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Abstract

Thinning is an important technique in apple growing which is used to reduce the number of fruits per plant and achieve commercial fruit size and quality. The objective of this work was to evaluate the efficacy of one and two applications of the chemical thinner Brevis in Gala apple applied at different fruit sizes and at different intervals between the first and second spray. The trials were conducted over two seasons from 2015 to 2016 in apple orchards of the IRTA experimental agricultural stations of Mas Badia and Lleida (Spain). One or two applications with Brevis were applied at different fruit sizes (king fruit diameter ranging between 7.5 and 13.5 mm) and at a rate of 1.65 kg/ha for all treatments. Under the trial conditions, a Brevis thinning effect was observed in all trials with a reduction in crop load, fruit set and number of fruits per tree which varied according to the number of applications. In addition, average fruit weight, color and diameter increased significantly with treatments in which Brevis reduced the number of fruits per tree. The degree of abscission of Brevis was highly dependent on night temperature and, for this reason, there was a high degree of variability between trials in terms of efficacy. Our results show that the number of days between applications was not as important a factor for Brevis efficacy as the difference in night temperature in the days immediately after its application.

Keywords

Night temperature; Metamitron; Fruit abscission; Carbohydrate deficit; Crop load

1. Introduction

Apple fruit trees can produce too many flower clusters and fruits to obtain high quality and regular marketable crops year-to-year. The main problems that result from too high a fruit set include low quality fruits and biennial bearing. Thinning is an important technique in apple growing and is used to reduce the number of fruits per plant in order to achieve the required commercial fruit size and quality. Hand, mechanical and chemical thinning are the strategies currently used on apple trees. Thinning by hand is generally not a feasible option owing to the costs involved (labor and time). Mechanical thinning can present different problems, including the need for special machinery and training systems, its lack of selectivity for fruit size, and potential damage to the plant (Besseling *et al.*, 2018; Byers, 2003; McClure and Cline, 2015). Chemical thinning is regarded as the most satisfactory method of thinning. It is carried out with standard spray equipment, is the most cost-effective, is relatively fast so it can be done at critical times (Costa *et al.*, 2018), and has the greatest positive effect on return bloom (Stopar, 2017). However, its efficacy varies as its use is dependent on climatic conditions and cultivar (Byers, 2003; Lordan *et al.*, 2018; Robinson and Lakso, 2004). Currently, in accordance with Spanish legislation, chemical thinning can be carried out during flowering (naphthalene acetamide (NAD)) and on fruitlets after flowering (using the hormones 6-benzyladenine (BA) and naphthyl acetic acid (NAA)).

Metamitron (commercial name Brevis) is the most recently released thinning agent in Spain for apple and pear. Brevis's action mode is different from that of other thinning products. The thinning activity of Brevis in apple is via inhibition of photosynthesis (Basak, 2011; Lafer, 2010). Brevis disrupts the photosynthetic apparatus after application and acts by blocking electron transfer between the primary and secondary quinones of PSII (McArtney *et al.*, 2012). The application rate can vary from 1.1 to 2.2 kg/ha in one or two

applications depending on cultivar, with a recommended interval of 5-10 days between applications. However, no studies have to date been published on the effect of different intervals between Brevis applications.

The abscission of fruitlets with thinning agents involves a complex interaction between environmental conditions, cultivar, fruit size and tree vigor (Rosa, 2016). Robinson and Lakso (2011) reported that conditions that favor good carbohydrate status are associated with less fruit abscission and a more difficult chemical thinning response (cool temperatures and sunny days). In addition, Byers (2003) concluded that low light conditions and periods of high night temperatures favour the abscission of fruitlets.

Gonzalez *et al.* (2019a) showed that night temperature was an important factor in explaining the efficacy of Brevis. When night temperatures are high, there is a resulting increase in the thinning effect (Costa et al., 2018). Stern (2014) concluded that higher night temperatures for 3 weeks after the application of Brevis increased respiration and caused assimilation deficiencies during that critical period of fruit development. Lakso *et al.* (2006) reported that hot spells of 3-5 days with maximum temperatures of 33-36°C caused significant fruit drop, especially in combination with a chemical thinner. Similarly, Parra-Quezada *et al.* (2005) showed that good fruit abscission could be associated with a 5 day period of intermediate and high temperatures after thinner application, independently of the chemical thinner applied, which resulted in a significant carbon deficit for fruit development. The data obtained from a study undertaken by Kviklys and Robinson (2010) were used to correlate the 4-day average carbohydrate balance (termed by the authors the 'thinning index') with fruit set and construct a predictive curve of thinning response at various carbohydrate levels (Robinson *et al.*, 2012).

87 The objective of the present study was to evaluate the efficacy of one and two
88 applications of Brevis at 1.65 kg/ha in Gala apple applied at different fruit sizes (king fruit
89 diameter ranging between 7.5 and 13.5 mm) and the effect of different intervals between the
90 first and second spray.

91 ## 2. Materials and methods

92 ### 2.1. Study site, plant material, weather data, chemical application and
93 experimental design

94 The trials were conducted over two seasons (2015 and 2016) in apple orchards at the
95 IRTA experimental agricultural stations in Mas Badia (Tallada d'Emporda, NE Spain) and
96 Lleida (Mollerussa, NE Spain). Trees were irrigated and fertilized using a drip irrigation
97 system. Fertilization, pruning, herbicide and phytosanitary treatments were applied following
98 standards normally used in commercial apple orchards in the region. Trees in the trial field
99 were uniform in terms of number of flower clusters and growth. Table 1 shows the principal
100 characteristics of the orchards used for the trials.

101 All trials used Brevis (ADAMA, Spain), a commercial chemical thinner containing
102 15% metamitron. One or two applications with Brevis were made at different fruit sizes and
103 at a rate of 1.65 kg/ha for all Brevis treatments. All thinning treatments were compared with
104 an untreated control. The time of application was determined by measuring king fruit
105 diameter, and water volume was equivalent to 1000 l/ha. Table 2 shows all the treatments
106 and fruit sizes in all the trials.

