Effect of thinning with Metamitron, NAA, BA and Naphthenic Acids on apple (*Malus domestica*) trees.

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Abstract

The successful use of chemical thinners on apples requires programs employing multiple chemistries over the thinning period. A thinning program can be designed using various active ingredients in single or multiple applications. The objective of this work was to compare that standard thinning program (COM-STD), with naphthalene acetamide (NAD), 6-benzyladenine (BA) and naphthyl acetic acid (NAA), to new programs involving Metamitron (ME). Five experiments were conducted over seven seasons, from 2013 to 2019 on Gala and Golden apples. Under the trial conditions, COM-STD (NAD/(BA+NAA)) and ME induced fruit abscission. However, the single applications of ME and COM-STD, made on the same days, showed the same level of thinning efficacy. NAD in petal fall with Tank mix (ME+NAA+BA) application at 11mm increased the thinning efficacy in comparison with applying ME and COM-STD alone. Dose effects were also observed with both ME applied alone and the Tank mix (ME+BA). In general, all combinations involving ME and COM-STD, and especially applying COM-STD after ME, produced greater thinning results than applying either product individually to 'Gala' and 'Golden' apples. Crop yields fell as the thinning efficacy increased, in all the experiments. There was also a negative quadratic relationship between thinning efficacy and average fruit weight, color and diameter.

Keywords

Metamitron, Benzyladenine, Naphthyl acetic acid, Apple thinning, fruit abscission, Crop load

1. Introduction

In intensive apple crop cultivation, nutrients and water are supplied to the optimum, hence management of light and crop load becomes the limiting factor in terms of fruit quality and marketability (Bosančić *et al.*, 2018). Apple trees need to produce many flower clusters and fruits to obtain regular, high-quality, marketable crops from year-to-year. Too many fruits/tree can result in small fruit size, poor quality, the breakage of limbs, the exhaustion of tree reserves, and reduced cold hardiness (Dennis, 2000). Partial removal of the plant sink organs is called thinning, and it is often performed in commercial orchards to increase final fruit size (Nuñez *et al.*, 2019). The objective of thinning is to obtain an optimum volume of fruit/tree. Appropriate thinning must be applied from year to year because of the benefits for yield, fruit size and distribution, and other aspects of fruit quality (including color, firmness, sugar, and acidity, etc.). Moreover, apple flowers bud in the year prior to bloom and appropriate thinning can help reduce biennial bearing effects. The efficacy of chemical thinning tends to be variable because it depends on climatic conditions and on the rate and timing of the application of the treatment (Gonzalez *et al.*, 2019a; Gonzalez *et al.*, 2019b; Lordan *et al.*, 2018; Yoon *et al.*, 2011). For all these reasons, apple thinning requires a complex management strategy, and this is a determining factor in the profitability of apple orchards (Dennis, 2000; Lordan *et al.*, 2019; Robinson *et al.*, 2013).

In Spain, chemical thinning can be carried out during flowering (with ammonium thiosulphate (ATS) and naphthalene acetamide (NAD)) and after fruit set (with Metamitron (ME), 6-benzyladenine (BA) and naphthyl acetic acid (NAA)). After fruit set, chemical thinners can be applied on young fruitlets with king fruit diameters ranging between 6 and 16 mm. The most common chemical thinning program in Spain is to apply NAD at petal fall (PF) and then a second spray of BA+NAA at 10-12mm fruit size. The objective of this work was to compare that standard thinning program (COM-STD) to new programs involving ME.

2. Materials and methods

2.1. Experiment 1:

Eight trials were conducted over four seasons, from 2014 to 2017, in apple orchards at the IRTA experimental agricultural stations of Mas Badia (MB) (Tallada d'Emporda), Mollerussa (MO) (Lleida) and Gimenells (GI) (Lleida), in Spain. We compared hand thinning (1 fruit/cluster and 15-20 cm separation between fruits) and Untreated Control (UTC) with applications of ME at 165 g ha^{-1}, commercial standard program (COM-STD)

(NAD 60 g ha^{-1} / BA 750 mL ha^{-1} + NAA 100 mL ha^{-1}) and NAD 60 g ha^{-1}/ Tank mix (BA 750 mL ha^{-1} + NAA 100 mL ha^{-1} + ME 165 g ha^{-1}) (Table 1). The water volume of spray was equivalent to 1000 l/ha in all trials. All NAD applications were sprayed at flowering time. The applications were sprayed when the king fruit diameter was between 10 and 11 mm on 'Gala' trees planted in 2003 at MO, in 2006 at GI and in 2000 at MB. The tree spacings were 4 m×1.5 m at MO and GI and 3.7 m x 1 m at MB (Table 1).

2.2. Experiment 2:

Three trials were conducted over two seasons, from 2015 to 2016, in apple orchards at the IRTA experimental agricultural stations at MB and MO. We compared an UTC with applications of ME at 165 g ha^{-1} at different moments, applying both alone and in combination with COM-STD (Table 1). Water volume was equivalent to 1000 l/ha. All COM-STD applications were sprayed with NAD (60 g ha^{-1}) at flowering time. The first application was made when the king fruit diameter was 8 mm. The second was made at a diameter of 11 mm and the third with king fruit at 13 mm. All trials involved 'Gala' were planted in 2003 at MO and in 2000 at MB. The tree spacings were 4 m ×1.5 m at MO and 3.7 m × 1 m at MB (Table 1).

