Universitat de Lleida

Metamitrona, una nueva herramienta para optimizar el aclareo químico en manzano

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TESIS DOCTORAL

Metamitrona, una nueva herramienta para optimizar el aclareo químico en manzano

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“No importa lo que hagas en esta vida
mientras seas buena persona y trabajador”

(mi madre)

A los de casa:
A Blau, XX/XY y Marta
A mis padres (Carmen y Luis)
A Javi, Irene,
Hugo, Claudia y Mar

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RESUMEN

El aclareo químico es una práctica habitual en manzano ya que significa una reducción temprana de la carga de frutos/árbol, lo que aporta una mejora en el tamaño del fruto y en su calidad, una disminución o incluso eliminación del aclareo manual y un incremento en la floración de retorno. Todo ello revierte en un incremento en la rentabilidad económica de las plantaciones de manzano. Un programa de aclareo puede estar diseñado por varias materias activas en aplicaciones únicas o combinadas, pero para todas ellas su eficacia está definida por multitud de factores, entre los que destacan claramente el tamaño del fruto en el momento de la aplicación, el número de aplicaciones, las condiciones climáticas y la dosis.

El objetivo general de la tesis fue determinar el modo de actuación de Brevis®, estudiando aquellos factores que afectan a la eficacia en el aclareo, como son las condiciones climáticas, la dosis de aplicación, el tamaño del fruto, el efecto de la reducción de radiación por el empleo de redes antigranizo, la comparativa entre una y dos aplicaciones, así como la separación entre dichas aplicaciones, y la comparativa entre el comportamiento de Brevis® en las variedades 'Gala' y 'Fuji'. Los ensayos se realizaron entre el 2013 y el 2016 en fincas IRTA-Estación Experimental de Lleida e IRTA-Estación Experimental de Mas Badia, en las variedades de manzana 'Gala', 'Fuji' y 'Pink Lady'.

En todos los ensayos realizados en los que se evaluó la respuesta de aclareo de Brevis® se observó reducciones significativas en la carga o número de frutos por árbol y en la producción, mientras que el color, el calibre y el peso del fruto fueron superiores al reducirse la carga. Brevis® mostró eficacia de aclareo en aplicaciones entre 7,5 y 19 mm del fruto central en estudios realizados en 'Gala' y 'Fuji', constatándose también que la máxima eficacia se registró en el rango de 11,5 a 14mm. Además, 'Gala' mostró mayor eficacia al aclareo con Brevis® que 'Fuji'. Del mismo modo, los valores de fluorescencia fueron mayores en 'Fuji' que en 'Gala' y, por lo tanto, la inhibición causada por Brevis® fue mayor en 'Gala' que en 'Fuji'. Los factores climáticos que afectan en mayor medida a la eficacia de Brevis® son las temperaturas medias y nocturnas 5 días antes y 3 días después de la aplicación. Brevis® mostró una relación lineal entre dosis y todos los parámetros de eficacia, calidad (calibre, color y pesos del fruto) y fluorescencia evaluados en 'Gala' y 'Fuji' entre 1,1 y 4,4 kg/ha. El modelo farmacológico a partir de la función biexponencial mostró un alto grado de ajuste y los valores calculados se correlacionaron con los valores reales. Los parámetros estudiados en la función biexponencial muestran relación directa entre eficacia e inhibición de fotosíntesis. Dichos parámetros actualmente solo pueden utilizarse para caracterizar la eficacia del año, ya que es necesario que el periodo de inhibición haya finalizado. En el caso de un programa de aclareo basado en dos aplicaciones de Brevis® entre 7,5 mm y 13,5 mm del fruto central, la eficacia del programa está definida por las condiciones climáticas que se dan en los dos momentos de aplicación, mientras que la separación entre las aplicaciones no afecta a la eficacia final. La reducción en la radiación ocasionada por las redes antigranizo no influyó en la eficacia de Brevis® en el aclareo, ni en la inhibición de la fluorescencia.

RESUM

L'aclarida química és una pràctica habitual en pomera, ja que significa una reducció primerenca de la càrrega de fruits/arbre, fet que aporta una millora en la mida del fruit i en la seva qualitat, una disminució o fins i tot eliminació de l'aclarida manual i un increment en la floració de retorn. Tot això reverteix en un increment en la rendibilitat econòmica de les plantacions de pomera. Un programa d'aclarida pot estar dissenyat per diverses matèries actives en aplicacions úniques o combinades, però per a totes elles la seva eficàcia està definida per multitud de factors, entre els quals destaquen clarament la mida del fruit en el moment de l'aplicació, el nombre d'aplicacions, les condicions climàtiques i la dosi.

L'objectiu general de la tesi va ser determinar la manera d'actuació de Brevis®, estudiant aquells factors que afecten a l'eficàcia de l'aclarida, com són les condicions climàtiques, la dosi d'aplicació, la mida del fruit, l'efecte de la reducció de radiació per l'ús de malles antipedra, la comparativa entre una i dues aplicacions, així com la separació entre aquestes aplicacions, i la comparativa entre el comportament de Brevis® en les varietats 'Gala' i 'Fuji'. Els assajos es van realitzar entre 2013 i el 2016 en finques IRTA-Estació Experimental de Lleida i IRTA-Estació Experimental de Mas Badia, en les varietats de poma 'Gala', 'Fuji' i 'Pink Lady'.

En tots els assajos realitzats en els quals es va avaluar l'efecte d'aclarida de Brevis® es va observar reduccions significatives en la càrrega o nombre de fruits per arbre i en la producció. Mentre que el color, el calibre i el pes del fruit van ser superiors quan es reduir la càrrega. Brevis® va mostrar eficàcia d'aclarida en aplicacions entre 7,5 i 19 mm del fruit central en estudis realitzats en 'Gala' i 'Fuji', constatant també que la màxima eficàcia es va registrar en el rang de 11,5 a 14 mm. A més, 'Gala' va mostrar major eficàcia a l'aclarida amb Brevis® que "Fuji". De la mateixa manera, els valors de fluorescència van ser majors en 'Fuji' que en 'Gala' i per tant la inhibició causada per Brevis® va ser major en 'Gala' que en 'Fuji'. Els factors climàtics que afecten en major mesura l'eficàcia de Brevis® són les temperatures mitjanes i nocturnes 5 dies abans i 3 dies després de l'aplicació. Brevis® va mostrar una relació lineal entre la dosi i tots els paràmetres d'eficàcia, qualitat (calibre, color i pes del fruit) i fluorescència avaluats en 'Gala' i 'Fuji' entre 1,1 i 4,4 kg/ha. El model farmacològic a partir de la funció biexponencial va mostrar un alt grau d'ajust i els valors calculats es van correlacionar amb els valors reals. Els paràmetres estudiats en la funció biexponencial mostren relació directa entre eficàcia i la inhibició de fotosíntesi. Aquests paràmetres actualment només es poden utilitzar per caracteritzar l'eficàcia de l'any, ja que és necessari que el període d'inhibició hagi finalitzat. En el cas d'un programa d'aclarida basat en dues aplicacions de Brevis® entre 7,5 mm i 13,5 mm, l'eficàcia del programa està definida per les condicions climàtiques que es donen en els dos moments d'aplicació, mentre que la separació entre les aplicacions no afecta l'eficàcia final. La reducció en la radiació ocasionada per les malles antipedra no va influir en l'eficàcia de Brevis® en l'aclarida, ni en la inhibició de la fluorescència.

ABSTRACT

Chemical thinning is a common practice used in apple orchards. It entails an early decrease in fruit tree load, resulting in enhanced fruit size and quality, a reduction or even the complete avoidance of manual thinning, and an increased return bloom. These effects translate into greater economic profitability for the apple grower. A thinning programme can be designed using various active ingredients in single or multiple applications. The efficacy of the programme will be defined by a number of factors, the most important of which are fruit size at the time of application, number of applications, meteorological conditions and dosage used.

The main aim of this thesis is to analyse the different factors affecting the thinning efficacy of Brevis®, a commercial chemical thinner, and the subsequent impact on various crop parameters in different apple varieties. These factors include action mode, meteorological conditions, application dosage, fruit size, the effect of using anti-hail netting to reduce solar radiation, the comparative impact of a single or double application, the time interval in double application treatments between the first and second application, and the comparative performance of Brevis® in the apple varieties 'Gala' and 'Fuji'. The tests were performed between 2013 and 2016 at the IRTA experimental stations in Lleida and Mas Badia (Spain) on the apple varieties 'Gala', 'Fuji' and 'Pink Lady'.

Significant reductions in crop load and yield were observed in all the tests that were conducted for the purpose of evaluating Brevis® thinning efficacy. In addition, fruit colour, size and quality increased as crop load decreased. Brevis® thinning efficacy was demonstrated in studies on 'Gala' and 'Fuji' varieties for applications made when king fruit diameter ranged between 7.5 mm and 19 mm, with maximum efficacy recorded in the 11.5-14 mm range. Thinning efficacy was also found to be greater in 'Gala' than in 'Fuji'. In the same way, fluorescence values were higher in 'Fuji' than in 'Gala', which means that the inhibition caused by Brevis® was higher in 'Gala' than in 'Fuji'. The most important meteorological factors affecting Brevis® efficacy were the mean temperature and mean night temperature in the period from 5 days before to 3 days after Brevis® application. A linear relationship was found between Brevis® dosage (applied at rates ranging between 1.1 and 4.4 kg/ha) and all the efficacy parameters of quality (fruit size, colour and weight) and fluorescence evaluated in 'Gala' and 'Fuji'. The pharmacological model based on the biexponential function showed a high degree of fit and the calculated values correlated closely to the real values. The parameters studied in the biexponential function revealed a direct correlation between Brevis® efficacy and photosynthesis inhibition. However, these parameters can presently only be used to characterise Brevis® efficacy of the year in question as it is necessary for the period of inhibition to have finished. In the case of a thinning programme based on two chemical thinner applications made when king fruit are 7.5-13.5 mm in diameter, Brevis® efficacy is defined by the meteorological conditions at the time of each application but is not affected by the time interval between application. The reduction in solar radiation as the result of the use of anti-hail netting did not affect Brevis® thinning efficacy or fluorescence inhibition.

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Acrónimos y abreviaturas

a.i.	Active ingredient
AIA	Ácido indol-3-acético
ANA	Ácido Naftil Acético
ATP	Trifosfato de adenosina
ATS	ammonium thiosulphate / tiosulfato de amonio
AUC	Area under curve
BA	6-benzyladenine/ Benciladenina
BBCH	Biologische Bundesanstalt, Bundessortenamt and CHemical industry
BR	Brevis®
ETo	Evapotranspiration
EU	Unión Europea
FAO	Organización de las Naciones Unidas para la Alimentación y la Agricultura
GLM	Generalized liner model
INIA	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria
IRTA	Instituto de Investigación y Tecnología Agroalimentaria
MAPA	Ministerio de Agricultura, Pesca y Alimentacion
NAA	naphthyl acetic acid
NAD	naphthalene acetamide/ naftaleno acetamida
NS	Not significant
PAR	Radiación fotosintéticamente activa/ Photosynthetically active radiation
PC	Polisulfuro de calcio
PLS	Partial least squares
PM-ETo	Penman-Monteith Evapotranspiration
PSII	Fotosistema II
Qy	Quantum yield

SG	Gránulos solubles en agua
TCSA	Trunk cross-sectional area (TCSA)
VIP	Variable importance in projection

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INTRODUCCIÓN GENERAL

1. El cultivo del manzano (*Malus domestica* Borkh).

El manzano (*Malus domestica*) es un árbol de caducifolio que pertenece a la familia de las Rosáceas, subfamilia *Pomoideae*. Aunque el origen preciso del manzano de hoy no está del todo claro, probablemente evolucionó en los bosques de Asia central, particularmente en Kazajstán (Ferree and Warrington, 2003). A lo largo de la historia se han registrado un gran número de variedades de manzanas en todo el mundo. Si bien, en la actualidad, la gran mayoría provienen de los diferentes programas de mejora varietal, existe un gran número de variedades autóctonas, locales y tradicionales.

La producción anual mundial de manzanas fue de 90 millones de toneladas en el periodo de 2012 a 2017 con 5 millones de hectáreas en producción en el 2017 (FAO, 2018). Asia produce el 64% de la producción mundial de manzanas, lo que se traduce en más de 50 millones de toneladas y una superficie en producción de 3,5 millones de hectáreas (Figura 1). En segundo lugar, se encuentra la Unión Europea, con una producción de 12 millones de toneladas y una superficie de 500.000 ha, que representa el 14% de la producción mundial, la siguen América del Norte (7%), América del Sur (5%) y África (3%) (Figura 1).

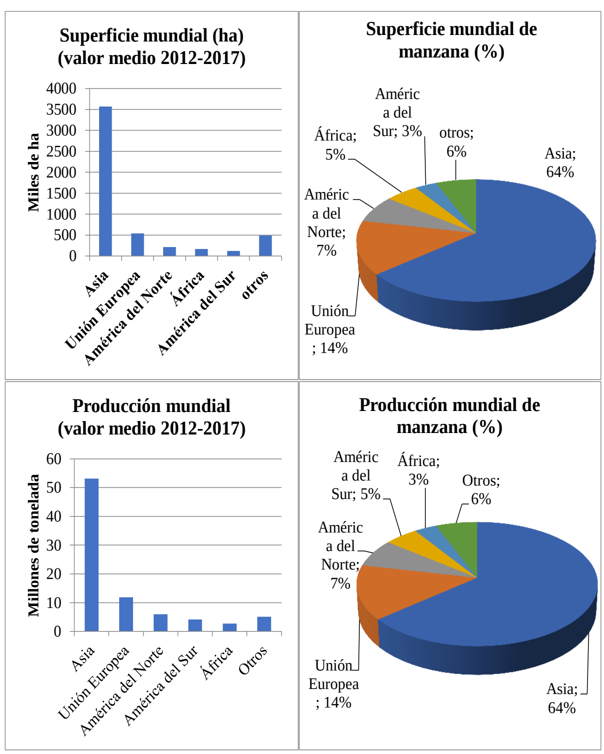

Figura 1: Elaboración propia a partir de los datos publicados de superficies y producciones FAO.

En el periodo de 2012 a 2017 se produjeron 12 millones de toneladas de manzana en los 28 países de la Unión Europea. Polonia es el primer productor de la Unión Europea (EU), con 3 millones de toneladas y el 26 % de la producción, seguida de Italia (19%), Francia (15%) Alemania (8%), Hungría (5%), y en sexto lugar se posiciona España con una superficie de 30.000 hectáreas y una producción de 500.000 toneladas que representan el 5% de la producción europea (Figura 2).