107 Meteorological data were collected from the weather station of the official
108 meteorological service of Catalonia. The stations were located in the Girona (Tallada
109 d'Emporda) and Mollerussa orchards. The weather data evaluated in this study was
110 downloaded in all years after the period of application. Night temperature, measured using a

111 Vaisala HUMICAP® HMP155 humidity and temperature probe (Helsinki, Finland), was
112 calculated as the average temperature in the period between 21:00 and 7:00.

113 All trials were arranged in a randomized block design with four replicates of four
114 uniform trees per elementary plot. On each plot, the central trees were used for the trial
115 assessments.

116 **2.2. Yield assessments**

117 In all trials, to assess the effect of the treatments on fruit set and fruit yield parameters,
118 the total number of flower clusters per tree was counted at bud break stage (BBCH 61-65)
119 before the treatments were applied. Homogeneous plants were selected for the trials based
120 on flowering intensity.

121 In each orchard, at harvest time, the number of fruits per tree was recorded. Crop load
122 was obtained from the number of fruits harvested per cm2 of trunk cross-sectional area
123 (TCSA) (number of fruits / TCSA). Fruit set was obtained as the relationship between number
124 of flower clusters and number of fruits at harvest time ([number of fruits / floral clusters] x
125 100). Total fruit yield (kg per tree), fruits per tree, fruit diameter (mm), weight (g) and blush
126 color (%) were measured with a commercial apple sorting and packing line machine; Calinda
127 (Caustier Ibérica, S.A. with Aweta Technology) in Mas Badia and Maf Roda (Agrobotic,
128 France) in Lleida. The criteria established for first class (Extra) products at harvest were fruit
129 color >60% of fruit surface with a good red color development, and fruit size >70 mm.

130 **2.3. Chlorophyll fluorescence**

131 Chlorophyll fluorescence measurements were carried out in Mollerussa 2016, in
132 treatments 1 (7.5 mm), 6 (7.5+9.5 mm), 7 (7.5+11.5 mm) and 8 (7.5+13.5 mm) (see Table
133 2). Measurements were made of Qy (quantum yield) with a handheld portable fluorimeter

(FluorPen FP100, Photon Systems Instruments, Czech Republic) to provide an indication of the effects of Brevis on the maximum potential quantum efficiency of PSII (Fv/Fm). Measurements were made on three recently fully expanded leaves (6 leaves per block and 24 leaves per treatment), under full daylight conditions in the shaded part between 10:00 and 16:00 and at a height of between 1-1.5 m. They were taken 0, 2, 4, 6 and 8 days after Brevis application, and subsequently repeated one day per week until the treatment values were the same as those of the Control.

2.4. Statistical analysis

Analysis of variance was preformed separately in each trial for yield, fruit size and fruit color according to a complete randomized block model with each block being a replication unit, using the Statistical Analysis System software SAS 9.2 (SAS Institute Inc., 2009). When the analysis was statistically significant (F-test), mean separation was carried out using Duncan's multiple range tests at P=0.05.

In addition, the linear relationship was determined between average night temperature (from day of application to four days after application) and percentage of abscission (final number of fruits per tree (treatment)/final number of fruits per tree (Control)). Data were analyzed using the JMP statistical software package (Version 13; SAS Institute Inc., Cary, North Carolina).

3. Results

3.1. Trial results

The orchards where the field trials were carried out showed a homogeneous bloom and TCSA in all trials. No significant differences regarding the initial number of flower clusters per tree and TCSA were observed (Table 3).

157 In Girona 2015, all chemical application treatments resulted in a significantly lower
158 number of fruits per tree, fruit set and crop load in comparison with the Control, except for
159 the single 9.5 mm application. Moreover, the double applications showed a significantly
160 higher efficacy than the single applications, except when one of the double applications was
161 made at 9.5 mm (Table 4). That is, the final thinning effect was the sum of two treatments
162 efficiency when both applications had a significant thinning effect. However, all double
163 treatments combined with the 9.5 mm strategy only showed the effect of the non-9.5 mm
164 application (Table 4). Moreover, the double application treatments showed the same efficacy
165 irrespective of the number of days between sprays. This situation can be observed when there
166 was a significant thinning effect with both treatments (7.5+11.5, 7.5+13.5, and 11.5+13.5
167 mm) and when there was a significant thinning effect with only one of the two applications
168 (7.5+9.5, 9.5+11.5 and 9.5+13.5 mm).

169 In Mollerussa 2016, significant differences were observed in the number of fruits per
170 tree, crop load and fruit set between the Control and the double applications (Table 4).
171 However, the single chemical application showed no significant differences with the Control
172 at any fruit size. That is, there was a higher effect of the second application in all double
173 sprays (Table 4). However, the double application treatments showed similar efficacy
174 irrespective of the number of days between sprays.

175 In Girona 2016, no significant differences were observed between treatments in
176 terms of the number of fruits per tree, fruit set or crop load (Table 4). However, all double
177 applications showed a tendency to higher efficacy than the single and Control treatments, as
178 the double application resulted in a lower (though not statistically significant) fruit set than
179 the Control and single treatments. However, this tendency was not so clear in crop load
180 (Table 4).

181 In Girona 2015, as can be seen in Table 5, the single applications at 11.5 and 13.5
182 mm resulted in significantly lower yield in comparison with the Control. The double
183 applications also showed significant differences in comparison with the Control in yield per
184 tree, except for the 7.5+9.5 mm and 9.5+11.5 mm treatments. That is, yield shows a negative
185 relationship with Brevis efficacy. However, no significant differences in yield (kg/tree) were
186 observed between the Control, and the single or double treatments in Girona and Mollerussa
187 2016. That is, fruit yield per tree at harvest did not show a negative relationship with Brevis
188 efficacy (Table 5).

189 In all trials, average fruit weight and fruit size increased significantly in the treatments
190 in which chemical thinning reduced the number of fruits per tree. That is, average fruit weight
191 and fruit size increased according to the thinning effect induced by Brevis. There were no
192 significant differences between the single application and the Control, except for the
193 treatments at 7.5 and 13.5 mm in Girona 2015 (Table 6).

