Effect of different application rates of metamitron as fruitlet chemical thinner on thinning efficacy and fluorescence inhibition in Gala and Fuji apple

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Abstract

Crop thinning is an important and difficult agricultural practice. Knowing the effect of the application dose of a product is a crucial element of any thinning program. The aims of this study were to investigate the effect of different metamitron doses on Gala and Fuji apples applied at fruit king diameters of between 8 and 10 mm and to determine fluorescence inhibition at the different application rates. Trials were conducted over two seasons from

2015 to 2016 in apple orchards in Lleida (Spain). Photosynthesis inhibition caused by metamitron was also analysed and measured, using chlorophyll fluorescence and biexponential pharmacokinetic models. Under the trial conditions, the application of metamitron reduced final fruit set, number of fruits per tree and crop load depending on the application rate. A dose effect was observed in all yield parameters. Moreover, when metamitron showed high efficacy, there was an improvement in fruit weight, coloration and diameter. The estimated parameters A, α and B using a biexponential equation were related with final fruit set, however the period of inhibition has to be finished before prediction can be made of metamitron efficacy in the year. The fluorescence analysis showed a dose effect, with metamitron dose increasing inhibition. Additionally, the same result was also observed in the area under curve analysis, with metamitron dose reducing the area and inhibition increasing. In all yield parameters, the fluorescence and area under curve analyses showed differences between cultivars, with the inhibition caused by metamitron higher in Gala than in Fuji. Moreover, differences between years were observed. 2015 was warmer than 2016, and the higher temperatures increased the thinning efficacy of metamitron.

Keywords

Crop load, Doses, Carbohydrate deficit, Fruit abscission, Photosynthesis, Brevis®

1. Introduction

Crop load management on apple trees remains a significant challenge to producers (Cline *et al.*, 2018). Crop thinning is a vitally important but difficult agricultural practice that has a significant impact on orchard profitability (Lordan *et al.*, 2018; Robinson *et al.*, 2016). Apple flowers are initiated the year prior to bloom, and inadequate thinning can result in biennial bearing (Cline et al., 2018). Good crop load management requires a sufficient reduction of crop load (yield) to achieve optimum fruit size and adequate return bloom, but without an excessive reduction of yield (Robinson et al., 2016).

Hand, mechanical and chemical thinning are the strategies currently used on apple. Hand thinning is costly in terms of labour and time-consuming. It requires waiting until the natural drop is complete, which often occurs late and may consequently affect fruit size and the return bloom (Lordan et al., 2018; McArtney *et al.*, 1996). Mechanical thinning can be a valuable tool to initially reduce crop load prior to chemical or hand thinning (McClure and Cline, 2015). However, this method can present different problems, requires special machinery, special training systems and is not selective for fruit size (Byers, 2003; McClure and Cline, 2015). Finally, chemical thinning is a commonly used practice because it acts early on the fruit and reduces production costs. However, its efficacy is variable as its use is dependent on climatic conditions and cultivar (Byers, 2003; Gonzalez *et al.*, 2019b; Lordan et al., 2018; Robinson and Lakso, 2004). Currently, in Spain, chemical thinning can be carried out during flowering (naphthalene acetamide (NAD)) and after fruit set on young fruitlets at the 6-16 mm stages (using the hormones 6-benzyladenine (BA) and naphthyl acetic acid (NAA)).

Brevis® was registered in Spain in 2015. As metamitron, its active ingredient at 15%, belongs to the triazinone family of herbicides, the mode of action of metamitron differs from that of other known bioregulators. Although the maximum permitted application commercial rate is 2.20 kg/ha, no studies are available to know the effect of applying higher dosages. The thinning activity of metamitron in apple is via inhibition of photosynthesis (Basak, 2011; Lafer, 2010). More specifically, it is a photosystem II (PSII) inhibitor that disrupts the photosynthetic apparatus (McArtney and Obermiller, 2012; Stern, 2014, 2015), and acts by blocking electron transfer between the primary and secondary quinones (McArtney *et al.*, 2012). This interruption of photosynthetic electron transport inhibits adenosine 5′-triphosphate production and carbon fixation (McArtney et al., 2012). One of the oldest approaches to testing photosynthesis is by measuring chlorophyll fluorescence, with Kautsky and Hirsch (1931) the first to determine the significant relationship between photosynthesis and chlorophyll fluorescence (Chen and Cheng, 2010). Chlorophyll fluorescence has been used as way of measuring photosystem activity, especially PSII (Fernandez *et al.*, 1997; Krause and Weis, 1984).

Knowing the effect of the application dose of a product is a crucial element of any thinning program. With this in mind, the aims of the current study were to investigate the effect of different metamitron doses on Gala and Fuji apples applied at fruit king diameters of between 8 mm and 10 mm and to determine fluorescence inhibition at the different application rates.

2. Materials and methods

2.1. Plant material and temperatures

The trials were conducted in an apple orchard of the Institute of Agrifood Research and Technology (IRTA) experimental station of Lleida (Mollerussa, NE Spain) during the seasons of 2015 and 2016, using mature, uniform “Brookfield Gala” and “Fuji Kiku 8” trees grafted onto M9 rootstock and planted in 2003 at 4 x 1.4 m spacing (1786 trees/ha). The training system was a central leader. The trees were irrigated and fertilized using a drip irrigation system. Fertilization, pruning, herbicide and phytosanitary treatments were applied following standards normally used in apple orchards in the region.

Meteorological data were collected from a weather station of the official meteorological service of Catalonia, situated 50 m away from the experimental area in the orchard of the IRTA facilities. The night temperature was calculated as average temperature when there was no solar radiation.

2.2. Experimental design and treatment

The trials tested the use of the commercial chemical thinner Brevis® (ADAMA, Spain), containing 15% metamitron. Brevis® was applied at five different commercial rates (1.10, 1.65, 2.20, 3.30, 4.40 kg/ha) and an untreated control was included in the study. The time of application was determined by measuring king fruit diameter which should be in the range of 8-10 mm, and water volume was equivalent to 1000 l/ha.

All trials were arranged in a randomized block design with four replicates of four uniform trees per elementary plot. On each plot, the 2 central trees were used for the trial assessments. All trees were selected by uniformity of initial number of flower clusters at full bloom.

2.3. Yield assessments

In each trial, the total number of flower clusters per tree was counted at bud break stage (BBCH 61-65), before the treatments were applied. Moreover, harvesting was performed during the commercial harvest season. Individual sample trees were harvested and evaluated separately. The criteria established for first class (Extra) products at harvest were fruit color >60% of fruit surface with a good red color development, and fruit size >70 mm. Fruit size distribution was based on fruit diameter categories (>70 mm and >75 mm). Fruit weight, diameter, blush color, total fruit yield (kg per tree) and fruits per tree were measured with a commercial apple sorting and packing line machine (MAF RODA AGROBOTIC, France). Crop load was obtained from the number of fruits harvested per cm2 of trunk cross-sectional area (TCSA) (number of fruits / trunk cross-sectional area). The final fruit set was obtained from the relationship between number of flower clusters and number of fruits at harvest time ([number of fruits / floral clusters] x 100).