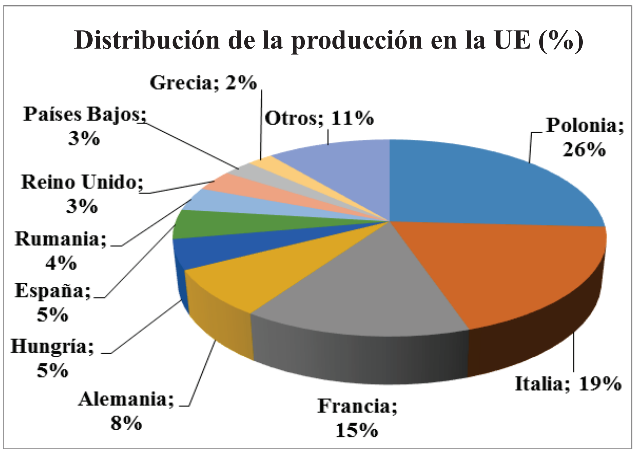

Figura 2: Elaboración propia a partir de los datos publicados de superficies y producciones FAO en el periodo de 2012 a 2017

El cultivo del manzano en España ocupaba una superficie aproximada de 35.000 ha en 2018, con una producción media de 485.000t en el período de 2013-2018 (MAPA, 2019). Las principales zonas productoras de manzanas se sitúan en las zonas bajas del Valle del Ebro. La principal productora de manzana en España es Cataluña, con una producción 273.000 toneladas que representan el 53% de la producción, seguida de Aragón (90.000 tonelada-18%), Galicia (12%) y Castilla León (6%) (Figura 3). La mayor parte de estas zonas se caracterizan por un clima mediterráneo con veranos calurosos y secos e inviernos fríos o muy fríos con un alto nivel de variabilidad a lo largo de las ubicaciones geográficas y los años (Funes *et al.*, 2016). A diferencia de las zonas productoras españolas, la producción mundial de manzana se encuentra principalmente en zonas templadas caracterizadas por climas húmedos, donde los inviernos son fríos y las primaveras y veranos templados.

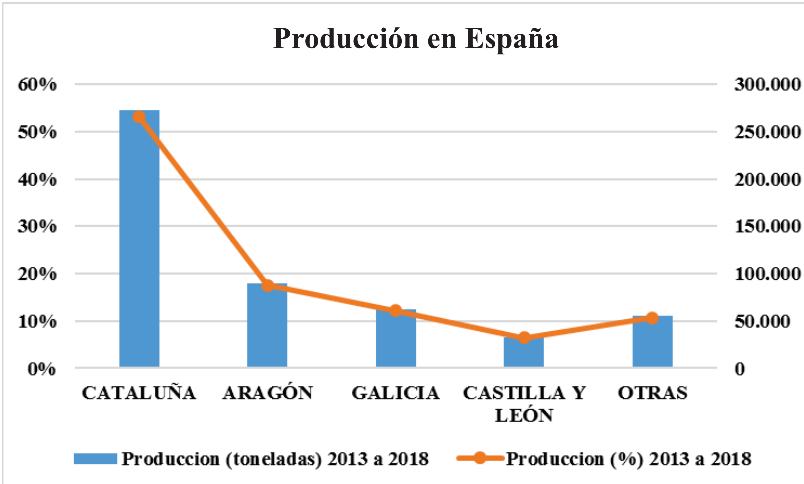

Figura 3: Elaboración propia a partir de los datos publicados de superficies y producciones MAPA (2019) en el periodo de 2013 a 2018

2. Fisiología del crecimiento y desarrollo del fruto

El valor productivo de la plantación está definido en gran medida por el desarrollo y crecimiento del fruto, unos procesos que están condicionados por una gran cantidad de factores que interrelacionan entre sí.

El fruto del manzano se deriva de la base de la flor de la manzana después de la polinización; posteriormente el ovario empieza a expandirse y desarrollarse hasta formar el fruto (Lakso and Goffinet, 2013).

La primera semana después de la fecundación de la flor (óvulos), el fruto crece exponencialmente por división celular (Figura 4). Después de esta semana, el crecimiento del fruto se produce por división y expansión celular (4 o 5 semanas después de la floración) (Figura 4). Pasado el período de división celular, el crecimiento del fruto durante el resto de la campaña se produce por expansión celular (Bain and Robertson, 1951; Lakso and Goffinet, 2013) (Figura 4).

De esta manera, el tamaño final del fruto depende principalmente del número de células y de su expansión, pero la base para obtener un buen tamaño del fruto es un elevado número de células que queda definido en el inicio de la campaña. Durante la primera parte de la fase de división celular, hasta la caída de pétalos, la demanda de carbohidratos del fruto es moderada y las reservas del árbol son capaces de suministrar un nivel de carbohidratos necesario para que no se vea afectada la división celular (Lakso and Goffinet, 2013, 2017)

(Figura 4). Sin embargo, pasado el periodo de caída de pétalos la demanda de carbohidratos por parte del fruto aumenta de manera exponencial al igual que la división celular (Figura 4). Además, en dicho periodo el árbol también realiza la mayor parte del crecimiento vegetativo necesario para realizar la fotosíntesis y obtener el suministro de carbohidratos necesario para abastecer las necesidades del árbol (Lakso and Goffinet, 2013, 2017). Por todo ello, en la segunda fase de la división celular existe competencia entre frutos, hojas y brotes vegetativos por los carbohidratos producidos en la fotosíntesis que pueden condicionar el número final de células del fruto, ya que el árbol se encuentra en una fase con poca superficie foliar, muchos frutos y poca producción de carbohidratos. Una vez finalizada la fase de división celular, cuando el fruto ha crecido hasta 20 mm éste pasa a ser prioritario para el árbol (Lordan, 2018), puesto que ya ha finalizado la fase de crecimiento vegetativo rápido de los brotes, y ya tiene el área foliar suficiente para abastecer de los carbohidratos necesarios a la fase de expansión celular.

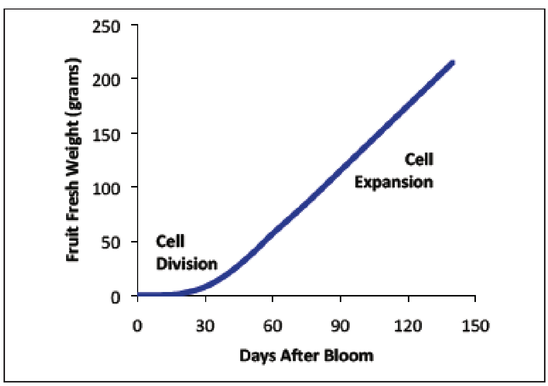

Figura 4: Patrón estacional del crecimiento y desarrollo del fruto del manzano sin limitaciones ambientales o de cultivo. El crecimiento curvilíneo inicial durante 4-6 semanas es por división celular y el crecimiento lineal posterior es por expansión celular (Lakso and Goffinet, 2013).

Asimismo, el número de células es el resultado de la división celular, que se posiciona en un momento crítico a nivel de demanda de carbohidratos. Dicha situación provoca que la única manera de reducir la competencia sea mediante la reducción del número de frutos, utilizando las diferentes estrategias de aclareo en esta fase. Además, el hecho de que a partir de los 20 mm la producción de carbohidratos priorice a los frutos, condiciona que a partir de dicho momento sea más difícil obtener resultados satisfactorios mediante aclareo químico.

Por otro lado, el número de células también está condicionado por las condiciones climáticas. De esta manera, la temperatura es muy influyente ya que, un aumento dentro del rango fisiológico, afecta exponencialmente a la velocidad de los procesos fisiológicos del fruto (Fischer, 2000). En términos generales, en zonas con climas cálidos los frutos presentan un desarrollo morfológico acelerado (Fischer, 2000), que provoca una mayor división celular y en consecuencia un mayor calibre en cosecha.

Otro factor a tener en cuenta es el riego en las diferentes fases; Parra-Quezada *et al.* (2008) observaron que la disponibilidad de humedad en el suelo es un factor determinante en el crecimiento del fruto, en especial durante la fase división celular, posteriormente en las siguientes fases en las que empieza la expansión celular los requerimientos de agua son mayores. En este sentido, el efecto del estrés hídrico en función de la severidad y el tiempo (Parra-Quezada et al., 2008), es factor que puede llegar a afectar tanto a la división como a la expansión celular y, en consecuencia, el tamaño final del fruto.

2.1. Fisiología de la abscisión

Abscisión puede definirse como la separación de una parte de la planta, tales como hoja, flor, fruto, semilla, tallo, u otro (Kolaric 2010). En el caso de flores o frutos en fase inicial de desarrollo en manzano hay tres períodos en los que su caída o abscisión tiene mayor importancia relativa:

- Después de floración: todas aquellas flores que no han sido fecundadas.
- 4-6 semanas después de la floración: es la caída más importante e intensa, también llamada “caída natural de frutos”, que en las diferentes zonas productoras de manzana de Cataluña suele producir-se a mediados o finales de mayo.
- Antes de la cosecha: suele ser normal en algunas variedades la caída de algunos frutos en los periodos próximos a la recolección.

La caída de flores se suele producir una semana después de plena floración; todas aquellas flores que no han sido fecundadas no se desarrollan y caen. Existen diferentes factores climáticos que favorecen la caída de flores y provocan un mal cuajado. Así, cuando se producen periodos prolongados de lluvias, viento o bajas temperaturas durante el periodo de floración causan inactividad de los polinizadores y en consecuencia una caída mayor de flores sin cuajar. La caída que suele ser menos importante es la que se produce antes de la cosecha, en algunas variedades como ‘McIntosh’ se produce una caída importante de frutos, debido un incremento en la síntesis de etileno endógeno antes de la recolección (Robinson *et al.*, 2010).

Por último, la caída natural de frutos, que se produce después del cuajado, es la más importante y la que más repercutirá en el tamaño del fruto y en el valor de la producción.

Asimismo, la caída natural de frutos se ve condicionada por factores como el balance de carbohidratos y diferentes cambios hormonales, como puntos que se abordaran a partir de ahora.

La zona de abscisión está formada por una o más capas de células parenquimáticas de pared delgada que se extienden a través del pecíolo y excluyen el haz vascular (Kolarič, 2010). La formación de la zona de abscisión también está regulada hormonalmente, el etileno induce la formación de dicha zona, mientras que el ácido indol acético (AIA) dicta dónde se ubica (Roberts *et al.*, 2002). El efecto fisiológico de la AIA en este proceso es reducir la sensibilidad de la zona de abscisión al etileno (Bangerth, 2000; van Doorn and Stead, 1997) . La baja concentración de AIA, provoca que el etileno active el tejido preformado donde se produce la abscisión (Bangerth, 2000). En este sentido, la caída natural de manzanas jóvenes depende de los cambios hormonales en la zona de abscisión, que se encuentra en la base del pedúnculo. Además, Kolarič (2010) indicó que el etileno, la auxina AIA y el equilibrio entre ambas es un factor clave que afectará la evolución de la abscisión de frutos.

La abscisión natural depende de varios factores. En primer lugar, es importante el grado de dominancia de un fruto sobre otro. Esta situación depende de la diferencia en el tiempo del cuajado de los diferentes frutos, el número de semillas/fruto, la proximidad y vigor de los brotes vegetativos y el número de frutos por corimbo (Kolarič, 2010). De esta manera, la flor central tiene ventaja en el desarrollo con respecto a las flores laterales porque florece antes. Además, todos los frutos con un mayor número de semillas son dominantes si los comparamos con aquellos que tienen menos (Bangerth, 2000; Kolarič, 2010). Otro factor es la proximidad y el vigor de los brotes vegetativos a los frutos que pueden inhibir el transporte de AIA a los frutos (Kolarič, 2010).

Por otro lado, el balance de carbohidratos del árbol es otro factor importante que afecta el grado de abscisión. Las condiciones que favorecen un buen estado del balance de los carbohidratos están asociadas a una menor abscisión natural de frutos (Robinson and Lakso, 2011). Por lo tanto, las condiciones que conducen a un mal estado del balance de carbohidratos también se asocian con una fuerte caída natural, como periodos calurosos y nublados, un número elevado de corimbos, arboles estresados, inhibidores de la fotosíntesis, periodos naturales o provocados de poca luz y altas temperaturas nocturnas causan o mejoran la abscisión de la fruta (Lakso *et al.*, 2006).

De esta manera, hay muchos factores que influyen en la abscisión natural de los frutos y es una interacción compleja entre condiciones ambientales, material vegetal (variedad y patrón), la floración, el número de frutos cuajados y el vigor del árbol.

3. El aclareo

Los manzanos producen muchos corimbos florales y, en consecuencia, demasiados frutos, lo cual dificulta obtener una producción regular y de alta calidad año tras año. Una elevada carga de frutos por árbol ocasiona un tamaño pequeño, mala calidad del fruto, rotura de ramas, agotamiento de las reservas de árbol y menor resistencia al frío (Dennis, 2000).

Con el fin de evitar estos problemas asociados a una carga excesiva e incrementar el valor de la producción es necesario eliminar frutos del árbol lo antes posible, y esta tarea clave en el proceso productivo es el aclareo. El objetivo del aclareo es alcanzar un nivel óptimo de frutos por árbol. De esta manera, se debe hacer un aclareo adecuado año tras año debido a los beneficios en el rendimiento, el tamaño de la fruta y otros aspectos de la calidad del fruto (color, firmeza, azúcar, acidez, etc.), los cuales determinaran el valor económico de la producción. La figura 5 muestra el efecto de aclareo en la producción de ‘Gala’, de una estrategia en la que se ha realizado 2 aplicaciones de Brevis® y otra sin tratar. En la estrategia en la que se han realizado 2 aplicaciones de Brevis® y el aclareo es el adecuado, la carga se distribuye de manera regular en el árbol, la coloración y calibres son adecuados, lo que se traduce en un alto porcentaje de producción con valor comercial. Por el contrario, la estrategia sin tratar muestra un numero de frutos mucho mayor, mal distribuidos, y con coloración y calibres deficientes. Además, en la estrategia de aclareo se podrá recolectar prácticamente toda la producción en una pasada, lo que reducirá los costes de recolección. De este modo, el aclareo es importante para maximizar el valor del de la producción (Byers, 2003). Para ello es importante tener en cuenta que el tamaño del fruto en el momento de la recolección está directamente relacionado con la precocidad y la intensidad en el aclareo de frutos, siempre que el número de yemas y el vigor sean adecuados (Williams, 1979).

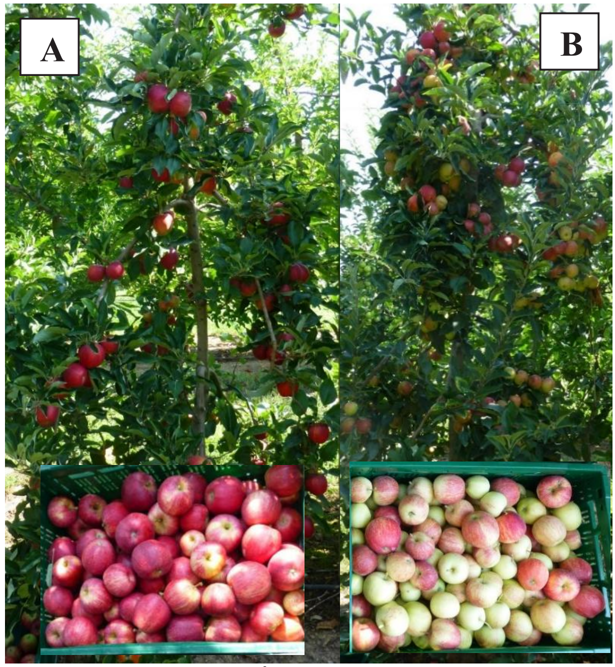

Figura 5: Arboles de manzano (Gala). **A.-** Árbol en que se ha realizado una estrategia de aclareo con Brevis®. **B.-** Árbol en que no se ha realizado aclareo.

La floración de retorno es otro factor que está influenciado por la carga del árbol. Las flores de manzana se diferencian el año anterior a la floración, y un aclareo inadecuado puede dar lugar a la vecería (Cline *et al.*, 2018). La vecería provoca una producción alternante de los árboles frutales que se ha descrito en documentos frutícolas antiguos y siempre se considera indeseable (Jonkers, 1979).

