Brevis thinning efficacy at different fruit size and fluorescence on Gala and Fuji apples

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Abstract

Brevis thinning efficacy depends on climatic and cultivar conditions. The objective of this work was to evaluate the efficacy of one application of Brevis in Gala and Fuji apple applied at different fruit sizes (fruit king diameter ranging between 6.5 and 21.5 mm) and to determine which fruit diameters were most sensitive to Brevis application. Trials were conducted over two seasons from 2015 to 2016 in apple orchards in Lleida (Spain). Photosynthesis inhibition caused by Brevis was also analysed and measured, using chlorophyll fluorescence and biexponential pharmacokinetic models. In 2016, for all Brevis treatments and an untreated control, quantum yield (Qy) was measured in all leaves in different shoots, with photosynthesis inhibition and its evolution analysed in three sections (closest to branch, mid-shoot and end of shoot). Under the trial conditions, Brevis thinning effect was observed at king fruit diameters from 9 to 19 mm, with maximum efficacy observed in the 11.5-14 mm range. However, susceptibility to Brevis differed between varieties and years. The fluorescence analysis using a biexponential equation showed adequate fits and the calculated values correlated well with the measured Qy(%) values. The area under curve per day analysis showed that, at the same application dose, fluorescence inhibition decreased with increasing fruit diameter. The fluorescence analysis of shoot sections four days after Brevis application showed differences between varieties, with the inhibition caused by Brevis higher in Gala than in Fuji. However, this analysis showed no significant differences in Gala, with all sections showing similar inhibition (27%-35%). By contrast, Fuji showed different inhibition values in the different sections. The vegetative section showed the significantly highest inhibition, and the zone nearest the branch the lowest.

Keywords

Crop load, Fruit weight, Carbohydrate deficit, Fruit abscission, Photosynthesis, Metamitron

1. Introduction

Apple fruit thinning is an important practice for the maximisation of crop value (Byers 2003). Appropriate thinning must be done year to year because of the benefits to fruit size, colour, and the regulation of alternance. Orchardists need to remove excessive flowers and fruitlets from apple fruit trees (Peifer et al. 2018).

Chemical thinning is a practice which helps to reduce production costs and time. However, the efficacy of chemical thinning depends on climatic and cultivar conditions (Byers 2003; Lordan et al. 2018; Robinson and Lakso 2004). Currently, in Spain, chemical thinning can be carried out at two different stages:

- During flowering to reduce fruit set at an early stage and enhance flower bud formation the following year. This can be achieved with ammonium thiosulphate (ATS) and naphthalene acetamide (NAD).
- After fruit set, on young fruitlets with king fruit diameters ranging between 6 and 16 mm. After Europe banned the widely used chemical thinner Carbaryl, the products registered for fruit thinning were the hormones 6-benzyladenine (BA) and naphthyl acetic acid (NAA).

Brevis was registered in Spain in 2015. Metamitron, its active ingredient at 15%, belongs to the triazinone family of herbicides and its mode of action differs from that of other known bioregulators. Brevis disrupts the photosynthetic apparatus after application and acts by blocking electron transfer between primary and secondary quinones of PSII (McArtney et al. 2012). This interruption of photosynthetic electron transport inhibits adenosine 5´-triphosphate production and carbon fixation (McArtney et al. 2012). The application of 1.1 to 2.2 kg/ha depends on the variety, as leaf susceptibility differs according to the cultivar. Golden, for example, is much more sensitive to Brevis application than Fuji (Brunner 2014). The dosage therefore needs to be regulated according to the sensitivity of each variety

Importantly, however, no studies have yet been conducted to define the moment of maximum fruit sensitivity.

The thinning activity of Brevis in apple is via inhibition of photosynthesis (Basak 2011; Lafer 2010), reducing carbohydrate production by the tree. This situation produces stress in the tree and the remaining carbohydrates are sent to shoots rather than fruit. Those carbohydrates that are sent to fruit are directed to the largest and dominant king fruitlets at the expense of the others. The smaller fruitlets stop growing and will drop, while the larger fruitlets continue growing (ADAMA New Zealand 2017).

One of the oldest approaches to test photosynthesis is chlorophyll fluorescence measurement. Kautsky and Hirsch (1931) were the first to report the significant relationship between photosynthesis and chlorophyll fluorescence. Chlorophyll fluorescence has been used as a way of testing photosystem activity, especially photosystem II (Fernandez et al. 1997; Krause and Weis 1984). Chlorophyll fluorescence can thus be used to analyze the photosynthesis inhibition caused by Brevis and hence as a tool to manage thinning decisions.

The decision as to when to apply the chemical thinner, based on fruit size and weather conditions, is a crucial element of any thinning program. The objective of this work was to evaluate the efficacy of one application of Brevis at 1.65 kg/ha in Gala and 2.2 kg/ha in Fuji (rates determined according to the sensitivity of each variety) applied at different fruit sizes (fruit king diameter ranging between 6.5 and 21.5 mm). Another aim was to determine which fruit diameters were most sensitive to Brevis application. Finally, a further aim was to analyze photosynthesis inhibition caused by Brevis and measured through chlorophyll fluorescence.

2. Material and methods

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

The trials were conducted in apple orchards of the IRTA Experimental Station of Lleida (Mollerussa and Gimenells, NE Spain) during the seasons of 2015 and 2016. The orchards are managed based on the standards normally used in commercial apple orchards in the region. Table 1 shows the principal characteristics of the orchards used for the trials.

Meteorological data were collected from the weather station of the official meteorological service of Catalonia, located 50 m away from the trials in the Mollerussa orchard of the IRTA-Experimental station of Lleida.

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.

2.2. Chemical application

The trials tested the use of the commercial chemical thinner Brevis (ADAMA, Spain). The rates of applications were 1.65 kg/ha on Gala and 2.2 kg/ha on Fuji. The moment of application was determined by measuring king fruit diameter (Table 2), and water volume was equivalent to 1000 l/ha. Table 2 shows the dates of application and actual fruit sizes in the different ranges at the moment of application.

2.3. Yield assessments

The assessments were carried out on two central trees of each elementary plot with the objective of assessing the effect of the treatments on fruit set and fruit yield parameters. The total number of flower clusters per tree was counted at bud break stage (BBCH 61-65). Homogeneous plants were selected for the trials based on flowering intensity.

In each orchard, harvesting was performed during the commercial harvest season for each selected tree separately. 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).

Crop load was obtained from the number of fruits harvested per cm^2 of trunk cross-sectional area (TCSA) (number of fruits / trunk cross-sectional area).

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). 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.

