mesh of 4 x 7 mm, installed as a flat frame over the rows with a gap of ~20 cm between the netting of the neighboring rows for eventual hail runoff. The region is classified as a Cfb climate, according to Köppen classification, with typically subtropical weather with mild-temperature summer, annual precipitation between 1600 and 1900 mm, mean annual temperatures between 12 and 14 °C (Alvares *et al.*, 2013).

The meteorological data was obtained from a meteorological station localized at 30 Km away from the orchard, in an uncovered area. In summary, from full bloom until

harvest, in the first growing season, total precipitation was 462 mm not regularly distributed, as the first half of the growing season was dry. The mean daily temperature amplitude was 13 °C, and total solar radiation was 2811 Mj.m⁻². In the second growing season, total precipitation was 462 mm not regularly distributed as the second half of the season was dry. The mean daily temperature amplitude was 13.56 °C, and total solar radiation was 3593 Mj.m⁻². The meteorological information is detailed in Figure 1.

The description of the treatments, growth stages, and concentrations are described in Table 1. The treatments were sprayed with a motorized backpack sprayer with a targeted output volume of 1000 L.ha⁻¹ of water, on climatic conditions of ascending temperature with clear or partially cloudy sky. In the first growing season, full bloom (FB) occurred on Sept 28th, 2020, and the growth stage of fruitlet Ø 7 mm (Ø: diameter) was 15 days after full bloom (DAFB); fruitlet Ø 15 mm was 23 DAFB; and fruitlet Ø 20 mm was

31 DAFB. In the second growing season, FB occurred on Sept 22nd, 2021, and the growth stage of fruitlet Ø 7 mm was 13 DAFB; fruitlet Ø 15 mm was 26 DAFB; and fruitlet Ø 20 mm was 33 DAFB. The experiment was arranged in a randomized complete block design with three blocks (rows) containing the experimental units that consisted of five sprayed trees, being evaluated the three central trees, and one tree in each end was left as guard trees, totalizing nine replications per treatment.

At the pink bud stage on both growing seasons, trees were selected based on bloom abundance and uniformity. In each growing season, different trees were used in the experimental site. To determine fruit set, three branches in opposite sides of each replication-tree along the top, middle, and lower scaffolds of the canopy were tagged with the total count of flower clusters of the branch. The treatments that begun the sprays at FB (treatments 3, 4, 5, and 7), the number of clusters was counted right before the FB sprays.

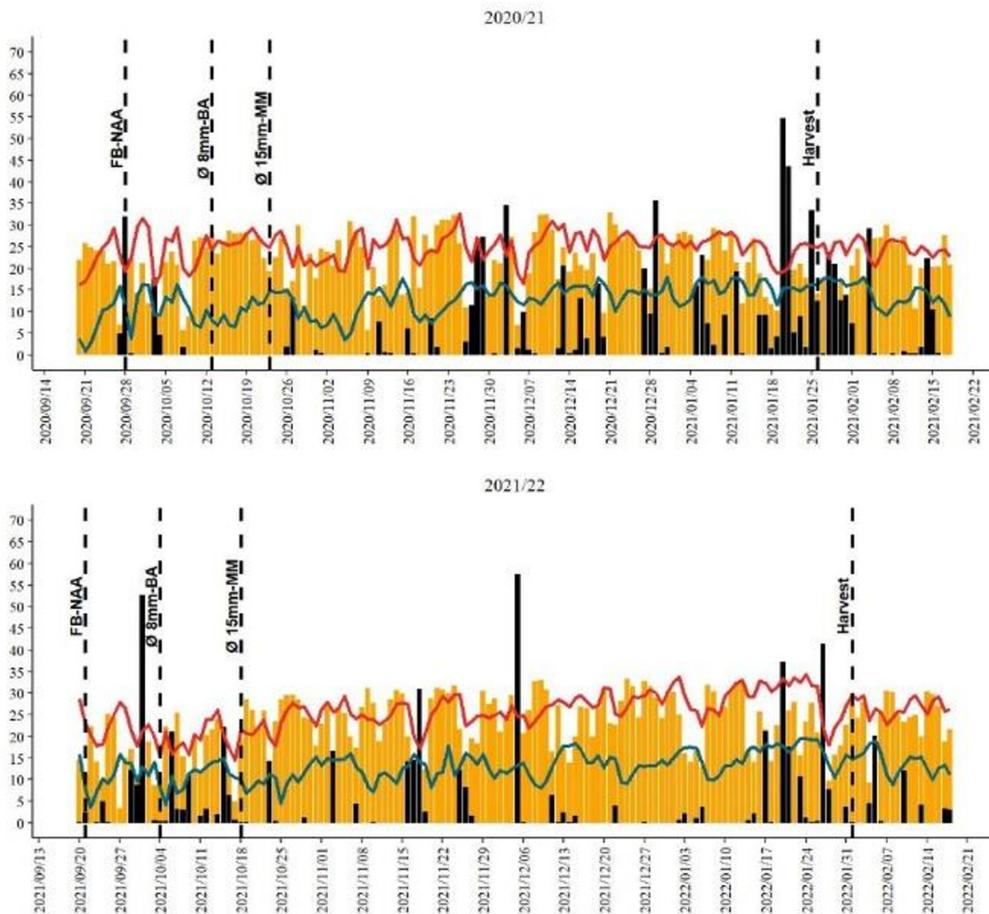


Figure 1: Precipitation, solar radiation, maximum and minimum temperature throughout the growing seasons of 2020/21 (above) and 2021/22 (below).