Existen numerosos estudios en los que se ha demostrado que tanto el tamaño del fruto como la floración de retorno mejora en aquellos árboles en los que se realiza un aclareo temprano y se reduce la carga considerablemente (Byers and Carbaugh, 2002; Iwanami *et al.*, 2018; Jones *et al.*, 1992; McArtney *et al.*, 1996; Serra *et al.*, 2016). Sin embargo, estos estudios se limitaron a probar diferentes dosis y numero de aplicaciones y no indicaron el momento óptimo del aclareo, a pesar de que es un factor importante que regula tanto el riesgo de entrar en alternancia como la calidad del fruto (tamaño, peso, color, firmeza, azúcar, acidez, etc.).

Por todas estas razones, el aclareo de frutos es una práctica agrícola complicada, pero de vital importancia y que tiene un impacto significativo en la rentabilidad del cultivo (Lordan *et al.*, 2018; Robinson *et al.*, 2016).

3.1. Aclareo manual

Antiguamente el aclareo se realizaba exclusivamente a mano. A pesar de ello, en la actualidad es la última opción a la que se recurre debido a sus altos costes en comparación con otras alternativas u opciones (Figura 6). El aclareo de frutos, junto con la poda y la recolección, integran las prácticas con más costes de mano de obra involucrados. Habitualmente se considera el aclareo manual como un complemento de la operación del aclareo químico cuando se han obtenido resultados deficientes (Figura 6).

Figura 6: Aclareo manual Estación experimental Lleida (Mollerussa) 21-junio-2019.

Una práctica habitual en el aclareo manual es dejar un fruto por corimbo, espaciados entre ellos aproximadamente unos 15 cm (Almanza *et al.*, 2000). A pesar de ello, el aclareo manual presenta serios problemas puesto que es necesario esperar hasta que finalice la caída natural, que a menudo ocurre demasiado tarde y, por lo tanto, afecta en menor medida el tamaño de la fruta y perjudica la inducción floral del año siguiente (Lordan et al., 2018; McArtney et al., 1996) (Figura 6).

Como se ha mencionado anteriormente, la caída natural en las condiciones españolas suele producirse a mediados o finales de mayo. Por esas fechas el tamaño de fruto habitual es superior a 25 mm, por lo que ya se ha producido el final del crecimiento vegetativo y la inducción floral del próximo año. Además, si el árbol ha presentado una floración normal es posible que la competencia entre hojas, brotes y frutos ya haya afectado la división celular. Por todo ello, el aclareo manual, aparte de requerir un gran número de horas hectárea con unos costes en mano de obra elevados, también tiene un efecto evidente el tamaño final del fruto y en la inducción floral. Además, es necesaria la supervisión de los trabajadores que a menudo suelen dejar más carga de la deseada y en consecuencia son necesarias diversas pasadas para conseguir un aclareo uniforme y deseado (Figura 6).

Sin embargo, el aclareo manual también tiene ciertas ventajas, ya que permite eliminar aquellas frutas dañadas por la presencia de defectos asociados a problemas en el control fitosanitario de ciertas enfermedades o plagas que afectan al fruto por ser un sistema absolutamente selectivo. Habitualmente se eliminan los frutos más pequeños y se seleccionan aquellos frutos que tienen un mayor tamaño, que están asociados a un mayor número de células que sirven como indicador del tamaño potencial del fruto en la madurez (Rosa, 2016). En el caso de variedades coloreadas, también se puede ser selectivo en el dosel del árbol, realizando un mayor aclareo en la zona sombreada, donde es más difícil obtener una buena coloración de los frutos.

3.2. Aclareo mecánico

El aclareo mecánico en frutales de pepita se ha desarrollado recientemente. Actualmente hay muchas investigaciones relacionadas con el aclareo mecánico con el objetivo de automatizar y agilizar el aclareo. En este sentido, el aclareo mecánico muestra ventajas como la intervención temprana, que favorecerá la floración de retorno y la división celular. Además, es rápido, no deja residuos, se puede realizar a cualquier hora del día y no se ve condicionado por la climatología puesto que se puede realizar incluso en condiciones de lluvia. Actualmente se está estableciendo como una práctica habitual para reducir la floración y la competencia inicial entre frutos en plantaciones de cultivo ecológico, en grandes fincas y en zonas más frías con eficacias erráticas de los productos de aclareo químico (Greene and Costa, 2013). A pesar de ello, en la actualidad el aclareo mecánico puede presentar diferentes problemas.

Actualmente existen dos equipos utilizados en el aclareo mecánico del manzano, Fuet® o Darwin®, los cuales son utilizados en floración (Figura 7). Dichos equipos constan de un eje vertical rotatorio al cual va unido un sistema de hilos que al girar golpean y desprenden las flores (Figura 7). Es importante adaptar en número de hilos, la separación entre ellos, la inclinación de la máquina y la velocidad de avance del tractor, para conseguir eficacias aceptables con un tiempo inferior a la hora y media por hectárea. Sin embargo, este método de aclareo a menudo requiere de un aclareo manual y/o químico, para lograr el nivel óptimo de carga.

Figura 7: Equipo de aclareo Fuet® (Torres, 2013). Estación experimental Lleida (Gimenells)

Para obtener una mayor eficacia son necesarios sistemas de formación que favorezcan un mayor número de ramas delgadas y flexibles, como el sistema Solaxe o sistemas de tipo bidimensionales como el muro frutal (Seehuber *et al.*, 2012). Estos sistemas de formación permiten que los latiguillos de la maquina puedan acceder mejor a todas las partes del árbol, por lo que se consigue un aclareo más regular. Otro factor a tener en cuenta es que el aclareo mecánico no es selectivo, por lo que se producen daños en las hojas, brotes y frutos (Besseling *et al.*, 2018; Byers, 2003; McClure and Cline, 2015). La figura 8 muestra los efectos después del aclareo con estos equipos. Se puede observar cómo en ocasiones los daños en hojas y brotes pueden ser importantes. Además, este tipo de equipos no es selectivo con los corimbos que hay que extraer, por lo que es habitual encontrar corimbos con todos los frutos arrancados y otros corimbos enteros (Figura 8).

Figura 8: Daños ocasionados por Fuet® o Darwin® en hojas, brotes y corimbos (Torres, 2013)

Por otro lado, el aclareo mecánico puede ser un vector de enfermedades a medida que la máquina avanza por fila (Greene and Costa, 2013). Durante la época de floración es muy habitual que enfermedades como el fuego bacteriano (*Erwinia amylovora*) aun estén en estado latente o empiecen a mostrar los primeros síntomas. El hecho de que la maquinaria ocasione heridas en las diferentes partes de la planta convierte a este tipo de maquinaria en un vector importante de enfermedades y no se recomienda su uso en fincas con dichos problemas.

Otros equipos de aclareo mecánico incluyen: chorros de agua a presión, vibradores, peines de púas y de cuerda (Dennis, 2000). Sin embargo, algunos de estos sistemas, como el peine de púas, solo se utilizan en frutales de hueso.

3.3. Aclareo químico

El aclareo químico es una práctica comúnmente utilizada porque actúa en las primeras fases de desarrollo del fruto, lo que supone una ventaja respecto al aclareo manual. El hecho de actuar en etapas tempranas de desarrollo del fruto se traduce en una reducción de la competencia por los carbohidratos movilizados de las reservas y producidos en la fotosíntesis a partir de la formación de las hojas. En este sentido, cuando con las estrategias químicas se obtienen resultados satisfactorios se obtienen frutos con mejor calibre, peso y coloración. El hecho de no tener tanta competencia beneficia a la división celular y los frutos tienen un potencial de crecimiento mayor, ya que disponen de más células. Además, no se ve afectada la inducción floral y en consecuencia se reduce el riesgo de alternancia, obteniendo una producción más regular año a año. Otra ventaja respecto al aclareo manual es que, al realizarse las aplicaciones con maquinaria, el tiempo de aplicación es mucho menor. Por lo tanto, cuando los resultados son satisfactorios el coste de aclareo se reduce de manera considerable. Si a esto se le suma que la producción es de más calidad, la rentabilidad de la plantación es mucho mayor.

Un inconveniente del aclareo químico es que en general es muy dependiente de las condiciones climáticas. Como se ha mencionado anteriormente en el apartado de la absición las condiciones climáticas que favorecen la caída de los frutos son: los días nublados con temperaturas nocturnas altas (Lakso et al., 2006; Robinson and Lakso, 2011): Dichas condiciones son coincidentes con las que favorecen la abscisión del fruto mediante aclareo químico.

Existen varios ingredientes activos, pero su eficacia en el aclareo depende en gran medida de la climatología, factores del cultivo, el tamaño del fruto y el vigor del árbol, lo que puede generar resultados inconsistentes (Byers, 2003; Iwanami *et al.*, 2012; Lordan et al., 2018; Robinson and Lakso, 2004). Actualmente en España el aclareo químico se puede realizar en dos etapas diferentes:

- **Durante la floración:** El único producto registrados para esta etapa es la hormona naftaleno acetamida (NAD). Sin embargo, en la bibliografía se ha trabajado en agricultura ecológica con diferentes productos que actúan reduciendo en número de flores como Caolín, jabón potásico, vinagre, mojante nonilfenol polietilenglicol de éter, aceite mineral de verano, aceite de oliva, aceite de panís, polisulfuro de calcio (PC) (Alins Valls, 2009) y tiosulfato de amonio (ATS).

- **Post-floración (justo después del cuajado en las etapas de 6-16 mm):** los productos actualmente registrados en España para el aclareo de frutos son dos hormonas (Benciladenina (BA) y Ácido Naftalén Acético (ANA)), y un inhibidor de la fotosíntesis (Brevis® (BR)). Por otro lado, existen también otros productos con actividad de aclareo registrados en diferentes países, como son el Ethephon y el Carbaryl.

Todos los productos mencionados anteriormente ven condicionada su eficacia por el estado fenológico del árbol, la variedad y las condiciones climáticas (Byers, 2003; Lordan et al., 2018; Robinson and Lakso, 2004). Actualmente hay investigaciones con modelos climáticos que pueden ayudar tomar la decisión de la aplicación.

De los productos mencionados anteriormente, que actúan como desecantes durante la floración, solo se han obtenido resultados satisfactorios con menores efectos de fitotoxicidad con ATS y PC. Estos productos actúan secando el tejido de las flores del manzano (Byers, 1997; Kon *et al.*, 2018) dañando los pétalos, pistilos y anteras (Milic *et al.*, 2011) y provocando que caigan las flores. La estrategia al utilizar estos productos es realizar la aplicación 24 horas después de que se abra la flor central, ya que en ese momento la flor no se ve afectada y evolucionará. A pesar de ello, su eficacia es limitada por lo que es necesaria otra actuación de aclareo pasado el cuajado.

NAD es una hormona sintética de la familia de las auxinas y se desarrolló como un análogo de ANA aunque su eficacia es menor. Presenta actividad de aclareo en las etapas de floración y caída de pétalos, siendo más sensibles en aquellas variedades de maduración temprana (Greene *et al.*, 2015). NAD es considerado un producto con poca actividad de aclareo (Stopar, 2006) y normalmente es utilizado como complemento de un tratamiento posterior con BA o ANA. NAD produce algunos efectos indeseables como un incremento del porcentaje de frutos pigmeos dependiendo del momento de aplicación y la tendencia varietal (Byers *et al.*, 1985; Dini Viñoly, 2013) Aquellas variedades con la que se tiene que prestar atención, ya que son muy sensibles a presentar frutos pigmeos, son 'Fuji', seguido de 'Red Delicious' y Braeburn' (Dini Viñoly, 2013; Reginato, 1997).

ANA fue el primer producto registrado y usado comercialmente para el aclareo del tipo hormonal (Burkholder and McCown, 1941; Greene, 2002). El transporte de auxinas polares en los frutos jóvenes es un factor clave que regula la caída natural e inducida de la fruta (Bangerth, 2000). ANA reduce el transporte de auxinas polares a los frutos laterales del corimbo (Milić *et al.*, 2017). Esta reducción provoca la inhibición de la fotosíntesis, que conduce a un breve estrés en el momento de la división celular del fruto y la expansión de la hoja (Milić et al., 2017). ANA muestra actividad de aclareo cuando se aplica en tamaños de fruto de 4 a 15 mm, sin embargo, el máximo efecto es de 4 a 8 mm (Marini, 1996). Se ha de prestar especial cuidado con las variedades mencionadas anteriormente que son sensibles a presentar frutos pigmeos (Dini Viñoly, 2013; Reginato, 1997) puesto que NAD es la análoga de ANA.

BA es una hormona que pertenece al grupo de las citoquininas y estimula la división celular. Los primeros trabajos en lo que se comentó la actividad en aclareo de BA fueron en la década de los 70 (Fallahi and Greene, 2010). Sin embargo, en España no fue registrada para el aclareo hasta el 2007. BA actua a través de la inhibición correlativa del transporte del AIA polar de los frutos laterales causado por el aumento en el transporte del AIA polar a las puntas de los brotes (Milić et al., 2017). Dicha situación estimula la competencia entre frutos del mismo corimbo, favoreciendo el crecimiento del fruto central mientras que los laterales caerán al disponer de menos recursos. Adema, BA tiene un efecto positivo en el tamaño del fruto, ya que favorece la división celular. De esta manera, ante la misma intensidad de aclareo BA muestra un tamaño de fruto mayor que ANA más allá del efecto atribuible a una disminución de la carga (Alegre *et al.*, 2008; Fallahi and Greene, 2010; Milić et al., 2017). BA muestra efectos de aclareo de 7 a 15 mm, sin embargo, la máxima eficacia es de 8 a 12 mm (Alegre et al., 2008; Greene, 2002).

3.4. Metamitrona

Metamitrona es el nombre común de 4-amino-4,5-dihidro-3-metil-6-fenil-1,2,4-triazin-5-ona (C10H10N4O) y se caracteriza por tener un grupo amino en posición 4 (Burnside *et al.*, 2008) (Figura 9).

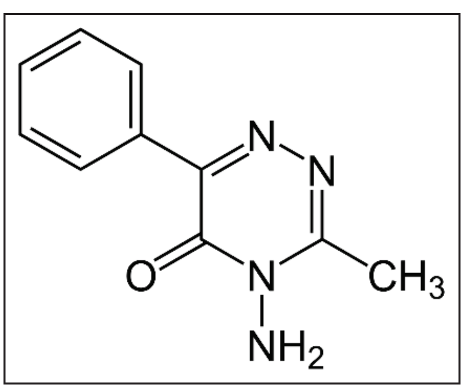

Figura 9: Estructura química de la metamitrona (Wikipedia).

La metamitrona es un herbicida selectivo del grupo de triazinonas (Burnside et al., 2008). Esta clase de herbicidas incluye metribuzin y metamitrona, y su modo de acción es la inhibición de la fotosíntesis en el fotosistema II (PSII). Se emplea mucho en la agricultura por su efecto herbicida selectivo (Belzunces, 2017). Es un herbicida con actividad tanto pre como post-emergencia con actividad tanto a nivel radicular como foliar (Mechant, 2011). Se utiliza en remolacha azucarera, remolacha forrajera, remolacha roja y en el cultivo de flores de bulbo (tulipanes, narcisos y lirios).

La primera mención del uso de metamitrona como herbicida en remolacha data de 1975 y probablemente se empezó a utilizar el mismo año (Belzunces, 2017). En el período 1989 a 2000, la remolacha azucarera fue un cultivo que tuvo una importante expansión en la Europa occidental (Belzunces, 2017), situación que provocó que la metamitrona fuera el herbicida más empleado en la remolacha azucarera en Alemania, Francia, Países Bajos, Reino Unido, España y Bélgica (Belzunces, 2017). En realidad, la metamitrona aún se vende actualmente en más de 25 países, y es producido por varios fabricantes bajo diferentes marcas.