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence measurements were carried out in the orchard of the trials for all Galaxy and Fuji test strategies (five Brevis® strategies vs. untreated control trees). Measurements were made on 3 recently fully expanded leaves per control tree (6 leaves per block and 24 leaves per treatment) using handheld portable fluorimeters (FluorPen FP100, Photon Systems Instruments, Czech Republic) under full daylight conditions in the shaded part between 10:00 and 16:00 and at a height of 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 treatment values stabilized at 90% of the control level. An analysis was made of Qy (quantum yield) to provide an indication of the effects of Brevis® on the maximum potential quantum efficiency of PSII (Fv/Fm).

2.4.1. Biexponential functions

Biexponential functions can be used in pharmacokinetics to study the absorption, distribution, biotransformation and elimination of drugs in man and animals (Urso *et al.*, 2002). Similar models have been used in agriculture to study the degradation of a pesticide in soil (Swarcewicz and Gregorczyk, 2013) and the same type of model has also been used to study the dissipation of pesticides in surface soil (Navarro *et al.*, 2009). In the present study, this model was used to evaluate the inhibition of photosynthesis caused by Brevis® in apple trees.

The parameter evaluated with this model was Qy percentage (Qy(%)). Calculated as Qy(Treatment)÷Qy(Control), Qy(%) allows correction for the natural fluctuation of fluorescence in the Control. The Qy(%) curves were fitted to the biexponential pharmacokinetic model (Urso et al., 2002) of type:

$$f(t) = A \times e^{-\alpha t} + B \times e^{-\beta t}$$

where $f(t)$ is the value of Qy(%) at time *t*, and *t* is the moment in time of the fluorescence measurement.

The parameters B and β in the biexponential analysis of Qy explain the reduction of Qy. These parameters represent from the time of application to the time of minimum Qy(%) value, which is the time of maximum inhibition (Fig. 1) (Gonzalez et al., 2019b). The parameters A and α explain the recuperation of Qy, representing from the time of maximum inhibition, Qy(%) minimum value to the end of the period of inhibition caused by Brevis® (Fig. 1). The parameters β and α are the slopes of the descent and ascent of the curve, respectively. When β is higher, the slope descends faster and the minimum value of the curve is earlier in time. When α is lower, the recuperation phase is slower and the inhibition period is longer. The origin of the function is A+B. A and B represent the y-intercepts (Gustafson and Bradshaw-Pierce, 2011). When $f(t)$)=1, the function starts in 1 and in this case the tree realizes 100% of fluorescence at the start of the trial (Fig. 1). The area under the curve (AUC) is the area in the 20 days after application (Fig. 1) (Gonzalez et al., 2019b). Table 1 shows the calculations of the parameters.

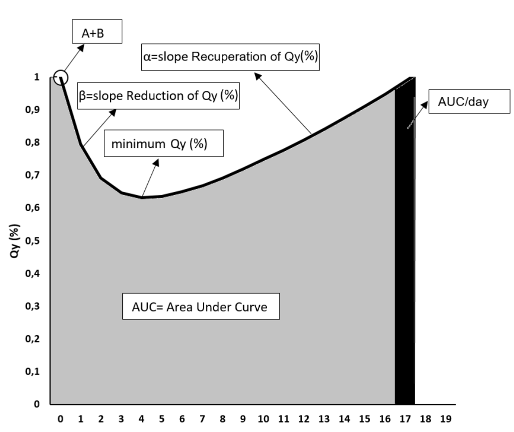

Fig. 1: Graphic representation of the parameters calculated with the biexponential pharmacokinetic model (AUC, AUC/day, A, α, B and β) (Gonzalez et al., 2019b).

Table 1: Parameters calculated

Parameter	Calculation
AUC/day (All AUC)	AUC ÷ inhibition 20 days
AUC reduction (0-min)	Area between day 0 and day of minimum Qy(%) value
Day of minimum Qy(%) value	Number of days between beginning of inhibition and day of minimum Qy(%) value
AUC recuperation (min-end)	Area between day of minimum Qy(%) value and 20 days after application

2.5. Statistical analysis

Analyses of crop load and AUC parameters were analyzed with a mixed model to assess the long-term effects of each production system using the PROC MIXED procedure of in SAS 9.2 (SAS Institute Inc., 2009). The mixed model included year (2015 and 2016), cultivar (Fuji and Gala), treatment, and their interactions as fixed effects for no. flower clusters per tree, no. fruits per tree, final fruit set, crop load, yield (kg/tree), average fruit weight, average fruit diameter (mm), yield >70 Ø , red blush (%) and yield (Kg) >60% red blush. Block was random effect. Main effects, interactions, and treatment effects within interactions were considered significant when $P \leq 0.05$. Moreover, a lineal regression analysis was made using JMP13 statistical analysis software (SAS institute, 2017), between commercial rates, crop load and AUC parameters for each experiment.

Chlorophyll fluorescence and AUC parameters were performed in JMP13 statistical analysis software (SAS institute, 2017). Data fitting of chlorophyll fluorescence and AUC (area under the curve) was performed using constrained nonlinear curve fitting in JMP13 statistical analysis software (SAS institute, 2017).

3. Results

3.1. Temperatures

The average 24h temperatures before and after the dates of Brevis® application were different in the two study years, with temperatures in 2015 higher than in 2016. In 2015, the temperature before application was generally higher than 15ºC, whereas in 2016 this temperature was never reached. Moreover, 4 days after Brevis® application, the average all day temperature in 2015 reached as high as 21ºC, whereas in 2016 it never reached 16ºC (Fig. 2).

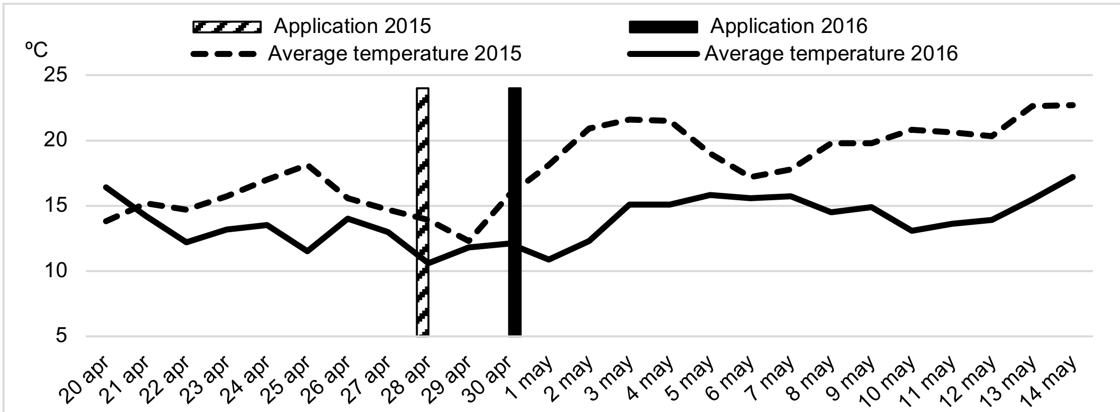

Fig. 2: Average 24h temperature and date of application in 2015 and 2016

Fig. 3 shows the average night temperature before and after Brevis® application. The average night temperature in 2015 was always higher than in 2016, except for 5 days. In 2015, the temperatures before and after application were higher than 11ºC, except for 2 days (day of application and day 1 after application). However, 11 days in the same period in 2016 had average night temperatures lower than 11ºC. Moreover, in 2015, the highest average night temperature after Brevis® application was 19ºC, but only 14ºC in 2016. These differences between 2015 and 2016 explain part of the differences in Brevis® efficacy.

