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1 Hail nets do not affect the efficacy of metamitron for chemical thinning of apple trees

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16 Abstract

Hail nets reduce photosynthetically active radiation (PAR) and alter the environment under 17 18 the netting in apple orchards. Thus, we investigated the effect of nets on the efficacy of metamitron, a short-term photosynthesis inhibitor used for fruit thinning. The objective of 19 this study was to evaluate the effect of the netting and metamitron on thinning efficacy, 20 yield, fruit quality and chlorophyll fluorescence in three apple cultivars. One or two 21 metamitron applications at 165, 248 and 330 g (ai)/ha were applied the tree under different 22 colored nets. The reduction of PAR was highest with black nets (19%-22%), followed by 23 green (13%-15%) and white nets (6%-11%). There were no significant differences 24 (P>0.05) in fruit weight or size with or without nets. Double applications of metamitron 25 increased average fruit fresh weight and reduced the fruit set over four experiments. In 26 contrast, single applications were less effective. In two experiments, thinning was 27 associated with lower yields. However, there was no effect in the other two experiments. 28 The double treatments tended to increase the percentage of the crop with fruit larger than 29 70 mm in diameter. All thinning strategies showed similar inhibition in fluorescence, with 30 31 the only observed significant differences between treatments occurring when using a single or double application. The results show that netting does not affect the response to thinning 32 33 with metamitron.

Keywords

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35 Brevis, Fluorescence, Quantum yield, PAR, Radiation, Netting

1. Introduction

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- 37 Thinning can be used in fruit tree to improve overall profitability, which depends on yield,
- fruit size distribution and fruit quality (color, firmness, sugar, acidity, etc.). Return bloom is
- 39 another factor that is influenced by fruit load. For all these reasons, fruit thinning is
- 40 essential for high yields and quality in apple orchards (Byers et al. 1990; Link 2000).
- Brevis (metamitron 15% SG) is a chemical thinning agent which has been available for use
- 42 in apple and pear in Spain since 2015. It inhibits photosynthesis and is different from other
- 43 thinning products. Metamitron disrupts photosynthesis by blocking electron transfer
- between the primary and secondary quinones of PSII (McArtney et al. 2012). It can be
- 45 applied at 165 to 330 g active ingredient (ai) per hectare in one or two applications,
- depending on the cultivar. The response to metamitron is affected by sunlight (Robinson et
- al. 2016), with a reduction in carbohydrates production as a result of lower photosynthesis
- 48 under shade enhancing fruit drop. Kviklys and Robinson (2010) conducted a greenhouse
- 49 study with potted trees and found that low temperatures and high light resulted in less
- 50 thinning, while high temperatures (especially at night) and low light resulted in more
- 51 thinning. They demonstrated that temperature and sunlight affected thinning, supporting the
- role of carbohydrates in the growth of fruitlets (Lakso 2011).
- Byers et al. (1985) reported that shading 16 to 26 days after full bloom, induced fruit drop
- 54 in 'Starkrimson' apple, demonstrating a relationship between light and carbohydrate
- 55 production. Shading decreases net CO₂ assimilation and reduces the amount of
- carbohydrates available for young fruitlets (Grappadelli et al. 1994), but the response is
- 57 dependent on the cultivar (Mathieu et al. 2016). In other experiments, Greene and Groome
- 58 (2010) showed that thinning agents such as carbaryl and naphthaleneacetic acid after
- shading does not modify their effect, but that when the chemicals are applied before
- shading the effect of thinning is greater. Research on shading has helped to clarify the
- 61 impact of photosynthesis on fruit abscission (Kockerols et al. 2008; Mathieu et al. 2016).
- Hailstorms are common in Spain's apple production zone, causing significant damage.
- 63 Currently, anti-hail nets are frequently used by Spanish growers. However, netting reduces
- the incidence of photosynthetically active radiation (PAR) above the trees (Ordonez et al.
- 65 2016). This situation, led us to consider the effects of anti-hail nets on the efficacy of
- 66 metamitron. The objective of this study was to evaluate the effect of the anti-hail netting
- and metamitron on thinning efficacy, yield, fruit quality and chlorophyll fluorescence in
- three apple cultivars.