194 In Girona 2015 and Mollerussa 2016, the double application of Brevis resulted in a
195 significant increase in fruit weight and fruit size compared to the Control. However, these
196 differences were not observed in Girona 2016 (Table 6). Moreover, there were significant
197 differences between the single and double application treatments, except the double
198 applications at 7.5+9.5 mm and 9.5+11.5 mm in Girona 2015 and 7.5+9.5 mm and 7.5+11.5
199 mm in Mollerussa 2016. These treatments coincided with lower fruit weight and fruit size in
200 the single applications (Table 6).

201 In all trials, no significant differences were found in fruit yield (% and kg) with >60%
202 blush area, except for the 9.5+11.5 mm, 9.5+13.5 mm and 11.5 +13.5 mm treatments in
203 Girona 2015. These treatments showed a higher Brevis fruit thinning efficacy. That is,

204 average fruit coloration increased according to the thinning effect induced by Brevis in
205 Girona 2015 (Table 7).

206 In general, the double applications showed higher efficacy than the single
207 applications and the Control. However, there was a high degree of variability between trials,
208 as chemical thinner efficiency depends on the dose and number of sprays. In this study, the
209 number of days between applications was not important.

210 **3.2. Chlorophyll fluorescence**

211 Fig. 1 shows the inhibition of chlorophyll fluorescence with different separation
212 between applications (4, 6 and 10 days after first application) in Mollerussa 2016. The single
213 Brevis application treatment showed maximum inhibition two days after spraying and then
214 recovered progressively from inhibition. However, when a second application was made 4
215 days after the first, this maximum inhibition value was maintained for a longer period (until
216 day 10 counting from the first application). In the treatments with a second application 6 and
217 10 days after the first, tree recovery had until the time of the second application been similar
218 to that of the single application. However, after the second application fluorescence inhibition
219 increased again for four or six day, respectively, before recovering progressively. That is, the
220 trees showed different variation in fluorescence inhibition depending on the number of days
221 between sprays. However, Brevis thinning efficacy was the same in all treatments. In all
222 double strategies, quantum yield decreased rapidly during 2 days after the foliar application
223 of Brevis, and the maximum Qy inhibition values were recorded between 2 and 10 days after
224 the treatment depending on the number of applications. The length of the period of inhibition
225 was the same in all treatments. That is, the together applications showed a higher area of
226 inhibition in comparison with the separate application. However, there was no difference in

227 thinning efficacy between treatments. That is, the increasing period of inhibition (4 days after
228 application) was more important than a long period of maximum inhibition.

229 **3.3. Night temperature**

230 Fig. 2 shows the night temperature in the application period of the Brevis chemical
231 thinner in all trials. There were important differences between years. Temperatures were
232 higher in the application period of 2015 than in 2016. Moreover, Girona 2015 had
233 temperatures above 14ºC at the time of all applications except the 9.5 mm treatment. This
234 situation explains the high efficacy of Brevis in all single applications except for 9.5 mm.
235 However, in all single applications in 2016 night temperatures never rose above 14ºC and,
236 correspondingly, the efficacy of single applications was lower in 2016. In the double
237 applications in Girona 2015, the second application was made 3, 4, 6, 7 or 10 days after the
238 first. Thinning efficacy increased with higher average night temperatures after Brevis
239 application. That is, when the climatology was favorable for the application, the number of
240 days between the first and second spray was not important. In the double applications in
241 Mollerussa 2016, the second application was made 2, 4, 6 or 10 days after the first. That is,
242 when the climatology was not favorable for the application, the period of days between the
243 first and second spray was also not important. These results show that night temperature is a
244 more important factor for thinning efficacy than the number of days between sprays.

245 **3.4. Night temperature (0/4) vs. % abscission**

246 In the single application treatments, when average night temperature was higher than
247 14ºC in the period of 0-4 days after Brevis application, Brevis efficacy was between 30% and
248 45%. However, when average night temperature was lower than 14ºC, Brevis efficacy was
249 less than 20% (Fig. 3). In the double application treatments, when average night temperature
250 after each application was around 14ºC, Brevis efficacy was higher than 50%. When average

night temperature of one application was around 14ºC and the other application temperature was lower than 13ºC, the efficacy of the double application treatment was between 30-40%. Finally, when the average night temperatures after each of the two applications was below 13ºC, efficacy was generally lower than 30% (Fig. 3).

4. Discussion

In the conditions of the trial, the spraying of apple trees with a chemical photosynthetic inhibitor induced fruit abscission, concurring with the results of Byers *et al.* (1990) and Gonzalez *et al.* (2019b). Brevis reduced crop load, fruit set and number of fruits per tree. In most cases, the effect was higher with two applications of Brevis than with a single application, concurring with the observations of Dorigoni and Lezzer (2007), Gonzalez et al. (2019a) and Stern (2014). Our results also suggest the sum effect of the number of applications. A similar effect on fruit set was reported by Stopar (2017), who found that final fruit set was mostly the sum of the two applications. Additionally, in Girona 2015 a single application was effective when compared to the Control treatment, again concurring with Deckers *et al.* (2010), Dorigoni and Lezzer (2007), Lafer (2010) and Reginato *et al.* (2017). However, in the other trials, where the climatology was not favorable for the application of a chemical thinner, there was no observed effect of the single applications, concurring with earlier observations of Byers (2003).

McArtney et al. (2012) reported a negative relationship between the application of a chemical thinner and fruit yield per tree at harvest, coinciding with the results obtained in Girona 2015. Yield fell with increasing Brevis thinning efficacy. However, in the 2016 experiments, fruit yield per tree at harvest did not show a negative relationship with Brevis efficacy.

Average fruit weight, diameter and coloration increased with the Brevis-induced thinning effect, which concurs with the observations of Brunner (2014), Gonzalez *et al.* (2019c) , Maas and Meland (2016) and McArtney *et al.* (1996). They reported a negative linear relationship between the number of fruits and their average weight, color and diameter, which increased significantly in the treatments in which the chemical thinner reduced the number of fruits per tree. For Gala apples to be marketable, they must have a minimum blush of 60%. In southern European countries, color development is a serious problem because climate conditions of hot and dry summers do not favor fruit color development (Iglesias and Alegre, 2006; Iglesias *et al.*, 2008). This circumstance in our study, with a hot and dry period before the harvest, explains the low rate of coloration in these trials.