Table 1: Description of the treatments with the corresponding growth stages, active ingredients, doses, and commercial products of plant growth regulators used as chemical thinners for ‘Maxi Gala’ apple trees grown under black anti-hail netting

Treatments	Growth stages			
	Full Bloom	7 mm	15 mm	20 mm
T1	Untreated Control (UTC)			
T2	Manual Thinning Only (MTO)			
T3	Promalin [®] , 2 L.ha ⁻¹		Maxcel [®] , 3 L.ha ⁻¹ + Ethrel 720 [®] , 1.5 L.ha ⁻¹	
T4	Promalin [®] , 2 L.ha ⁻¹	ANA Técnico [®] , 20 g.ha ⁻¹ + Sevin 480 SC [®] , 0.6 L.ha ⁻¹		
T5	Promalin [®] , 2 L.ha ⁻¹		Maxcel [®] , 3 L.ha ⁻¹ + Sevin 480 SC [®] , 0.6 L.ha ⁻¹	
T6			Ethrel 720 [®] , 1.5 L.ha ⁻¹ + Sevin 480 SC [®] , 0.6 L.ha ⁻¹	Goltix WG [®] , 280 g.ha ⁻¹ + Ethrel 720 [®] , 1.5 L.ha ⁻¹
T7	Promalin [®] , 2 L.ha ⁻¹	Maxcel [®] , 3 L.ha ⁻¹		Goltix WG [®] , 280 g.ha ⁻¹
T8		Goltix WG [®] , 170 g.ha ⁻¹		Goltix WG [®] , 280 g.ha ⁻¹

Note: From treatments 3 to 8 it was added the surfactant adjuvant Break Thru[®] at 0.015% vol/vol. Spray volume used: 1000 L of water.ha⁻¹. **Promalin[®]:** Benzyladenine+GA₄₊₇ (37.6 + 37.6 g ai ha⁻¹); **Maxcel[®]:** Benzyladenine (60 g ai ha⁻¹); **Ethrel 720[®]:** Ethephon (1080 g ai ha⁻¹); **ANA Técnico:** Naphthalene Acetic Acid (19 g ai ha⁻¹); **Sevin 480 SC[®]:** Carbaryl (288 g ai ha⁻¹); **Goltix WG[®]:** Metamitron (119 g ai ha⁻¹ at Ø 7 mm, or 196 g ai ha⁻¹ at Ø 20 mm).

For treatment 8, the initial count for the Untreated Control (UTC) was counted with fruitlets at Ø 7 mm, and treatment 6 at Ø 15 mm, to eliminate the influence of natural fruitlet drop on their UTCs, until the moment of the first spray. Fruit set was defined as the ratio between the total amount of remaining fruitlets on the flower clusters present on November 19th, 2020, and November 22nd, 2021, (52 and 61 DAFB, respectively) before proceeding to the manual thinning, in relation to the tagged number of clusters.

The manual thinning was done following the determination of the fruit set, looking to leave one fruitlet in leafless spurs, two fruitlets in leafed spurs, and three fruitlets in brindles. It was assessed the number of removed fruitlets on each tree.

The assessment of the reduction of the Photosynthetically Active Radiation (PAR) by the black anti-hail netting was done on December 24th, 2021, with a radiometer that consisted of an Arduino Uno[®] controller equipped with a datalogger shield Adafruit[®] v.1.0, and an ambient light sensor BH1750FVI manufactured by ROHM Semiconductor[®] to quantify the PAR (400-700 nm; $\mu\text{mol m}^{-2} \text{s}^{-1}$). The measurements were taken hourly from 9 AM to 3 PM, doing one reading every two seconds in a total of 435 readings each hour per ambient (with and without anti-hail netting). Raw data was converted from Lumens.m⁻² to Watts.m⁻² according to Michael *et al.* (2020) and from Watts.m⁻² to $\mu\text{mol m}^{-2} \text{s}^{-1}$ according to Reis & Ribeiro (2020).

Fruit harvest occurred on February 8th, 2021, and March 3rd, 2022. The criteria to determine the harvest moment was when most fruits were predominantly red, and the starch iodine test of a sample was rating on average 3.4. At this moment it was determined the number of fruits per tree, yield, mean fruit weight, and trunk cross sectional area at 15 cm above the graft union. A sample of 50 fruits per tree was collected to be sorted out in a commercial grader (Prodol[®]), in which fruits > 90 g were considered as > 6 mm of diameter to determine the percentage of fruits into this classification. A subsample of 30 fruits was picked up to visually assess percentage blush coverage (red color), into the classes of 0-25%, 25-50%, 50-75%, and 75-100% of the fruit area; also, skin russetting coverage was visually assessed in the classes of 0%, < 10%, 10-30%, 30-50%, and > 50% of the skin surface area. Then, a subsample of 20 fruits was collected to assess fruit length (cm), diameter (cm), flesh firmness (N) with a penetrometer with a 11 mm tip on opposite sides of the fruit, total soluble solids (°Brix) with a digital refractometer Atago[®], and number of seeds.