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2. Materials and methods

2.1. Study site, plant material, temperatures, chemical application and experimental design

- 72 The experiments were conducted from 2014 to 2016 in apple orchards at the IRTA
- 73 Experimental Agricultural Station of Mas Badia (Tallada d'Emporda, NE Spain). The trees
- 74 were irrigated and fertilized using drip-irrigation. Fertilization, pruning, herbicide and
- 75 phytosanitary treatments were applied following standards in the region.

The experimental unit comprised four rows and two guard rows. The rows were divided into two sections (one in front of the other). One half-row section was covered with an anti-hail net, while the other section was kept open (without net). The net was mounted on a fixed structure in the planting year in all rows. Both areas used the same cultivars and management. For all experiments and half-row areas (with and without net), a completely randomized block design was used, with four blocks per treatment. Each replication comprised a four-tree unit with the central trees as the experimental unit and the end trees as guards. Each treatment (chemical thinning and untreated control) was repeated with and without net, with the same experimental design.

Meteorological data were collected from the weather station of the official meteorological service of Catalonia, located 50 m in the Tallada d'Emporda orchard of the IRTA experimental agricultural station of Mas Badia. Night temperature was calculated as the average temperature recorded by the weather station between 2000 and 0700 h. Average temperature was calculated as the average temperature recorded by the weather station between 0000 and 2400 h.

All experiments used Brevis (ADAMA, Spain containing 15% Metamitron) applied with a customized air blast sprayer, to simulate commercial application. This was equivalent to 1000 L/ha of volume applied before run-off.

2.1.1. Experiments 1 and 2

Experiments 1 and 2 were carried out on 'Galaxy Gala' and 'Fuji Zhen®', respectively in 2014. 'Galaxy' and 'Fuji' were planted in 1994 and 2006, respectively, trained to a central leader and spaced at 3.75 m x 1 m (2,666 trees/ha). 'Galaxy' and 'Fuji' trees were grafted on M.9 PAJAM® rootstocks. The netting was white for 'Gala' and green for 'Fuji'. The study analyzed two chemical thinning strategies, with and without nets. One or two applications of metamitron at a 248 g (a.i.)/ha were compared against an untreated control. The first spray was applied when the fruit were 7-9 mm wide and the second when the fruit were 10-12 mm wide.

2.1.2. Experiments 3 and 4

Experiments 3 and 4 were carried out on 'Fuji Zhen®' in 2015 and 'Pink Lady' in 2016. 'Fuji' and 'Pink Lady' were planted in 2006 and 2004, trained to a central leader and spaced at 3.75 m x 1 m (2,666 trees/ha). 'Fuji' was grafted on M.9 PAJAM® rootstocks and 'Pink Lady on M.9T337 rootstocks. Netting was green for 'Fuji' and black for 'Pink Lady'. There were four thinning strategies, with and without nets. One or two applications of metamitron were applied at 248 or 330 g/ha in 'Gala', and 165 or 248 g/ha in 'Pink Lady'. All treatments were compared with an untreated control. The chemical was applied at the same stages as in the earlier experiments.

2.2. Photosynthetically active radiation (PAR)

PAR was measured in an experimental orchard (IRTA-Mollerussa) in 2008 using an SS1-UM-1.05 Sun Scan ceptometer (Delta-T Devices Ltd, Cambridge, UK) with a 64-sensor photodiode linearly sorted in a 100 cm sword. The measurements were taken from other anti-hail net experiments (unpublished data). PAR was measured outside the nets (full

- light) and under black, green or white nets, 1.10 m above the ground. PAR was measured in
- spring under full sun at regular intervals, between 1200 and 1500 h.