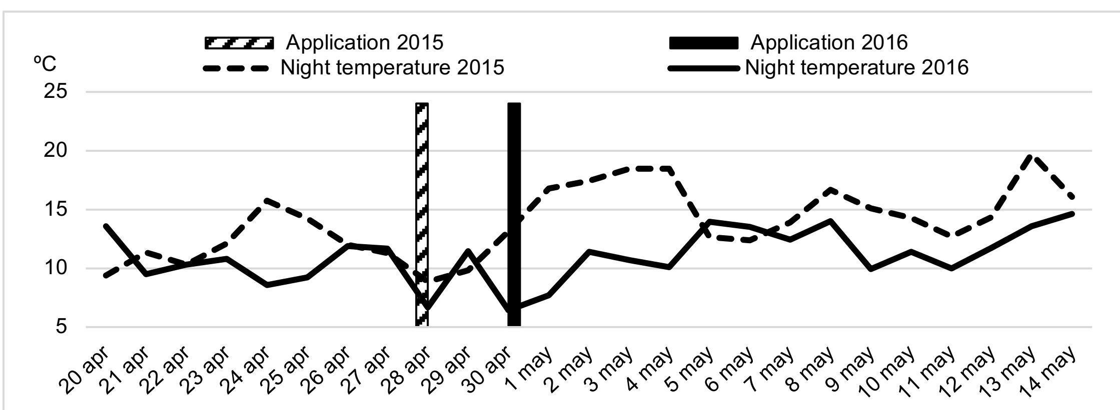

Fig. 3: Average night temperature (average temperature when there was no solar radiation) and date of application in 2015 and 2016.

3.2. Final fruit set and yield

In the two cultivars, all rates and years, the number of flower clusters per tree was uniform at the start of the trials (data not presented). All crop load parameters showed a significant differences between thinning rates. The values for average number of fruits per tree, final fruit set and yield (kg/tree) were significantly lower in Gala than in Fuji. However, average crop load in Fuji was significantly lower than in Gala. All productive parameters in 2015 were significantly lower compared to 2016 (Table 2). The interaction between year and cultivar was significant in the case of final fruit set, crop load, number of fruits per tree and yield. In yield and number of fruits per tree, there was significant interaction between thinning rate and year (Table 2). The triple interaction between year, cultivar and thinning rate was significant in number of fruits per tree and final fruit set (Table 2). With this in mind, Fig. 4 shows analysis of regression for each trial and parameters.

Table 2: Effect of thinning with Brevis® on final fruit set and yield in Gala and Fuji trees (avg. 2015-2016).

	No. fruits per tree	Final fruit set (No. fruits per 100 flowers clusters)	Crop load (No. of fruits per cm2 of TCSA)	Yield (kg/tree)
Thinning rate (Br)	***	***	***	***
Cultivar (C)	***	***	***	***
Gala	295	109	7.5	38
Fuji	365	134	5.5	63
Year (Y)	***	***	***	***
2015	253	94	4.5	41
2016	405	147	8.5	60
Significant interactions				
Br x C	ns	ns	ns	ns
Br x Y	*	ns	ns	**
C x Y	***	***	***	*
Br x C x Y	**	**	ns	ns

*, **, and *** denote means significantly different at $P< 0.05$, 0.01, or 0.001, respectively.
ns - not significant at $P<0.05$

All Brevis® strategies showed a reduction in number of fruits per tree, final fruit set, crop load and yield in comparison with the Control treatment, except for Fuji 2016 (Fig. 4). A Brevis® lineal dose effect was observed, with an increase in the dose rate accompanied by a decrease in fruit number per tree, final fruit set, crop load and yield. Minimum Brevis® efficacy was at 1.10 kg/ha, and maximum Brevis® efficacy at 4.40. However, Fuji 2016 showed lower efficiency in all treatments and dose effect was not observed (Fig. 4). Moreover, Brevis® thinning efficacy varied from year to year (Fig. 4)