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence 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 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 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). In addition, in 2016, for all Brevis treatments and the Control treatment, Qy was measured in all leaves per shoot per control tree (two shoots per elementary plot and 8 shoots per treatment). The measurements were taken four days after Brevis application. For the analysis, the shoots were divided into 3 sections: section 1/3 closest to branch, 2/3 mid-shoot and 3/3 vegetative section. The Qy of a section was the average of all the leaves for that section in all shoots.

2.4.1. Biexponential functions

The use of biexponential pharmacokinetic models has been proposed to study the absorption, distribution, biotransformation and elimination of drugs in man and animals (Urso et al. 2002). The same type of model has also been used to study the dissipation of pesticides in surface soil (Navarro et al. 2009), and similar models have been used in agriculture to study the degradation of a pesticide in soil (Swarcewicz and Gregorczyk 2013). In our trials, the 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 (Gustafson and Bradshaw-Pierce 2011; Urso et al. 2002) of type:

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

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 moment of application to the moment of minimum Qy(%) value, which is the moment of maximum inhibition (Figure 1). The parameters A and α explain the recuperation of Qy, representing from the moment of maximum inhibition, Qy(%) minimum value, to the end of the period of inhibition caused by Brevis (Figure 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 (Figure 1). The area under the curve (AUC) is the area in all periods of inhibition (Figure 1). Table 3 shows the calculations of the parameters.

2.5. Statistical analysis

Chlorophyll fluorescence was measured and analyzed in all treatments except in Hand Thinning because the values are the same as in Control. 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). Analyses of chlorophyll fluorescence and AUC parameters for the two years separately were performed in SAS 9.2 (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 variety and king fruit size as main factor. The analysis of shoots was performed using constrained quadratic linear regression fitting in JMP13 statistical analysis software (SAS institute, 2017).

Analyses of crop load were performed in SAS 9.2 (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 year, variety and king fruit size as main factor and the interaction terms.

3. Results

3.1. Temperature

Figure 2 shows the average temperature in the application period of the Brevis chemical thinner. There were important differences between years. In the application period of 2015, the temperature was higher than 16ºC every day except for 3 days. In 2016, the temperature was lower than 16ºC every day, except for 3 days at the end of the period.

Figure 3 shows the average night temperature during the period of Brevis application in the two years of the study. Night temperatures in 2015 were always higher than in 2016, except for 6 days. In 2015, there were 14 days with a night temperature higher than 14ºC, whereas in 2016 this was the case on only 1 day.

3.2. Fruit set and yield

In all fruit sizes, the number of flower clusters per tree was uniform at the start of the trials. However, Fuji flowering was significantly lower than Gala (275 and 299 flower clusters per tree, respectively), and the flowering of 2015 was significantly lower compared with 2016 (Table 4).

The maximum reduction in number of fruits, the moment of maximum fruit sensitivity, was produced by the Brevis treatment at 11.5-14 mm, with a significantly lower number of fruits per tree than in the Control (341 vs. 414 fruits per tree, respectively). The other fruit sizes showed a non-significant fruits/tree ratio in comparison with the Control. In relation to the number of fruits/cm^2 of TCSA (crop load), Brevis at 9-11.5 mm, 11.5-14 mm and 16.5-19 mm registered a significant reduction of fruits in comparison with the Control (7.2, 6.8, 7.3 and 8.9 fruits/cm^2 of TCSA, respectively), while there were no significant differences between the other Brevis treatments and the Control. All Brevis treatments showed a significantly lower efficacy in comparison with Hand Thinning. There was no significant effect of application of Brevis on yield (Table 4).

The values for average number of fruits per tree, 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 4).

For yield, fruit set, and number of fruits per tree, there were significant interactions between moment of application and year (Table 4, Figure 4A). Figure 4A shows different Brevis efficacy between years and treatments, with climate conditions in 2015 more favorable to the Brevis effect. The interaction between year and variety was significant in the case of fruit set, crop load and number of fruits (Table 4, Figure 4B). The effect of Brevis on

Gala in 2015 was higher than on Fuji in 2015, showing that sensitivity to thinning differs according to variety (Figure 4B). Other interactions were not significant.

Figure 5 shows fruit set in Gala in 2015. This trial obtained the maximum Brevis efficacy. For the treatments from 9 to 19 mm. there was a significant thinning effect in comparison with the Control. Maximum Brevis efficacy was at 11.5-14 mm fruit stage, with this strategy showing over-thinning as a significantly lower fruit set was recorded than in Hand Thinning (47 and 69 fruits per 100 flower clusters respectively). The other Brevis treatments were significantly equal in comparison with Hand Thinning.

3.3. Fruit quality (fruit weight, fruit size and blush color)

The greatest fruit weight and diameter were obtained in the 11.5-14 mm Brevis treatment diameter range (150 g and 72 mm), coinciding with maximum thinning efficacy. The lowest fruit weight and diameter were in the Control (131 g and 68 mm). Brevis application from 9 to 19 mm showed a significant effect in comparison with the Control and increased average fruit weight. Brevis sprayed between 9 and 16.5 mm increased average diameter. All Brevis treatments showed significantly lower fruit weight and diameter in comparison with the Hand Thinning treatment (162 g and 74 mm). Average fruit weight and diameter in 2016 were significantly lower than in 2015, and these parameters in Gala were significantly lower than in Fuji (Table 5). Average fruit diameter and weight increased as a function of thinning efficacy, and were significantly higher in the treatments with higher thinning efficacy.

The 11.5-14 mm (30 kg per tree) strategy also showed maximum % and kg of fruit yield >70 mm, coinciding with maximum thinning efficacy, maximum average fruit weight and maximum average diameter (Table 5). This strategy was significantly equal in comparison with the Hand Thinning treatment (30 kg per tree) in kg of fruit yield >70 mm. Brevis applied from 9 to 16.5 mm showed a significant effect in comparison with the Control and increased kg of fruit yield >70 mm. No significant differences were found between the remaining

Brevis strategies and the Control (Table 5). Gala yielded significantly lower % and kg of fruit >70 mm compared with Fuji, and % of fruit >70 mm was significantly higher in 2015 than in 2016 (Table 5).

There was no significant difference among treatments in fruit color distribution. However, Brevis applied at 11.5-14 mm showed a significantly higher average percentage blush area compared with the Control (28% and 20%, respectively), coinciding with maximum thinning efficacy, maximum average fruit weight and maximum average diameter (Table 5). This Brevis strategy was significantly equal to Hand Thinning (32%) (Table 5). There were significant differences between varieties, and the average % of blush area was significantly higher in 2015 compared to 2016 (Table 5).

Average fruit diameter, weight and % of blush area showed a trend, increasing as a function of thinning efficacy. However, there was no linear relationship between fruit quality parameters and fruit yield parameters (Tables 4 and 5).

The interaction between year and variety was significant in all fruit quality parameters, however other interactions were not significant (Table 5).