Entre los años del 2002 y el 2005 en el centro de investigación Fruit Research Station en Jork empezaron a estudiar los efectos de aclareo de la Metamitrona basándose en estudios con herbicidas inhibidores de la fotosíntesis. Dichos estudios comprobaron que el grupo Uracilo, como Terbacil, Bromacil, Metribucin y la Metamitrona eran inhibidores de la fotosíntesis y potenciales productos de aclareo, ya que afectan a las reacciones químicas del PHII y podían inducir a la abscisión de frutos cuando se aplicaban después de la floración (Byers *et al.*, 1986; Byers *et al.*, 1984; Byers *et al.*, 1990a; Byers *et al.*, 1990b; Byers et al., 1985; DelValle *et al.*, 1985; Lafer, 2009; Schröder, 2001; Villeneuve and Ferre, 1988). En el año 2006 la Metamitrona ya se posicionó como uno de los mejores inhibidores de la fotosíntesis con efecto de aclareo y la empresa que poseía la patente de la molécula (Makhteshim Agan Industries actualmente conocida como ADAMA) decidió apostar por el desarrollo del producto junto con los centros de investigación pioneros en Europa, entre ellos IRTA.

3.5. Brevis®

De la investigación mencionada anteriormente ADAMA reformuló la metamitrona con el nombre de Brevis® (metamitrona 15% y formiato de calcio) y empezaron tramites de registro en los principales países productores de mazana. El primer país donde se registró Brevis® fue en Serbia en el 2013, y posteriormente en el 2014 se unieron Italia. Grecia, Suiza e Israel. En 2015 se registró Brevis® en España para aclareo químico en manzana y pera. Actualmente está registrado en más de 23 países de todo el mundo.

Brevis® es un inhibidor fotosintético (Basak, 2011; Lafer, 2010) cuyo modo de acción es diferente del de otros biorreguladores conocidos. En condiciones normales, el receptor de electrones plastoquinona QA acepta electrones fotosinteticos, sin embargo no puede aceptar otro hasta que electrón haya pasado al receptor siguiente, plastoquinona QB (Rosa, 2016). De esta manera, Brevis® actúa bloqueando la transferencia de electrones entre los receptores QA y QB del fotosistema II (PSII) (McArtney *et al.*, 2012). Esta interrupción del transporte de electrones fotosintéticos inhibe la producción de adenosina 5´-trifosfato y la fijación de carbono (McArtney et al., 2012) e induce automáticamente el cierre de los centros de reacción reduciendo la eficacia de la fotosíntesis (Rosa, 2016). Dicha inhibición en fijación de carbono y la producción de ATP provoca una reducción de la producción de carbohidratos en el árbol, y en consecuencia un estrés. Esta situación causa que los carbohidratos producidos se envíen a los brotes en lugar de los frutos (ADAMA, New Zealand 2017) y los que se envían a la frutos se dirigen al fruto dominante provocando que los frutos más pequeños dejan de crecer y caigan (ADAMA, New Zealand 2017).

La dosis máxima de aplicación según el registro es de 2.2 kg/ha para una y dos aplicaciones, sin embargo, es importante regular la dosis y el número de dependiendo de la variedad, ya que la susceptibilidad de las variedades al aclareo es diferente. La decisión de cuándo aplicar un producto de aclareo químico está condicionada por el tamaño de fruto, las dosis, el número de aplicaciones y las condiciones climáticas. Todos estos condicionantes son elementos cruciales para cualquier programa de aclareo y condicionarán la eficacia de la aplicación.

Además, Stern (2014) demostró que Brevis® es más eficaz en los países cálidos debido a las altas temperaturas nocturnas y la mayor intensidad de la respiración oscura en el momento crítico del crecimiento de los frutos en comparación con los países más fríos. Las altas temperaturas nocturnas aumentan la respiración y la sensibilidad de los frutos a la deficiencia de fotoasimilados (Lakso, 2011; Stern, 2015). Investigaciones anteriores demostraron que temperatura, humedad y radiación son factores importantes antes y después de la aplicación de cualquier producto de aclareo (Stover and Greene, 2005). Además, las altas temperaturas nocturnas favorecen e incrementan el efecto de aclareo (Costa *et al.*, 2018). Por otro lado, Parra-Quezada *et al.* (2005) mostraron que un buen aclareo puede asociarse con un periodo de calentamiento de 5 días después de la aplicación del producto de aclareo. Sin embargo, no hay trabajos para identificar los factores climáticos clave que expliquen la eficacia de Brevis® año a año.

En la actualidad cada vez son más comunes las tormentas de granizo en las zonas productoras de manzanas de España, causando daños significativos en los frutos y árboles. Actualmente, las redes contra el granizo son utilizadas frecuentemente por los productores españoles. Sin embargo, la red reduce la incidencia de radiación fotosintéticamente activa (PAR) y, por lo tanto, altera el medio ambiente debajo de la red (Ordonez *et al.*, 2016). Esto reduce y modifica la luz que llega al dosel del árbol (Kalcsits *et al.*, 2017). Esta situación junto a la aplicación de Brevis® que causa la inhibición de la fotosíntesis a un corto periodo de tiempo, llevó a considerar los posibles efectos de las redes contra el granizo en la eficacia de Brevis®.

Objetivos de la tesis

Objetivos de la tesis

El aclareo de frutos en manzano es uno los procesos productivos clave porque tiene una influencia determinante en la calidad del fruto y en la floración de retorno de la campaña siguiente. Ambos factores son fundamentales a la hora de establecer la rentabilidad económica de las plantaciones de manzano. A pesar de que con anterioridad se habían realizado numerosos estudios sobre el efecto de aclareo de Brevis®, en general se limitaban a estudiar su eficacia, el número óptimo de aplicaciones y el modo de acción.

El objetivo general de la tesis fue evaluar la eficacia en el aclareo de Brevis® en diferentes variedades, momentos de aplicación, dosis, número de aplicaciones y condiciones meteorológicas. Además, también se analizó el efecto de Brevis® sobre la producción y la calidad (color, calibre y peso) del fruto, y la floración de retorno.

Para alcanzar este fin los objetivos específicos de la tesis fueron:

1. Evaluar el efecto de aclareo de una aplicación de Brevis® a 1.65 kg/ha en 'Gala' y 2.2 kg/ha en 'Fuji' aplicada a diferentes tamaños de fruto (diámetro del fruto central de 6.5 a 21.5 mm), para determinar en qué tamaños de fruto Brevis® muestra eficacia de aclareo y en qué momento la susceptibilidad del fruto es mayor (Capítulo I).

2. Identificar los factores climáticos que afectan a la eficacia de Brevis® en el aclareo (Capítulo II).

3. Determinar la relación entre dosis de Brevis® (1.1 a 4.4 kg/ha) y su eficacia en aclareo en las variedades 'Gala' y 'Fuji' (Capítulo III).

4. Analizar la inhibición de la fotosíntesis causada por Brevis® mediante la fluorescencia clorofílica en diferentes momentos (diámetro de fruto central entre 6.5 y 21.5 mm) y dosis (1.1 a 4.4 kg / ha) (Capítulo I y III).

5. Evaluar la eficacia de aclareo de una y dos aplicaciones de Brevis® y la inhibición de la fotosíntesis en 'Gala' en aplicaciones a 1.65 kg/ha en diferentes tamaños del fruto (diámetro de fruto central entre 7.5 y 13.5 mm) (Capítulo IV).

6. Evaluar el efecto del sombreo ocasionado por las redes antigranizo y la inhibición causada por Brevis® en la eficacia del aclareo, la producción, la calidad del fruto y la fluorescencia en 'Gala', 'Fuji' y 'Pink Lady' (Capítulo V).

Capítulo I.

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

Gonzalez, L., Bonany, J., Alegre, S., Àvila, G., Carbó, J., Torres, E., Recasens, I., Martin, B., Asin, L. (2019). 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

Highlights

- Maximum efficacy of Brevis® was observed in the 11.5-14 mm range.
- Brevis® thinning effect was observed at king fruit diameters from 9 to 19 mm.
- Susceptibility to Brevis® differed between varieties and years.
- The fluorescence analysis using a biexponential equation showed adequate fits.
- The different sections of shoots showed different fluorescence inhibition.

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.

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)

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.

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	

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:

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

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.

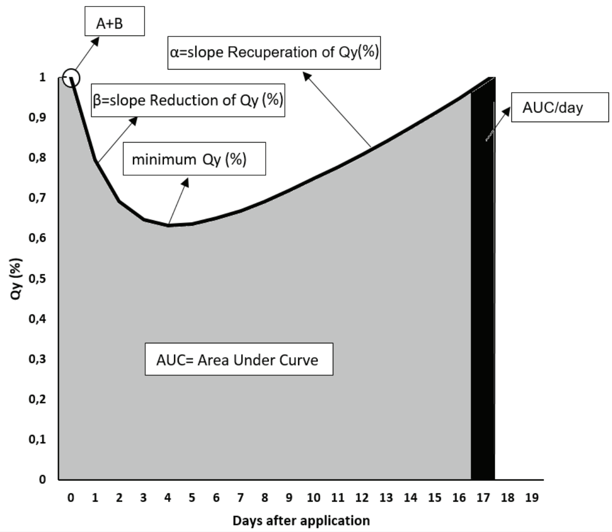


Figure 1: Graphic representation of the parameters calculated with the biexponential pharmacokinetic model (AUC, AUC/day, A, α, B and β).

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

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.

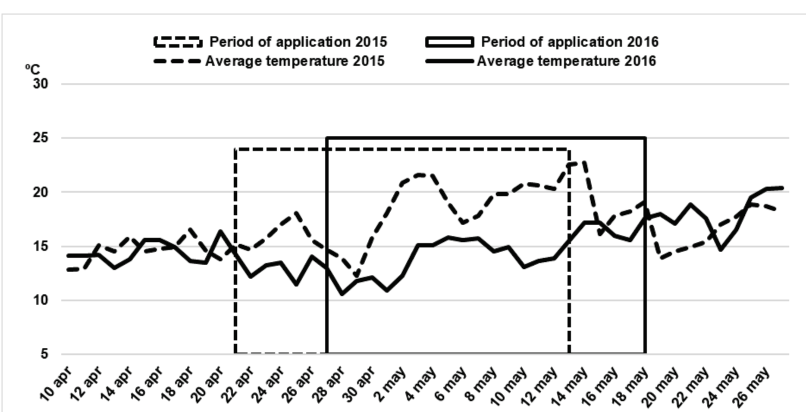


Figure 2: Average temperatures in the period of application in 2015 and 2016.

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.

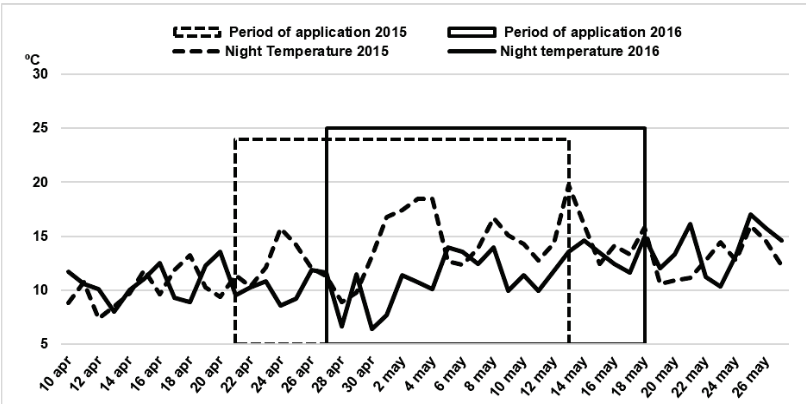


Figure 3: Average night temperature in the period of application in 2015 and 2016.

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).

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 $P<0.05$

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 favourable 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.

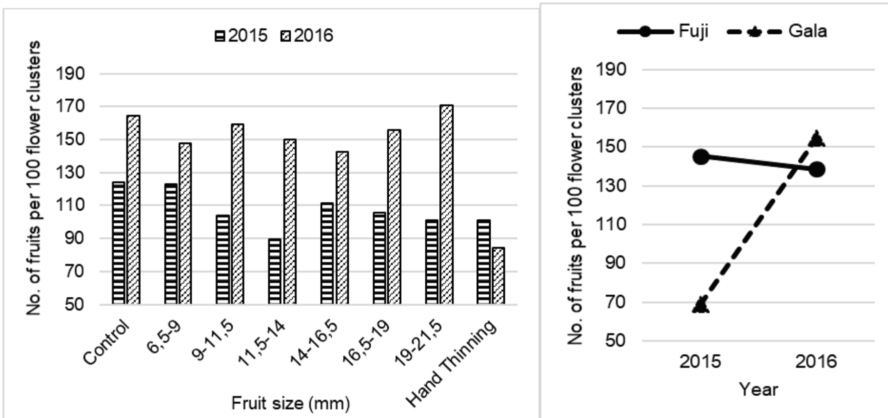


Figure 4: Effect of treatments in 2015 and 2016 on fruit set (A); Effect of fruit set in different years and varieties (B)

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.

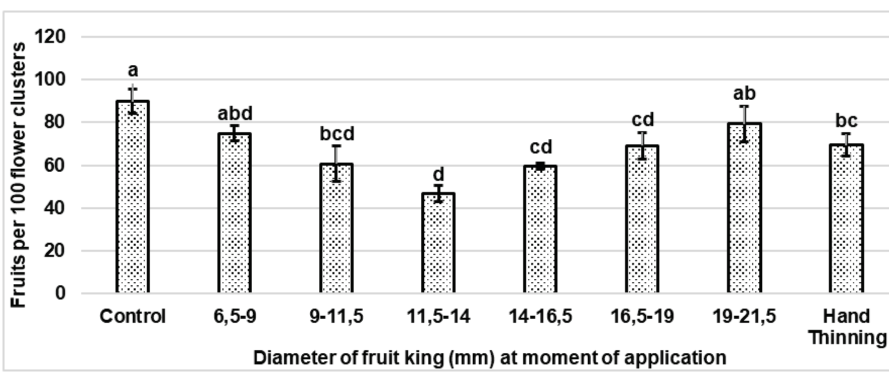


Figure 5: Average fruit set (fruits per 100 flower clusters) on ‘Gala’ in 2015. Different letters denote significant differences (Duncan's range test at P<0.05).

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).

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

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).

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

Year	Fruit Size (FS)	'Fuji'		'Gala'	
		Qy(%)		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

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).

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

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).

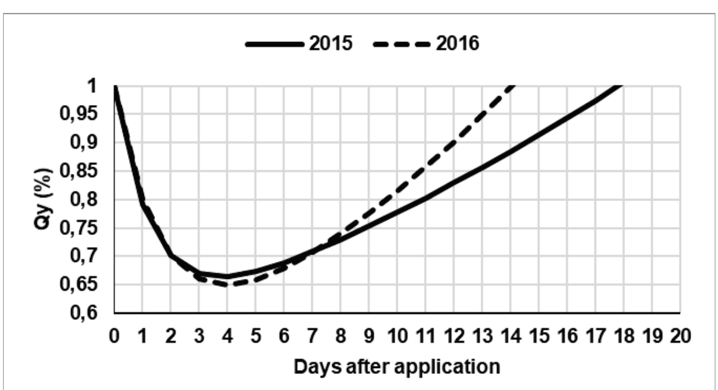


Figure 6: Graphic representation of the parameters estimated with the biexponential pharmacokinetic model (A, α, B and β) for the years 2015 and 2016

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 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).