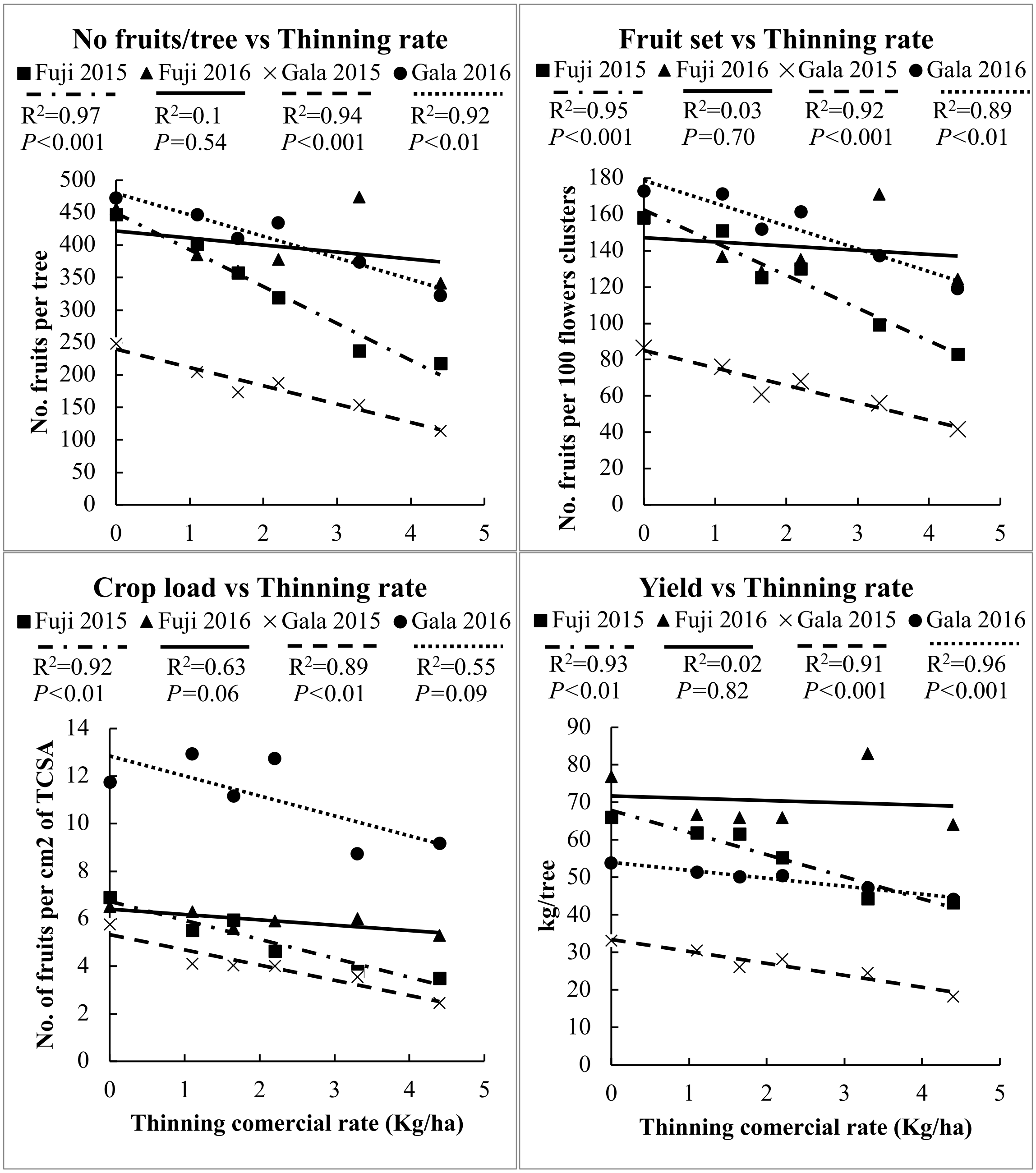

Fig. 04: Relationships between Brevis® comerial rates and the number of fruit per tree, final fruit set, crop load and yield in Gala and Fuji trees.

3.3. Fruit quality

All quality parameters showed a significant difference between thinning rates. Average fruit weight, diameter, and percentage of fruit >70 mm and >60% red blush in 2016 were significantly lower than in 2015. Gala yielded significantly lower fruit weight, diameter and percentage of fruit >70 mm and >75 mm compared with Fuji (Table 3). While red blush percentage showed no significant differences between cultivars, it did between years, with 2015 having a significantly higher percentage than 2016. The interaction between year and cultivar was significant in all fruit quality parameters. Average fruit weight, diameter, red blush and percentage of fruit >60% red blush were significant in the interaction between Brevis® rate and year. The triple interaction between year, cultivar and thinning rate was significant in the case of average fruit weight and yield percentage > 70 mm diameter, but not in the other parameters (Table 3). With this in mind, Fig. 5 and 6 shows analysis of regression for each trial and parameters.

Table 3: Effect of thinning with Brevis® on fruit weight, fruit size and fruit color in Gala and Fuji trees (avg. 2015-2016).

	Average fruit weight (g)	**Average fruit diameter (mm)**	**Yield >70 Ø (% of total)**	**Red blush (%)**	**Yield (Kg) >60% red blush**
Thinning rate (Br)	***	***	***	***	***
Cultivar (C)	***	***	***	ns	***
Gala	138	69	53	23	18
Fuji	177	74	67	23	10
Year (Y)	***	***	***	***	***
2015	162	73	71	31	23
2016	151	70	49	14	5
Significant interactions					
Br x C	ns	ns	ns	ns	ns
Br x Y	*	**	ns	**	**
C x Y	***	***	***	***	***
Br x C x Y	*	ns	**	ns	ns

*, **, and *** denote means significantly different at P< 0.05, 0.01, or 0.001, respectively.
ns - not significant at P<0.05

All Brevis® rates increased fruit weight and diameter in comparison with the Control treatment. Moreover, when the chemical rate increased, the fruit weight, diameter, fruit size distribution and fruit color also increased. That is, all these parameters showed a lineal dose effect and a direct relation with crop load reduction. Maximum Brevis® efficacy was at 4.40 kg/ha (Fig 5), with this treatment giving the highest fruit weight, diameter, and red blush percentage. Moreover, minimum Brevis® efficacy was at 1.10 kg/ha, with minimum fruit weight, diameter and red blush percentage (Fig 5).

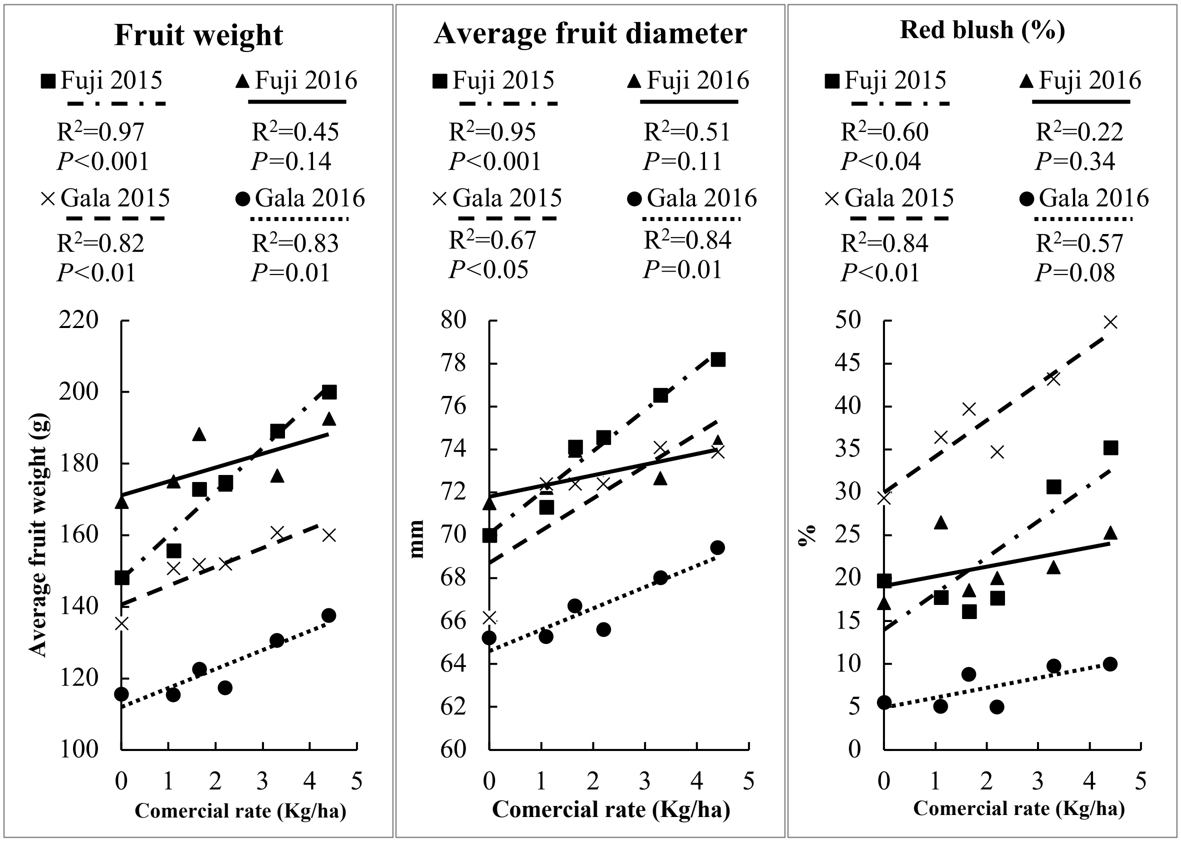

Fig. 5: Relationships between Brevis® comerial rates and fruit weight average fruit diameter and average red blush (%) in apple in Mollerussa, Spain.

There were significant (P < 0.05) positive relationships between percentage of fruit >70 mm and different rates in all the experiments, except for Fuji 2016. If so, percentage of fruit >70 mm increased as rates increased. That is, the treatment with highest percentage of fruit >70 mm, the 4.40 kg/ha treatment, had a higher Brevis® efficiency (Fig 6).