2.3. Experiment 3:

Nine trials were conducted over five seasons (from 2015 to 2019) in apple orchards at the IRTA experimental agricultural stations at MB and MO. We compared an UTC with applying COM-STD alone at a king fruit diameter of 10 to 11 mm and in two sprays: a 1st of COM-STD at 10 to 11 mm and a 2nd of ME at 13 to 14 mm. All the treatments were sprayed with NAD at flowering time and water volume was equivalent to 1000 l/ha. The first application was made when the king fruit diameter was 8 mm, the second at 11 mm, and the third at 13 mm. All the trials were conducted on 'Gala' planted in 2003 at MO and in 2000 at MB. The tree spacings were 4 m ×1.5 m at MO and 3.7 m × 1 m at MB (Table 1).

2.4. Experiment 4:

Three trials were carried out on 'Gala' over three different seasons (from 2017 to 2019) in orchards at the IRTA experimental agricultural stations at MB and MO. The tree spacings were 4 m ×1.5 m at MO and 3.7 m × 1 m at MB. We compared an UTC with applications of only COM-STD to fruit that was from 10 to 11 mm in diameter. There were two sprays, the first of COM-STD at diameters of 10 to 11 mm and the second of ME at two different rates (165 and 220 g ha^{-1}) at diameters of 13 to 15 mm respectively.

The water volume of spray was equivalent to 1000 l/ha in all trials. All the treatments were sprayed with NAD at flowering time (Table 1).

2.5. Experiment 5:

One trial was conducted in 2013 at MB, on 'Golden Crielaard' planted in 2003, with a tree spacing of 3.8 m × 1.1 m. We compared an UTC and hand thinning with single applications of ME at different rates (110 to 220 g ha^{-1}), sprayed both alone and in combination with BA (500 g ha^{-1}), and a double application of ME at 165 g ha^{-1}. The single application was sprayed at a king fruit diameter of 9 mm and the second at 13 mm. Water volume was equivalent to 1000 l/ha (Table 1).

2.6. General information

All the orchards were managed according to the standards normally used in commercial apple orchards in the region. The trees were irrigated and fertilized using a drip irrigation system. The trees in the field trials were uniform in terms of the number of their flower clusters and growth. All trials were designed as randomized complete blocks with four replicates of four uniform trees per elementary plot. Within each plot, the two central trees were used for the trial assessments and two border trees as guards.

2.7. Type of assessment

In all trials, the assessments were carried out on two centrally located tree from each elementary plot. This was done with the objective of assessing the effect of the different treatments on fruit set, yield and quality parameters (fruit weight, size and coloration).

2.7.1. Yield and fruit set

The total number of flower clusters/tree was counted at bud break (BBCH 61-65), before the treatments were applied and the next following season (return bloom). Return bloom was determined in all trials except for MB2015, MO2019 and the experiment 5. At harvest, individual sample trees were separately harvested and evaluated. In each orchard, all the fruit was harvested through a single pick that was carried out during the commercial harvest season. Fruit weight, fruit size, fruit blush area, total fruit yield (kg/tree) and fruits/tree were registered using commercial apple sorting and packing line machinery. The commercial sorting machines were a Calinda (Caustier Ibérica, S.A. with Aweta Technology) at MB and a Maf Roda (Agrobotic, France) at MO and GI. The fruit set percentage was calculated as 100*(No. fruits/(No. flower clusters/tree)).

Table 1. List of experiment numbers, locations, years, cultivars, treatments and timings for the different thinning trials. All trials used Amid Thin (8.2% NAD), Brevis® (containing 15% metamitrona), MaxCel® (1,98% 6-Benziladenina) and Rhodofix (1% NAA).

Exp.	Location	Years	Cultivar	Treatments (Abbreviation treatment; Timing (mm))
1	Mollerussa, Lleida, Spain (MO) Gimenells, Lleida, Spain (GI) La Tallada d'Empordà, Girona, Spain (MB)	2014 - 2017 2014 & 2016 2015 & 2016	Gala	- Control (UTC)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD;10/11 mm)
				- ME 165 g ha^{-1} (10/11 mm)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} + ME 165 g ha^{-1} (Tank mix; 10/11 mm) (MB15, MB16 & MO16)
				- Hand thinning
2	Mollerussa, Lleida, Spain La Tallada d'Empordà, Girona, Spain	2015&2016 2015	Gala	- Control (UTC)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD;11 mm)
				- NAD 60 g ha^{-1} (PF) - ME 165 g ha^{-1} (8 mm) & BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD;11 mm)
				- NAD 60 g ha^{-1} (PF) - ME 165 g ha^{-1} (8 mm) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD; 11 mm) - ME 165 g ha^{-1} (13 mm)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD; 11 mm) - ME 165 g ha^{-1} (13 mm)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} + ME 165 g ha^{-1} (Tank mix; 13 mm)
3	Mollerussa, Lleida, Spain La Tallada d'Empordà, Girona, Spain	2015 - 2018 2015 - 2017& 2019	Gala	- Control (UTC)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD; 10/11 mm)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD; 10/11 mm) - ME 165 g ha^{-1} (13/14 mm)
4	Mollerussa, Lleida, Spain La Tallada d'Empordà, Girona, Spain	2017 & 2018 2019	Gala	- Control (UTC)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD; 10/11 mm)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD; 10/11 mm) - ME 165 g ha^{-1} (13/15 mm)
				- NAD 60 g ha^{-1} (PF) - BA 750 mL ha^{-1}+ NAA 100 mL ha^{-1} (COM-STD; 10/11 mm) - ME 220 g ha^{-1} (13/15 mm)
5	La Tallada d'Empordà, Girona, Spain	2013	Golden Crielaard	- Control (UTC)
				- ME 110 g ha^{-1} (9 mm)
				- ME 165 g ha^{-1} (9 mm)
				- ME 220 g ha^{-1} (9 mm)
				- ME 110 g ha^{-1} + BA 500 mL ha^{-1} (Tank mix; 9 mm)
				- ME 165 g ha^{-1} + BA 500 mL ha^{-1} (Tank mix; 9 mm)
				- ME 220 g ha^{-1} + BA 500 mL ha^{-1} (Tank mix; 9 mm)
				- ME 165 g ha^{-1} (9 mm) - ME 165 g ha^{-1} (13 mm)
				- Hand thinning