All data analysis was performed using R software v. 4.0.2. The data was analyzed through mixed model ANOVA where the factors growing seasons and treatments (GS*T) were set as fixed, and the interaction GS*block, blocks, and plots (trees) were set as random effects using the package “nlme”. In case of significance, treatment means were separated through the Tukey’s HSD test

($p \leq 0.05$). For fruit set, the treatment means were compared through linear contrasts. The factor growing seasons were compared through confidence intervals ($p \leq 0.05$). All mean comparisons were done with the package “emmeans”. Data that violated the ANOVA assumptions were transformed accordingly, and the data that still violated the assumptions were analyzed through the Kruskal-Wallis test ($p \leq 0.05$), and in case of significance the means were separated through the Tukey-Kramer-Nemenyi test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Overall incident PAR reduction (Figure 2) on each measured hour was between 20 and 24% due to the black anti-hail netting, and at the end of the measurements, it was found a total sum of 12286 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of cumulative PAR in the uncovered area, whereas in the covered area it was found a total sum of 9571 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of cumulative PAR, representing a reduction of 22% in the total light supply by the black anti-hail netting during the measured period.

The treatments similarly modulated fruit set in both years (Table 2), although there was bearing alternance in the second year, and consequently the initial load of flower buds was lower (est. 678 vs 384 buds in the first and second

growing seasons, respectively). The treatment 4 (BA+GA₄₊₇ at full bloom followed by Naphthalene Acetic Acid + Carbaryl at fruitlet Ø 7 mm), and the treatment 6 (Ethephon + Carbaryl at fruitlet Ø 15 mm followed by Metamitron + Ethephon at fruitlet Ø 20 mm) significantly reduced fruit set compared to their Untreated Controls (UTCs), while the treatment 3 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Ethephon at fruitlet Ø 15 mm), and the treatment 5 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Carbaryl at fruitlet Ø 15 mm) had a trend to have reduced fruit set ($p = 0.0766$, $p = 0.0624$, respectively) in comparison to their UTCs.

In 2020/21, all PGRs treatments reduced crop load in relation to the UTC, although the treatments 3 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Ethephon at fruitlet Ø 15 mm) and treatment 8 (Metamitron at fruitlet Ø 7mm followed by Metamitron at fruitlet Ø 20 mm) also did not differ from the MTO (Table 3). In 2021/22, the treatment 5 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Carbaryl at fruitlet Ø 15 mm) and 7 (BA+GA₄₊₇ at full bloom followed by Benzyladenine at fruitlet Ø 7 mm followed by Metamitron at fruitlet Ø 20 mm), mostly reduced crop load, whereas all other treatments did not differ from the UTC.