For Fuji and Gala 2015 showed a significant positive relationship between percentage of red blush >60%. These trials a lineal dose effect was observed, with an increase in the rate accompanied by an increase percentage of red blush >60%. Fuji and Gala 2016 showed a lower color development because climate conditions of hot and dry summers do not favor fruit color development. However, these trials showed a dose effect tendency (Fig 6).

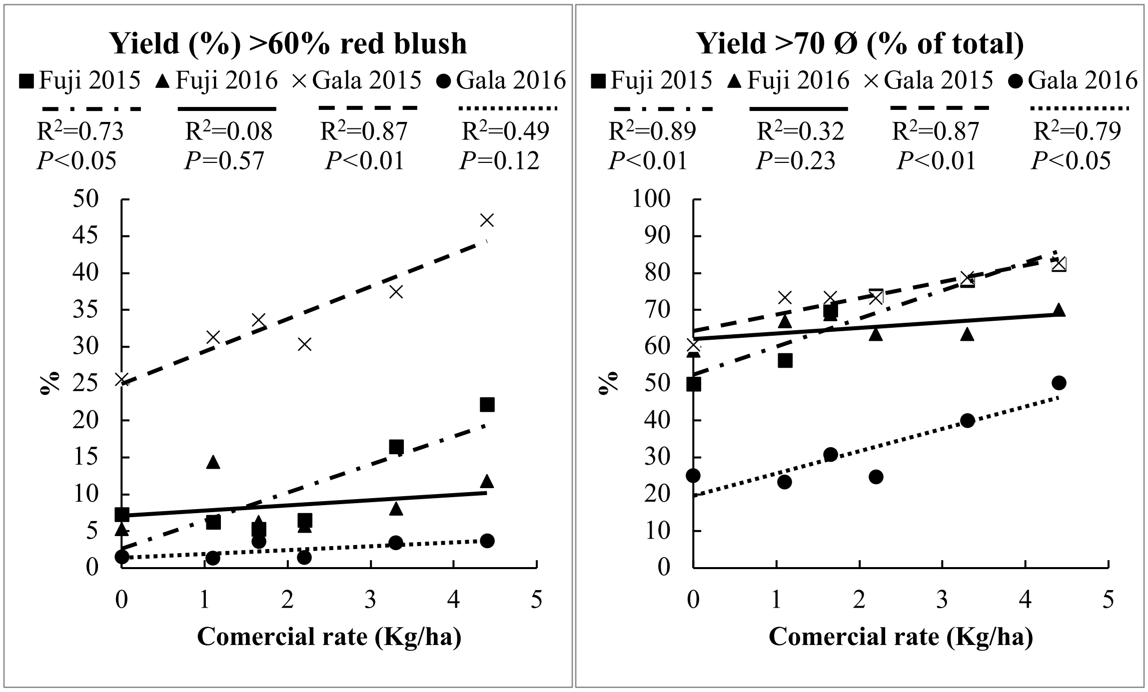

Fig. 6: Relationships between Brevis® comerial rates and yield >70 mm and percentage of red blush >60%.

3.4. Biexponential pharmacokinetic model

The p-value was significant at <0.001 in all models. Moreover, the R^2 values were between 0.7 and 0.98 in the biexponential pharmacokinetic model of the Qy(%) (Table 4). Thus, the biexponential equation provided adequate fits to the data, and the values calculated from the biexponential fits correlated very closely with the real values of Qy(%).

Table 4: Biexponential pharmacokinetic model results (p-value and R^2) for the evolution of Qy(%) in time at different doses.

Year		**2015**					**2016**				
Thinning rate (Kg/ha)		**1.10**	**1.65**	**2.20**	**3.30**	**4.40**	**1.10**	**1.65**	**2.20**	**3.30**	**4.40**
Gala	**R^2**	0.922	0.871	0.849	0.977	0.837	0.805	0.706	0.775	0.772	0.89
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fuji	**R^2**	0.904	0.861	0.839	0.918	0.957	0.857	0.851	0.859	0.796	0.922
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The parameters B and β of the biexponential analysis of Qy(%) explain from the start of the inhibition period until the day of maximum inhibition of the product, and the parameters A and α explain from the day of maximum inhibition until the end of the period of inhibition. The Qy(%) values showed significant differences between treatments. Brevis® inhibition at 4.40 kg/ha was significantly different to the other treatments in the parameters A, α and B (Table 5). There were significant differences between cultivars in all the productive and quality parameters. However, the estimated parameters showed no differences between cultivars, except for the parameter β. On the other hand, all parameters showed significant differences between years, as in all productive and quality parameters. Parameter β showed significant interaction between cultivar and year. The other interactions were not significant (Table 5).

Table 5: Parameters estimated with the biexponential pharmacokinetic model (A, α, B and β) for Qy(%) evolution in time at different doses on Gala and Fuji trees in 2 years (2015 and 2016).

	A	**α**	**B**	**β**
Thinning (Br)	*	*	*	ns
Cultivar (C)	ns	ns	ns	*
Gala	0.518	-0.024	0.495 a	0.386
Fuji	0.530	-0.027	0.482 a	0.467
Year (Y)	**	**	**	***
2015	0.588	-0.020	0.416 b	0.679
2016	0.460	-0.030	0.561 a	0.173
Significant interactions				
Br x C	ns	ns	ns	ns
Br x Y	ns	ns	ns	ns
C x Y	ns	ns	ns	*

Means within a column followed by different letters denotes significant differences (t-test).
*, **, and *** denote means significantly different at P< 0.05, 0.01, or 0.001, respectively.
ns - not significant at P<0.05

All Qy(%) parameters, except β, were related to final fruit set and crop load reduction. The parameters A, α and B had significant p values, however parameter β was not significant. The R^2 values ranged between 0.97 and 0.74. When final fruit set increased, A and α increased and B decreased (Table 5 and Fig. 7). The parameter β explained the reduction period, and there were no significant differences between doses because this period was the same in all doses (Table 5 and Fig. 7). This situation is also observed in the analysis of the AUC reduction (Table 6). However, parameters A and α, which explained the recuperation period, did show differences between doses. These parameters were significantly lower in the 4.40 kg/ha dose (0.4 and -0.034, respectively) in comparison with the other doses (A between 0.5 and 0.6, and α between -0.021 and 0.025) with inhibition values of between 10% and 15%. This difference caused the period of Brevis® inhibition to be longer in the 4.40 kg/ha dose than the other doses.