3.4. Biexponential pharmacokinetic model

In both varieties, the p-value was significant and showed high R^2 in all representations of Qy(%) with the nonlinear biexponential pharmacokinetic model. There were no significant differences between varieties, years and fruit size with the R^2 values (Table 6).

There were no significant differences in any of the parameters evaluated in the different fruit sizes. However, parameters A and α showed a trend to increase and B to decrease with increasing fruit size. However, no trend was observed with the β parameter. In all productive and quality parameters there were significant differences between varieties and years. However, the estimated parameters showed no differences between varieties. Parameters A, B and β also showed no significant differences between years. There were only significant differences in parameter α between years (2015 = -0.032 and 2016 = -0.051) (Table 7). These results showed no correlation between the estimated parameters (Qy%) and the yield and quality parameters (Table 7).

Figure 6 shows the difference in α slopes between years. In 2016 the α slope was -0.051, significantly different to 2015 when the slope was -0.032. This difference caused the period of Brevis inhibition of Brevis to be longer in 2015 than in 2016 (18 and 14 days, respectively).

The analysis of AUC, reduction AUC (0-min.) and recuperation AUC (min.-end) showed no significant differences between moments of application, varieties, years and interactions (Table 8).

The minimum Qy(%) value showed a significant effect on fruit size, with fruit size increasing with minimum Qy(%) value. The minimum Qy(%) value was 0.6 in the 6.5-9 mm range, with this value corresponding to 40% of fluorescence inhibition. The highest Qy(%) values were 0.76 and 0.75 in fruit sizes 16.5-19 and 19-21.5 mm, respectively, with these values corresponding to 24% and 25% of inhibition, respectively. There were no significant differences between varieties and years (Table 8).

There were no significant differences in the number of days between beginning and end of inhibition (when the Qy(%) value was 90% of the Control) and fruit size, although the values ranged between 10 and 16 days. There were no significant differences between day of minimum Qy(%) value and number of days between minimum Qy(%) value and end of application period. There were no significant differences between varieties and years.

There was a significant difference between fruit size and AUC/day, reduction AUC/day and recuperation AUC/day. These differences were equal in the three parameters. When fruit size increased, the parameter values increased. These three parameters varied significantly between the minimum value (0.7 in 6.5-9 mm) and maximum value (>0.8 in 16.5-19 and 19-21.5 mm). These results from the analysis of Qy(%) show that fluorescence inhibition caused by Brevis decreased with increasing fruit size. AUC/day and recuperation AUC/day showed lower Qy(%) values in Gala than in Fuji. These values show that fluorescence inhibition was higher in Gala than Fuji (Table 8). These results show there was no correlation between the AUC parameters and the productive and quality parameters, because maximum fruit thinning efficacy and maximum fluorescence inhibition were different strategies.

The interaction between year and variety was significant in the case of AUC/day, minimum Qy(%) value and reduction AUC/day. The interaction between fruit size and year was significant in AUC/day, minimum Qy(%) value, reduction AUC/day and recuperation AUC/day. The other interactions were not significant (Table 8).

Table 9 shows the differences between Control Qy in different shoot sections. Fuji showed significant differences between the three sections. The highest Qy value was for section 1/3 (closest to branch), followed by section 2/3 (mid-shoots) and the lowest value of Qy in section 3/3 (vegetative section). The Control Qy values in Gala were different compared with Fuji. There were no significant differences between sections 1/3 and 2/3 (higher values) in Gala. However, section 3/3 showed a significantly lower Qy value. The interaction between section and measurements was significant in the case of Control Gala. However, this interaction was not significant in Fuji.

For the Control Qy measurements on Gala, there was a significant interaction between section and measurements (Table 9, Figure 7). Figure 7 shows how the Control Qy values in the sections differed on different dates. While there were differences in the three measurements, all measurements showed the same behavior. That is to say, the lowest Qy values were always in section 3/3 and the highest values were always in sections 1/3 and 2/3.

Gala showed lower Qy and Qy(%) values in comparison with Fuji. Therefore, the inhibition caused by Brevis was higher in Gala four days after application. There were significant differences between fruit size and Qy values in Gala and Fuji, and Qy(%) in Gala. The maximum significant inhibition four days after application in Fuji was in the 11.5-14 mm range. However, Brevis applied to Gala between 6.5 and 19 mm showed similar Qy values, and the significantly highest value was in the 19-21.5 mm range. Qy(%) values showed no significant differences in Gala (Table 10).

The analysis of the sections showed no significant differences in Gala, with all sections showing similar inhibition (27%-35%). By contrast, Fuji showed different inhibition values in the different sections. Section 3/3 (vegetative section) showed the significantly highest inhibition (Qy 0.41 and Qy(%) 0.85). In the zone nearest the branch (section 1/3), inhibition was lowest (15% of inhibition). There were no significant interactions (Table 10).

4. Discussion

The moment of application is a key factor for the use of plant bioregulators (Mathieu et al. 2016). The maximum efficacy of a chemical thinner depends on the diameter of the developing fruit, the application dose, the crop variety and climatology (Byers 2003). In this study, there were significant differences in flowering, production and quality parameters between the analyzed apple varieties and between years. The differences in the parameter values show that Gala is more sensitive to Brevis thinning than Fuji. Moreover, in most of these parameters there was a significant interaction between variety and year, suggesting that these cultivars are genetically distinct in vegetation material and react specifically to the meteorological conditions of the year. Stern (2015) reported that high night temperatures increase respiration and may increase the sensitivity of fruitlets to photoassimilate deficiency caused by Brevis. Therefore, in hot years (2015) the thinning effect caused by Brevis is increased. Moreover, this explains the year-to-year difference in efficacy when using the same dose.

In the present study, maximum Brevis efficacy was obtained in the 11.5-14 mm king fruit diameter range, confirming the results in Brunner (2014). However, this result differs from

Reginato et al. (2017) who obtained maximum efficacy at 16 mm. Many authors have reported Brevis efficacy at different fruit stages with fruit diameters ranging between 8 and 20 mm (Brunner 2014; Deckers et al. 2010; Greene and Costa 2013; Greene 2014; Mathieu et al. 2016; McArtney and Obermiller 2012; Petri et al. 2016; Reginato et al. 2014). Their results concur with the observations of this study, in which Brevis thinning effect on crop load was observed at king fruit diameters from 9 to 14 mm and from 16.5 to 19 mm, and specifically in Gala 2015 from 9 to 19 mm.