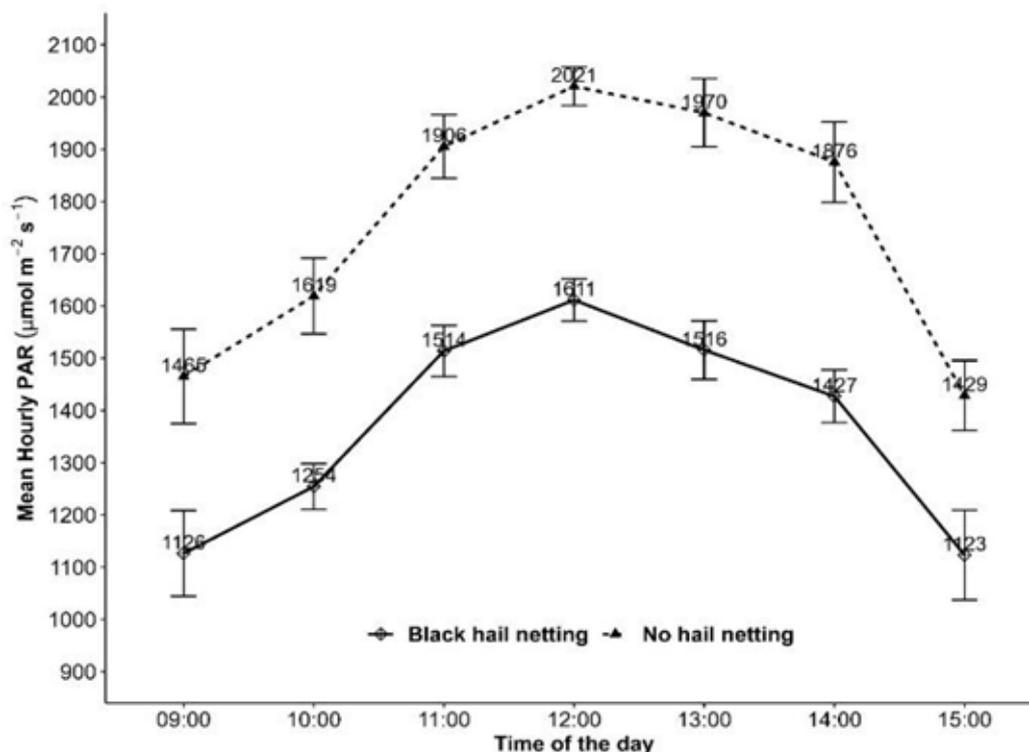


Figure 2: Photosynthetically active radiation (PAR 400-700nm) from 9:00 until 15:00 between rows at 2 meters above the ground under black anti-hail netting and no anti-hail netting. n = 435 measurements for each hour in each environment. Bars represent the mean standard deviation.

Table 2: Fruit set of 'Maxi Gala' apple trees grown under black anti-hail netting as a function of different combinations of plant growth regulators used as fruitlet thinners over two growing seasons

Treatments		Fruit set (fruits/flower clusters) ¹		
		Growing seasons		
		20/21	21/22	Mean
UTC 1 (T3, T4, T5, T7)	-	0.54	0.54	0.54
UTC 2 (T6)	-	0.97	1.01	0.99
UTC 3 (T8)	-	0.82	0.64	0.73
T3	BA+GA,FB / BA+ETH,15	0.45	0.35	0.40
T4	BA+GA,FB / NAA+CB,7	0.29	0.29	0.29
T5	BA+GA,FB / BA+CB,15	0.37	0.39	0.38
T6	ETH+CB,15 / MM+ETH,20	0.56	0.54	0.55
T7	BA+GA,FB / BA,7 / MM,20	0.47	0.58	0.52
T8	MM,7 / MM,20	0.57	0.61	0.59
Mean		0.56	0.55	
			p-value	
Growing season (GS)			ns	
Treatment (T)			< 0.0001	
GS*T			ns	
C.V. (%)			46.72	
Contrasts				p-value
T3 vs UTC 1				0.0766
T4 vs UTC 1				0.0006
T5 vs UTC 1				0.0624
T6 vs UTC 2				< 0.0001
T7 vs UTC 1				0.8929
T8 vs UTC 3				0.2481

Note: ¹Data transformed through $\ln(x+1)$. ns: non-significant. UTC: Untreated Control; BA+GA₄₊₇: Benzyladenine+Gibberellic Acid₄₊₇; BA: Benzyladenine; ETH: Ethephon; NAA: Naphthalene Acetic Acid; CB: Carbaryl; MM: Metamitron. FB: Full Bloom; 15: Fruitlet Ø 15 mm; 20: Fruitlet Ø 20 mm. Non-bold: simple effects; Bold: main effects.

In this experiment, all treatments that contained Carbaryl showed strong fruitlet thinning, that is, it potentialized the thinning effects of Benzyladenine, Ethephon, and Naphthalene Acetic Acid; thus, the use of Carbaryl on vigorous trees under black anti-hail netting proved to be unsafe. This is the case when in both growing seasons the mean yield of the treatment 3 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Ethephon at fruitlet Ø 15 mm) and treatment 8 (Metamitron at fruitlet Ø 7mm followed by Metamitron at fruitlet Ø 20 mm) bore a yield around 60 t.ha⁻¹, which was also the targeted level of the producer.

The synergic effect of Carbaryl on enhancing the fruitlet thinning capacity of other PGRs agrees with Stover *et al.* (2001) for NAA, with Cline *et al.* (2019) for BA, and with Marini (1996) for Ethephon, wherein it was reported that, in uncovered areas, the mix of these PGRs

with Carbaryl promoted strong synergistic effect and increased fruitlet thinning. In addition, the reduction of 22% of the PAR promoted by the netting may have enhanced the thinning effect of these PGRs, especially Carbaryl, as Byers *et al.* (1990) reported that a four-days 92% shading increased the thinning effect of Carbaryl, up to a level of overthinning the trees.

Natural fruitlet abscission in apple trees is driven by the threshold of assimilates available for cell multiplication and growth; that is, the source-sink relationship plays the utmost role in natural fruitlet drop regulation, e.g., when the initial flower bud load is high, the greater is the natural fruitlet drop, and vice-versa (Lakso, 2011; Morandi *et al.*, 2011; Lordan *et al.*, 2019). Concerning the pattern of natural fruitlet abscission in areas covered with anti-hail netting, there are several studies depicting that the reduction of the PAR leads to a reduction in the number of fruits and

Table 3: Crop load, number of fruits removed at the manual thinning per tree, and yield of 'Maxi Gala' apple trees grown under black anti-hail netting as a function of different combinations of plant growth regulators used as fruitlet thinners over two growing seasons