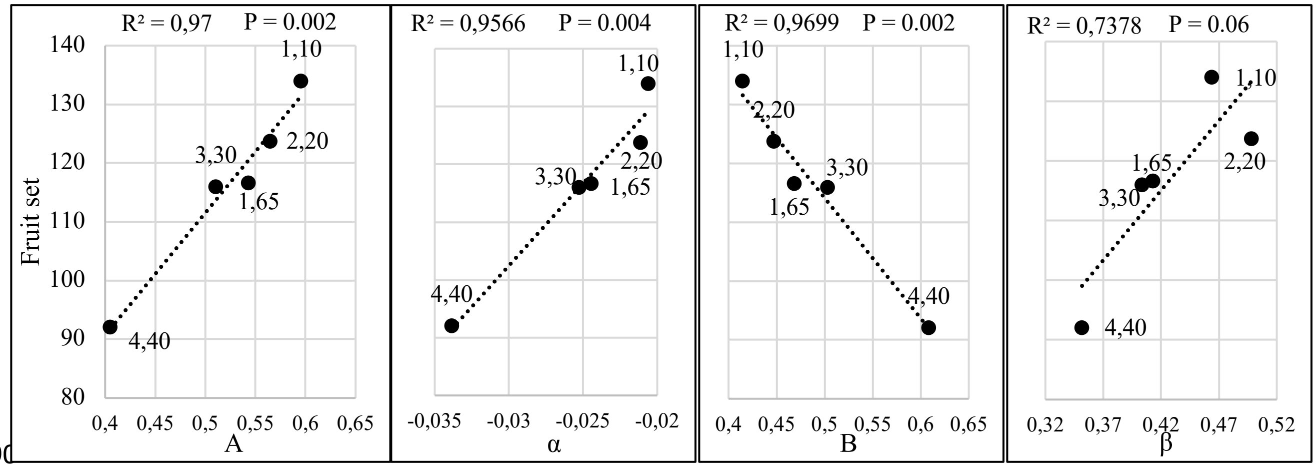

Fig. 7: Correlation between final fruit set and the parameters estimated with the biexponential pharmacokinetic model of Qy(%) (A, α, B and β) for the different application doses (kg/ha).

Table 6: Area under the curve (AUC), Qy(%) predicted minimum (Qy(%) min), day of minimum Qy(%) value (number of days from day 0 to minimum Qy(%) value), AUC reduction (AUC between day 0 to minimum Qy(%) value), AUC recuperation (AUC between day of minimum Qy(%) value to end of inhibition period) and AUC/day (all AUC), for the evolution of Qy(%) in time at different doses on Gala and Fuji trees in 2 years (2015 to 2016).

	All AUC	**Qy(%) min**	**Day of minimum Qy(%) value**	**AUC reduction (0-min)**	**AUC recuperation (min-final)**	**AUC/day (All AUC)**
Thinning (Br)	*	*	ns	ns	*	*
Cultivar (C)	*	ns	ns	ns	*	*
Gala	15.1	0.68	8	5.9	9.2	0.75
Fuji	15.8	0.71	7	5.5	10.3	0.79
Year (Y)	*	*	***	***	**	*
2015	15.0	0.66	5	3.5 b	11.6	0.75
2016	15.8	0.73	10	7.9 a	7.9	0.79
Significant interactions						
Br x C	ns	ns	ns	ns	ns	ns
Br x Y	ns	ns	ns	ns	ns	ns
C x Y	ns	ns	ns	ns	ns	ns

Means within a column followed by different letters denotes significant differences (t-test).
*, **, and *** denote means significantly different at $P< 0.05$, 0.01, or 0.001, respectively.
ns - not significant at $P<0.05$

The AUC, value of Qy(%) min, AUC recuperation (min-final) and AUC/day (All AUC) showed a significant differences between thinning rates (Table 6). A lineal dose effect was observed in the analysis of the AUC and fluorescence inhibition (Fig. 8). When chemical dose increased, the AUC, value of Qy(%) min, AUC recuperation (min-final) and AUC/day (All AUC) decreased, except for Fuji 2016 (Fig. 8). However, there were no differences in the day of minimum Qy(%) value and AUC reduction (0-min) at different doses (Table 6).

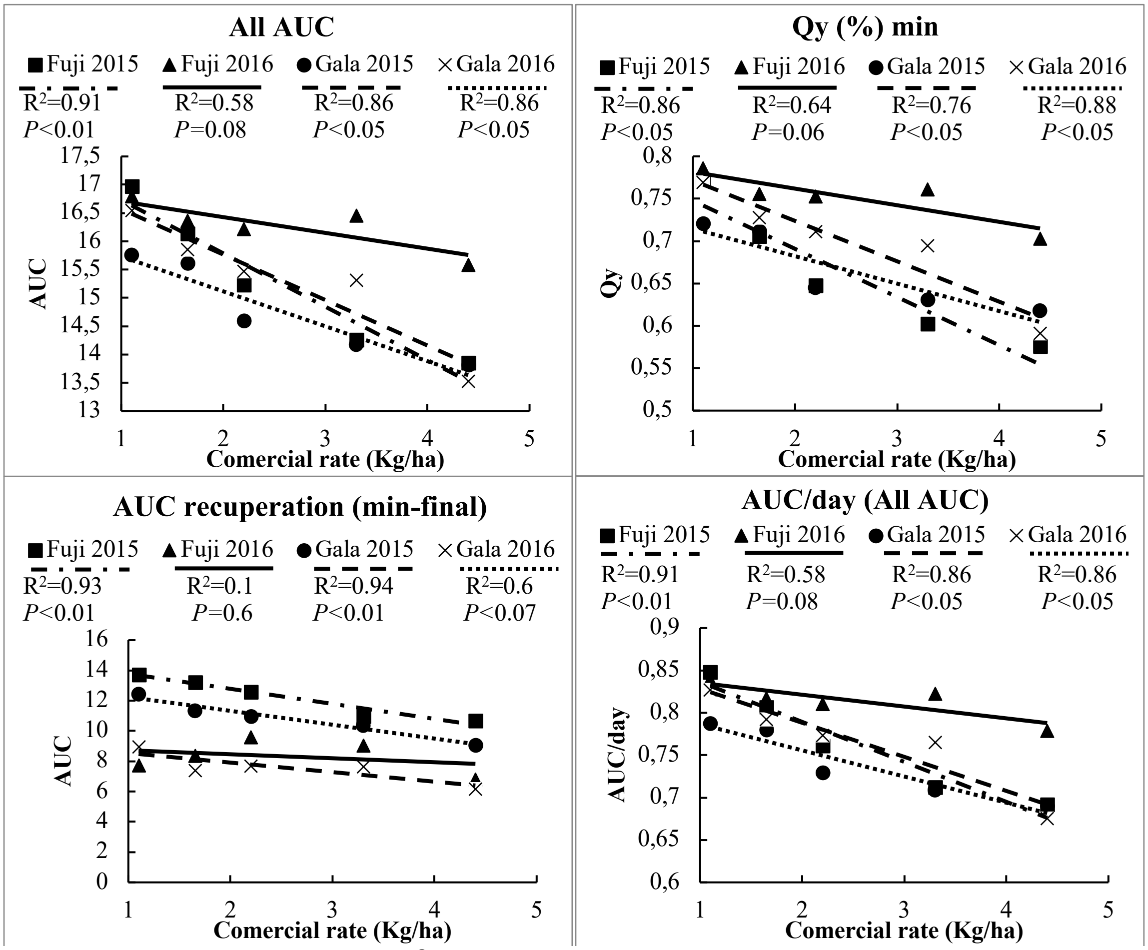

Fig. 8: Relationships between Brevis® comerial rates and Area under the curve (AUC), Qy(%) predicted minimum (Qy(%) min), AUC recuperation (AUC between day of minimum Qy(%) value to end of inhibition period) and AUC/day (all AUC), in Gala and Fuji trees in 2 years (2015 to 2016).