Measuring chlorophyll fluorescence to test photosynthesis is an approach that was first considered by Kautsky and Hirsch (1931) who detected a significant relationship between photosynthesis and chlorophyll fluorescence (Chen and Cheng, 2010). Chlorophyll fluorescence has therefore been used as a measure of photosystem activity, especially photosystem II (Fernandez *et al.*, 1997; Krause and Weis, 1984). In Mollerussa 2016, the maximum Qy inhibition values were recorded between 2 and 10 days after the treatment depending on whether the treatment involved a single or double spray and on the number of days between sprays in the double application treatments. These results concur with earlier observations by Brunner (2014) and McArtney et al. (2012). The interval between the first and second spray in the double application treatments in Mollerussa 2016 varied between 4 and 10 days, with differing fluorescence inhibition rates observed in these periods. When the two sprays were separated by just 4 days, maximum inhibition was maintained for 10 days. However, when the two applications were further apart in time, inhibition began to progressively recover after reaching its maximum value after the first application and increased again after the second application. Nonetheless, although inhibition varied

299 depending on the number of days between the first and second application, Brevis efficacy
300 was the same in all the double application treatments. This suggests that the number of days
301 between the first and second sprays and the subsequent different fluorescence inhibition
302 patterns were not important factors in Brevis thinning efficacy.

303 The degree of abscission of Brevis is highly dependent on environmental factors
304 (Basak, 2011; Lordan et al., 2018; Mathieu *et al.*, 2016), and for this reason efficacy varied
305 considerably between trials. Many authors have reported that temperature plays an important
306 role in apple chemical thinning efficacy with different products (Kviklys and Robinson,
307 2010; Lakso et al., 2006; Li and Cheng, 2011; Lordan *et al.*, 2019; Parra-Quezada et al.,
308 2005; Pretorius *et al.*, 2011). Their results concur with the observations of this study, which
309 show that temperature is an important factor in determining Brevis efficiency. According to
310 Lakso et al. (2006), hot temperatures (especially high night temperatures) and cloudy (low
311 light periods) conditions cause or enhance fruit abscission. These conditions, which lead to
312 poor carbohydrate status, are associated with heavy drop and easier thinning. That is, carbon
313 assimilation increases when night temperature is low. Such conditions intensify competition
314 among competing sinks at a time when metabolic demand is highest in the tree (Lakso et al.,
315 2006; Yoon *et al.*, 2011). As a result, the smaller fruitlets stop growing and will drop,
316 consequently increasing the Brevis effect. The above described effects explain the
317 importance of night temperature, and concur with the observations of this study in which
318 Brevis thinning efficacy was enhanced with increasing night temperature.

319 According to the manufacturer, an interval of 5-10 days between applications is
320 recommendable for Brevis. In Girona 2015, the number of days between sprays in the double
321 application treatment ranged between 3 and 10 days. Thinning efficacy was higher when
322 average night temperature after application was higher, and the number of days between the

first and second spray was not important. In Mollerussa 2016, the number of days between sprays in the double application treatment ranged between 2 and 10 days. The climatology was not favorable at the time of application. However, the efficacy of the double applications was similar in all treatments, indicating that the number of days between the first and second spray was not important. That is, the results suggest that an appropriate climatology is more important for Brevis efficacy than the number of days between applications.

5. Conclusions

A Brevis thinning effect was observed in all trials, with the reduction in crop load, fruit set and number of fruits per tree varying according to the number of applications. Efficacy with two Brevis applications was higher than with a single application and, in most cases, a single application was effective when compared to the Control treatment.

Yield fell with increasing Brevis thinning efficacy in the 2015 trail but not in the 2016 trials. In addition, there was a negative linear relationship between Brevis efficacy and average fruit weight, color and diameter. That is, average fruit weight, color and diameter increased significantly in the treatments in which Brevis reduced the number of fruits per tree.

In the double application treatments in Mollerussa 2016, although fluorescence inhibition rates varied depending on the number of days between the first and second application, Brevis thinning efficacy was the same in all the double application treatments. That is, the different inhibition rates and the number of days between the first and second spray were not important factors for the thinning efficacy of Brevis.

The degree of abscission of Brevis is highly dependent on night temperature, and for this reason Brevis efficacy varied considerably between trials. The regression analysis suggests that night temperature after Brevis application was an important factor, with higher

347 average night temperatures in the days immediately after spraying coinciding with greater
348 Brevis efficacy.

349 Importantly, our results show that the number of days between applications (which
350 depended on king fruit diameter) was not an important factor in explaining Brevis efficacy.
351 That is, it is not necessary to wait 5 days between treatments when the climatology is
352 favorable.

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356 **References**

357 Basak, A., 2011. Efficiency of fruitlet thinning in apple "Gala Must" by use of metamitron
358 and artificial shading. J. Fruit Ornam. Plant Res 19, 51-62.

359 Besseling, T., Bonany, J., Costa, G., 2018. Method for fruit thinning. Google Patents.

360 Brunner, P., 2014. Impact of Metamitron as a Thinning Compound on Apple Plants, In:
361 McArtney, S.J., Spann, T. (Eds.), XII International Symposium on Plant Bioregulators in
362 Fruit Production. Int Soc Horticultural Science, Leuven 1, pp. 173-181.

363 Byers, R.E., 2003. Flower and fruit thinning and vegetative: fruiting balance. CABI
364 Publishing, Wallingford, Oxon, UK.

365 Byers, R.E., Barden, J.A., Polomski, R.F., Young, R.W., Carbaugh, D.H., 1990. Apple
366 thinning by photosynthetic inhibition. J. Am. Soc. Hortic. Sci. 115, 14-19.

367 Chen, L.S., Cheng, L.L., 2010. The acceptor side of photosystem II is damaged more severely
368 than the donor side of photosystem II in 'Honeycrisp' apple leaves with zonal chlorosis. Acta
369 Physiol. Plant. 32, 253-261.

370 Costa, G., Botton, A., Vizzotto, G., 2018. Fruit thinning: advances and trends. Horticultural
371 Reviews 46, 185-226.