2.3. Data collection and statistical analyses

- The total number of flower clusters per tree was counted at bud break (BBCH 61-65),
- before the treatments were applied. At harvest, the number of fruits per tree was recorded.
- Fruit set was calculated as 100*(No. fruit/No. flower clusters per tree).
- Fruit were harvested with in a single pick during the commercial harvest for each tree. Fruit
- weight, total fruit yield (kg per tree) and number of fruit per tree were measured with a
- commercial apple sorting and packing line (Calinda, Caustier Ibérica, S.A. with Aweta
- 126 Technology).
- First class fruit were >70 mm. Fruit size distribution, based on fruit diameter categories of
- >70 mm, was determined for each tree. Fruit size and coloration were measured with the
- 129 commercial sorting machine.
- 130 Chlorophyll fluorescence was measured in Experiments 1 and 2, for 'Galaxy' and 'Fuji'.
- Measurements were made on three recently fully-expanded leaves per control tree (6 leaves
- per block and 24 leaves per treatment), under full daylight in the shaded of the tree part
- between 1000 and 1600 h and at a height of 1-1.5 m. The measurements were taken once
- per week until values stabilized at 90% of the control.
- QY (quantum yield) was measured with a handheld portable fluorimeter (FluorPen FP100,
- Photon Systems Instruments, Czech Republic) to provide an indication of the effect of the
- chemical on the maximum potential quantum yield efficiency of PSII.
- Each experiment was analyzed individually because the nets were different colours and
- average PAR values were different. The cultivars and application doses were also different.
- 140 Statistical analyses were performed in SAS 9.3 (SAS Institute Inc., 2009). Means were
- separated with the general linear model using Duncan's multiple range tests at P<0.05 by
- one-way or factorial analysis of variance (Proc GLM), considering netting and chemical
- application as the main factors.

144 **3. Results**

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3.1. Photosynthetically active radiation (PAR)

- Netting reduced PAR values in comparison with the controls (Table 1). The reduction of
- 147 PAR was highest with black nets (19%-22%), followed by green (13%-15%) and white
- 148 nets (6%-11%).

3.2. Temperatures

- Temperatures were highest in 2015 (fruit 5 mm after 4 days) than in 2014 (fruit 5 mm after
- 5 days) and 2016 (fruit 5 mm after 8 days). Moreover, fruit growth was positively related to
- temperatures because the days between applications were different. That is, when
- temperature was higher, fruit growth was faster. In 2014 and 2015, average daily and night
- temperatures increased after the fruit had a diameter of 12 mm. This situation increased the
- efficacy of the second application. In 2016, average daily and night temperatures during the

second application (12 mm) were highest. This situation also increased the efficacy of the second application.

3.3. Growth, yield and fruit quality

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Thinning had no significant (P > 0.05) effect on flower production in any of the experiments (data not presented). The effect of netting on flower production was small, with significantly more flowers under the nets (188 flower clusters per tree) than in the control (159 flower clusters per tree) but only in the 2016 experiment with 'Pink Lady' (P < 0.05). There was no significant (P > 0.05) interaction between thinning and netting on

164 flower production in any of the experiments (data not presented).