Treatments ²	Crop load (Fruits.cm ⁻² TCSA) ¹				Number of fruits removed at the manual thinning per tree ³				Yield (t.ha ⁻¹) ¹			
	Growing season		Growing season		Growing season		Growing season		Growing season		Growing season	
	20/21	21/22	Mean	20/21	21/22	Mean	20/21	21/22	Mean	20/21	21/22	Mean
T1	10.24 a	5.77* a	8.00	-	-	-	79.1	44.4	61.7 A			
T2	7.89 ab	4.15* ab	6.02	41 a	5 a	23	85.9	33.3	59.6 AB			
T3	6.21 bc	4.11* ab	5.16	9 b	3 a	6	63.9	34.1	48.9 ABC			
T4	3.74 d	4.19 ^{ns} ab	3.97	6 b	2 a	4	50.4	35.1	42.7 BC			
T5	5.42 cd	3.29 ^{ns} b	4.35	10 b	1 a	6	55.0	26.3	40.7 C			
T6	5.29 cd	4.15 ^{ns} ab	4.72	10 b	2 a	6	50.2	34.0	42.1 C			
T7	5.13 cd	3.61 ^{ns} b	4.37	15 ab	1 a	8	60.1	26.5	43.3 BC			
T8	5.41 bcd	4.45 ^{ns} ab	4.93	12 ab	3 a	8	60.3	33.7	47.0 ABC			
Mean	6.17	4.22		15	2		63.1	33.4*				
Growing season (GS)	p-value			p-value			p-value		p-value			
Treatment (T)	0.0125			-	-		0.0008		0.0008			
GS*T	<0.0001			0.0013 ⁴	ns ⁴		0.0003		0.0003			
C. V. (%)	0.0017			-	-		ns		ns			
	21.89			159.56			25.24		25.24			

Note: ¹Data transformed through \sqrt{y} . ²Treatment means followed by different letters in a column are significantly different according to the Tukey's HSD test ($p \leq 0.05$). ³Growing season means followed by * and ^{ns} in a row are significant and non-significant, respectively according to confidence intervals ($p \leq 0.05$). ⁴Treatment means followed by different letters in a column are significantly different according to the Tukey-Kramer-Nemenyi test ($p \leq 0.05$). ⁵Significance according to the Kruskal-Wallis test ($p \leq 0.05$). TCSA: trunk cross-sectional area; UTC: Untreated Control; MTO: Manual Thinning Only; BA+GA₄₊₇: Benzyladenine+Gibberellic Acid₄₊₇; BA: Benzyladenine; ETH: Ethephon; NAA: Naphthalene Acetic Acid; CB: Carbaryl; MM: Metamitron. FB: Full Bloom; 15: Fruitlet Ø 15 mm; 20: Fruitlet Ø 20 mm. Non-bold lowercase data: simple effects; Bold uppercase data: main effects.

yield, and enhanced natural fruitlet drop (Amarante *et al.*, 2009; Brglez Sever *et al.*, 2021). In addition, the thinning effect of the PGRs is on modulating the source-sink relationship creating a temporary shortage of assimilates to the fruitlets leading to an increment of the competition among leaves, branches, and fruitlets, as these PGRs act either on inhibiting PPSII, halting the translocation of assimilates, stimulating whole plant cell multiplication, increasing respiration, or inducing ethylene evolution directly (Eccher *et al.*, 2013; Morandi *et al.*, 2011; Yuan, 2007; Zhu *et al.*, 2011). Thus, the PAR limitation imposed by the netting may be an enhancer of the effect of the PGRs on promoting fruitlet thinning.

The effectiveness of the PGRs treatments required significantly less complement of manual thinning (Table 3), and, in comparison with the treatment MTO, the treatment 3 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Ethephon at fruitlet Ø 15 mm, the treatment 4 (BA+GA₄₊₇ at full bloom followed by Naphthalene Acetic Acid + Carbaryl at fruitlet Ø 7 mm, the treatment 5 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Carbaryl at fruitlet Ø 15 mm, and the treatment 6 (Ethephon + Carbaryl at fruitlet Ø 15 mm followed by Metamitron + Ethephon at fruitlet Ø 20 mm required significantly less manual thinning. In 2021/22 due to bearing alternance, and lower flower bud load, it was found no difference among treatments, thus there was no need for intense manual thinning, and the number of fruitlets removed was negligible for all treatments.

There was significant bearing alternance between both growing seasons (Table 3). In 2020/21, the yield of the UTC was 79.1 tonnes per hectare, and in 2021/22 it was 44.4 tonnes per hectare, corresponding to a reduction of 44%. In the first year, initial flower bud load was 687, and in the second year it was 394 buds per tree, which explains the lower overall cropping yield in the second year. The treatments 4 (BA+GA₄₊₇ at full bloom followed by Naphthalene Acetic Acid + Carbaryl at fruitlet Ø 7 mm), 5 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Carbaryl at fruitlet Ø 15 mm, 6 (Ethephon + Carbaryl at fruitlet Ø 15 mm followed by Metamitron + Ethephon at fruitlet Ø 20 mm), and 7 (BA+GA₄₊₇ at full bloom followed by Benzyladenine at fruitlet Ø 7 mm followed by Metamitron at fruitlet Ø 20 mm) mostly reduced yield, although all PGRs treatments were significantly equivalent. However, the treatment 3 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Ethephon at fruitlet Ø 15 mm) and the

treatment 8 (Metamitron at fruitlet Ø 7mm followed by Metamitron at fruitlet Ø 20 mm) also did not differ from the UTC or MTO.