	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)**
Fruit Size (FS)										
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 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'.

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

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.

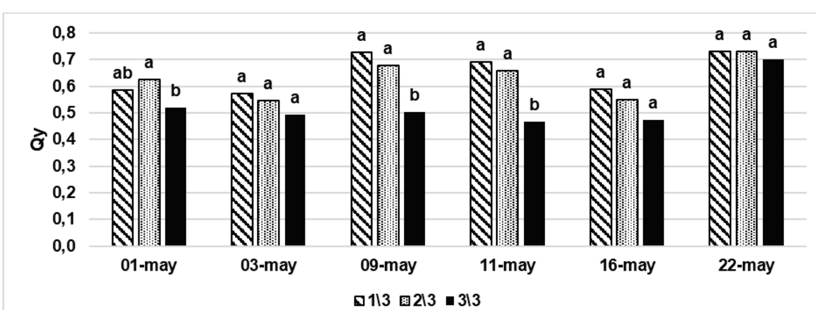


Figure 7: Value of Control Qy in 'Gala' 2016. The Control measurements were taken four days after Brevis® application. The shoots were divided into 3 sections: section 1/3 closest to branch, 2/3 mid-shoot and 3/3 vegetative section Different letters denote significant differences (Duncan's range test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

'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).

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

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 favouring 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.

Capítulo II.

Determination of main weather conditions which influence Brevis® efficacy in apple fruit thinning.

Gonzalez, L., Àvila, G., Carbó, J., Bonany, J., Alegre, S., Torres, E., Recasens, I., Martin, B., Asin, L. **(in press)**. Determination of main weather conditions which influence Brevis® efficacy in apple fruit thinning.
Scientia Horticulturae.

Abstract

The efficacy of chemical thinning and the degree of abscission are highly dependent on environmental and cultivar factors. Brevis® chemical thinning fruit abscission varies significantly year-to-year. The objective of this work was to identify the key environmental data that explain Brevis® efficacy year-to-year. Trials were conducted over four seasons from 2013 to 2016 in 'Gala' apple orchards in Lleida and Girona. The commercial chemical thinner Brevis® (metamitron) was applied at a rate of 1.65 kg/ha, with fruit size at time of application ranging between 8 and 16 mm. The weather data evaluated were evapotranspiration (Penman-Monteith-FAO formula: PM-ETo), relative humidity, radiation, average 24-h, day-time and night-time temperatures, and average 24-h, day-time and night-time wind speeds. These weather data were analyzed in 3- and 5-day periods before and after Brevis® application. Multiple regression models were built to predict fruit number per tree with the weather variables and number of flower clusters per tree as covariate. Our results suggest that wind speed and day-time, night-time and 24-h temperatures were important before and after Brevis® application, and that PM-ETo was important after Brevis® application. However, in the Spanish conditions of the study, radiation and humidity had no impact on Brevis® efficacy. The weather models to predict Brevis® efficacy showed, in descending order, highest sensitivity to night-time temperature and 24-h temperature, followed by day-time temperature, PM-ETo, night-time wind speed and, finally, 24-h wind speed. Nevertheless, the joint analysis model which considered all weather variables selected 24-h and night-time temperatures both before and after Brevis® application as the best weather variables to predict final fruit number per tree.

1. Introduction

As apple trees can produce an excessive number of fruitlets, fruit thinning is important to ensure crop value maximisation (Byers, 2003). Appropriate thinning must be done on a year-to-year basis because of the benefits to fruit size, colour, and the regulation of alternance. However, this involves a complex management strategy which is a major determining factor in the profitability of apple orchards (Dennis, 2000; Lordan *et al.*, 2019; Robinson *et al.*, 2013). Since hand thinning is an expensive and laborious practice, the use of chemicals to thin flowers or fruitlets is customary (Iwanami et al., 2012). However, the efficacy of chemical thinning and the degree of abscission are strongly dependent on environmental and cultivar factors, which can create inconsistent results (Byers, 2003; Iwanami et al., 2012; Lordan et al., 2018; Robinson and Lakso, 2004). The objective of chemical thinning is to reduce tree crop load and obtain a more uniform production over the years.

The abscission of fruitlets with thinning agents is a complex interaction between environmental conditions, cultivar, fruit size and tree vigour (Rosa, 2016). Temperature, humidity and radiation levels are also important factors that need to be taken into account. Byers (2003) reported that low light conditions and periods of high night-time temperatures favour the abscission of fruitlets. Such conditions stimulate a carbohydrate deficit in the fruit. The tree favours the carbohydrate demand, and hence the growth, of shoots over that of fruit, in this way determining sensitivity to chemical thinning (Iwanami et al., 2012; Lakso et al., 2006).

Brevis® (metamitron 15% SG) is a photosynthetic inhibitor (Basak, 2011; Lafer, 2010) whose mode of action is different from that of other thinning products. 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 inhibition reduces carbohydrate production by the tree, producing stress in the tree and resulting in carbohydrates being sent to shoots rather than fruit (ADAMA, New Zealand 2017). Those carbohydrates that are sent to fruit are directed to the dominant king fruit and the smaller fruitlets stop growing and will drop (ADAMA, New Zealand 2017). The application rate can be varied from 1.1 to 2.2 kg/ha in one or two applications depending on the variety. According to Stern (2014), Brevis® is more effective in warm countries because of high night-time temperatures and the higher intensity of dark respiration at the critical time of fruitlet growth in comparison with colder countries. High night-time temperatures increase respiration and fruitlet sensitivity to photoassimilate deficiency (Lakso, 2011; Stern, 2015).

While numerous studies have been carried out on fruit abscission with Brevis, these have generally been limited to studying its efficacy, optimal number of applications and mode of action. For this reason, the objective of the present study was to identify the key environmental data that explain Brevis® efficacy on a year-to-year basis.

2. Material and methods

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

The trials were conducted in apple orchards of the IRTA Experimental Station of Lleida (Mollerussa and Gimenells, NE Spain) and the IRTA experimental agricultural station of Mas Badia (Tallada d'Emporda, NE Spain) during the seasons of 2013 and 2016. The strategies tested the use of the commercial chemical thinner Brevis® (ADAMA, Spain). The application rate was 1.65 kg/ha and water volume was equivalent to 1000 l/ha. The time of application was determined by measuring the king fruit diameter, with fruit sizes between 8 and 16 mm (Table 1). 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.

Table 1. Principal characteristics of the orchards used for the trials and king fruit diameters at time of Brevis® application.

Location	Variety	Rootstock	Density plantation	Training system	Planted	No. of treatments and year	Diameter at application (mm)
Gimenells	'Brookfield 'Gala''	M9	1786 trees/ha (4m x 1.4m)	Central leader	2006	1 (2013)	8
						1 (2014)	8
						6 (2016)	8, 10, 11, 13 and 16
Mas Badia	'Galaxy 'Gala''	M9	2666 trees/ha (3.75m x 1m)	Central leader	1994	1 (2014)	8
					2000	1 (2014)	8
						4 (2015)	8, 10, 11 and 14
						4 (2016)	9, 11 and 13
Mollerussa	'Brookfield 'Gala''	M9	1786 trees/ha (4m x 1.4m)	Central leader	2003	1 (2014)	8
						6 (2015)	8, 10, 13, 15 and 16
						5 (2016)	8, 9, 10, 12 and 14

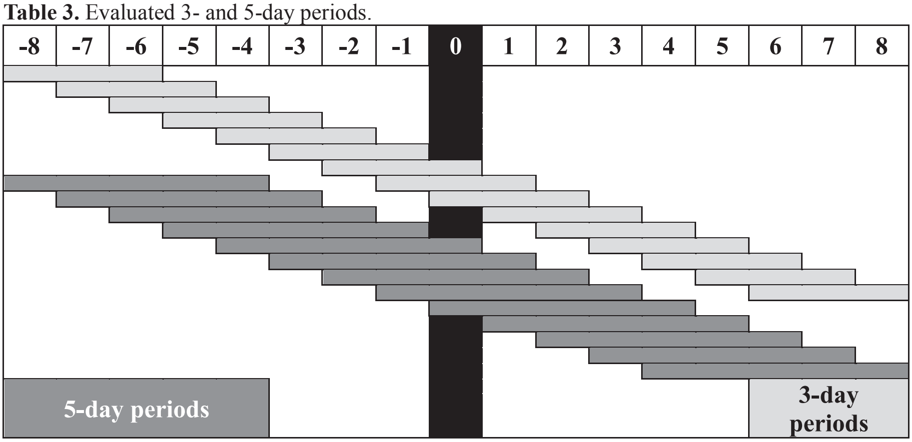
Meteorological data were collected from weather stations of the official meteorological service of Catalonia. The stations were located in the Mas Badia, Mollerussa and Gimenells orchards. In all the study years, the recorded meteorological parameters evaluated in this study were downloaded and analyzed after the period of (Table 2). Table 2 also shows the period used to calculate the average value.

Table 2. Weather data evaluated and period of day used to calculate the average value.

Weather data evaluated	Period	Sensor used or calculated variable
Evapotranspiration (PM-ETo) (mm/day)	00:00-24:00	Penman-Monteith-FAO formula with temperature, relative humidity, radiation and wind speed
Relative humidity (%)	00:00-24:00	Sensor Vaisala HUMICAP® HMP155 (Helsinki, Finland)
Radiation (MJ/m^2)	08:00 to 20:00	Sensor Kipp&Zonen SMP6-V Pyranometer (Delft, Netherlands)
Day-time temperature (ºC)	08:00 to 20:00	Sensor Vaisala HUMICAP® HMP155 (Helsinki, Finland)
24-h temperature (ºC)	00:00-24:00	Sensor Vaisala HUMICAP® HMP155 (Helsinki, Finland)
Night-time temperature (ºC)	21:00 to 7:00	Sensor Vaisala HUMICAP® HMP155 (Helsinki, Finland)
24-h wind speed (m/s)	00:00-24:00	Sensor Young 05103 (Traverse City, Michigan USA)
Day-time wind speed (m/s)	8:00 to 20:00	Sensor Young 05103 (Traverse City, Michigan USA)
Night-time wind speed (m/s)	00:00-24:00	Sensor Young 05103 (Traverse City, Michigan USA)

Preliminary work at the IRTA facilities showed that the maximum inhibition caused by a single application of Brevis® was maintained for 8 days. For this reason, it was decided to extend our analysis from 8 days before to 8 days after application. Day *0* is the day of application (Table 3). The 8 days before application (negative numbers) and after application (positive numbers) correspond to the overall time frame. The average value of each parameter in Table 2 was calculated for series of 3- and 5-day periods within this time frame. Table 3 shows the separate 3- and 5-day periods that were used for the analysis. For example, the 3-day period *-2/0* covers the two days before application and the day of application. In this way, it was possible to evaluate which weather data and which periods are the most important for Brevis® efficacy.

Table 3. Evaluated 3- and 5-day periods.



All trials were arranged with four replicates of four uniform trees per elementary plot. Each replicate was located in different zones of the same orchard with the objective of homogenizing orchard variability. On each plot, the central trees were used for the trial assessments.

2.2. Yield assessments

The assessments were carried out on central trees of each elementary plot with the objective of assessing the effect of the treatments on fruit yield parameters. The total number of flower clusters per tree was counted at bud break stage (BBCH 61-65). In each orchard, harvesting was performed during the commercial harvest season separately for each selected tree. Fruit weight and fruits per tree were measured with a commercial apple sorting and packing line machine, Maf Roda (Agrobotic, France) in Lleida and Calinda (Caustier Ibérica, S.A. with Aweta Technology) in Mas Badia.

2.3. Data analysis

A partial least squares (PLS) regression was applied to determine the importance of each weather variable in the model. All the weather variables were analyzed together for the 3-day periods and again separately for the 5-day periods. The two PLS outputs used were the variable importance in projection (VIP) scores and the standardized model coefficients (Guo et al. 2015b). Based on these PLS outputs, important weather variables were identified when VIP values were greater than 1 and the standardized model coefficients were higher than +0.02 and lower than -0.02. Table 4 shows the variables analyzed for the PLS and the variables selected for analysis with a multiple regression model.

Scatter plots were then generated to identify the relationships between Brevis® efficacy and the weather variables. Linear terms for all weather variables for the different 3- and 5-day periods and flower cluster number per tree were considered as regressor variables in the multiple regression model to explain the variability observed in the final fruit number per tree after Brevis® application.

Each weather variable was analyzed individually, but in this case the 3- and 5-day periods were included together. So, for example, in the night-time temperature model the 3- and 5-day periods were analyzed together.

Finally, for the joint analysis model, all the 3- or 5-day period weather variables selected in the individual models were analyzed together to identify which were the most important.

The multiple regression model was run iteratively, with the most complex interaction term with the highest P value deleted from the model before being run again. This manual backward elimination continued until only significant ($P < 0.05$) terms remained in the model (Milliken and Johnson, 2001). Data were analyzed using the JMP statistical software package (Version 13; SAS Institute Inc., Cary, North Carolina).

Table 4. Weather data evaluated in the PLS and multiple regression models.

Weather variable evaluated	**PLS**	**Multiple regression**
PM-ETo	X	X
Relative humidity	X	
Radiation	X	
24-h temperature	X	X
Day-time temperature		X
Night-time temperature		X
24-h wind speed	X	X
Day-time wind speed		X
Night-time wind speed		X

3. Results

3.1. Number of flower clusters and fruit number per tree

For 'Gala', the number of flower clusters per tree ranged from 163 to 321 and fruit number per tree from 134 to 534 (Figure 1). A positive linear relationship was found between these two variables, with P<0.0033 (Figure 1). Fruit number per tree increased with flower clusters, although the correlation had a low R^2 value of 0.27. This is due to differing tree performance after Brevis® application, as Brevis® efficacy is conditioned by the moment of application. This result confirmed the need to conduct further studies on the relationship between apple load management and Brevis® to gain a better understand of the interaction between the degree of abscission of Brevis® and environmental factors.

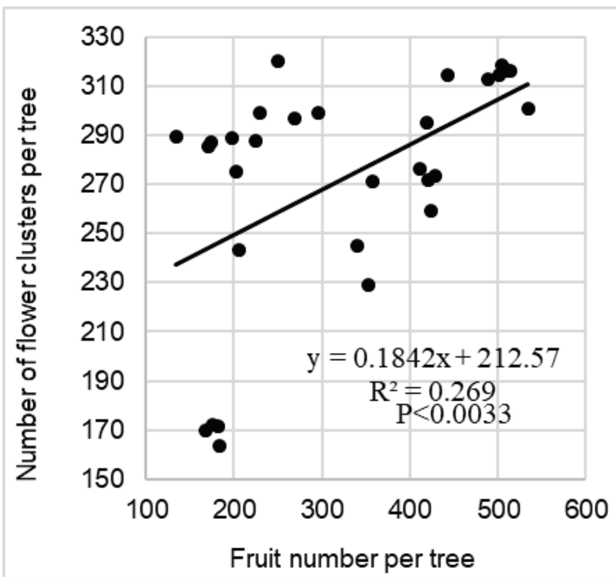


Figure 1: Scatter plot showing the relationship between number of flower clusters per tree and final fruit number per tree for 'Gala'. Each symbol represents 1 tree per treatment (2013–2016).