Fruit yield per tree at harvest did not show a negative relationship with Brevis efficacy, unlike in McArtney et al. (2012). McArtney et al. (1996), Brunner (2014) and Maas and Meland (2016) reported a negative linear relationship between number of fruits and average weight, color and diameter, with average fruit weight, color and diameter increasing significantly for treatments in which Brevis reduced the number of fruits per tree. This concurs with the observations of the present study. Moreover, in our study, there were significant differences between the Control and the 9 to 19 mm treatments in average fruit size, though these differences were not significant in fruit set and number of fruits per tree. This result corroborates the maximum Brevis thinning effect at 11.5-14 mm and a Brevis thinning effect from 9 to 19 mm. Fruit size distribution improved with fruit reduction, concurring with earlier observations of Bergh (1990) Dorigoni and Lezzer (2007) and Lafer (2010), with improvements observed in % and kg of yield <70 mm.

For Gala and Fuji 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.

An interesting development in this field has been the use of pharmacokinetic models for the study of the behaviour or effect of phytosanitary products in plants. These models can be used to appraise product concentration in the plant (leaf, fruit...), the efficacy, absorption, distribution and elimination after application of insecticides, herbicides, fungicides, acaricides, bactericides, phytoregulators and other products, and to study 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 in parameter α. In the biexponential model, when parameter α was lower the period of inhibition was longer and better for thinning efficacy. However, the period of inhibition has to be finished before prediction can be made of Brevis efficacy in the year. When the estimated parameters α and β were analysed in the different fruit size applications, no correlation with the crop load parameters was found, which means that these parameters cannot be used to predict Brevis thinning efficacy in the different fruit sizes.

The AUC/day analysis increased with fruit diameter, and consequently fluorescence inhibition was lower in the same application dose. In the shoot analyses, Gala showed lower values of Qy and Qy(%) in comparison with Fuji. Therefore, four days after Brevis application, inhibition in Gala was different to that in Fuji, with Gala more sensitive to Brevis thinning.

Possible reasons and hypotheses to explain this circumstance include the following:

- Studies by Olesen and Muldoon (2009) found that the elongation of vegetative shoots is continuous from spring until the follow winter. This concurs with our observations which showed that the number of leaves per tree increases with fruit diameter (unpublished data), resulting in a lower amount of product per leaf and hence lower fluorescence inhibition.
- When the apple leaf is developing, there are important cuticle and wax changes, as reported by Bringe et al. (2006) who explained that during the ontogenetic development of apple leaves, leaf area increases and wax mass per unit of area tends to decrease. This situation causes the hydrophobicity of upper leaf surfaces to decrease during the ontogenetic development of apple leaves. This hydrophilic increase is associated with a decrease in the total amount of extractable surface waxes as well as with modifications in the composition of wax compounds.
- The AUC/day increase may be caused by leaf ageing. Results reported by Lakso et al. (1999) suggest that the photosynthetic rate of apple leaves is maximal shortly after full expansion, but declines only slowly over the season if the leaf remains healthy and fully exposed. The photosynthetic ability does decline, however, in the shade and shows little recovery upon re-exposure (Lakso et al. 1999). The significance of slow photosynthetic aging may be because the apple tree canopy can remain productive without continually producing young leaves over the entire season (Lakso et al. 1999). In this study, the differences between Qy(%) and Qy reduction in different shoot sections four days after Brevis application may be due to leaf ageing, as the section closest to the branch (old leaf) showed lower fluorescence inhibition.

More research is required on leaf evolution and physiology changes during the vegetative period to help determine the reasons for the reduction in Brevis inhibition with increasing fruit size.

5. Conclusions

Brevis thinning effect was observed at king fruit diameters from 9 to 19 mm, with maximum efficacy observed in the 11.5-14 mm range. However, susceptibility to Brevis differed between varieties, with Gala more sensitive to Brevis thinning than Fuji. In addition, the thinning efficacy of Brevis varied between years, with the hotter year favoring Brevis thinning efficacy. Using a biexponential equation, the fluorescence analysis showed adequate fits and the calculated values correlated well with the measured Qy(%) values. However, the estimated parameters of the model cannot be used to predict Brevis thinning efficacy in different fruit sizes. The AUC/day analysis showed that, at the same application dose, fluorescence inhibition decreased with increasing fruit diameter. This can be explained partly as the result of the number of leaves per tree increasing with increasing fruit diameter, meaning that the amount of product per leaf is lower and inhibition is reduced, partly as the result of cuticle and wax changes during apple leaf development, and partly as the result of leaf ageing. For all these reasons, the inhibition caused by Brevis was different at different fruit size applications.

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

Variety	Rootstock	Planted	Density plantation	Training system	Location	Trials: No. and year
Brookfield Gala	M9	2006	1786 trees/ha (4m x 1.4m)	Central leader	Gimenells	1 (2016)
Brookfield Gala	M9	2003	1786 trees/ha (4m x 1.4m)	Central leader	Mollerussa	1 (2015)
Fuji kiku 8	M9	2003	1786 trees/ha (4m x 1.4m)	Central leader	Mollerussa	2 (2015 and 2016)

Table 2. Date of applications and fruit size in the different ranges

		2015		2016	
	Strategy	Date of application	Real fruit size (mm)	Date of application	Real fruit size (mm)
GALA	Control	-	-	-	-
	6.5-9 mm	21-Apr	6.7	27-Apr	8.1
	9-11.5 mm	28-Apr	10.4	29-Apr	10.1
	11.5-14 mm	2-May	13.5	5-May	13.1
	14-16.5 mm	5-May	14.9	7-May	15.5
	16.5-19 mm	7-May	16.7	12-May	18.1
	19-21.5 mm	11-May	20.3	18-May	21.5
	Hand Thinning	10-Jun		6-Jun	
FUJI	Control	-	-	-	-
	6.5-9 mm	23-May	6.8	30-Apr	8.3
	9-11.5 mm	2-May	9.2	2-May	10.4
	11.5-14 mm	5-May	13.2	6-May	13.2
	14-16.5 mm	7-May	14.7	8-May	14.8
	16.5-19 mm	11-May	18.5	13-May	18.7
	19-21.5 mm	13-May	20.6	17-May	21.6
	Hand Thinning	10-Jun		7-Jun	

Table 3. Parameters calculated

Parameter	Calculation
AUC/day (All AUC)	AUC ÷ all inhibition days.
Days of inhibition All period	Number of days between beginning of inhibition and end of inhibition (when value of Qy(%) is 90% of Control).
Reduction AUC (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.
Reduction AUC/day (0-min)	Reduction AUC ÷ number of days until minimum Qy(%) value.
Recuperation AUC (min-end)	Area between day of minimum Qy(%) value and end of inhibition period.
Days of min final	Number of days between day of minimum Qy(%) value and end of inhibition (when value of Qy(%) is 90% of Control).
Recuperation AUC/day (min-end)	Recuperation AUC (min-end) ÷ number of days between minimum Qy(%) value and end of inhibition period .