A good chemical thinning program must take into consideration the crop load adjustment at a level that improves fruit size without compromising cropping yield. The treatments that fitted these criteria were: BA+GA₄₊₇ at full bloom followed by Benzyladenine + Ethephon at Ø 15 mm (Treatment 3), and Metamitron at Ø 7 mm followed by Metamitron at Ø 20 mm (Treatment 8), in a condition of vigorous rootstocks under black anti-hail netting as in this experiment.

The PGR Metamitron is reported as having no synergic thinning effect with shading, as under anti-hail nets (19-22% PAR reduction) there is no increase of fruitlet thinning, thus, the effect of this PGR is not affected by the level of solar radiation, but by the night temperatures, as in the following days after the application under night temperatures below 14 °C the thinning effect is impaired, and higher night temperatures potentialize fruitlet thinning (Reginato *et al.*, 2014; Gonzalez *et al.*, 2020a; Gonzalez *et al.*, 2020b; Clever, 2022). However, Metamitron has a cumulative thinning effect for apples, that is, two sequential sprays have a stronger effect than one (Gonzalez *et al.*, 2020b).

In our experiment, the night temperatures seven days after Metamitron sprays were below 14 °C in both years, and this may have contributed to a safer effect of treatment 8 (Metamitron at fruitlet Ø 7mm followed by Metamitron at fruitlet Ø 20 mm) in such conditions. In contrast, Clever (2022) found that the effectiveness of Metamitron was influenced by hail netting only when the fruitlets had Ø 15 and 18 mm, as it promoted thinning under hail netting and no thinning in the open sky condition, although, when the sprays happened on smaller fruitlets, no difference was found. It is likely that under netting the availability of assimilates is further limiting and during the transition from the assimilates supply from the stored carbohydrates to the assimilates currently being synthesized by leaves takes longer and subjects the fruitlets to a more stressing condition (Lordan *et al.*, 2019; 2020).

Under hail netting the lower sunlight incidence creates an unfavorable condition for fruit set, and even the thinning effect of the PGRs may be affected. Benzyladenine did not promote thinning of 'Pinova' apples, when applied under natural conditions, but under hail netting, the thinning was strong (Clever, 2022). The effectiveness of Ethephon in

promoting fruitlet thinning is dependent on the temperature after the application, as the peak of ethylene evolution happens faster when the night temperatures are higher, and vice-versa on cooler nights (Yuan, 2007).

In our experiment, treatment 3 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Ethephon at fruitlet Ø 15 mm) promoted a mild thinning and was safe to use on a condition of shaded environment and vigorous trees. Similarly, Elfving & Cline (1993), and Clever (2022) found that when using Benzyladenine alone or mixed with Ethephon had the same thinning intensity of Benzyladenine alone, although the authors attribute the lower efficacy of the Ethephon to the cooler temperatures. On both years the mean temperatures were cool in the three days following Ethephon sprays, being in the first year, 18, 19, and 19 °C for Ø 15 mm, and 13, 12, and 13 °C for Ø 20 mm; while in the second year, it was 13, 14, and 16 °C for Ø 15 mm, and 17, 18, and 17 °C for Ø 20 mm (Figure 1).

Fruit weight was affected by treatment only in 2020/21 (Table 4). All PGRs treatments improved fruit weight in relation to the treatments MTO and UTC, although, the treatment 6 (Ethephon + Carbaryl at fruitlet Ø 15 mm followed by Metamitron + Ethephon at fruitlet Ø 20 mm) also did not differ from the treatments UTC and MTO, and

the treatment 8 (Metamitron at fruitlet Ø 7mm followed by Metamitron at fruitlet Ø 20 mm) also did not differ from the treatment MTO. In addition, the treatments UTC, MTO, and Treatment 6 did not differ from the results found in 2021/22, where the fruits were lighter.

Fruit shape (L:D ratio) was affected by treatments in both years, and in 2020/21, with treatment 4 (BA+GA₄₊₇ at full bloom followed by Naphthalene Acetic Acid + Carbaryl at fruitlet Ø 7 mm), the fruits had a less flattened shape than the other treatments (Table 4). In 2021/22, the treatment 5 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Carbaryl at fruitlet Ø 15 mm) induced the longest fruits, after that treatment 7 (BA+GA₄₊₇ at full bloom followed by Benzyladenine at fruitlet Ø 7 mm followed by Metamitron at fruitlet Ø 20 mm). Overall, on the second year the fruits were longer. There was no effect of treatments on the yield of fruits > 60 mm, although in the second growing season, the distribution into this class was lower than the previous growing season (Table 4). The smaller fruits in the second growing season could be due the poor distribution of the precipitation, which happened to be high at early season, and low during December and mid-January, even though total precipitation of both growing seasons was virtually the same (Figure 1).