3.2. PLS

Figure 2A shows the important parameters in the 3-day periods: flower clusters per tree, evapotranspiration, 24-h temperature and 24-h wind speed. With respect to the 5-day periods, the important parameters were flower clusters per tree, 24-h temperature and 24-h wind speed (Figure 2B). With respect to the other weather parameters included in the PLS, radiation and relative humidity in the Spanish conditions of the study were considered not important in explaining Brevis® efficacy as their VIP scores were lower than 1 and the standardized model coefficients were between -0.02 and 0.02.

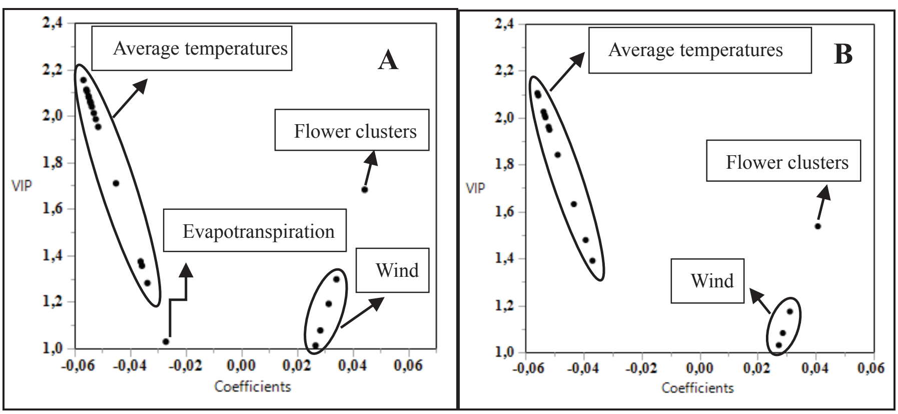


Figure 2: Plot A showing VIP vs Coefficients for centered and scaled data for 3-day periods. Plot B showing VIP vs Coefficients for centered and scaled data for 5-day periods. Parameters analyzed were flower clusters per tree, evapotranspiration, relative humidity, radiation, 24-h temperature and 24-h wind speed.

3.3. Multiple regression model

3.3.1. Evapotranspiration (PM-ETo)

A multiple regression model was constructed using the 3- and 5-day periods of PM-ETo values to predict fruit number per tree at harvest after Brevis® application (Figure 3). The final model had an adjusted R^2 value of 0.39 and the statistically significant regressive variables ($P<0.05$) were number of flower clusters per tree, 3-day PM-ETo (0/2) and 3-day PM-ETo (6/8). The prediction profiler shows fruit number per tree was positively related to number of flower clusters and negatively related to both the PM-ETo parameters. That is, as PM-ETo increased, fruit number per tree decreased and Brevis® efficacy was higher (Figure 3).

PM-ETo model for No. fruits per tree

Summary of Fit

RSquare	0,466864
RSquare Adj	0,397325
Root Mean Square Error	101,0013
Mean of Response	326,0028
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	205463,65	68487,9	6,7137
Error	23	234629,26	10201,3	Prob > F
C. Total	26	440092,91		0,0020*

Parameter Estimates

Term	Std Error	t Ratio	Prob>|t|
Intercept	166,111	1,85	0,0769
Flower clusters per tree	0,453356	3,66	0,0013*
(3 days) PM-ETo (0/2)	25,72886	-2,38	0,0261*
(3 days) PM-ETo (6/8)	20,12956	-2,76	0,0112*

Prediction Profiler

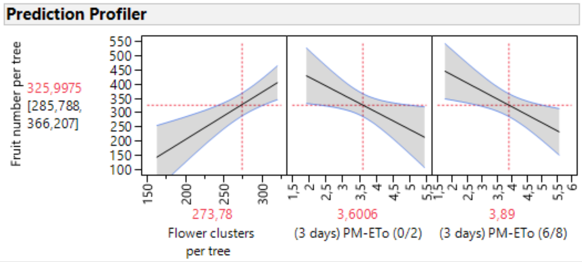


Figure 3: Summary of fit, analysis of variance, parameter estimates, and prediction profiler of 'Gala' model built to predict fruit number per tree at harvest after Brevis® applications using 3- and 5-day PM-ETo periods. Model coefficients are initial number of flower clusters per tree, 3-day average PM-ETo between day of Brevis® application and day 2 after application (0/2), and 3-day average PM-ETo between days 6 and 8 after Brevis® application (6/8).

3.3.2. 24-h temperature

The model that was built to predict fruit number per tree with average 24-h temperature gave an adjusted R^2 value of 0.72 (Figure 4). For this model, the significant regressor variables were number of flower clusters per tree, and average 24-h temperature for the 5-day periods of *0/4* and *-5/-1*. The prediction profiler shows fruit number per tree was positively related to number of flower clusters per tree and negatively related to average 24-h temperature both before and after Brevis® application. That is, as average 24-h temperature increased, fruit number per tree decreased and Brevis® efficacy was higher (Figure 4).

24-h temperature model for No. fruits per tree

Summary of Fit

RSquare	0,753336
RSquare Adj	0,721163
Root Mean Square Error	68,70067
Mean of Response	326,0028
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	331537,92	110513	23,4148
Error	23	108554,99	4720	Prob > F
C. Total	26	440092,91		<,0001*

Parameter Estimates

Term	Std Error	t Ratio	Prob>|t|
Intercept	117,8491	4,49	0,0002*
Flower clusters per tree	0,305772	5,33	<,0001*
(5 days) 24-h temperature (-5/-1)	8,419587	-2,11	0,0462*
(5 days) 24-h temperature (0/4)	8,46233	-3,13	0,0046*

Prediction Profiler

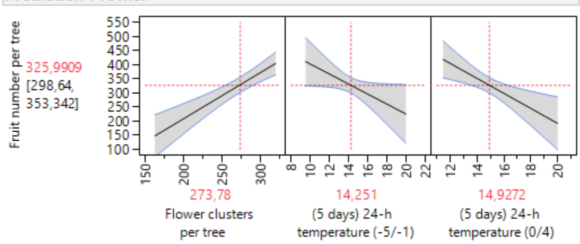


Figure 4: Summary of fit, analysis of variance, parameter estimates, and prediction profiler of 'Gala' model built to predict fruit number per tree at harvest after Brevis® applications using 3- and 5-day 24-h temperature periods. Model coefficients are initial number of flower clusters per tree, 5-day 24-h temperature between day of application and day 4 after Brevis® application (0/4), and 5-day average temperature between days 5 and 1 before Brevis® application (-5/-1).

3.3.3. Day-time and night-time temperature

For 'Gala', the night-time temperature model to predict fruit number had a higher adjusted R^2 value than the day-time temperature model and the same adjusted R^2 value as the 24-h temperature model (0.72, 0.67 and 0.72 respectively) (Figs. 4, 5A and B). For the night-time temperature model, the significant variables were number of flower clusters per tree, the 5-day period before application encompassing days *-5/-1*, and the 3-day period after Brevis® application encompassing days *1/3* (Figure 5B). For the day-time temperature model, the significant variables were number of flower clusters per tree and the 5-day period encompassing days *-1/3* (Figure 5A). In both models, the correlation was positive between number of flower clusters per tree and final fruit number per tree. Fruit number per tree was negatively related to average day-time and night-time temperature both before and after Brevis® application (Figure 5A & B). The average 24-h temperature (Figure 4) was calculated from the average day-time and night-time temperatures. Given that the adjusted R^2 values of the night-time and 24-h temperature models are the same and higher than that of the day-time model, night-time temperature has more importance than day-time temperature in the 24-h temperature and, hence, it can be concluded that night-time temperature has more importance in predicting Brevis® efficacy.

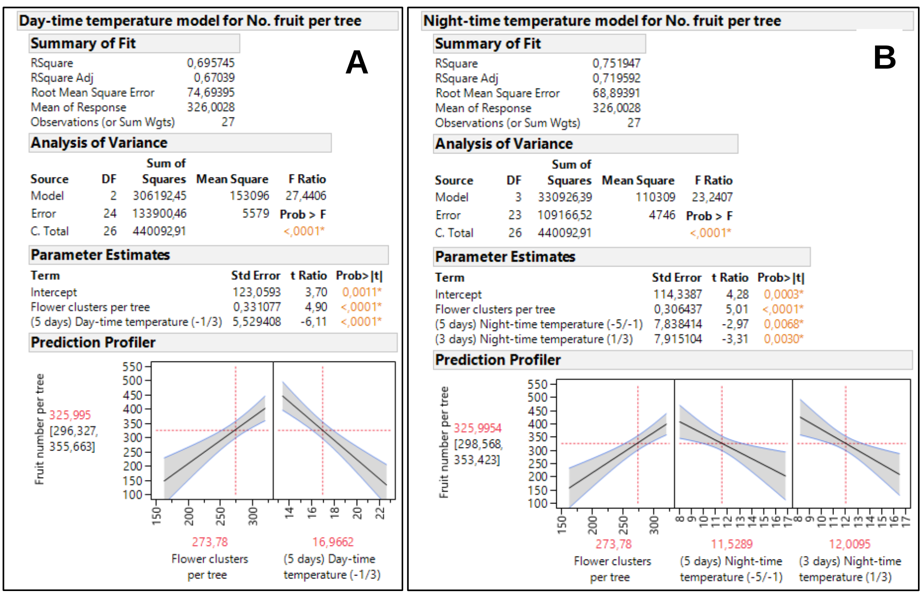


Figure 5: Summary of fit, analysis of variance, parameter estimates, and prediction profiler of 'Gala' model built to predict fruit number per tree at harvest after Brevis® applications using day-time temperature **(A)** and night-time temperature **(B)** for 3- and 5-day periods. **A.-** Model coefficients are initial number of flower clusters per tree and 5-day average day-time temperature between 1 day before application and day 3 after application (-1/3). **B.-** Model coefficients are initial number of flower clusters per tree, 3-day average night-time temperature between days 1 and 3 after Brevis® application (1/3), and 5-day average night-time temperature between days 5 and 1 before Brevis® application (-5/-1).

3.3.4. Wind speed

The 24-h wind speed model to predict fruit number had a lower adjusted R^2 value (0.30) than the other weather variable models (Figure 6A). The significant variables were number of flower clusters per tree and the 5-day period encompassing days *-1/3*. The correlation was positive between fruit number per tree and both number of flower clusters per tree and 24-h wind speed. That is, as wind speed after Brevis® application increased, fruit number per tree increased and Brevis® efficacy was lower (Figure 6A).

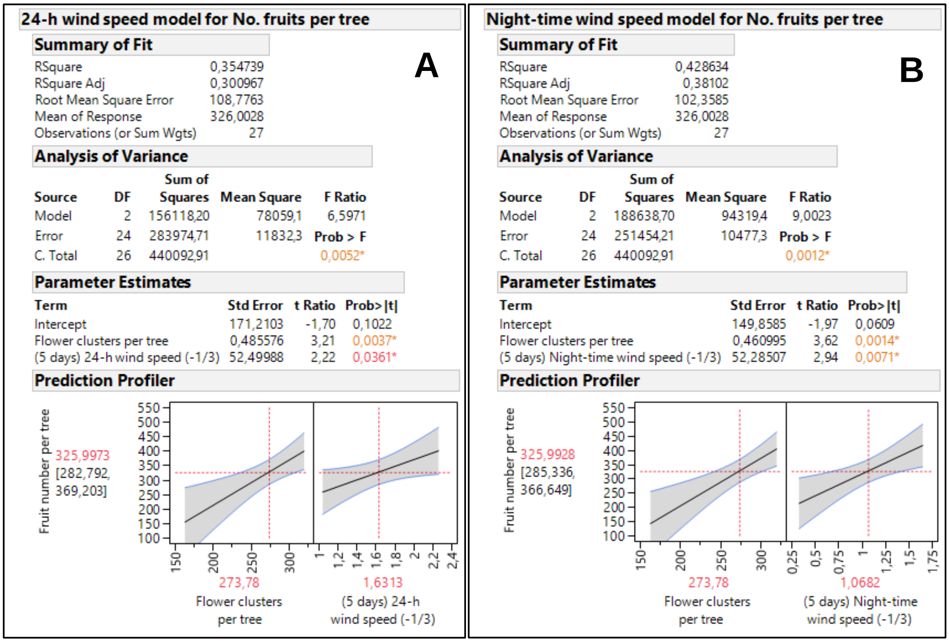


Figure 6: Summary of fit, analysis of variance, parameter estimates, and prediction profiler of 'Gala' model built to predict fruit number per tree at harvest after Brevis® applications using 24-h wind speed **(A)** and night-time wind speed **(B)** for 3- and 5-day periods. **A.-** Model coefficients are initial number of flower clusters per tree, 5-day period wind speed between day 1 before Brevis® application and day 3 after Brevis® application (-1/3). **B.-** Model coefficients are initial number of flower clusters per tree, 5-day period wind speed between day 1 before Brevis® application and day 3 after Brevis® application (-1/3)**.**

As the night-time wind speed (Figure 6B) model had a higher adjusted R^2 (0.38) than the 24-h wind speed model (0.3) it was concluded that night-time wind speed was better correlated with fruit number per tree than day-time wind speed. The significant variables were number of flower clusters per tree and the 5-day period encompassing days *-1/3*. The correlation was positive between final fruit number per tree and both number of flower clusters per tree and night-time wind speed. That is, as night-time wind speed increased, fruit number per tree increased and Brevis® efficacy decreased (Figure 6B).

3.3.5. All parameters selected

Figure 7 shows all the weather parameters selected for the joint analysis. The best model to predict fruit number included, number of flower clusters per tree, 24-h temperature before application and night-time temperature after application (Figure 7). This model had the same adjusted R^2 value as the 24-h and night-time temperature models (0.72) to predict fruit number (Figure 4, 5B & 7). For this model, the significant variables were number of flower clusters per tree, 5-day period of 24-h temperature before Brevis® application encompassing days *-5/-1* and the 3-day period of night-time temperature after Brevis® application encompassing days *1/3* (Figure 7).

In this joint analysis, 5-day periods were selected for all weather variables, except for night-time temperature and evapotranspiration after application where 3-day periods were selected. Temperatures were the most important weather variables for prediction of Brevis® efficacy in the Spanish conditions of the study, with all temperature models having high adjusted R^2 values. Final fruit number per tree was negatively related with temperature before and after Brevis® application. That is, when temperature increased, fruit number per tree decreased and Brevis® efficacy was higher (Figure 4, 5A, 5B & 7). The most important temperature variables were average 24-h temperature before application and average night-time temperature after application (Figure 7).