Gala showed significantly lower AUC, AUC recuperation (min-final) and AUC/day compared with Fuji. Therefore, the inhibition was higher in Gala that Fuji. Moreover, the reduction period was the same in Gala and in Fuji, and the recuperation period or the period of Brevis® inhibition was longer in Gala than in Fuji, with Gala showing 18% of inhibition 20 days after application and Fuji 10% (Fig. 9A). However, the other AUC parameters showed no significant differences between cultivars because the reduction period was the same in both cultivars (Fig. 9A). Moreover, the biexponential analysis of Qy(%) and all AUC parameters showed significant differences between years (Table 6 and Fig. 9B). The period of inhibition was the same in both years and 20 days after application showed 15% of inhibition. However, the day of minimum Qy(%) was faster in 2015 in comparison with 2016 (5 and 10 days after application, respectively) (Table 6 and Fig. 9B). Moreover, the maximum inhibition (Qy(%) min) was higher in 2015 than in 2016 (34% and 27%, respectively). For all these reasons, the recuperation period was longer in 2015 than 2016 (15 and 10 days, respectively) (Fig. 9B). There were no significant interactions (Table 6).

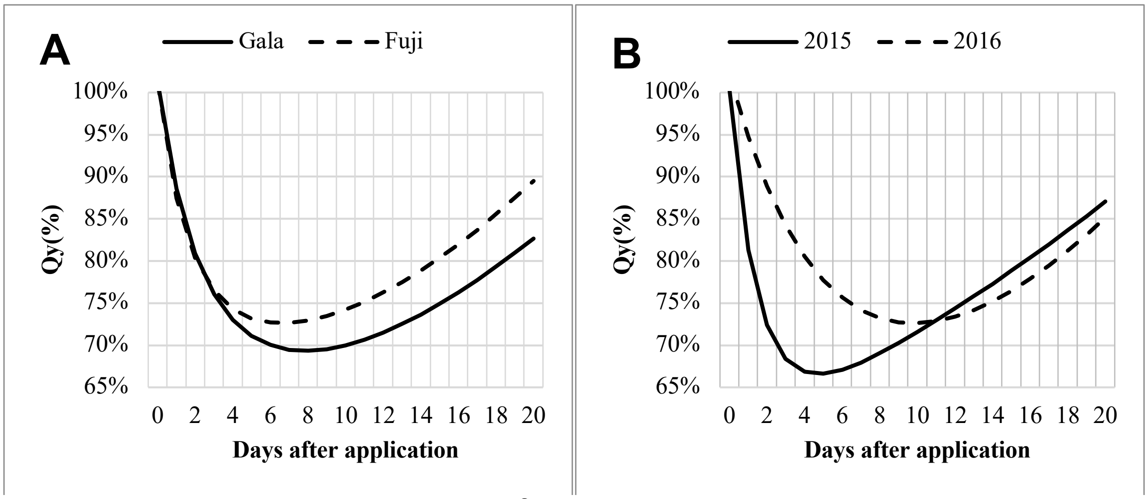

Fig. 9: Graphic representation of the Brevis® inhibition 20 days after application estimated with the biexponential pharmacokinetic model of Qy(%) (A, α, B and β) for cultivars (A) and years (B).

4. Discussion

The maximum effectiveness for chemical thinner is based on the diameter of the developing fruit, the application rate, the cultivar and climatology (Byers, 2003). In all trials, the spraying of apple trees with chemical photosynthetic inhibitors induced fruit abscission, as also reported by Byers *et al.* (1990). The application of Brevis® reduced final fruit set, number of fruits per tree and crop load depending on the application rate, which concurs with the observations of Brunner (2014), Deckers *et al.* (2010),Gonzalez *et al.* (2019a), Mathieu *et al.* (2016) and McArtney et al. (2012). Final fruit set, number of fruits per tree, crop load and yield showed differences between Gala and Fuji cultivars because product susceptibility differs according to cultivar because the meteorological conditions differed between years.

McArtney et al. (2012) reported a negative relationship between fruit yield per tree at harvest and metamitron concentration, which concurs with the results of this study. Moreover, average fruit weight, diameter and coloration increased with the Bevis® induced thinning effect, with the highest values for these parameters detected in those treatments in which crop load and final fruit set were significantly reduced. These results again concurring with earlier observations made by McArtney et al. (1996), Brunner (2014), Gonzalez et al. (2019a) and Maas and Meland (2016). Fruit size and fruit color distribution improved with yield reduction, also concurring with earlier observations of Bergh (1990), Dorigoni and Lezzer (2007) and Lafer (2010), as did the various % values of the fruit harvested at first pick (% of yield >70 mm, >75 mm and >60% blush area), as also reported by Mathieu et al. (2016).

In this study, differences between years were observed, which concurs with the observations of Brunner (2014) and Gonzalez et al. (2019b), who argued that the same amounts of metamitron applied in different years might not always reduce final fruit set to the same extent. In this respect, the results of this study also concur with those of previous studies made by Robinson and Lakso (2004) and Robinson et al. (2016) with naphthaleneacetic acid (NAA), 6-benzyladenine (BA) and carbaryl, which reported significant variation in chemical thinning efficacy from year to year and within year. Byers (2003) indicate that cool temperatures may delay or interfere with abscission and that increasing temperatures may promote it. According to Jackson (2003), high night temperatures increase respiration and, according to Yoon *et al.* (2011), warm temperatures intensify competition among competing sinks at a time when metabolic demand is highest in the tree. This concurs with the observations of the present study (the year 2015 was warmer than 2016). The carbohydrate balance can also play a significant role in apple tree response to fruit abscission; if the carbohydrate supply is abundant it may limit fruit development and abscission (Lordan *et al.*, 2019). Other factors may also explain year-to-year Brevis® efficacy, including the weather of the previous year, carbohydrate ratios from the previous year, temperature and sunlight from bud break to bloom or post bloom, tree vigor, leaf area, or the sensitivity of the tree itself. Lordan et al. (2019) reported how these factors can affect natural fruit abscission. The significant interactions between year and cultivars in most of the parameters evaluated can be attributed to the different efficacy between years and cultivars. The triple interaction between year, cultivar and thinning rate was significant in number of fruits per tree and yield, because the results of the previously explained factors were significant, and the Brevis® dose at 3.30 kg/ha in Fuji in 2016 had no thinning effect. However, the triple interaction was not significant in crop load and final fruit set because these parameters were calculated with the trunk diameter and number of flower clusters.

Many authors have reported the day of maximum Brevis® induced inhibition at between 2 and 6 days after application (Brunner, 2014; McArtney et al., 2012; Rosa, 2016; Stern, 2015). Their results differ from the observations of this study, with maximum inhibition Qy(%) values observed 5-10 days after treatment. Moreover, Brevis® reduced electron transport rates by up to 40%, with similar observations reported by McArtney and Obermiller (2012), Stern (2014) and Stern (2015).