Table 4. Effect of thinning with Brevis on fruit set and yield in Gala and Fuji trees (avg. 2015-2016).

	No. of flower clusters per tree	No. of fruits per tree	Fruit set (No. of fruits per 100 flower clusters)	Crop load (No. of fruits per cm^2 of TCSA)	Yield (kg/tree)
Moment of application (M)					
Control	286 a	414 a	144 a	8.9 a	52 a
6.5-9 mm	286 a	372 ab	133 a	7.8 ab	47 a
9-11.5 mm	286 a	367 ab	132 a	7.2 b	50 a
11.5-14 mm	282 a	341 b	120 a	6.8 b	49 a
14-16.5 mm	290 a	358 ab	127 a	7.5 ab	49 a
16.5-19 mm	291 a	376 ab	131 a	7.3 b	50 a
19-21.5 mm	291 a	386 ab	136 a	7.8 ab	49 a
Hand Thinning	285 a	262 c	93 b	4.9 c	43 a
Variety (V)					
Fuji	275 b	386 a	142 a	5.9 b	64 a
Gala	299 a	333 b	112 b	8.5 a	34 b
Year (Y)					
2015	273 b	287 b	106 b	5.3 b	43 b
2016	302 a	431 a	147 a	9.2 a	54 a
Significant interactions					
M x V	ns	ns	ns	ns	ns
M x Y	ns	*	*	ns	*
V x Y	ns	**	**	**	ns
M x V x Y	ns	ns	ns	ns	ns

* Means within a column followed by different letters denotes significant differences (Duncan's range test at $P<0.05$).
** Means within a column followed by different letters denotes significant differences (Duncan's range test at $P<0.001$).
ns - not significant at $P<0.05$

Table 5. 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 >70Ø (kg of total)**	**Yield >70 Ø (% of total)**	**Average % blush area**	**Yield > 60% blush area (% of total)**	**Yield > 60% blush area (kg of total)**
Moment of application (M)							
Control	131 d	68 d	24 bc	45 d	20 c	11 a	5 a
6.5-9 mm	135 cd	69 cd	22 c	49 cd	25 abc	14 a	5 a
9-11.5 mm	143 bc	71 bc	28 ab	57 bc	26 abc	15 a	6 a
11.5-14 mm	150 b	72 b	30 a	62 b	28 ab	18 a	6 a
14-16.5 mm	142 bc	70 bc	25 bc	55 bc	26 abc	17 a	6 a
16.5-19 mm	141 bc	69 cd	23 c	49 cd	25 abc	15 a	6 a
19-21.5 mm	137 cd	69 cd	24 c	50 cd	23 bc	13 a	5 a
Hand Thinning	162 a	74 a	30 a	70 a	32 a	22 a	7 a
Variety (V)							
Fuji	169 a	72 a	38 a	61 a	22 b	9 b	5 b
Gala	117 b	68 b	15 b	46 b	30 a	22 a	6 a
Year (Y)							
2015	151 a	72 a	26 a	63 a	29 a	19 a	6 a
2016	134 b	69 b	26 a	46 b	22 b	13 b	6 a
Significant interactions							
M x V	ns	ns	ns	ns	ns	ns	ns
M x Y	ns	ns	ns	ns	ns	ns	ns
V x Y	**	**	**	**	**	*	*
M x V x Y	ns	ns	ns	ns	ns	ns	ns

* Means within a column followed by different letters denotes significant differences (Duncan's range test at P<0.05).
** Means within a column followed by different letters denotes significant differences (Duncan's range test at P<0.001).
ns - not significant at P<0.05

Table 6. Biexponential pharmacokinetic model results (p-value and R^2) for the evolution of Qy(%) in time.

Year	Fruit Size (FS)	Fuji Qy(%)		Gala Qy(%)	
		R^2	p-value	R^2	p-value
2015	**6.5-9 mm**	0.982	<0.003	0.949	<0.001
	9-11.5 mm	0.842	<0.001	0.996	<0.001
	11.5-14 mm	0.992	0.023	0.971	<0.001
	14-16.5 mm	0.976	0.019	0.938	0.001
	16.5-19 mm	0.928	0.001	0.961	0.029
	19-21.5 mm	0.983	<0.001	0.780	0.006
2016	**6.5-9 mm**	0.945	<0.001	0.923	0.049
	9-11.5 mm	0.914	0.003	0.975	<0.001
	11.5-14 mm	0.944	0.003	0.982	<0.001
	14-16.5 mm	0.966	0.033	0.999	0.005
	16.5-19 mm	0.998	0.004	0.968	0.042
	19-21.5 mm	0.999	0.001	0.972	<0.001

Table 7. Parameters estimated with the biexponential pharmacokinetic model (A, α, B and β), for Qy(%) evolution in time on Gala and Fuji trees in 2 years (2015 and 2016).

	Parameters estimated (Qy(%))			
Fruit Size (FS)	**A**	**α**	**B**	**β**
6.5-9 mm	0.424	-0.054	0.579	0.985
9-11.5 mm	0.458	-0.041	0.543	0.371
11.5-14 mm	0.494	-0.047	0.508	0.836
14-16.5 mm	0.604	-0.038	0.395	0.778
16.5-19 mm	0.605	-0.039	0.393	0.559
19-21.5 mm	0.572	-0.033	0.428	0.402
Variety (V)				
Fuji	0.528	-0.044	0.472	0.686
Gala	0.524	-0.040	0.476	0.624
Year (Y)				
2015	0.563	-0.032 a	0.436	0.730
2016	0.489	-0.051 b	0.512	0.580
Significant interactions				
FS x V	ns	ns	ns	ns
FS x Y	ns	ns	ns	ns
V x Y	ns	ns	ns	ns

* Means within a column followed by different letters denotes significant differences (Duncan's range test at P<0.05).
ns - not significant at P<0.05

Table 8. Area under the curve (AUC), days of inhibition in all the period, AUC/day (all AUC), Qy(%) predicted minimum (Qy(%) min), reduction AUC day 0 to minimum Qy(%) value, day of minimum Qy(%) value (number of days from day 0 to minimum Qy(%) value), reduction AUC/day (0-min), recuperation AUC (day of minimum Qy(%) value to end of inhibition period), number of days between minimum Qy(%) value and end of inhibition period), and recuperation AUC/day (day of minimum Qy(%) value to end of inhibition period), for the evolution of Qy(%) in time on Gala and Fuji trees in 2 years (2015 to 2016).