All parameters selected model for No fruits per tree

SSE	DFE	RMSE	RSquare	RSquare Adj	Cp	p	AICc	BIC
107747,76	23	68,444759	0,7552	0,7232	-1,931354	4	313,356	316,9781

Current Estimates

Lock	Entered	Parameter	nDF	SS	"F Ratio"	"Prob>F"
☑	☑	Intercept	1	0	0,000	1
☐	☑	Flower clusters per tree	1	129911,3	27,731	2,42e-5
☐	☐	(3 days) PM-ETo (0/2)	1	1995,369	0,415	0,52605
☐	☐	(3 days) PM-ETo (6/8)	1	76,90534	0,016	0,90138
☐	☑	(5 days) 24-h temperature (-5/-1)	1	43317,34	9,247	0,00581
☐	☐	(5 days) 24-h temperature (0/4)	1	1938,93	0,403	0,53202
☐	☐	(5 days) Day-time temperature (-1/3)	1	754,3718	0,155	0,69749
☐	☐	(5 days) Night-time temperature (-5/-1)	1	946,5696	0,195	0,66311
☐	☑	(3 days) Night-time temperature (1/3)	1	47185,47	10,072	0,00424
☐	☐	(5 days) 24-h wind speed (-1/3)	1	637,0036	0,131	0,72102
☐	☐	(5 days) Night-time wind speed (-1/3)	1	1501,982	0,311	0,58269

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	332345,15	110782	23,6476
Error	23	107747,76	4685	Prob > F
C. Total	26	440092,91		<,0001*

Prediction Profiler

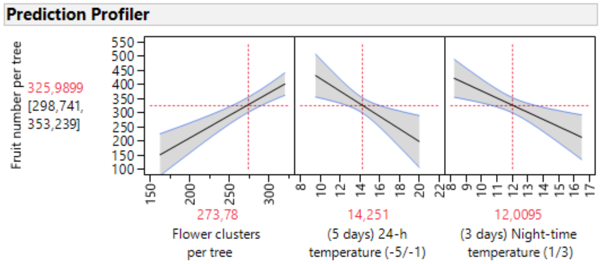


Figure 7: 'Gala' model built to predict fruit number per tree at harvest after Brevis® applications using all the selected weather variables and periods. The parameters selected were those used for the best model for prediction of Brevis® efficacy. Model coefficients are initial number of flower clusters per tree, 5-day average temperature between days 5 and 1 before Brevis® application (-5/-1) and 3-day average night-time temperature between days 1 and 3 after Brevis® application (1/3).

4. Discussion

Over the period of this study there were important differences in the number of flower clusters per tree, with 'Gala' flowering ranging between 163 and 321 flower clusters per tree. Flowering variability is dependent on the fruit yield and weather variables of the previous season. In this regard, Anthony *et al.* (2019) concluded that return bloom and flower bud development can be affected by temperature, rootstock, branch habit, hormones, light interception, pruning, spur leaf area and crop load.

The degree of abscission of Brevis® is highly dependent on environmental factors (Basak, 2011; Lordan et al., 2018; Mathieu et al., 2016). Conditions that lead to poor carbohydrate status are also associated with heavy drop and easier thinning: cloudy conditions, a heavy initial set on many weak spurs, stressed trees, photosynthesis inhibitors, natural or imposed low light periods, and high night-time temperatures can all cause or enhance fruit abscission (Lakso et al., 2006). Variations in the above factors explain the low correlation between the number of flower clusters and the number of fruits per tree at harvest.

Lakso and Robinson (2015) and Robinson et al. (2013) concluded that humidity was an important factor at the time of application. Many authors have also reported that radiation is an important weather factor in explaining chemical thinning efficacy (Byers et al., 1985; Grappadelli *et al.*, 1994; Greene and Groome, 2010; Lakso, 2011; Robinson et al., 2016). However, in the Spanish conditions of the present study, radiation and humidity had no effect on Brevis® efficacy. Nonetheless, results obtained by Gonzalez (unpublished data) when covering trees with shading nets during 4 days after Brevis® application caused strong apple fruitlet abscission. This shading, with a 50% radiation reduction, simulated 4 days of cloudy weather. That is, a reduction in radiation during various days after Brevis® application was found to be an important factor in Brevis® efficacy. In the experimental conditions of the present study, no application period was followed by 4 cloudy days, with 80% of such time periods including only 1 cloudy day. This explains why radiation had no impact in the trial conditions in the period of Brevis® application.

In all models, the final fruit number per tree was positively related to the initial number of flower clusters per tree. That is, final fruit number per tree increased with the number of flower clusters per tree, concurring with earlier observations of Lordan et al. (2019). Since initial number of flower clusters per tree was an important predictor variable of final number of fruits per tree, it was decided to use this parameter as a covariate to assess the impact of all the weather variables. This allowed in all models the normalizing of flowering intensity to assess the effect of the other variables as predictor variables.

The wind speed model to predict final fruit number per tree showed a low coefficient of determination. The most important period was the 5-day wind speed period encompassing days *-1/3*. This period, together with flowering, explained 30% of the final fruit number per tree. Ouma *et al.* (2005) concluded that wind speed during the time of spraying and immediately after had an effect on the efficacy of chemical thinners (NAD, ATS, Etefon and Urea). Moreover, according to Catania *et al.* (2011) wind speed is one of the most significant climatic factors influencing the efficiency of chemical distribution in vineyards. They concluded that when wind speed was equal to or greater than 2 m/s treatment efficiency was reduced by 40%, and when wind speed was 5 m/s the reduction was approximately 70%. Such an effect was possible in our study given that the average wind speed (in the 5-day period selected by the wind speed model) ranged between 1 and 2.3 m/s.

Comparing the day-time and night-time wind speed analyses, night-time wind speed was found to better explain final fruit number per tree, with the night-time wind speed model having a coefficient of determination 0.08 higher than the 24-h wind speed model). Hamacher *et al.* (1994) observed slow photosynthetic and respiration rates due to weak global radiation values below 1.3 Mj/m2 and wind speeds of 1.2-1.5 m/s. This concurs with the observations of the present study as in the selected period night-time wind speed ranged between 0.35-1.6 m/s. It is possible that night-time wind speed in this range can influence and reduce respiration and, consequently, photoassimilate consumption, making a carbohydrate deficit situation more difficult. The results in this respect in our study suggest that Brevis® efficacy is lower when night-time wind speed is in this range.

Evapotranspiration (ETo) and flowering explained 39% of the final fruit number per tree. ETo was calculated with the Penman-Monteith FAO formula (PM-ETo). This formula considers temperature, relative humidity, radiation and wind speed. However, radiation and humidity were generally constant throughout the trial periods, with more than one cloudy day being the exception. When considering the average radiation or relative humidity of the 3- or 5-day periods, the values were similar in all trials, with temperature and wind speed thus gaining in importance. Results published by Pirkner *et al.* (2014) for ETo models of table grape showed, in descending order, highest sensitivity to radiation, temperature and wind speed. Paltineanu and Chitu (2006) observed that initial fruit set was hampered during June by increases in PM-ETo and mean and maximum air temperature. Their results concur with the observations of this study, with higher average temperatures accompanied by higher ETo and greater Brevis® efficacy. The important period of PM-ETo was after Brevis® application.

Many authors have reported that temperature plays an important role in apple thinning efficacy with various chemical products (Kviklys and Robinson, 2010; Lakso et al., 2006; Li and Cheng, 2011; Lordan et al., 2019; Parra-Quezada et al., 2005; Pretorius *et al.*, 2011). Their results concur with the observations of this study, which indicate that temperature is an important factor in determining Brevis® efficacy. Brevis® efficacy increased with higher temperatures and final fruit number per tree decreased. The temperature models to predict Brevis® efficacy showed, in descending order, highest sensitivity to night-time temperature, 24-h temperature and day-time temperature.

The average 24-h temperature and flowering model explained 72% of final fruit number per tree. Our study results indicate that the important temperature period ranged between 5 days before to 4 days after application. That is, average temperatures both before and after application were important, concurring with earlier observations of Parra-Quezada et al. (2005). It should be noted however that Kviklys and Robinson (2010) reported that temperatures for the 5 days after chemical thinner application have a much greater impact on thinning efficacy than temperatures before application.

The most important period for the day-time temperature regression model encompassed the *-1/3* period, explaining 67% of final fruit number per tree. The results suggest that when day-time temperature increases, fruit number per tree decreases and Brevis® efficacy is higher. This coincides with Stern (2014), who concluded that higher metamitron efficacy in Israel was correlated with higher day-time temperatures, which can increase the efficiency of photosynthesis inhibition by metamitron compared to the lower temperatures in Europe. Previous reports by Parra-Quezada et al. (2005) with BA, Carbaryl and NAD also concluded that high temperatures following chemical thinner application increased the thinning effect.

The results of the night-time temperature model indicate that the most important period extends from 5 days before to 3 days after Brevis® application. The night-time temperature model explained 73% of final fruit number per tree. As night-time temperature increased, fruit number per tree decreased and Brevis® efficacy increased, which concurs with the observations of Costa et al. (2018), Stern (2014) and Robinson et al. (2016).

Our field study results indicate that night-time, 24-h and day-time temperature as well as 24-h and night wind speed were important both before and after Brevis® application. In addition, PM-ETo was important after Brevis® application. The weather models to predict Brevis® efficacy showed, in descending order, highest sensitivity to night-time temperature and all-day temperature, followed by day-time temperature, PM-ETO, night-time wind speed and finally 24-h wind speed. The best model in the joint analysis selected the 5-day 24-h temperature period before application and the 3-day night-time temperature period after application. This result suggests that night-time and average 24-h temperatures both after and before Brevis® application are the dominant factors influencing the response to the Brevis® thinner. Moreover, the other parameters (day-time temperature, PM-ETo and wind speed) were not selected in the final model. The average 24-h temperature was calculated with day-time and night-time temperature, with night-time temperature having more importance as carbon assimilation increases when night-time temperature is low. That is, increasing night-time temperatures may induce enhanced respiration during the night, which may in turn affect the overall carbon balance of the tree (Costa et al., 2018) and consequently increase the Brevis® effect. Competition is thus intensified among competing sinks at a time when metabolic demand is highest in the tree (Lakso et al., 2006; Yoon *et al.*, 2011). Moreover, Brevis® reduces carbohydrate production by inhibiting photosynthesis. Typically, warm nights increase respiration during the night-time, decreasing the overall carbon balance of the tree (Costa et al., 2018). Remaining carbohydrates are sent to shoots rather than fruit and the smaller fruitlets stop growing and will drop (ADAMA, New Zealand 2017). All of the above explain the importance of temperature and concur with the observations of this study, in which Brevis® thinning efficacy increased with temperature, especially the 24-h temperature during the 5-day period before Brevis® application and the night-time temperature in the 3-day period after.

5. Conclusions

For 4 years, we assessed the response of 'Gala' apple trees to Brevis® chemical thinning with respect to flower cluster intensity and various weather variables to better understand fruit abscission caused by Brevis®. Multiple regression models were built to predict fruit number per tree with weather variables and number of flower clusters per tree. In all models, the final fruit number per tree was positively related to initial flower cluster number per tree. Our results suggest that wind speed, day-time, night-time and average 24-h temperature were all important factors in Brevis® efficacy both before and after its application. In addition, PM-ETo was important after Brevis® application. However, in the study zone, radiation and humidity had no effect on Brevis® efficacy. The weather models to predict Brevis® efficacy showed the highest sensitivity to temperature. Nevertheless, the joint analysis model with all the weather variables selected average 24-h temperature before Brevis® application and night-time temperature after application as the best weather variables for prediction of the final fruit number per tree after Brevis® application.

In summary, although many factors have been reported to affect final fruit number per tree, in our study this variable could be relatively well modeled with number of flower clusters and average 24-h temperature and night-time temperature before and after Brevis® application.

Capítulo III.

Effect of different application rates of Brevis® as fruitlet chemical thinner on thinning efficacy and fluorescence inhibition in ‘Gala’ and ‘Fuji’ apple

Gonzalez, L., Torres, E., Carbó, J., Alegre, S., Bonany, J., Àvila, G., Martin, B., Recasens, I., Asin, L., (2019). 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 'Gala'xy 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 (Figure 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® (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 the 20 days after application (Figure 1) (Gonzalez et al., 2019b). Table 1 shows the calculations of the parameters.

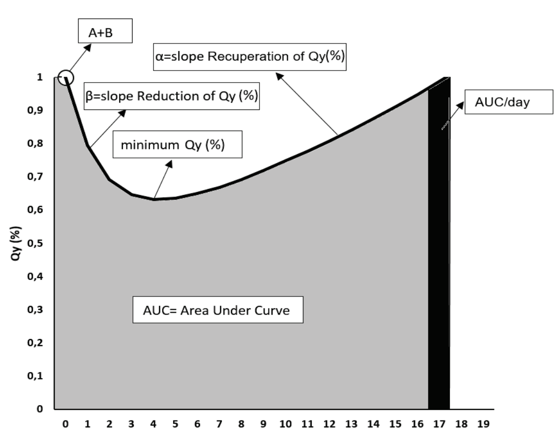

Figure 1: Graphic representation of the parameters calculated with the biexponential pharmacokinetic model (AUC, AUC/day, A, α, B and β) (Gonzalez et al., 2019b).

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 (Figure 2).

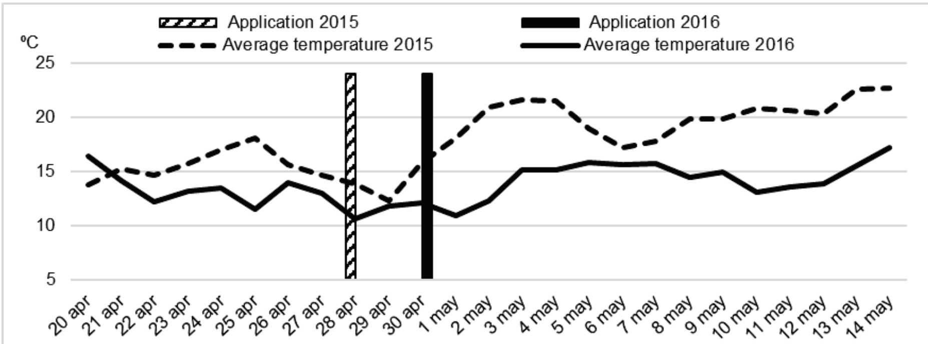

Figure 2: Average 24h temperature and date of application in 2015 and 2016

Figure 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.

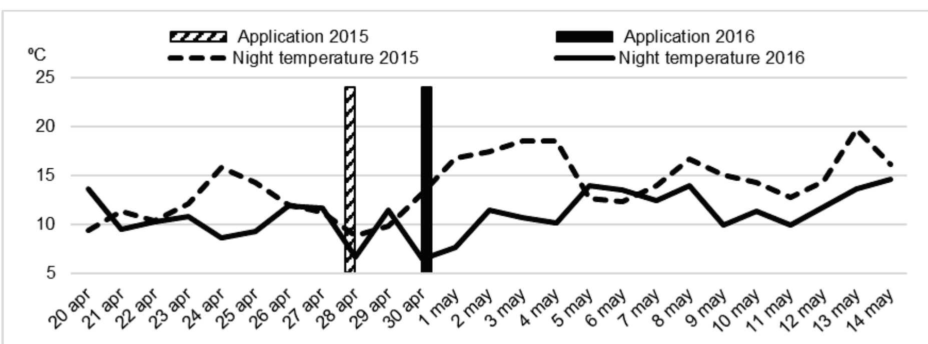

Figure 3: Average night temperature (average temperature when there was no solar radiation) and date of application in 2015 and 2016.