Table 4: Fruit weight, fruit length/diameter ratio, and yield of fruits > than 60 mm of diameter of 'Maxi Gala' apple trees grown under black anti-hail netting as a function of different combinations of plant growth regulators used as fruitlet thinners over two growing seasons

Treatments ²	Fruit weight (g)						Ratio Length:Diameter				Yield of fruits Ø > 60mm (%) ¹				
	Growing season						Growing season				Growing season				
	20/21		21/22		Mean	20/21		21/22		Mean	20/21	21/22	Mean		
T1	UTC		94.8	c	91.1 ^{ns}	a	93.0	0.94	b	1.03*	b	0.98	70	37	54A
T2	MTO		98.8	bc	92.8 ^{ns}	a	95.8	0.94	b	1.03*	b	0.99	73	45	59A
T3	BA+GA,FB / BA+ETH,15		120.7	a	90.5*	a	105.6	0.96	ab	1.03*	b	0.99	87	45	66A
T4	BA+GA,FB / NAA+CB,7		116.1	a	88.0*	a	102.0	0.99	a	1.03 ^{ns}	b	1.01	78	40	59A
T5	BA+GA,FB / BA+CB,15		120.2	a	93.6*	a	106.9	0.96	ab	1.07*	a	1.02	79	45	62A
T6	ETH+CB,15 / MM+ETH,20		105.6	abc	88.2 ^{ns}	a	96.9	0.95	b	1.03*	b	0.99	66	40	53A
T7	BA+GA,FB / BA,7 / MM,20		115.8	a	94.9*	a	105.4	0.97	ab	1.05*	ab	1.01	86	48	67A
T8	MM,7 / MM,20		112.0	ab	90.0*	a	101.0	0.95	b	1.03*	b	0.99	82	39	60A
Mean			110.6		91.1		0.96			1.04		0.96	78	42*	
					p-value					p-value					p-value
Growing season (GS)					0.0157					0.0004					< 0.0001
Treatment (T)					0.0002					0.0003					ns
GS*T					0.0004					0.0029					ns
C.V. (%)					15.96					5.09					22.25

Note: ¹Data transformed through . ²Treatment means followed by different letters in a column are significantly different according to the Tukey's HSD test (p ≤ 0.05). ¹Growing season means followed by * and ^{ns} in a row are significant and non-significant, respectively according to confidence intervals (p ≤ 0.05). UTC: Untreated Control; MTO: Manual Thinning Only; BA+GA₄₊₇: Benzyladenine+Gibberellic Acid₄₊₇; BA: Benzyladenine; ETH: Ethephon; NAA: Naphthalene Acetic Acid; CB: Carbaryl; MM: Metamitron. FB: Full Bloom; 15: Fruitlet Ø 15 mm; 20: Fruitlet Ø 20 mm. Non-bold data: simple effects; Bold data: main effects.

Table 5: Seed number per fruit, flesh firmness, and total soluble solids of 'Maxi Gala' apple trees grown under black anti-hail netting as a function of different combinations of plant growth regulators used as fruitlet thinners over two growing seasons

Treatments ²	Seed number per fruit ¹			Flesh firmness (N)			Total soluble solids (°Brix)			Skin russet 10-30% of fruit surface area (%)			
	Growing season			Growing season			Growing season			Growing season			
	20/21	21/22	Mean	20/21	21/22	Mean	20/21	21/22	Mean	20/21	21/22	Mean	
T1	UTC	5.47	3.17	AB	67.48	78.94	73.21A	10.42cd	11.62* ^b	11.02	46	64	55A
T2	MTO	5.54	3.86	A	66.01	76.90	71.46A	10.40d	12.11* ^{ab}	11.26	48	64	56A
T3	BA+GA,FB / BA+ETH,15	4.63	2.45	CD	68.53	76.45	72.49A	11.21abc	12.00* ^{ab}	11.61	40	61	51AB
T4	BA+GA,FB / NAA+CB,7	4.58	3.00	BCD	68.08	74.74	71.41A	10.83bcd	11.89* ^{ab}	11.36	28	56	42B
T5	BA+GA,FB / BA+CB,15	4.14	2.32	D	68.74	80.19	74.47A	10.77bcd	12.43* ^a	11.60	37	55	46AB
T6	ETH+CB,15 / MM+ETH,20	5.13	2.83	ABC	70.35	77.94	74.14A	11.70a	12.19* ^{ab}	11.94	45	70	58A
T7	BA+GA,FB / BA,7 / MM,20	4.99	3.16	ABC	70.73	77.74	74.23A	11.24ab	11.71* ^{ab}	11.48	41	59	50AB
T8	MM,7 / MM,20	5.32	3.59	AB	67.63	78.20	72.91A	10.62bcd	12.04* ^{ab}	11.33	42	69	56A
Mean		4.98	3.05*		68.44	77.64*		10.90	12.00		41	62*	
Growing Season (GS)			p-value			p-value			p-value			p-value	
			0.0345			0.0001			0.0003			0.0005	
Treatment (T)			< 0.0001			0.0257			0.0001			0.0009	
GS*T			ns			ns			0.0014			ns	
C.V.(%)			19.06			7.91			7.25			21.29	

Note: ¹Data transformed through... ²Treatment means followed by different letters in a column are significantly different according to the Tukey's HSD test ($p \leq 0.05$). Growing season means followed by * and ns in a row are significant and non-significant, respectively according to confidence intervals ($p \leq 0.05$). UTC: Untreated Control; MTO: Manual Thinning Only; BA+GA₄₊₇: Benzyladenine+Gibberellic Acid₄₊₇; BA: Benzyladenine; ETH: Ethephon; NAA: Naphthalene Acetic Acid; CB: Carbaryl; MM: Metamitron. FB: Full Bloom; 15: Fruitlet Ø 15 mm; 20: Fruitlet Ø 20 mm. Non-bold data: simple effects; Bold data: main effects.