372 Deckers, T., Schoofs, H., Verjans, W., 2010. Looking for Solutions for Chemical Fruit
373 Thinning on Apple, In: Costa, G. (Ed.), XI International Symposium on Plant Bioregulators
374 in Fruit Production. Int Soc Horticultural Science, Leuven 1, pp. 237-244.

375 Dorigoni, A., Lezzer, P., 2007. Chemical thinning of apple with new compounds. Erwerbs-
376 Obstbau 49, 93-96.

377 Fernandez, R.T., Perry, R.L., Flore, J.A., 1997. Drought response of young apple trees on
378 three rootstocks .2. Gas exchange, chlorophyll fluorescence, water relations, and leaf abscisic
379 acid. J. Am. Soc. Hortic. Sci. 122, 841-848.

380 Gonzalez, L., Àvila, G., Carbó, J., Bonany, J., Alegre, S., Torres, E., Martin, B., Recasens,
381 I., Asin, L., 2019a. Hail nets do not affect the efficacy of metamitron for chemical thinning
382 of apple trees. J. Horticult. Sci. Biotechnol.

383 Gonzalez, L., Bonany, J., Alegre, S., Àvila, G., Carbó, J., Torres, E., Recasens, I., Martin,
384 B., Asin, L., 2019b. Brevis thinning efficacy at different fruit size and fluorescence on 'Gala'
385 and 'Fuji' apples. Sci. Hortic. 256.

386 Gonzalez, L., Torres, E., Carbó, J., Alegre, S., Bonany, J., Àvila, G., Martin, B., Recasens,
387 I., Asin, L., 2019c. Effect of different application rates of metamitron as fruitlet chemical
388 thinner on thinning efficacy and fluorescence inhibition in Gala and Fuji apple. Plant Growth
389 Regul.

390 Iglesias, I., Alegre, S., 2006. The effect of anti-hail nets on fruit protection, radiation,
391 temperature, quality and profitability of 'Mondial Gala'apples. Journal of Applied
392 Horticulture 8, 91-100.

393 Iglesias, I., Echeverría, G., Soria, Y., 2008. Differences in fruit colour development,
394 anthocyanin content, fruit quality and consumer acceptability of eight 'Gala' apple strains.
395 Sci. Hortic. 119, 32-40.

396 Kautsky, H., Hirsch, A., 1931. Neue versuche zur kohlensäureassimilation.
397 Naturwissenschaften 19, 964-964.

398 Krause, G.H., Weis, E., 1984. Chlorophyll fluorescence as a tool in plant physiology .2.
399 interpretation of fluorescence signals. Photosynth. Res. 5, 139-157.

400 Kviklys, D., Robinson, T., 2010. Temperature before and after Application of Chemical
401 Thinners Affects Thinning Response of 'Empire' Apple Trees., In: Costa, G. (Ed.), XI

International Symposium on Plant Bioregulators in Fruit Production. Int Soc Horticultural Science, Leuven 1, pp. 525-530.

Lafer, G., 2010. Effects of Chemical Thinning with Metamitron on Fruit Set, Yield and Fruit Quality of 'Elstar'. In: Costa, G. (Ed.), XI International Symposium on Plant Bioregulators in Fruit Production. Int Soc Horticultural Science, Leuven 1, pp. 531-536.

Lakso, A.N., Robinson, T.L., Greene, D.W., 2006. Integration of environment, physiology and fruit abscission via carbon balance modeling - Implications for understanding growth regulator responses, 727 ed. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 321-326.

Li, P., Cheng, L., 2011. Varietal Difference of Apple Peel in Response to High Temperature and High Light Stress as Evaluated by Chlorophyll Fluorescence, In: Robinson, T.L. (Ed.), IX International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems. Int Soc Horticultural Science, Leuven 1, pp. 1049-1054.

Lordan, J., Alins, G., Àvila, G., Torres, E., Carbó, J., Bonany, J., Alegre, S., 2018. Screening of eco-friendly thinning agents and adjusting mechanical thinning on ‘Gala’,‘Golden Delicious’ and ‘Fuji’apple trees. Sci. Hortic. 239, 141-155.

Lordan, J., Reginato, G.H., Lakso, A.N., Francescatto, P., Robinson, T.L., 2019. Natural fruitlet abscission as related to apple tree carbon balance estimated with the MaluSim model. Sci. Hortic. 247, 296-309.

Maas, F.M., Meland, M., 2016. Thinning response of 'Summerred' apple to Brevis (R) in a northern climate, In: Costa, G. (Ed.), Eufrin Thinning Working Group Symposia. Int Soc Horticultural Science, Leuven 1, pp. 53-59.

Mathieu, V., Lavoisier, C., Bouniol, M., Saint Hilary, J.F., 2016. Apple thinning by photosynthesis inhibition, In: Costa, G. (Ed.), Eufrin Thinning Working Group Symposia. Int Soc Horticultural Science, Leuven 1, pp. 19-26.

McArtney, S., Palmer, J.W., Adams, H.M., 1996. Crop loading studies with 'Royal Gala' and 'Braeburn' apples: Effect of time and level of hand thinning. N. Z. J. Crop Hortic. Sci. 24, 401-407.

McArtney, S.J., Obermiller, J.D., Arellano, C., 2012. Comparison of the Effects of Metamitron on Chlorophyll Fluorescence and Fruit Set in Apple and Peach. Hortscience 47, 509-514.

McClure, K.A., Cline, J.A., 2015. Mechanical blossom thinning of apples and influence on yield, fruit quality and spur leaf area. Can. J. Plant Sci. 95, 887-896.

Parra-Quezada, R., Robinson, T., Osborne, J., Lakso, A., Parra-Bujanda, L., 2005. Temperature and chemical thinner affect fruit set, and growth of Empire apple, X International Symposium on Plant Bioregulators in Fruit Production 727, pp. 327-336.

Pretorius, J.J.B., Theron, K.I., Midgley, S.J.E., 2011. The Effect of Temperature and Developmental Stage on Carbon Dioxide Exchange of Attached 'Royal Gala', 'Fuji' and 'Cripps' Pink' Apple Fruits, In: Robinson, T.L. (Ed.), IX International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems. Int Soc Horticultural Science, Leuven 1, pp. 1077-1084.