2.8. Statistical analysis

The crop load parameters were analyzed using a mixed model to assess the long-term effects of each production system using SAS 9.2 (SAS Institute Inc., 2009). The mixed model included the trial and treatment and their interaction as the fixed effects for flower clusters/tree, return bloom, fruits/tree, fruit set and kg/tree. When the interaction was significant, each experiment was analyzed individually. The block was a random effect. For all the models, when the main effects (treatment and trial) were significant, comparisons between treatments were made using Tukey's HSD test at P values of ≤ 0.05. Average fruit weight, average fruit diameter (mm), and average red blush (%) were recorded. When their interaction was significant, each experiment was individually analyzed in the same way to assess the crop load parameters. However, when the interaction was not significant, a quadratic relationship was determined between all quality parameters and final fruits/tree.

3. Results

3.1. Experiment 1:

The orchards where the field trials were carried out showed homogeneous bloom in all the trials. Overall, the chemical applications with ME and COM-STD produced significantly lower fruit numbers/tree, yield and fruit set than the UTC, except in the cases of GI2016, MB2016, MO2016 and MO2017 (Table 2). These treatments also showed no significant differences between trials, except at MO2014. There were no significant differences between ME, COM-STD and hand thinning in terms of fruit number/tree, yield and fruit set in any of the trials, except for MO2014 and MO2016 (Table 2). The hand thinning in these trials produced a significantly low number of fruits/tree and fruit set in comparison with the ME treatment. In all of the trials, the Tank mix provided the most efficient thinning treatment. For example, the Tank mix applied in MB2015 and MO2016 produced the same crop load parameters as hand thinning. However, this treatment produced excessive thinning in MO2015 (Table 2). There were no significant treatment differences in the return bloom in all trials except for GI2014. All experiments showed a tendency to increase the return bloom when efficiency of thinning was higher. However, the hand thinning treatment did not show this tendency. In all of the trials, there was a negative relationship between number of fruits and average weight, color and diameter. This way, average fruit weight, fruit size and red blush area increased when the crop load was reduced (Table 2).

Table 2. Effects of COM-STD (BA+NAA), ME, Tank mix (BA+NAA+ME) and hand thinning on ‘Gala’ trees at GI2014, GI2016, MB2015, MB2016 and MO2014–2017. All treatments were sprayed when the king fruit diameter was between 10 and 11 mm. The target thinning effect was the hand thinning treatment (Experiment 1).

Location	**Year**	**Treatment**	**Flowering (clusters/ tree)**	**Crop load (fruits/ tree)**	**Fruit set (fruits/100 clusters)**	**Yield (kg/tree)**	**Average fruit weight (g)**	**Average fruit diameter (mm)**	**Average red blush (%)**	**Return bloom (clusters/ tree)**
GI	2014	UTC	257	519 a	216 a	50 a	105 b	62 c	56 b	216 b
		COM-STD	269	296 b	113 b	38 ab	145 a	68 b	61 b	249 b
		ME	243	205 c	87 b	29 b	160 a	72 a	88 a	357 a
		Hand thinning	255	296 b	120 b	36 b	133 ab	68 b	79 a	177 b
		P	**ns**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
	2016	UTC	314	509	171 a	35	68 c	61 b	12 b	155
		COM-STD	312	449	140 ab	46	101 ab	68 a	22 b	188
		ME	319	505	163 ab	39	77 bc	64 b	17 b	137
		Hand thinning	316	237	75 b	25	110 a	71 a	43 a	159
		P	**ns**	**ns**	**0.04**	**ns**	**<0.01**	**<0.01**	**<0.01**	**ns**
MB	2015	UTC	313	420 a	141 a	44 a	107 b	65 b	10 b	86
		COM-STD	327	242 b	74 b	30 ab	122 ab	67 ab	16 b	158
		ME	312	262 b	87 b	30 ab	118 ab	67 ab	14 b	141
		Hand thinning	312	199 b	64 b	24 b	118 ab	66 ab	19 b	157
		Tank mix	323	233 b	75 b	33 ab	141 a	70 a	30 a	83
		P	**ns**	**<0.01**	**<0.01**	**0.02**	**0.03**	**0.02**	**<0.01**	**ns**
	2016	UTC	168	197	121	26	132	66	70	176
		COM-STD	168	165	101	22	137	66	68	234
		ME	171	182	112	28	152	68	69	236
		Hand thinning	168	175	112	24	142	67	66	183
		P	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
MO	2014	UTC	222	367 a	168 a	50	137 c	69 b	35	233
		COM-STD	226	264 b	118 b	45	171 a	74 a	46	282
		ME	229	353 a	153 a	50	142 bc	70 ab	43	250
		Hand thinning	227	264 b	120 b	43	163 ab	73 a	60	271
		P	**ns**	**0.02**	**<0.01**	**ns**	**<0.01**	**0.01**	**ns**	**ns**
	2015	UTC	269	243 a	90 a	33	137 c	70 b	31 b	254
		COM-STD	280	147 ab	53 bc	25	171 ab	76 a	51 ab	280
		ME	287	174 ab	60 bc	26	151 c	72 b	39 ab	259
		Hand thinning	277	191 ab	69 ab	30	154 bc	73 b	35 ab	217
		Tank mix	280	101 b	36 c	19	185 a	78 a	54 a	275
		P	**ns**	**<0.01**	**<0.01**	**ns**	**<0.01**	**<0.01**	**0.02**	**ns**
	2016	UTC	278	472 a	173 a	54 a	116 d	65 d	6	294
		COM-STD	274	348 abc	130 ab	48 ab	138 bc	70 bc	12	337
		ME	277	441 ab	152 a	50 a	123 cd	67 cd	9	214
		Hand thinning	260	227 c	91 b	34 b	151 ab	72 ab	13	352
		Tank mix	284	264 bc	96 b	42 ab	161 a	74 a	26	268
		P	**ns**	**<0.01**	**<0.01**	**0.01**	**<0.01**	**<0.01**	**ns**	**ns**
	2017	UTC	300	318	109	42	136	68	22	354
		COM-STD	294	230	78	34	153	71	36	434
		ME	293	284	98	41	147	70	27	447
		Hand thinning	298	234	79	35	150	71	24	427
		P	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**