Fruit Size (FS)	All AUC	Days of inhibition All period	AUC/day (All AUC)	Qy(%) min	Reduction AUC (0-min)	Day of minimum Qy(%) value	Reduction AUC/day (0-min)	Recuperation AUC (min-final)	Days: min-final	Recuperation AUC/day (min-final)
6.5-9 mm	9 a	13 a	0.70 c	0.60 c	3.1 a	4 a	0.70 d	5.9 a	9 a	0.70 d
9-11.5 mm	12 a	16 a	0.76 b	0.67 bc	4.8 a	6 a	0.76 bc	7.4 a	10 a	0.75 bc
11.5-14 mm	10 a	13 a	0.73 bc	0.64 bc	2.9 a	4 a	0.73 cd	6.7 a	9 a	0.72 cd
14-16.5 mm	8 a	10 a	0.77 ab	0.71 ab	2.8 a	4 a	0.78 abc	5.2 a	6 a	0.77 ab
16.5-19 mm	9 a	11 a	0.82 a	0.76 a	3.8 a	5 a	0.83 a	5.2 a	6 a	0.81 a
19-21.5 mm	13 a	16 a	0.81 a	0.75 a	4.5 a	6 a	0.81 ab	8.3 a	10 a	0.81 a
Variety (V)										
Fuji	10 a	13 a	0.78 a	0.71 a	3.6 a	5 a	0.78 a	6.4 a	8 a	0.78 a
Gala	10 a	14 a	0.75 b	0.67 a	3.7 a	5 a	0.76 a	6.5 a	9 a	0.74 b
Year (Y)										
2015	11 a	15 a	0.76 a	0.68 a	3.5 a	5 a	0.76 a	7.5 a	10 a	0.75 a
2016	9 a	12 a	0.77 a	0.70 a	3.8 a	5 a	0.78 a	5.3 a	7 a	0.76 a
Significant interactions										
FS x V	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
FS x Y	ns	ns	*	*	ns	ns	*	ns	ns	*
V x Y	ns	ns	*	*	ns	ns	*	ns	ns	ns

* Means within a column followed by different letters denotes significant differences (Duncan's range test at P<0.05).
ns - not significant at P<0.05

Table 9. Value of Control Qy in Gala and Fuji shoots in 2016. The shoots were divided into 3 sections: section 1/3 closest to branch, 2/3 mid-shoot and 3/3 vegetative section. This value was calculated with six control measurements that coincide with measurements of treated shoots.

	Control (Fuji)	Control (Gala)
Section	*	*
1/3	0.695 a	0.650 a
2/3	0.644 b	0.631 a
3/3	0.589 c	0.526 b
Section x Measurements	ns	*

* Means within a column followed by different letters denotes significant differences (Duncan's range test at P<0.05).
ns - not significant at P<0.05

Table 10. Value of Qy and Qy(%) four days after Brevis application in Gala and Fuji shoots in 2016. The shoots were divided into 3 sections: section 1/3 closest to branch, 2/3 mid-shoot and 3/3 vegetative section.

Fruit Size (FS)	**Qy**		**Qy(%)**	
	Fuji	**Gala**	**Fuji**	**Gala**
6.5-9	0.543 a	0.419 b	0.856 ab	0.773 a
9-11.5	0.530 a	0.380 b	0.775 abc	0.717 a
11.5-14	0.392 b	0.378 b	0.673 c	0.620 a
14-16.5	0.481 a	0.361 b	0.715 bc	0.613 a
16.5-19	0.557 a	0.385 b	0.864 a	0.722 a
19-21.5	0.485 a	0.489 a	0.846 ab	0.680 a
Section (S)				
1//3	0.581 a	0.421 a	0.853 a	0.651 a
2//3	0.506 b	0.405 a	0.812 a	0.662 a
3//3	0.405 c	0.378 a	0.706 b	0.738 a
FS x S	ns	ns	ns	ns

* Means within a column followed by different letters denotes significant differences (Duncan's range test at P<0.05).
ns - not significant at P<0.05

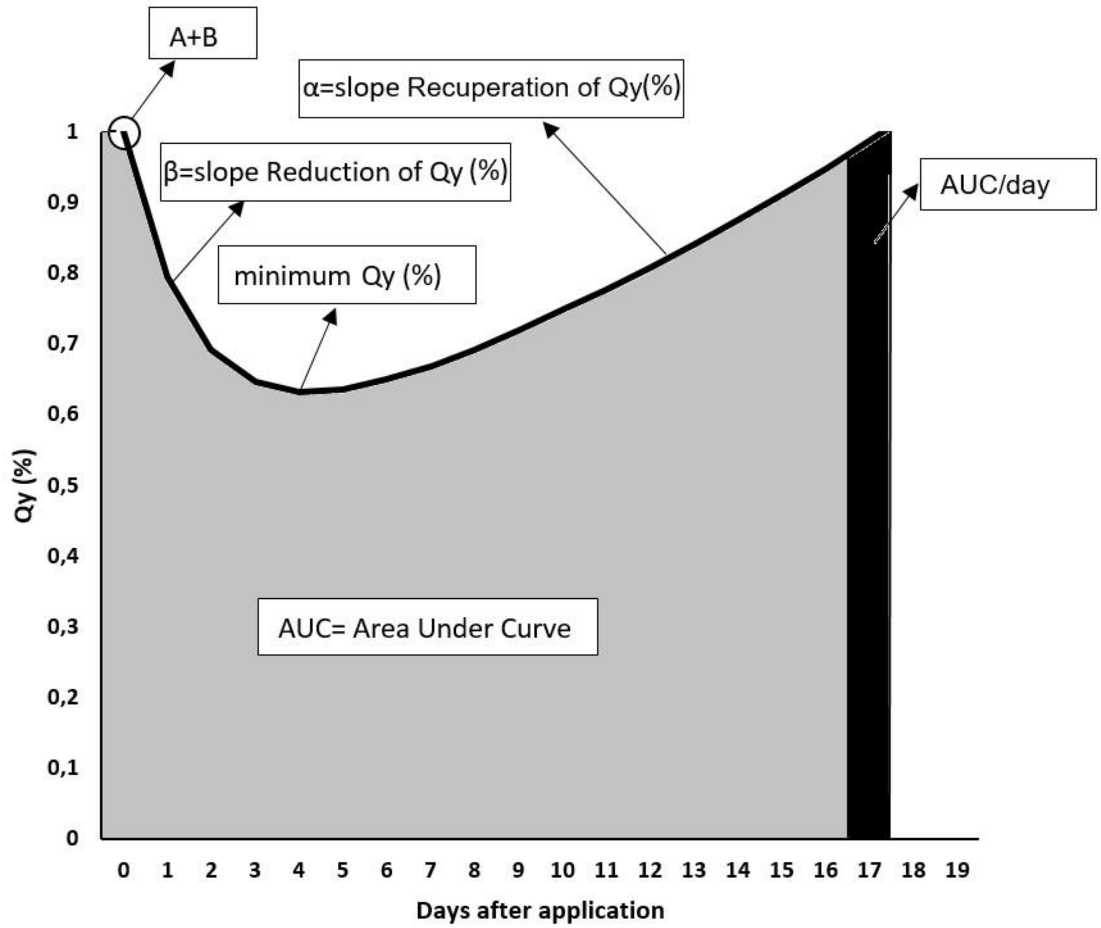

Figure 1. Graphic representation of the parameters calculated with the biexponential pharmacokinetic model (AUC, AUC/day, A, α, B and β).