- There were mixed effects of thinning on fruit production and fruit set (Table 2). In the 2014 165 experiments with 'Gala' and 'Fuji', metamitron decreased fruit production and fruit set 166 compared with the controls, with a greater response with the double applications in the first 167 experiment. In the 2015 experiment with 'Fuji' and the 2016 experiment with 'Pink Lady' 168 only the double applications decreased fruit production and fruit set (Table 2). Netting had 169 170 only a small effect on fruit production and fruit set, with netting increasing these 171 parameters compared with the control no net plots only in the 2014 experiment with 'Gala' (Table 2). There was no significant (P > 0.05) interaction between thinning and netting on 172 173 fruit production and fruit set in any of the experiments (data not presented).
- 174 There were mixed effects of thinning on total yield and average fruit fresh weight (Table 3). The double applications of metamitron decreased yields compared with the control in the 175 first two experiments, whereas there was no significant (P > 0.05) of the chemical in the 176 last two experiments. The single applications of the chemical increase average fruit weight 177 compared with the control in the first and second experiments (two out of two cases), while 178 179 the double applications of the chemical increased average fruit fresh weight in five out of six cases in all the experiments (Table 3). There was no significant (P > 0.05) effect of 180 netting on total yield and average fruit weight, and no interaction between thinning and 181 netting (data not presented). 182
- There were significant (P > 0.05) negative relationships between fruit weight and the number fruit per tree in the experiments. There was a negative relationship between fruit weight and the number of fruit per tree (Fig. 2). Fruit weight decreased as fruit production increased.
- The effect of thinning on the yield of premium fruit and the percentage of total yield that 187 included fruit greater than 70 mm varied in the different experiments (Table 4). The double 188 applications increased premium yield compared with the controls in all experiments except 189 the last, while the single applications increased premium yield only in the second 190 191 experiment. Thinning increased the percentage of total yield in the larger fruit category in seven out of twelve cases (Table 4). There was no significant (P > 0.05) effect of netting and 192 no interaction between thinning and netting on premium fruit production in any of the 193 194 experiments (data not presented).
- Thinning had no significant (P > 0.05) effect on the yield of fruit that were highly coloured (60% of fruit surface coloured) (Table 5). There was no consistent effect of thinning on the percentage of yield that had coloured fruit. The double applications of metamitron

increased the percentage of coloured fruit compared with the control in the first and third

experiments, while none of the applications had an effect in the second experiment (Table

5). Netting had at best a small effect on fruit colour development (Table 5), while there was

no significant (P > 0.05) interaction between thinning and netting (data not presented).

3.4. Chlorophyll fluorescence

The netted and un-netted control trees tended to have similar values of quantum yield (QY)

over the experiments (Figs. 3 and 4). In contrast, the trees sprayed with the thinning agent

- had lower values for most of the experiments, and recovered fully or almost fully after
- about 35 or 40 days. The maximum inhibition was three days after first application in any
- of the experiments. The maximum inhibition was maintained for a longer period (11 days)
- in all strategies with double applications than with single applications (3 days) (Figs. 3 and
- 209 4).

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4. Discussion

- Netting can modify plant water status, light interception, photosynthesis and carbohydrate
- accumulation in crop plants (Mupambi et al. 2018). The response of the leaves and the
- canopy to light is the major factor affecting carbohydrate production in apple trees (Lakso
- 214 1994). For a whole canopy, as well as total light, the distribution of light between direct and
- 215 diffuse components may be important since many leaves may be dependent on diffuse light
- 216 (Lakso 1994). At Girona, the PAR was reduced by 19%-22% by black nets, by 13%-15%
- by green nets and 6%-11% by white nets. These values were similar to those reported by
- 218 Dussi et al. (2005), Iglesias and Alegre (2006), Blanke (2009) and Ordonez et al. (2016).
- 219 While this would not affect leaf photosynthesis on sunny summer days, photosynthesis
- under the nets would be reduced in autumn, spring or on overcast days (Widmer 2001).
- Many authors have reported that temperature plays an important role in chemical thinning
- 222 (Kviklys and Robinson 2010; Lakso et al. 2006; Li and Cheng 2011; Lordan et al. 2019;
- Parra-Quezada et al. 2005; Pretorius et al. 2011). Typically warm nights increase
- respiration during the night, decrease overall carbon balance of the tree (Costa et al. 2018),
- and increase the efficacy of thinning. Metamitron was generally more effective after the
- second application under warmer weather. Byers (2003) indicate that the efficacy of a
- chemical depended on the diameter of the fruit, application dose, cultivar and weather.
- Double applications of metamitron tended to reduce fruit set and number of fruit per tree
- more than single applications as shown previously (Dorigoni and Lezzer 2007; Stern 2014).
- 230 Single application typically reduce fruit set compared with control untried, again
- concurring with earlier studies (Deckers et al. 2010; Dorigoni and Lezzer 2007; Lafer 2010;
- 232 Reginato et al. 2017).
- In 2014, thinning was associated with lower yields whereas these were no effect in 2015
- and 2016. McArtney et al. (2012) reported that thinning decreased yields in 'Gala' in New
- 235 Zealand. Average fruit weight trended to increase with thinning as shown by Brunner
- 236 (2014), Maas and Meland (2016) and McArtney et al. (1996). These authors reported a
- 237 negative relationship between average fruit weight and the number of fruit per tree. Fruit
- size moved to the larger category as the number fruit per tree decrease, as shown by Bergh
- 239 (1990), Dorigoni and Lezzer (2007), Lafer (2010) and Mathieu et al. (2016).