Means within a column followed by different letters denote significant differences (Tukey’s honestly significant difference, P<0.05). ns - not significant at P<0.05

3.2. Experiment 2:

No significant difference between treatments were observed in the number of flower clusters/tree. However, in MB2015, flowering was significantly higher than in MO2015 and MO2016 (336, 279 and 275 flower clusters/tree, respectively) (Table 3). All the chemical thinning treatments produced significant reductions in the number of fruits/tree, fruit set and yield, in comparison with the UTC treatment. The lowest level of efficacy was observed with COM-STD (240 fruits/tree, 81 fruits/100 flower clusters and 35 kg/tree) and the highest with the triple application of ME/COM-STD/ME (135 fruits/tree and 46 fruits/100 flower clusters and 24 kg/tree) (Table 3). However, ME/COM-STD/ME produced excessive thinning (Table 3). All the chemical applications involving first COM-STD and then ME produced higher levels of efficiency and lower numbers of fruits/tree than any other chemical treatments. Moreover, the ME/COM-STD (217 fruits/tree, 77 fruits/100 flower cluster and 32 kg/tree) and Tank mix (294 fruits/tree, 66 fruits/100 flower cluster and 32 kg/tree) treatments had the same thinning efficiency as applying COM-STD alone (240 fruits/tree, 81 fruits/100 flower cluster and 35 kg/tree). However, these treatments showed a tendency to be greater thinning than the single COM-STD application (Table 3). Return bloom was not significantly affected by treatments.

Table 3. Effect of thinning with a combination of COM-STD (BA+NAA), ME and Tank mix (BA+NAA+ME) on 'Gala' in MB2015 and MO2015 and MO2016. Numbers in parentheses show the king fruit diameter in the moment of spray. The target thinning effect was 200 fruits/ tree in MO and 150 fruits/ tree in MB (Experiment 2).

	Flowering (clusters/tree)	**Crop load (fruits/tree)**	**Fruit set (fruits/100clusters)**	**Yield (kg/tree)**	**Return bloom**
Treatment (TRT)	**ns**	*****	*****	*****	**ns**
UTC	297	362 a	124 a	42 a	274
COM-STD (11)	307	240 b	81 b	35 ab	304
ME (8)/COM-STD (11)	291	217 bc	77 b	32 b	265
ME (8)/COM-STD (11)/ME (13)	299	135 d	46 c	24 c	297
COM-STD (11)/ME (13)	295	167 cd	58 bc	27 bc	320
Tank mix (13)	297	194 bcd	66 b	30 bc	308
Trial	*****	*****	*****	*****	*****
MO2015	279 b	124 c	45 c	21 c	280 b
MO2016	275 b	314 a	116 a	44 a	310 a
MB2015	336 a	233 b	71 b	32 b	
TRT*Trial	**ns**	**ns**	**ns**	**ns**	**ns**

* Means in a given column followed by different letters denote significant differences (Tukey's honestly significant difference, P<0.05).

The values for the average number of fruits/tree, fruit set, yield (kg/tree) and return bloom were significant different between trials. The observed levels of efficacy, in descending order, were: MO2015 (124 fruits/tree), MB2015 (233 fruits/tree) and MO2016 (314 fruits/tree). MO2015 trial showed lower fruit/tree compared to the target fruits/tree (124 fruits/tree and 200 fruits/tree, respectively). Return bloom was significantly higher in MO2015 (310 flower clusters/tree) that in MO2016 (270 flower clusters/tree). It should be noted, however, that all values of return bloom were high. However, the interaction between Trial and Treatment was not significant for all the crop load parameters (Table 3).

Figure 1 shows a negative quadratic relationship between the number of fruits/tree at harvest and average fruit weight (R^2=0.71, P<0.0001), fruit size (R^2=0.71, P<0.0001) and red blush area (R^2=0.79, P<0.0001). In other words, as the number of fruits/tree decreased, the average fruit weight, diameter and color all increased. Thus, when the effect of the thinning treatment increased, average fruit weight, diameter and color also increased.