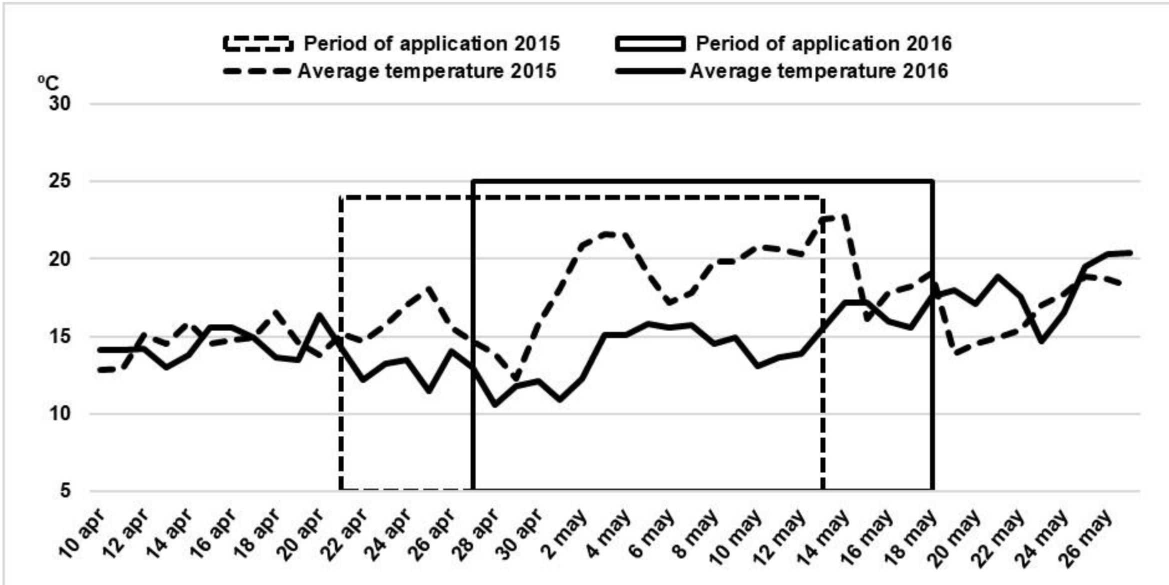

Figure 2. Average temperatures in the period of application in 2015 and 2016.

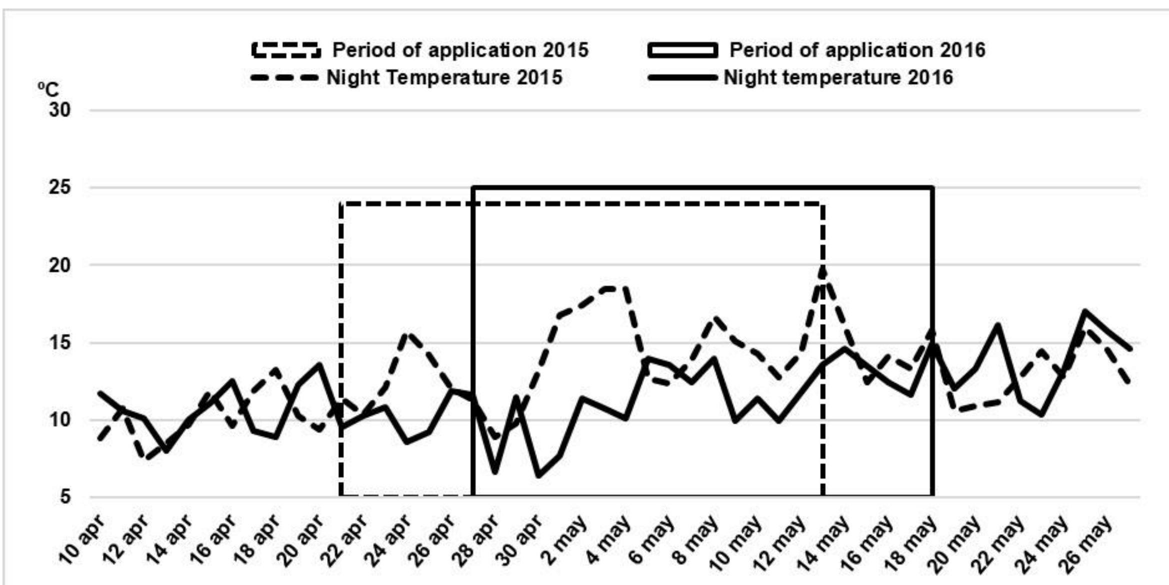

Figure 3. Average night temperature in the period of application in 2015 and 2016.

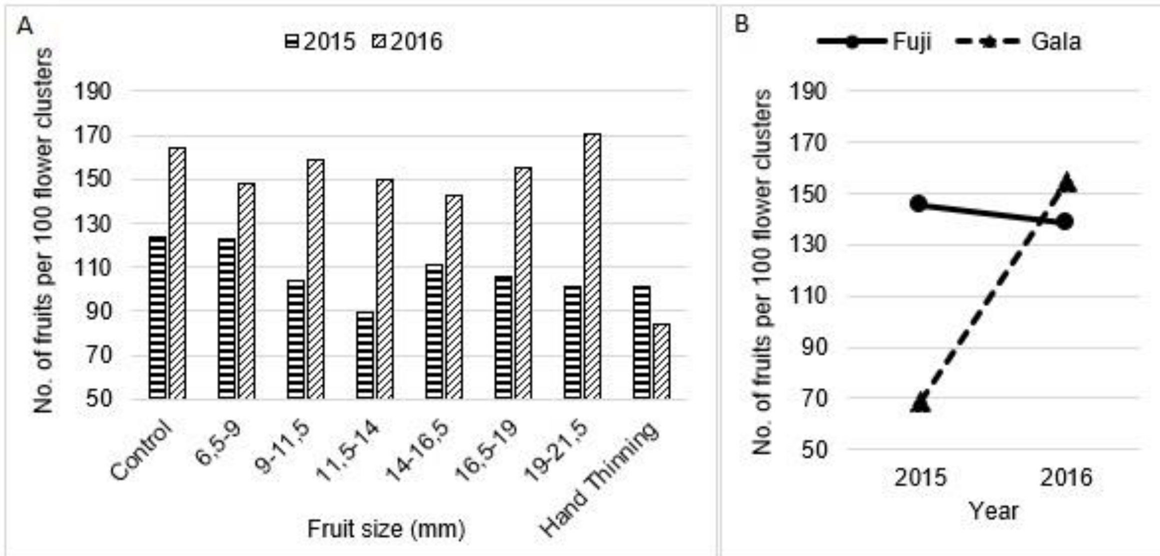

Figure 4. Effect of treatments in 2015 and 2016 on fruit set (A); Effect of fruit set in different years and varieties (B).