Fruit weight was highly impacted by the timing that the PGRs were first sprayed in our experiment. All treatments that contained BA+GA₄₊₇ at full bloom presented higher fruit weight, after that the treatment 8 (Metamitron at fruitlet Ø 7mm followed by Metamitron at fruitlet Ø 20 mm), which the first PGR spray was on fruitlets at Ø 7 mm, and treatment 6 (Ethephon + Carbaryl at fruitlet Ø 15 mm followed by Metamitron + Ethephon at fruitlet Ø 20 mm), with the first spray at Ø 15 mm. Although the latter two treatments reduced crop load, the enhancement of fruit size was not so pronounced. The treatment MTO also played little influence on the fruit weight, as fruitlet removal occurred ~50 DAFB.

The growth pattern of apple fruitlets throughout the growing season is divided into three parts: following pollination the growth is exclusively by cell multiplication, then multiplication and expansion, and finally only through expansion of the cells; thus, the earlier the competition is removed, the higher is the potential for increasing cell number and fruit size (Lakso & Goffinet, 2013). In addition, Benzyladenine also acts directly on cell multiplication, and increases fruit size independently from crop load (Wismer *et al.*, 1995).

The treatments modulated fruit seed number similarly in both years, and in 2021/22, seed number was significantly lower than in 2020/21 (Table 5). The treatment 5 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Carbaryl at fruitlet Ø 15 mm) mostly reduced seed number, after that the treatment 3 (BA+GA₄₊₇ at full bloom followed by Benzyladenine + Ethephon at fruitlet Ø 15 mm) and treatment 4 (BA+GA₄₊₇ at full bloom followed by Naphthalene Acetic Acid + Carbaryl at fruitlet Ø 7 mm). The unfavorable meteorological conditions in the beginning of the second growing season struggled pollination and led to a lower seed number.

Regarding fruit skin russetting, treatments only impacted in the class of 10-30% of the surface area whereas the other classes were not significant (data not shown) (Table 5). No effect of treatment was found for fruit red color coverage (data not shown)

All treatments with BA+GA₄₊₇ sprayed at full bloom had slightly lower incidence of skin russetting. Skin russetting is formed as a consequence of tensile forces (especially caused by high humidity and chemicals) on the cuticle membrane and fruit epidermis, which cause microcracks on the fruit surface that are filled with phenolic compounds for repairing, leaving a harsh aspect; although, GA₄₊₇ on

early fruitlet growth stages increases the elasticity of the hypodermal tissues, which decreases the tensile forces and ameliorate the incidence of this disorder (Knoche *et al.*, 2011).

Treatments containing Benzyladenine alone or in combination reduced seed number. This is a feature of this PGR and one of the reasons it promotes fruitlet thinning (Greene *et al.*, 1992) as it promotes intense cell multiplication in the whole canopy making available less assimilates to the least developing embryos, and also antagonizing the embryo synthesis of auxin, leading to its abortion (Schröder *et al.*, 2013). This was similar to what was found by Stover *et al.* (2001) in which the authors reported that Benzyladenine alone only reduced the seed number of apples when sprayed at Ø 15 mm, but when mixed with Carbaryl, the range was wider: from Ø 10 to 15 mm.

Flesh firmness was not affected by treatment on both growing seasons, although, on the second, overall, the fruits were firmer (Table 5). On the other hand, total soluble solids (TSS) was impacted by treatments each growing season, being treatments 3, 6, and 7 the ones with higher TSS in the first growing season, and T5 in the second growing season. Also, except T7, all other treatments had higher TSS in the second growing season. Accordingly, Bound & Wilson (2007) found that the treatments which reduced crop load, also increased TSS, and flesh firmness, although the latter was not impacted by treatments in our experiment.

CONCLUSIONS

Our results suggest that in the conditions of this experiment in which 'Maxi Gala' apple trees are grown under black anti-hail netting, the thinning programs: BA+GA₄₊₇ at full bloom followed by Benzyladenine + Ethephon at fruitlet diameter of 15 mm, or Metamitron at fruitlet diameter of 7 mm followed by Metamitron at fruitlet diameter of 20 mm, are safe and effectively reduce crop load without compromising crop yield, besides enhancing fruit quality.

In the conditions of 'Maxi Gala' apple trees growing under black anti-hail netting, the use of Carbaryl potentialized the thinning effect of other PGRs turning out to be unsafe to use as fruitlet thinner.

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