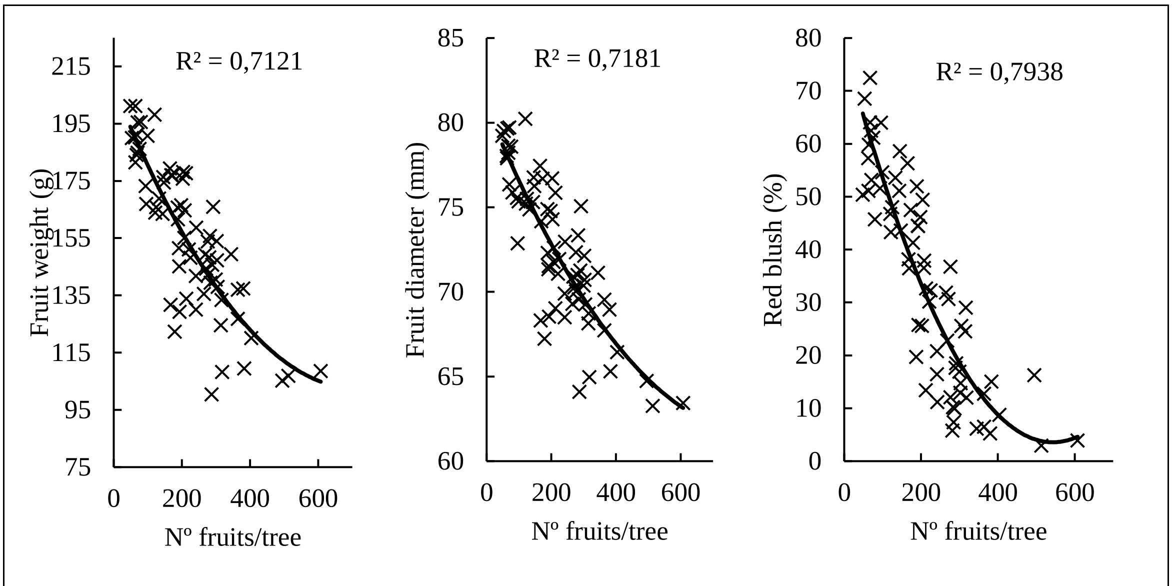

Figure 1: Scatter plot showing the relationship between final fruit numbers/tree and fruit weight, average fruit diameter and average red blush (%), for 'Gala' in MB2015 and MO2015 & MO2016. Each symbol represents 1 block in 1 year (Experiment 2).

3.3. Experiment 3:

The number of flower clusters/tree was uniform at the start of the trials. However, the flowering showed differences between trials. The average level of flowering observed in the trials, presented in descending order, was: MO2018 (538 cluster/tree), MB2015 (344 cluster/tree), MB2019 (305 cluster/tree), MO2017 (295 cluster/tree), MO2015 (276 cluster/tree), MO2016 (272 cluster/tree) and MB2017 (224 cluster/tree) (Table 4).

Table 4. Effects of COM-STD (BA+NAA) and COM-STD/ME on ‘Gala’ in MB2015, MB2017 & MB2019 and MO 2015, MO2016, MO2017 & MO2018. The COM-STD application was sprayed at a king fruit diameter between 10 and 11 mm and ME at 13 and 14 mm. The target thinning effect was 200 fruits/tree (average all trials) (Experiment 3).

	Flowering (clusters/tree)	**Crop load (fruits/tree)**	**Fruit set (fruits/100clusters)**	**Yield (kg/tree)**	Return bloom
Treatment (TRT)	**ns**	*	*	*	*
UTC	325	356 a	118 a	41 a	233 b
COM-STD	321	266 b	90 b	35 b	267 ab
COM-STD/ME	326	209 c	68 c	30 b	301 a
Trial	*	*	*	*	*
MB2015	344 b	271 bc	80 bc	35 bc	
MB2017	224 d	307 abc	140 a	32 cd	180 c
MB2019	305 bc	257 c	84 bc	30 cd	91 d
MO2015	276 bcd	151 d	55 d	23 d	284 b
MO2016	272 cd	363 a	136 a	49 a	315 b
MO2017	295 bc	257 c	88 b	37 bc	419 a
MO2018	538 a	342 ab	64 cd	43 ab	
TRT*Trial	**ns**	**ns**	**ns**	**ns**	**ns**

* Means within a given column followed by different letters denote significant differences (Tukey’s honestly significant difference, P<0.05).

The chemical applications produced significantly lower numbers of fruits/tree, fruit sets and yields than the UTC (356 fruits/tree, 118 fruits/100 flower cluster and 41 kg/tree). Moreover, the chemical applications with COM-STD and after ME (209 fruits/tree, 68 fruits/100 flower cluster and 30 kg/tree) were greater thinning and produced fewer fruits/tree and fruit set than applying COM-STD alone (266 fruits/tree, 90 fruits/100 flower cluster and 35 kg/tree) (Table 4). Return bloom was enhanced on all thinned ‘Gala’ trees. This way, return bloom was inversely proportional to the yield from the previous season, so that trees with high yield had the lowest return bloom for the following season.

There were significant differences between trials in terms of the number of fruits/tree, fruit set, yield and return bloom. However, the interaction between treatment and trial was not significant (Table 4).

Figure 2, we can observe a quadratic relationship between number of fruits/tree at harvest and average fruit weight (R^2=0.77, P<0.0001), fruit size (R^2=0.80, P<0.0001) and red blush area (R^2=0.68, P<0.0001). In other words, when the effect of the thinning treatment increased, the fruits/tree decreased and the average fruit weight, diameter and color all increased (Figure 2).