Reginato, G., Riquelme, C., Robinson, T.L., 2017. Evaluation of timing for spraying Metamitron or NAA plus BA for thinning 'Brookfield Gala' apple fruitlets, 1177 ed. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 229-234.

Robinson, T., Lakso, A., 2004. Between year and within year variation in chemical fruit thinning efficacy of apple during cool springs, XXVI International Horticultural Congress: Key Processes in the Growth and Cropping of Deciduous Fruit and Nut Trees 636, pp. 283-294.

Robinson, T.L., Lakso, A.N., 2011. Predicting Chemical Thinner Response with a Carbohydrate Model, In: Robinson, T.L. (Ed.), IX International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems. Int Soc Horticultural Science, Leuven 1, pp. 743-750.

Robinson, T.L., Lakso, A.N., Hoying, S.A., 2012. Advances in Predicting Chemical Thinner Response of Apple Using a MaluSim Carbon Balance Model, In: Palmer, J.W., Herrero, M., Hormaza, I., Tustin, D.S., Talon, M., Tadeo, F.R., Chaves, M., Webster, A.D. (Eds.), XXVIII International Horticultural Congress on Science and Horticulture for People. Int Soc Horticultural Science, Leuven 1, pp. 223-229.

459 Rosa, N., 2016. Comparison between benzyladenine and metamitron as chemical thinning
460 agents in Gala, Kanzi, Pink Lady and Red Delicious apple cultivars. ISA-Ul.

461 Stern, R.A., 2014. The photosynthesis inhibitor Metamitron is an effective fruitlet thinner for
462 'Gala' apple in the warm climate of Israel, In: Wunsche, J.N., Tranbarger, T.J. (Eds.), XXIX
463 International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and
464 Landscapes. Int Soc Horticultural Science, Leuven 1, pp. 15-23.

465 Stopar, M., 2017. Photosynthesis Inhibition as a Tool for Apple Fruitlet Thinning.

466 Yoon, T.M., Robinson, T.L., Reginato, G.H., 2011. Effects of Temperature and Light Level
467 on Efficiency of Chemical Thinner on 'Empire' Apple Trees, In: Robinson, T.L. (Ed.), IX
468 International Symposium on Integrating Canopy, Rootstock and Environmental Physiology
469 in Orchard Systems. Int Soc Horticultural Science, Leuven 1, pp. 1085-1093.

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471

472 **Table 1.** Principal characteristics of the orchards used for the trials

Trials: No. and year	Cultivar	Rootstock	Planted	Density plantation	Training system	Location
2 (2015 and 2016)	Galaxy Gala	M9	2000	2666 trees/ha (3.75m x 1m)	Central leader	Girona (Tallada d'Emporda)
1 (2016)	Brookfield Gala	M9	2003	1786 trees/ha (4m x 1.4m)	Central leader	Mollerussa

473

474 **Table 2.** Chemical application

Treatments No.	Rate (kg/ha) and moment of application (fruit size)			
	Ø ≈ 7.5 mm	**Ø ≈ 9.5 mm**	**Ø ≈ 11.5 mm**	**Ø ≈ 13.5 mm**
1	Control			
2	1.65			
3		1.65		
4			1.65	
5				1.65
6	1.65	1.65		
7	1.65		1.65	
8	1.65			1.65
9		1.65	1.65	
10		1.65		1.65
11			1.65	1.65

475

476 **Table 3:** Average number of flower clusters per tree and trunk cross-sectional area (TCSA)
477 in all trials.

Treatments No.	Fruit size of application (mm)	Girona 2015		Girona 2016		Molleussa 2016	
		No. of flower clusters per tree	TCSA (cm^2)	No. of flower clusters per tree	TCSA (cm^2)	No. of flower clusters per tree	TCSA (cm^2)
1	**Control**	313 a	30 a	168 a	25 a	278 a	42 a
2	**7.5 mm**	279 a	28 a	170 a	30 a	272 a	43 a
3	**9.5 mm**	277 a	28 a	163 a	31 a	277 a	39 a
4	**11.5 mm**	275 a	26 a	171 a	29 a	274 a	45 a
5	**13.5 mm**	275 a	28 a	172 a	35 a	259 a	42 a
6	**7.5 + 9.5 mm**	284 a	29 a	168 a	27 a	272 a	45 a
7	**7.5 + 11.5 mm**	291 a	26 a	169 a	30 a	275 a	43 a
8	**7.5 + 13.5 mm**	292 a	28 a	171 a	28 a	276 a	44 a
9	**9.5 + 11.5 mm**	282 a	35 a	171 a	33 a	275 a	51 a
10	**9.5 + 13.5 mm**	273 a	30 a	167 a	30 a	274 a	45 a
11	**11.5 + 13.5 mm**	296 a	35 a	171 a	31 a	274 a	49 a

Means within a column followed by different letters denotes significant differences (Duncan's range test at $P<0.05$).

478

479

480 **Table 4:** Effect of thinning with Brevis on final number of fruits per tree, fruit set (final fruit
481 number/100 flower clusters) and crop load (number of fruits per tree/TCSA) in all trials

Treatments No.	Fruit size of application (mm)	Girona 2015			Girona 2016			Molleussa 2016		
		No. of Fruits per tree	Fruit set	Crop load	No. of Fruits per tree	Fruit set	Crop load	No. of Fruits per tree	Fruit set	Crop load
1	**Control**	420 a	141 a	14.1 a	197 a	121 a	8.1 a	472 a	173 a	11.7 a
2	**7.5 mm**	296 b	107 b	10.7 b	167 a	105 a	5.7 a	420 abc	156 ab	9.9 abc
3	**9.5 mm**	403 a	149 a	14.5 a	184 a	115 a	6.4 a	411 abc	152 abc	11.2 a
4	**11.5 mm**	271 bc	101 b	10.5 b	182 a	112 a	6.4 a	429 ab	159 ab	9.9 abc
5	**13.5 mm**	230 bcd	85 cd	8.2 bcd	176 a	103 a	5.6 a	423 abc	168 a	10.2 ab
6	**7.5 + 9.5 mm**	262 bc	93 bc	9.1 bc	159 a	96 a	5.9 a	319 cd	121 bcd	7.2 bcd
7	**7.5 + 11.5 mm**	178 d	61 d	6.9 cd	151 a	90 a	5.1 a	329 bcd	126 bcd	7.9 bcd
8	**7.5 + 13.5 mm**	168 d	59 d	6.1 d	170 a	101 a	5.9 a	302 d	119 bcd	6.8 cd
9	**9.5 + 11.5 mm**	274 bc	100 b	8.0 bcd	166 a	98 a	5.2 a	333 bcd	121 bcd	6.9 cd
10	**9.5 + 13.5 mm**	213 cd	81 bcd	7.5 cd	138 a	83 a	4.9 a	301 d	110 cd	7.2 bcd
11	**11.5 + 13.5 mm**	205 cd	70 cd	5.8 d	148 a	89 a	4.7 a	276 d	105 d	5.9 d