- Netting generally had no effect on production, fruit size or fruit quality as demonstrated by
- Iglesias and Alegre (2006) and Ordonez et al. (2016). Netting also had no effect in the
- response to the thinning agent. Shade provided by nets has been shown to reduce net CO₂
- assimilation, increase vegetative growth and, therefore, reduce yield and fruit size in apple
- 244 (Amarante et al. 2011; Amarante et al. 2007; Middleton and McWaters 2002; Romo-
- 245 Chacón et al. 2007). Moreover, there was a fewer and smaller cell in the fruit of apple
- under net, leading to a lower fruit size count (Amarante et al. 2011).
- Metamitron disrupted the photosynthetic apparatus for 41 to 43 days after he chemical was
- 248 applied, although this differs from some published studies in which shorter inhibition
- periods have been reported (McArtney et al. 2012; Stern 2014; Stern 2015). Quantum yield
- 250 decreased rapidly for the first three days, and the maximum inhibition of QY values were
- recorded for three to ten days after the treatment, depending on the number of applications.
- 252 These results concur with earlier observations by Brunner (2014) and McArtney et al.
- 253 (2012).

254 **5. Conclusions**

- 255 Double applications of the thinning agent metamitron typically increased average fruit fresh
- 256 weight in apple trees over four experiments in Spain. In contrast, single application were
- less effective. The double treatments also tended to increase the percentage of the crop with
- 258 fruit larger than 70 mm in diameter. These treatments had lower total yields than the control
- in the first two experiments, and similar yields as the controls in the last two experiments.
- Overall, netting that decreased PAR by up to 22% had no effect of yield, fruit size or fruit
- 261 quality. Netting also did not affect the response to thinning. It can be concluded that double
- applications of metamitron can be used to increase fruit size in apple trees growing under
- 263 hail nets.

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Table 1: Effect of netting on photosynthetic active radiation, PAR (µmol m⁻² s⁻¹) over four experiments in apple in Girona, Spain. Data are the means of 24 measurements per treatment.

	1200 h	1300 h	1400 h	1500 h
Control	1576 (100%)	1770 (100%)	1901 (100%)	1900 (100%)
White	1402 (89%)	1657 (94%)	1767 (93%)	1746 (92%)
Green	1335 (85%)	1536 (87%)	1646 (87%)	1611 (85%)
Black	1248 (80%)	1377 (78%)	1540 (81%)	1521 (80%)

Table 2: Effect of thinning and netting on fruit production and fruit set (final number fruit/100 flower clusters) in apple trees in Girona, Spain. There was no significant (P<0.05) interaction between thinning and netting on fruit production as fruit set.

	Gala 2	2014	Fuji 2	2014	Fuji 2	2015	Pink Lad	ly 2016
	No. of		No. of		No. of		No. of	
Treatment	fruit per	Fruit set						
	tree		tree		tree		tree	
Control	415 a	132 a	379 a	134 a	360 a	173 a	214 a	128 a
165 g/ha							181 ab	109 ab
248 g/ha	307 b	96 b	221 b	76 b	328 a	162 a	171 ab	102 abc
330 g/ha					302 a	150 a		
165+165 g/ha							162 b	94 bc
248+248 g/ha	156 c	50 c	170 b	59 b	211 b	103 b	132 b	77 c
330+330 g/ha					219 b	107 b		
Nets	295 a	92 a	254 a	94 a	285 a	135 a	173 a	95 a
Control	242 b	78 b	260 a	84 a	283 a	144 a	171 a	109 a

Means within a column followed by different letters are significantly different (Duncan's range test at P<0.05).