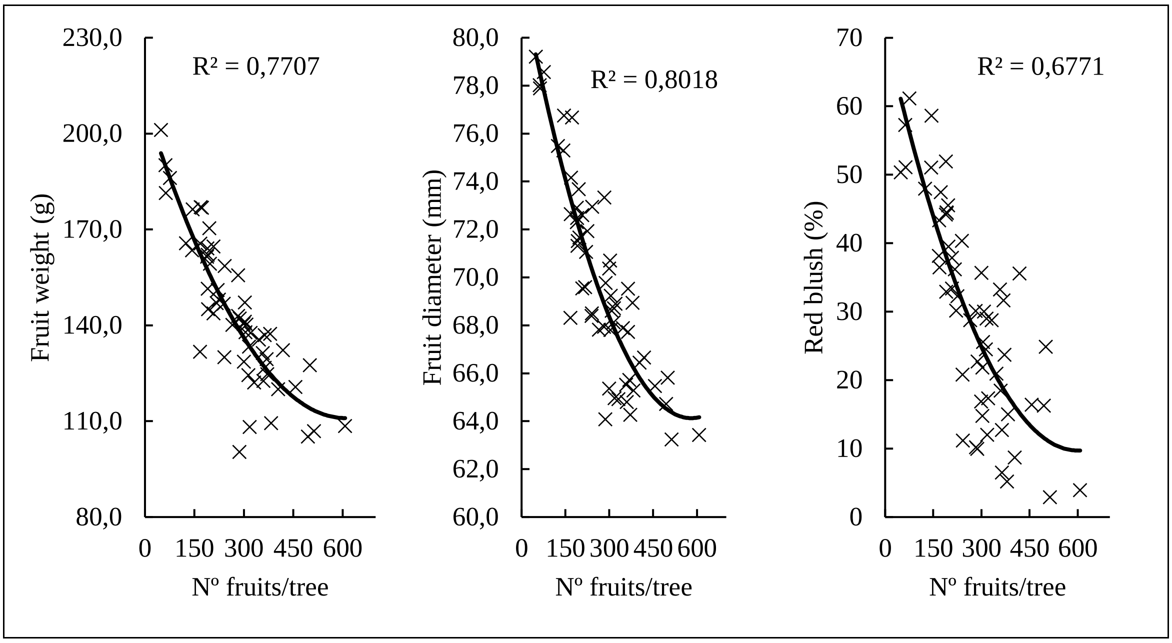

Figure 2: Scatter plot showing the relationship between final fruit numbers/tree and fruit weight, fruit diameter and red blush (%) for 'Gala' in MB2015 and MO2015, MO2016, MO2017 & MO2018. Each symbol represents 1 block in 1 year (Experiment 3).

3.4. Experiment 4:

The orchards where the field trials were carried out had homogeneous bloom in all the trials. There were no significant differences between treatments in terms of flower clusters. The trials did, however, exhibit different flowering patterns (Table 5).

All the chemical thinning treatments produced significant reductions in the number of fruits/tree, in fruit set and in yield, in comparison with the UTC treatment. A dose effect was also observed, with an increase in the rate of ME and with decreases in the number of fruits/tree and in fruit set, crop load and yield. The lowest chemical thinning efficacy was observed with COM-STD alone (277 fruits/tree, 81 fruits/100 flower clusters and 35 kg/tree). This was followed by the first COM-STD application and after ME at 165g/ha (236 fruits/tree, 63 fruits/100 flower clusters and 34 kg/tree). The greatest thinning efficacy was observed in the COM-STD application and after ME at 220 g/ha (200 fruits/tree and 57 fruits/100 flower clusters and 31 kg/tree) (Table 5). There were no significant differences between treatment in the return bloom. Nevertheless, there was a trend that increase the return bloom when the efficiency of thinning was higher.

All the productive parameters and the return bloom exhibited significant differences between trials. Even so, the interaction between treatment and trial was not significant (Table 5).

Table 5. Effects of COM-STD (BA+NAA) and COM-STD/ME applied at two different rates to 'Gala' in MB2019 and MO2017 & MO2018. The number after ME was the dose of application in g/ha. The COM-STD application was sprayed at a king fruit diameter between 10 and 11 mm and ME at 13 and 15 mm. The target thinning effect was 200 fruits/ tree in MO and 150 fruits/ tree in MB (Experiment 4).

	Flowering (clusters/tree)	**Crop load (fruits/tree)**	**Fruit set (fruits/100clusters)**	**Yield (kg/tree)**	**Return bloom**
Treatment (TRT)	**ns**	*	*	*	**ns**
UTC	394	345 a	93 a	42 a	223
COM-STD	366	277 b	81 a	35 ab	262
COM-STD / ME 165	393	236 bc	63 b	34 ab	326
COM-STD / ME 220	368	200 c	57 b	31 b	339
Trial	*	*	*	*	*
MB2019	300 b	231 b	78 a	28 c	119 b
MO2017	296 b	237 b	82 a	35 b	436 a
MO2018	537 a	323 a	61 b	42 a	
TRT*Trial	**ns**	**ns**	**ns**	**ns**	**ns**

* Means within a given column followed by different letters denote significant differences (Tukey's honestly significant difference, P<0.05).