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

482

483 **Table 5:** Effect of thinning with Brevis on yield (kg/tree) in all trials

Treatments No.	Fruit size of application (mm)	Yield (kg/tree)		
		Girona 2015	Girona 2016	Molleussa 2016
1	**Control**	44 a	26 a	32 a
2	**7.5 mm**	37 ab	24 a	33 a
3	**9.5 mm**	42 a	26 a	34 a
4	**11.5 mm**	32 bc	28 a	34 a
5	**13.5 mm**	30 bc	23 a	32 a
6	**7.5 + 9.5 mm**	36 ab	24 a	37 a
7	**7.5 + 11.5 mm**	27 c	23 a	37 a
8	**7.5 + 13.5 mm**	27 c	25 a	39 a
9	**9.5 + 11.5 mm**	38 ab	26 a	40 a
10	**9.5 + 13.5 mm**	33 bc	21 a	40 a
11	**11.5 + 13.5 mm**	31 bc	21 a	42 a

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

484

485 **Table 6:** Effect of thinning with Brevis on average of fruits size and weight in all trials.

Treatments No.	Fruit size of application (mm)	Girona 2015		Girona 2016		Molleussa 2016	
		Fruit weight (g)	Fruit size (mm)	Fruit weight (g)	Fruit size (mm)	Fruit weight (g)	Fruit size (mm)
1	**Control**	107 fg	65 fg	132 a	66 a	116 d	65 e
2	**7.5 mm**	126 de	68 de	142 a	67 a	121 d	67 de
3	**9.5 mm**	105 g	65 g	144 a	68 a	123 cd	67 de
4	**11.5 mm**	118 ef	66 ef	152 a	68 a	124 cd	67 de
5	**13.5 mm**	132 cd	69 cd	142 a	68 a	125 cd	67 cde
6	**7.5 + 9.5 mm**	138 c	69 cd	149 a	68 a	137 bc	70 abc
7	**7.5 + 11.5 mm**	150 ab	71 ab	158 a	69 a	136 bc	69 bcd
8	**7.5 + 13.5 mm**	161 a	72 a	153 a	69 a	140 ab	70 ab
9	**9.5 + 11.5 mm**	139 bc	70 bc	157 a	69 a	145 ab	71 ab
10	**9.5 + 13.5 mm**	154 a	71 a	155 a	69 a	145 ab	71 ab
11	**11.5 + 13.5 mm**	152 a	71 a	144 a	67 a	153 a	73 a

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

Table 7: Effect of thinning with Brevis on fruit color (60% blush area in % and kg of total) in all trials

Treatments No.	Fruit size of application (mm)	Yield > 60% blush area					
		Girona 2015		Girona 2016		Molleussa 2016	
		kg of total	% of total	kg of total	% of total	kg of total	% of total
1	**Control**	4 d	10 d	21 a	84 a	1 a	2 a
2	**7.5 mm**	7 bcd	19 bcd	20 a	87 a	1 a	3 a
3	**9.5 mm**	6 bcd	16 cd	22 a	82 a	2 a	5 a
4	**11.5 mm**	5 cd	16 cd	23 a	83 a	2 a	6 a
5	**13.5 mm**	7 bcd	24 bcd	21 a	87 a	1 a	3 a
6	**7.5 + 9.5 mm**	8 bcd	23 bcd	16 a	69 a	1 a	4 a
7	**7.5 + 11.5 mm**	7 bcd	27 bc	20 a	87 a	2 a	6 a
8	**7.5 + 13.5 mm**	7 bcd	27 bc	21 a	82 a	3 a	7 a
9	**9.5 + 11.5 mm**	9 abc	24 bcd	19 a	73 a	3 a	7 a
10	**9.5 + 13.5 mm**	11 ab	34 ab	18 a	84 a	3 a	7 a
11	**11.5 + 13.5 mm**	13 a	44 a	20 a	93 a	3 a	7 a

Means within a column followed by different letters denotes significant differences (Duncan's range test at $P<0.05$).