Table 3: Effect of thinning and netting on yield and fruit weight in apple trees in Girona, Spain. There was no significant (P<0.05) interaction between thinning and netting on yield

as fruit weight.

	Gala	2014	Fuji	2014	Fuji	2015	Pink Lady 2016	
Treatment	Yield (kg/tree)	Fruit weight (g)	Yield (kg/tree)	Fruit weight (g)	Yield (kg/tree)	Fruit weight (g)	Yield (kg/tree)	Fruit weight (g)
Control	47 a	113 b	38 a	101 c	31 a	87 b	26 a	125 b
165 g/ha							25 a	136 ab
248 g/ha	39 a	125 b	33 ab	149 b	30 a	90 b	24 a	142 ab
330 g/ha					29 a	98 b		
165+165 g/ha							23 a	144 ab
248+248 g/ha	26 b	168 a	29 b	177 a	28 a	138 a	20 a	156 a
330+330 g/ha					29 a	134 a		
Nets	38 a	135 a	33 a	142 a	29 a	106 a	23 a	139 a
Control	33 a	143 a	35 a	146 a	30 a	112 a	23 a	142 a

Means within a column followed by different letters are significantly different (Duncan's range test at P<0.05).

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Table 4: Effect of thinning and netting on fruit size (yield >70 mm in percent and kg of total) in apple trees in Girona, Spain. There was no significant (P<0.05) interaction between thinning and netting on fruit size.

	Yield >70 mm							
	Gala 2014		Fuji 2014		Fuji 2015		Pink Lady 2016	
Treatment	Percent of total	kg of total						
Control	19 c	9 b	8 c	3 b	4 b	1 b	14 b	4 a
165 g/ha							18 b	5 a
248 g/ha	33 b	14 b	59 b	20 a	6 b	2 b	27 ab	6 a
330 g/ha					9 b	3 b		
165+165 g/ha							25 ab	5 a
248+248 g/ha	80 a	20 a	78 a	23 a	47 a	12 a	46 a	9 a
330+330 g/ha					43 a	13 a		
Nets	45 a	15 a	50 a	16 a	20 a	5 a	24 a	5 a
Control	54 a	16 a	52 a	17 a	24 a	7 a	28 a	6 a

Means within a column followed by different letters are significantly different (Duncan's range test at P<0.05).

Table 5: Effect of thinning and netting on fruit colour (60% blush area in percent and kg of total) in apple trees in Girona, Spain. There was no significant (P<0.05) interaction between thinning and netting on fruit colour.

	Yield > 60% blush area							
	Gala	Gala 2014		2014	Fuji 2015			
	Percent of	Percent of		1ra of total	Percent of	leg of total		
Treatment	total	kg of total	total	kg of total	total	kg of total		
Control	23 b	11 a	18 a	7 a	10 c	2 a		
248 g/ha	31 b	11 a	26 a	9 a	13 bc	3 a		
330 g/ha					13 bc	2 a		
248+248 g/ha	50 a	12 a	29 a	8 a	23 a	4 a		
330+330 g/ha					17 b	3 a		
Nets	39 a	13 a	21 a	6 b	14 a	2 b		
Control	39 a	11 a	27 a	9 a	17 a	3 a		

Means within a column followed by different letters are significantly different (Duncan's range test at P<0.05).