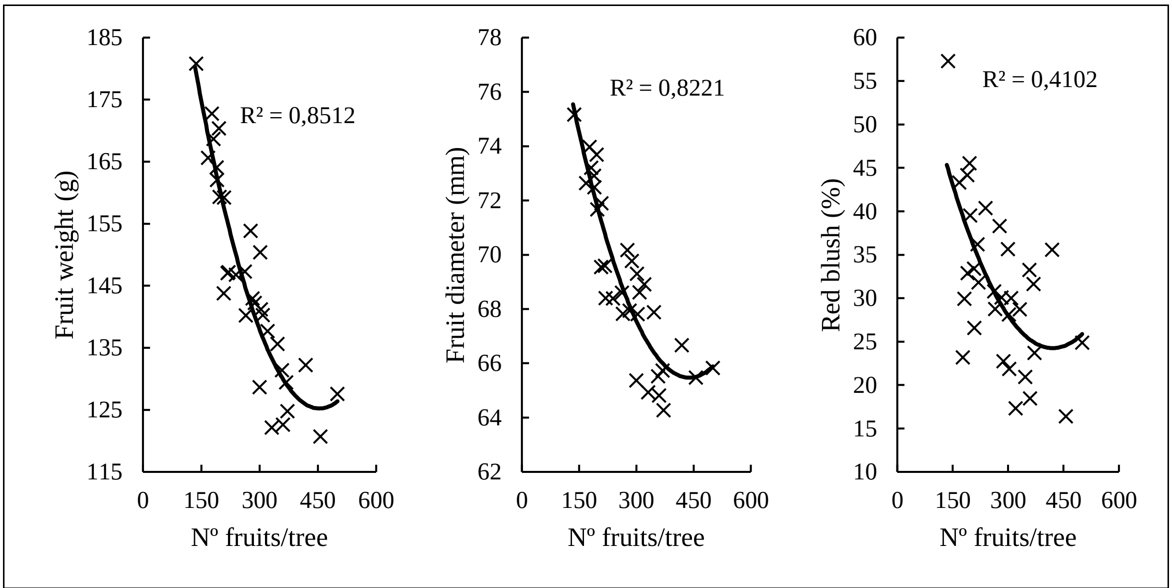

Figure 3: Scatter plot showing the relationship between final fruit number/tree and fruit weight, fruit diameter and red blush (%) for 'Gala' in MO2017 & MO2018. Each symbol represents 1 block in 1 year (Experiment 4).

As expected, the final number of fruits/tree was negatively related to average fruit weight (R^2=0.85, P<0.0001), fruit size (R^2=0.82, P<0.0001) and red blush area (R^2=0.41, P=0.0005). As a result, when the effects of the thinning treatment increased, the fruits/tree decreased and the average weight, diameter and color of the fruit all increased (Figure 3).

3.5. Experiment 5:

The elemental plots produced a similar number of flower clusters/tree at bloom, before the treatments were applied. The average for the trial was 273 cluster/tree, with a high degree of homogeneity between treatments (Table 6).

Table 6. Effects of ME applied at different rates both alone and in combination with BA to 'Golden' in MB2013. The number after ME was the dose of application in g/ha. Numbers in parentheses show the king fruit diameter in the moment of spray. The target thinning effect was the hand thinning treatment (Experiment 5).

Treatment	Flowering (clusters/tree)	Crop load (fruits/tree)	Fruit set (fruits/100clusters)	Yield (kg/tree)
UTC	274	137 a	50 a	19 a
ME 110 (9)	276	87 b	32 b	15 ab
Tank mix (ME 110+ BA) (9)	269	82 b	31 b	14.7 abc
ME 165 (9)	270	58 bc	23 bcd	11.5 bcd
Tank mix (ME 165+BA) (9)	270	67 bc	25 bc	12.4 abcd
ME 220 (9)	271	40 c	15 cd	8 d
Tank mix (ME 220+BA) (9)	274	29 c	10 d	6 d
ME 165 (9) - ME 165 (13)	278	47 bc	19 bcd	9 cd
Hand thinning	274	54 bc	20 bcd	10 bcd

Means within a given column followed by different letters denote significant differences (Tukey's honestly significant difference, $P<0.05$).

All the treatments showed significant reductions in the number of fruits/tree and in fruit set in comparison with the UTC. When ME was applied alone and in combination with BA (Tank mix), a dose effect was observed: an increase in the ME dose rate was accompanied by a decrease the final fruits/tree, fruit set and yield. The lowest level of efficacy was observed at ME 110 g/ha (87 fruits/tree, 32 fruits/100 flower clusters and 15 kg/tree) and with the Tank mix (ME 110 & BA) (82 fruits/tree, 31 fruits/100 flower clusters and 14.7 kg/tree). The highest levels of efficacy were obtained with ME 220 g/ha (40 fruits/tree, 15 fruits/100 flower clusters and 8 kg/tree) and the Tank mix (ME 220 & BA) (29 fruits/tree, 10 fruits/100 flower clusters and 6 kg/tree). However, the Tank mix (ME 220 & BA) treatment produced excessive thinning in comparison with Hand thinning. The double application of ME produced the same level of thinning efficacy as the other thinning treatments. Moreover, there were no significant differences between any of the different chemical thinning treatments and hand thinning in terms of the number of fruits/tree, yield and fruit set (Table 6).