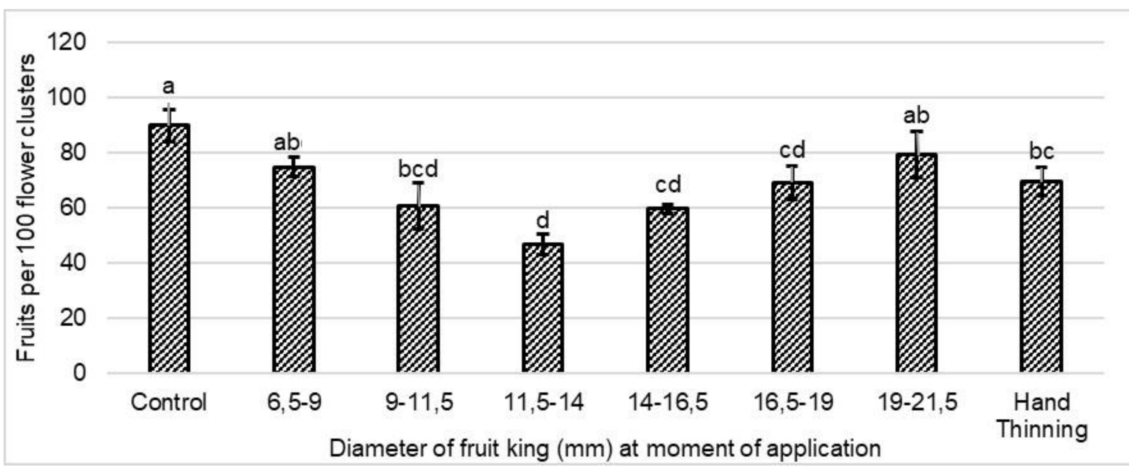

Figure 5. Average fruit set (fruits per 100 flower clusters) on Gala in 2015. Different letters denote significant differences (Duncan's range test at P<0.05).

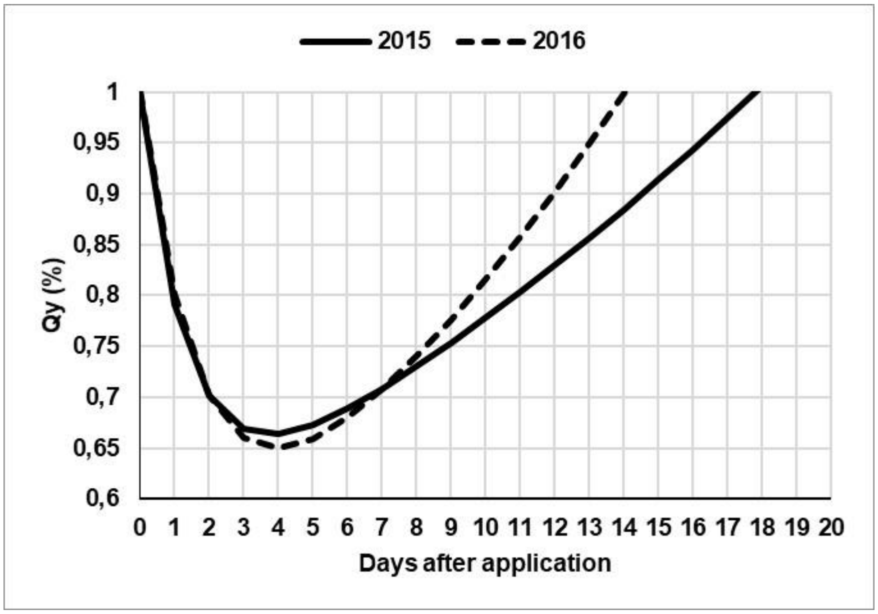

Figure 6: Graphic representation of the parameters estimated with the biexponential pharmacokinetic model (A, α, B and β) for the years 2015 and 2016

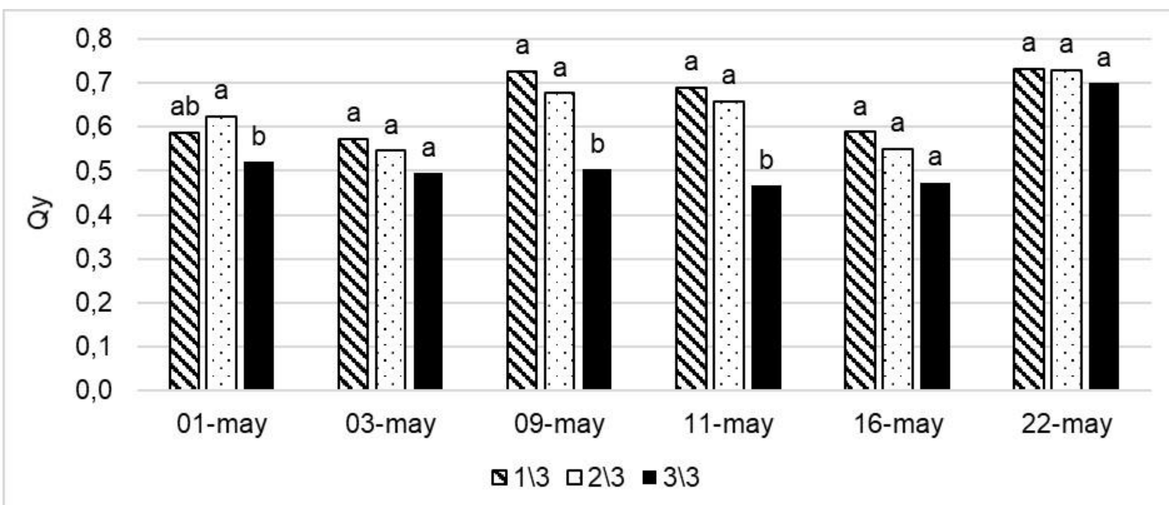

Figure 7: Value of Control Qy in Gala 2016. The Control measurements were taken four days after Brevis application. The shoots were divided into 3 sections: section 1/3 closest to branch, 2/3 mid-shoot and 3/3 vegetative section Different letters denote significant differences (Duncan's range test at P<0.05).