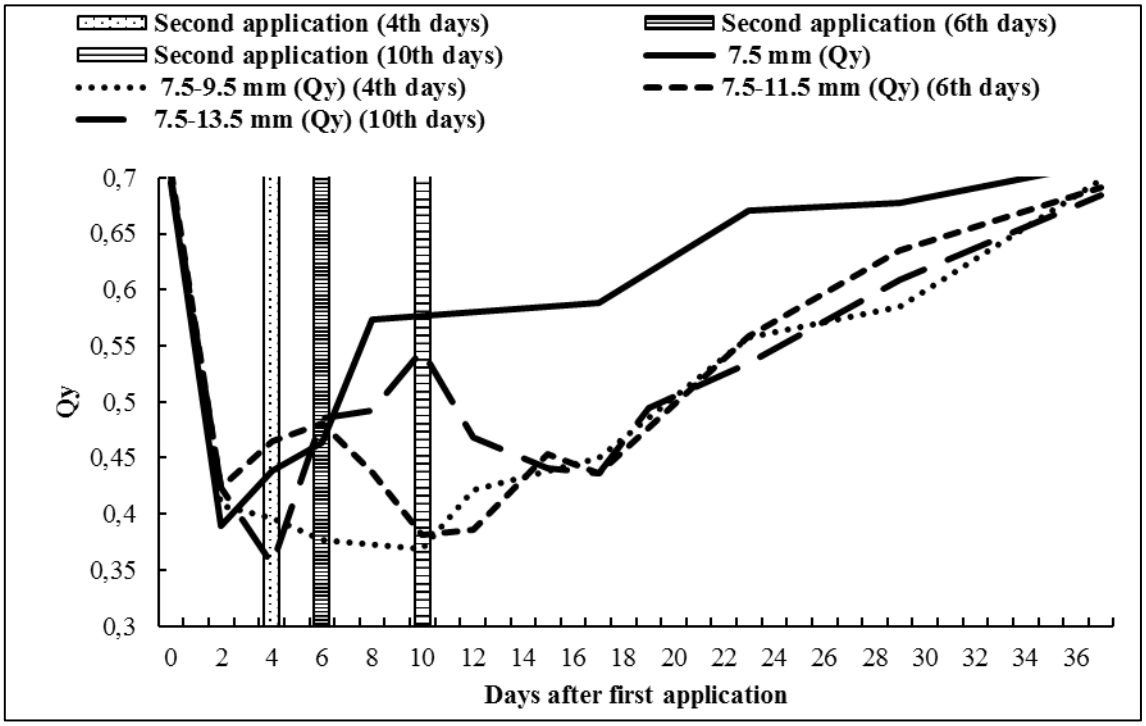

Fig. 1: Effect of application of the photosystem II (PSII) inhibitor Brevis with one and two applications on chlorophyll fluorescence (Qy) in leaves of 'Gala' apple in Mollerussa 2016. The 7.5 mm application was on 26 April, the 9.5 mm application on 30 April, the 11.5 mm application on 2 May, and the 13.5 mm application on 6 May.

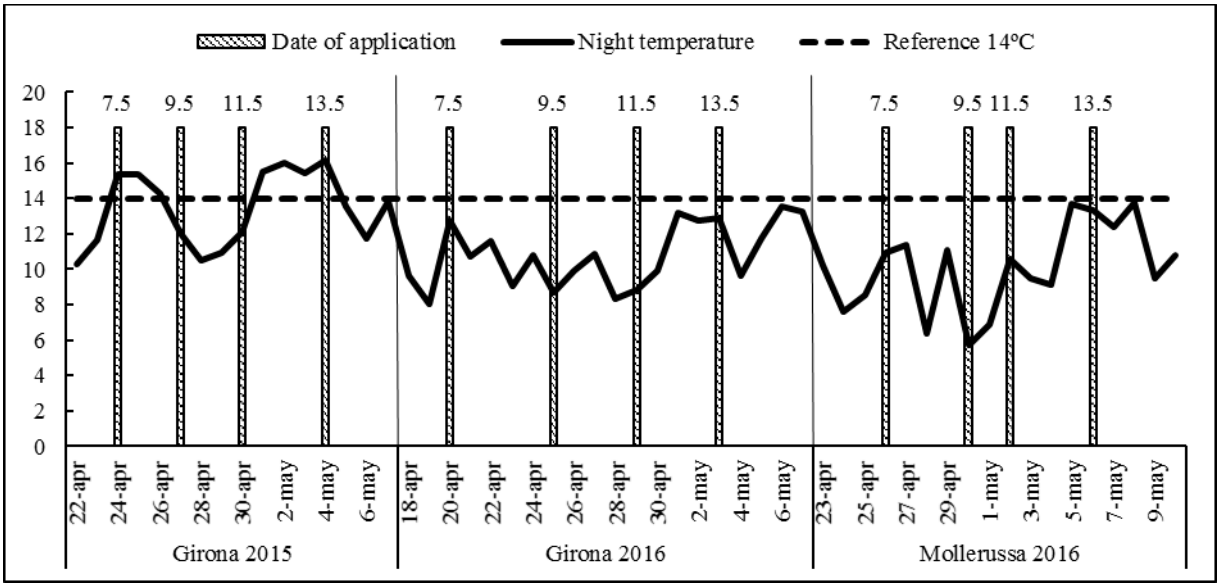

Fig.2: Average night temperatures and periods of king fruit diameter (mm) in apple trees over three trials.

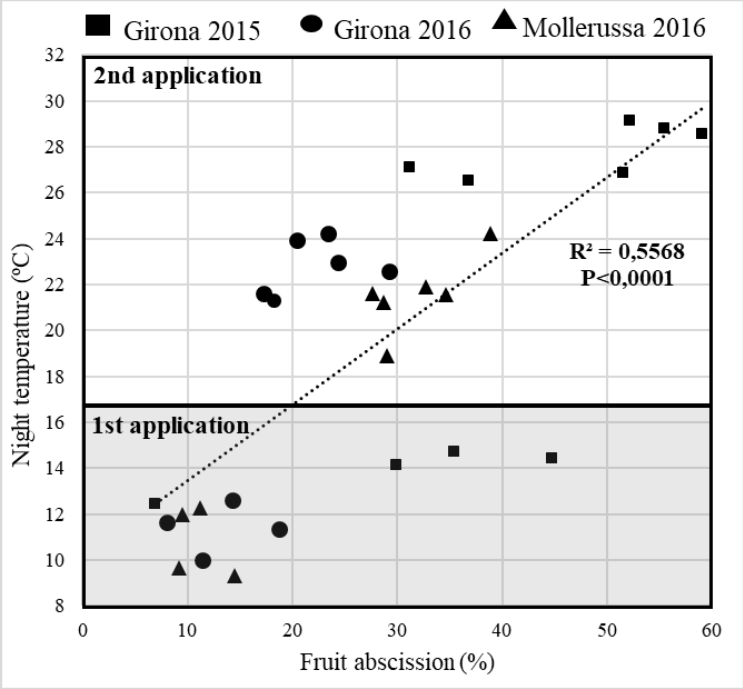

Fig. 3. Scatter plot showing the relationship between night temperature (0/4) and fruit abscission (%) for 'Gala' (2015–2016). Night temperature for the single application treatments was calculated as the average night temperature (21:00 to 7:00) between the day of application and 4 days after application. Night temperature for the double application treatments was calculated as the sum of the average of the two periods after application. Fruit abscission (%) was obtained from the relationship between final number of fruits per tree (treatment)/final number of fruits per tree (Control). Each symbol represents 1 treatment.