All experiments showed a significant reduction in the number of fruits/tree and fruit set with respect to the UTC. Final fruit numbers/tree were also negatively related to average fruit weight, fruit size and red blush area. In contrast, the Tank mix treatment

(Exp. 1, 2 and 5) and COM-STD followed by ME (Exp. 2, 3 and 4) produced the greatest thinning efficiencies. In all the experiments, the combination between COM-STD and ME was a greater thinning than single applications of ME and COM-STD. Overall, all experiments in 'Gala' showed homogeneous return bloom in the following season. However, there was a positive tendency with higher thinning efficacy to improve return bloom, in some experiments significant.

4. Discussion

The successful use of chemical thinners on apple crops requires programs that employ multiple chemistries during the thinning period. It is, therefore, necessary to find new alternatives that can increase the chemical thinning efficacy (Reginato *et al.*, 2017). Consistent enhancement of fruit weight, diameter and red color development are the most important considerations when evaluating a chemical thinning program (Stover *et al.*, 2001). One of the most important effects of chemical thinners on fruit weight, diameter and coloration is their ability to reduce crop load because this, in turn, also reduces inter-fruit competition (Stover et al., 2001).

In the present study, spraying apple trees with ME and COM-STD (BA+NAA) induced fruit abscission. The findings concurred with the results of Gonzalez *et al.* (2020b), who used ME and Basak (2004) in conjunction with a COM-STD application. The thinning efficiency of single applications of ME and COM-STD produced no significant differences when they were applied on the same day, confirming results also reported by Goulart *et al.* (2017). However, our results differed from those of Rosa *et al.* (2017), who concluded that ME was a more effective thinner than BA applied alone. In addition, the Tank mix application, increased the thinning efficacy in comparison with individual applications of ME and COM-STD, and had similar effects on fruit yield parameters to those previously observed by Radivojevic *et al.* (2019) and Petri *et al.* (2016). Furthermore, this treatment can produce excessive thinning. In this line, previous studies made Lafer (2010) and Cline *et al.* (2022) with ME alone reported over-thinning with higher rates of ME. However, in our study, ME only showed over-thinning in tank mix with BA and NAA.

Combinations of ME and COM-STD used for thinning, in particular the application of COM-STD after ME, showed higher efficiency than applying either of these products individually. This suggests an additive effect based on carbohydrate stress, in line with similar observations by McArtney and Obermiller (2012). However, these other authors suggested that a combination of metamitron plus 1-Aminocyclopropane Carboxylic Acid could have additive effect on fruit abscission. Moreover, Eccher *et al.* (2013) have explained that the nutritional stress caused by both BA and MET indirectly reduces the already low assimilate availability to the sinks (i.e. the fruitlets). On the other hand, Petri et al. (2016) reported that a chemical mixture of thinning agents with different mechanisms of action may enhance the thinning effect. Previous reports by Dennis (2003) and Cortens and Cline (2019) shown that fruit thinning improves flower initiation and hence return bloom for the following season. This concurs with our results which showed that the high thinning efficiency can improve the return bloom.

In experiment 5, ME was applied alone a dose effect was observed; this was in line with the observations of Deckers *et al.* (2010), Gonzalez *et al.* (2020a) Mathieu *et al.* (2016) and McArtney *et al.* (2012). It was also possible to observe this effect in Tank mix applications.

All the experiments showed a negative relationship between efficiency and crop yield. Yield fell with increased thinning efficacy and, as also reported by Reginato *et al.* (2014), this was independent of the product applied. Moreover, in all the experiments conducted, the average fruit weight, diameter and coloration increased as a result of the thinning effect. There was therefore a negative relationship between the number of fruits and their average weight, color and diameter. These results again concurred with earlier observations made by Bergh (1990) and Dorigoni and Lezzer (2007).

Our results suggested that all thinning programs evaluated in this study showed differences between years. Tank Mix showed the greatest program thinning on some years and possible overthinning on others. Moreover, it was observed that a dose of ME, alone and in combination with other products, influence thinning. ME allows to adjust the rate when lower year efficiency is expected. The greatest thinning program was the combination COM-STD at 11mm and after ME due to additive effect among these products. This way, the fruit growth model (Greene *et al.*, 2013) could be used to predict the treatment efficiency and decision making, if a second application will be necessary and the rate of ME. In this line, the Malusim model (Lakso and Robinson, 2011) and BreviSmart model (ADAMA, 2022) could be used to explain the variability between years and predict the treatment efficiency.

5. Conclusions

In this study, we evaluated different thinning programs, using several products registered in Spain, to offer an alternative to conventional thinning programs. Overall, the results showed that spraying apple trees with ME and COM-STD induced fruit abscission and improve the return bloom. However, the thinning efficiency of single applications with ME and COM-STD, administered on the same day, did not exhibit any significant differences between them. The Tank mix application did, however, increase the thinning efficacy in comparison with applying ME and COM-STD as single treatments. However, this treatment showed excessive thinning in two trials. All the different combinations involving ME and COM-STD, and especially the COM-STD application applied after ME, proved greater thinning than applying either product alone; this would suggest an additive effect. In experiment 5, a dose effect was observed with single spray with ME and with the Tank mix (ME&BA). When doses of ME increase, the final fruit set and crop load tend to be reduced.

Yield fell with increases in the efficacy of the thinning programs in all the experiments involving 'Gala' and 'Golden'. In addition, there was a negative quadratic relationship between thinning efficacy and average fruit weight, color and diameter. In other words, average fruit weight, color and diameter increased in the treatments in which the thinning program reduced the number of fruits/tree.

These results suggest that the combination between COM-STD at 11mm and ME was the greatest thinning program due to their additive effect. However, it is necessary tools to predict the treatment efficiency and decision making, if a second application will be necessary and the rate of ME.

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