An interesting development in this field has been the use of pharmacokinetic models for the study of the behavior or effect of phytosanitary products in plants, and studies on how these products affect plants at physiological level. In the present study, the biexponential function of the pharmacokinetic model was adapted for inhibition of fluorescence caused by Brevis® in time. The biexponential equation provided adequate fits to the data, and the values calculated from the biexponential fits correlated very closely with the real values of Qy(%). Bringe *et al.* (2006) reported that the tolerance of plants toward triazines may be influenced by differing environmental conditions. This could explain the result in this study which showed differences between years. The estimated parameters A, α and B were related with final fruit set, however the period of inhibition has to be finished before prediction can be made of Brevis® efficacy in the year. Moreover, in trials performed by Gonzalez et al. (2019b) with applications at different fruit size, these parameters were not related with final fruit set. With these results, the parameters can be related with crop load when the applications are made at the same time and at different doses. Additionally, this model showed high differences between years and the parameters were different each year, making it more difficult to use these parameters to predict Brevis® efficacy. Future studies in this respect are therefore recommended.

Previous research has shown an increasing negative effect of Brevis® concentration on the maximum potential quantum efficiency of PSII in apple leaves (McArtney and Obermiller, 2012), which concurs with the AUC, Qy(%) min and AUC/day results reported in this study. Gala showed a significantly higher AUC compared with Fuji because the period of inhibition was longer in Gala, indicating that Gala is more sensitive to Brevis® than Fuji. This difference between cultivars was also observed by Brunner (2014) and Gonzalez et al. (2019b), they reported that leaf susceptibility differs according to cultivar. This result suggests that Brevis® absorption rates could differ between cultivars because of differing leaf structure and/or leaf wax concentration. On the other hand, Lordan et al. (2019) studied natural fruit drop, suggesting that some cultivars could be more susceptible than others to carbohydrate deficit and that thinning windows may depend on the cultivar.

This study found higher AUC, Qy(%) min, day of Qy(%) min and AUC-day values in 2016 than 2015, because in 2016 the cool temperatures reduced the period of inhibition caused by Brevis®, as also reported by Byers (2002) and Kviklys and Robinson (2010). In their greenhouse studies with potted trees, it was observed that, for the same application concentration, cool temperatures with high sunlight after chemical application resulted in less thinning efficacy, while high temperatures (especially high night temperatures) with low light levels after chemical application resulted in greater thinning efficacy. The combined effects of temperature and sunlight on thinning efficacy indicate that carbohydrate supply to the young fruitlets influences fruitlet retention or abscission (Lakso, 2011; Robinson et al., 2016). Moreover, Stern (2014) concluded that the higher efficacy of metamitron in Israel than Europe was due to the higher average 24 hours temperatures in Israel, which can increase the efficiency of photosynthesis inhibition by metamitron. This result in concordance with those obtained in the present study and could explain the differences between years in all the parameters evaluated.

5. Conclusions

A dose effect was observed, with Brevis® dose reducing final fruit set and crop load. Additionally, when Brevis® showed high efficacy, there was an improvement in fruit weight, coloration and diameter.

The fluorescence analysis showed a dose effect, with Brevis® dose increasing inhibition. Additionally, the same result was also observed in the AUC analysis, with Brevis® dose reducing the area and inhibition increasing. The biexponential equation provided adequate fits to the data, and the values calculated from the biexponential fits correlated very closely with the real Qy(%) values.

Thinning efficacy varied between cultivars, with Gala more sensitive to Brevis® than Fuji. Moreover, the year 2015 was warmer than 2016, and the higher temperatures increased the thinning efficacy of Brevis®. Thus, the efficacy of the thinning agent Brevis® is conditioned by dose rate, cultivar and temperature.

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References

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

Bergh, O., 1990. Effect of time of hand-thinning on apple fruit size. South African Journal of Plant and Soil 7, 1-10.

Bringe, K., Schumacher, C.F., Schmitz-Eiberger, M., Steiner, U., Oerke, E.-C., 2006. Ontogenetic variation in chemical and physical characteristics of adaxial apple leaf surfaces. Phytochemistry 67, 161-170.

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

Byers, R., 2002. Influence of temperature and darkness on apple fruit abscission and chemical thinning. Journal of tree fruit production 3, 41-53.

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

Byers, R.E., Barden, J.A., Polomski, R.F., Young, R.W., Carbaugh, D.H., 1990. Apple Thinning by Photosynthetic Inhibition. J. Am. Soc. Hortic. Sci. 115, 14-19.

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

Cline, J., Bakker, C.J., Gunter, A., 2018. Response of ‘Royal Gala’ apple to multiple applications of chemical thinners and the dynamics of fruitlet drop. Can. J. Plant Sci.

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

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

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

Gonzalez, L., Àvila, G., Carbó, J., Bonany, J., Alegre, S., Torres, E., Martin, B., Recasens, I., Asin, L., 2019a. Hail nets do not affect the efficacy of metamitron for chemical thinning of apple trees. The Journal of Horticultural Science and Biotechnology.

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

Gustafson, D.L., Bradshaw-Pierce, E.L., 2011. Fundamental Concepts in Clinical Pharmacology, In: Garrett-Mayer, E. (Ed.), Principles of Anticancer Drug Development. Springer New York, New York, NY, pp. 37-62.

Jackson, J.E., 2003. Biology of apples and pears/John E. Jackson. Biology of Horticultural Crops.

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

Krause, G.H., Weis, E., 1984. CHLOROPHYLL FLUORESCENCE AS A TOOL IN PLANT PHYSIOLOGY .2. INTERPRETATION OF FLUORESCENCE SIGNALS. Photosynth. Res. 5, 139-157.

Kviklys, D., Robinson, T., 2010. Temperature before and after Application of Chemical 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., 2011. Early Fruit Growth and Drop - the Role of Carbon Balance in the Apple Tree., 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. 733-742.

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., 2012. Use of 1-Aminocyclopropane Carboxylic Acid and Metamitron for Delayed Thinning of Apple Fruit. Hortscience 47, 1612-1616.

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.

Navarro, S., Bermejo, S., Vela, N., Hernandez, J., 2009. Rate of Loss of Simazine, Terbuthylazine, Isoproturon, and Methabenzthiazuron during Soil Solarization. J. Agric. Food Chem. 57, 6375-6382.

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., Greene, D., Reginato, G., Rufato, A.D., 2016. Managing fruit abscission in apple part 1, In: Wunsche, J.N., Tranbarger, T.J. (Eds.), Xxix International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes. Int Soc Horticultural Science, Leuven 1, pp. 1-13.

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

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

Stern, R.A., 2015. The photosynthesis inhibitor metamitron is a highly effective thinner for 'Golden Delicious' apple in a warm climate. Fruits 70, 127-134.

Swarcewicz, M.K., Gregorczyk, A., 2013. Atrazine degradation in soil: effects of adjuvants and a comparison of three mathematical models. Pest Manag. Sci. 69, 1346-1350.

Urso, R., Blandi, P., G., G., 2002. A short introduction to pharmacokinetics. European Review for Medical and Pharmacological Sciences 6, 33-44.

Yoon, T.M., Robinson, T.L., Reginato, G.H., 2011. Effects of Temperature and Light Level on Efficiency of Chemical Thinner on 'Empire' Apple Trees, 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. 1085-1093.