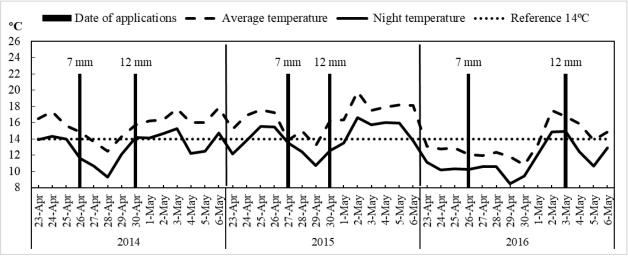


Fig. 1: Average temperatures, average night temperatures and periods of king fruit diameter in apple trees over three years in Girona, Spain.

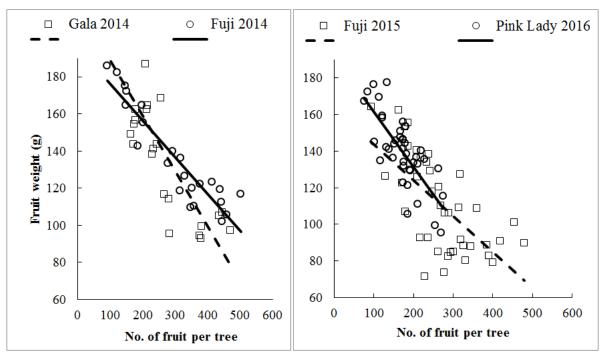


Fig. 2. Relationships between fruit weight (g) and the number of fruit per tree in apple in Girona, Spain. Each symbol represents the average fruit weight per tree and number of fruit per tree. For Gala 2014 y=-0.30*No. fruit+218 (R^2 =0.71, P > 0.001), Fuji 2014 y=-0.20* No. fruit+196 (R^2 =0.86, P > 0.001), Fuji 2015 y=-0.20* No. fruit +163(R^2 =0.43, P > 0.001) and Pink Lady 2016 y=-0.30* No. fruit+192 No. fruit (R^2 =0.58, R > 0.001),

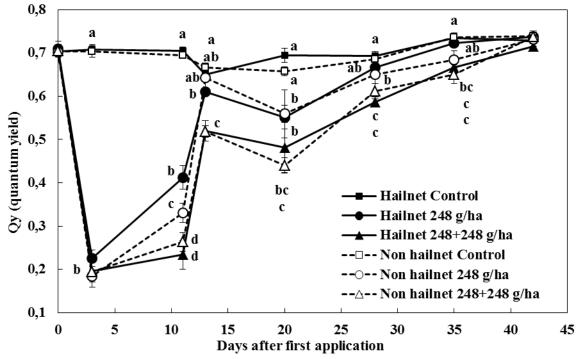


Fig. 3. Effect of thinning and netting on quantum yield (QY) of chlorophyll fluorescence applied in leaves of 'Gala' apple in Girona, Spain. Metamitron was applied on 26 April and 30 April. Vertical bars indicate standard error of the means; n = 24. Means at the same time followed by different letters are significantly different (Duncan's range test at P < 0.05).

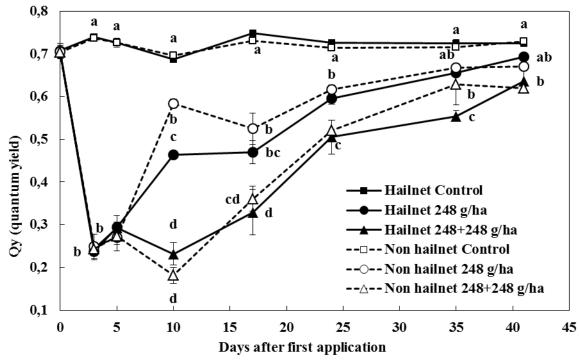


Fig. 4. Effect of thinning and netting on quantum yield (QY) of chlorophyll fluorescence applied in leaves of 'Fuji' apple in Girona, Spain. Metamitron was applied on 26 April and 30 April. Vertical bars indicate standard error of the means; n = 24. Means at the same time followed by different letters significantly different (Duncan's range test at P < 0.05).