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, Figure 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 cm^2 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 (Figure 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 (Figure 4). Moreover, Brevis® thinning efficacy varied from year to year (Figure 4)

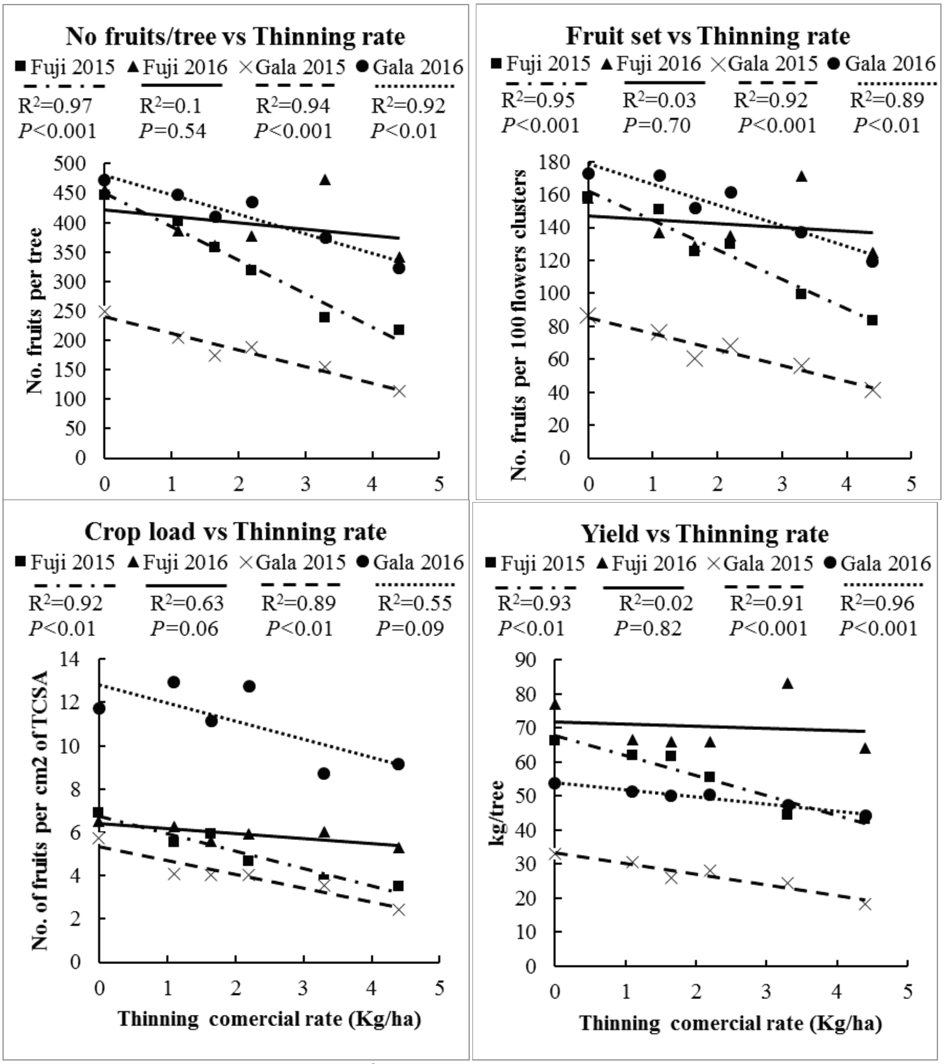

Figure 4: Relationships between Brevis® comerial rates and the number of fruit per tree, final fruit set, crop load and yield in 'Gala' and 'Fuji' trees.

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, Figure 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).

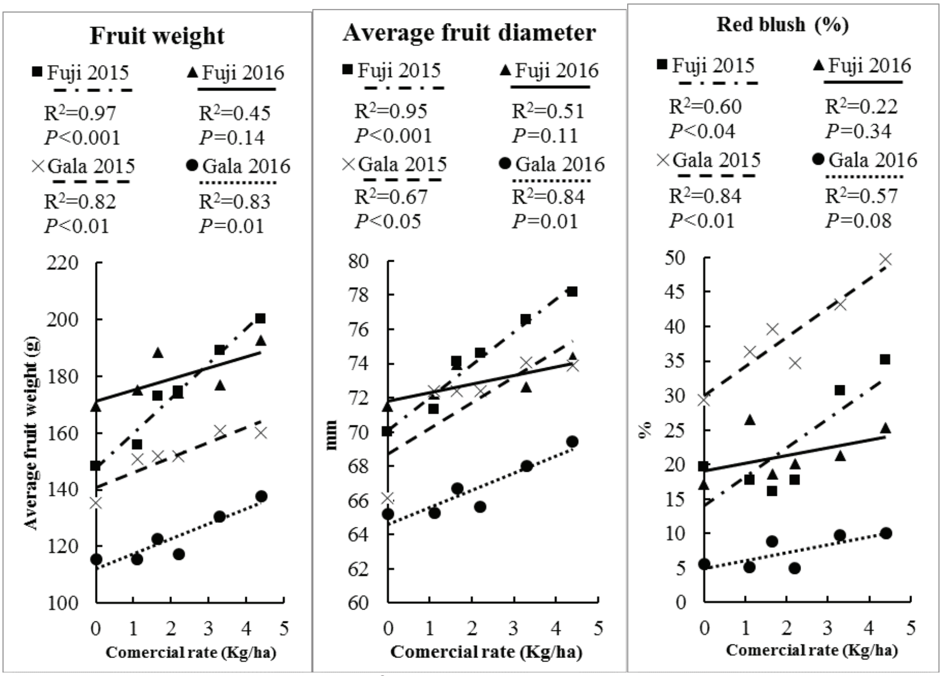

Figure 5: Relationships between Brevis® comerial rates and fruit weight average fruit diameter and average red blush (%) in apple in Mollerussa, Spain.

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).

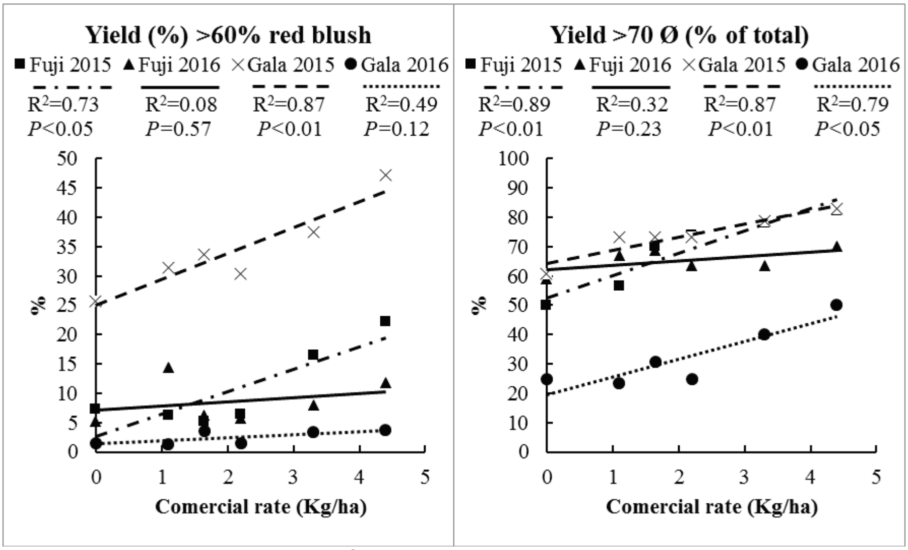

Figure 6: Relationships between Brevis® comerial rates and yield >70 mm and percentage of red blush >60%.

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

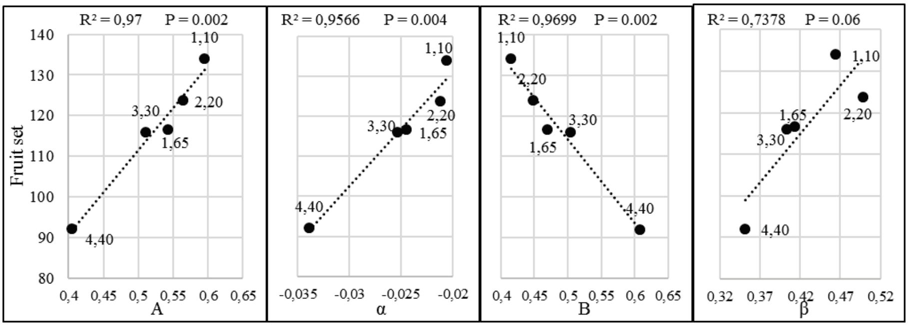

Figure 7: Correlation between final fruit set and the parameters estimated with the biexponential pharmacokinetic model of Qy(%) (A, α, B and β) for the different application doses (kg/ha).

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 (Figure 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 (Figure 8). However, there were no differences in the day of minimum Qy(%) value and AUC reduction (0-min) at different doses (Table 6).

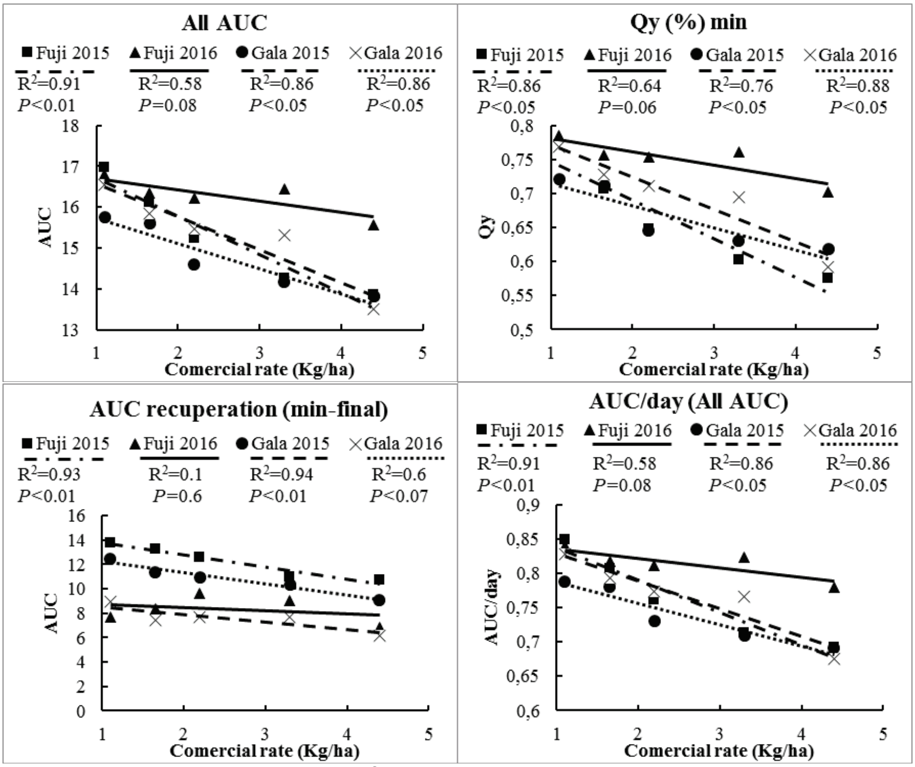

Figure 8: Relationships between Brevis® comerial rates and Area under the curve (AUC), Qy(%) predicted minimum (Qy(%) min), AUC recuperation (AUC between day of minimum Qy(%) value to end of inhibition period) and AUC/day (all AUC), in 'Gala' and 'Fuji' trees in 2 years (2015 to 2016).

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% (Figure 9A). However, the other AUC parameters showed no significant differences between cultivars because the reduction period was the same in both cultivars (Figure 9A). Moreover, the biexponential analysis of Qy(%) and all AUC parameters showed significant differences between years (Table 6 and Figure 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 Figure 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) (Figure 9B). There were no significant interactions (Table 6).

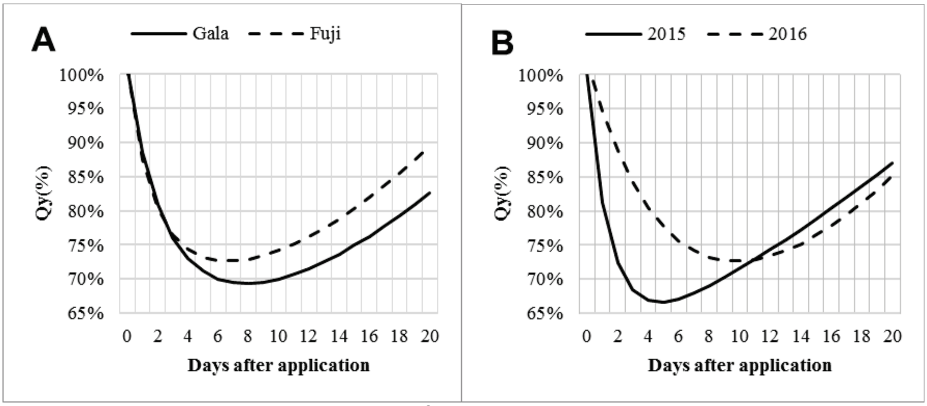

Figure 9: Graphic representation of the Brevis® inhibition 20 days after application estimated with the biexponential pharmacokinetic model of Qy(%) (A, α, B and β) for cultivars (A) and years (B).

4. Discussion

The maximum effectiveness for chemical thinner is based on the diameter of the developing fruit, the application rate, the cultivar and weather conditions (Byers, 2003). In all trials, the spraying of apple trees with chemical photosynthetic inhibitors induced fruit abscission, as also reported by Byers et al. (1990b). 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.

Capítulo IV.

Evaluation of chemical fruit thinning efficiency using Brevis® (Metamitron) on apple trees ('Gala') under Spanish conditions.

Gonzalez, L., Torres, E., Àvila, G., Bonany, J., Alegre, S., Carbó, J., Martin, B., Recasens, I., Asin, L. **(In press)**. Evaluation of chemical fruit thinning efficiency using Brevis® (Metamitron) on apple trees ('Gala') under Spanish conditions.

Scientia Horticulturae.

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

Highlights

- Fruit set varying according to the number of applications.
- Fruit weight and diameter increased when Brevis® reduced the fruit set.
- The degree of abscission of Brevis® is highly depends of night temperature.
- Higher night temperatures after spraying coinciding with greater Brevis® efficacy.
- Number of days between application was not relevant for Brevis® thinning effect

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).

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

2. Materials and methods

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

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

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

Trials: No. and year	Variety	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

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

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

Meteorological data were collected from the weather station of the official meteorological service of Catalonia. The stations were located in the Girona (Tallada d'Emporda) and Mollerussa orchards. The weather data evaluated in this study was downloaded in all years after the period of application. Night temperature, measured using a Vaisala HUMICAP® HMP155 humidity and temperature probe (Helsinki, Finland), was calculated as the average temperature in the period between 21:00 and 7:00.

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

2.2. Yield assessments

In all trials, to assess 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) before the treatments were applied. Homogeneous plants were selected for the trials based on flowering intensity.

In each orchard, at harvest time, the number of fruits per tree was recorded. Crop load was obtained from the number of fruits harvested per cm2 of trunk cross-sectional area (TCSA) (number of fruits / TCSA). Fruit set was obtained as the relationship between number of flower clusters and number of fruits at harvest time ([number of fruits / floral clusters] x 100). Total fruit yield (kg per tree), fruits per tree, fruit diameter (mm), weight (g) and blush color (%) were measured with a commercial apple sorting and packing line machine; Calinda (Caustier Ibérica, S.A. with Aweta Technology) in Mas Badia and Maf Roda (Agrobotic, France) in Lleida. 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.

2.3. Chlorophyll fluorescence

Chlorophyll fluorescence measurements were carried out in Mollerussa 2016, in 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 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 analysed 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).

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

Treatments No.	Fruit size of application (mm)	Girona 2015		Girona 2016		Mollerussa 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).

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

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

Table 4: Effect of thinning with Brevis® on final number of fruits per tree, fruit set 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**			**Mollerussa 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 $P<0.05$).

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

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

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**	**Mollerussa 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 $P<0.05$).

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

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

No.	Fruit size of application (mm)	Girona 2015		Girona 2016		Mollerussa 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 $P<0.05$).

In Girona 2015 and Mollerussa 2016, the double application of Brevis® resulted in a significant increase in fruit weight and fruit size compared to the Control. However, these differences were not observed in Girona 2016 (Table 6). Moreover, there were significant differences between the single and double application treatments, except the double 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 mm in Mollerussa 2016. These treatments coincided with lower fruit weight and fruit size in the single applications (Table 6).

In all trials, no significant differences were found in fruit yield (% and kg) with >60% blush area, except for the 9.5+11.5 mm, 9.5+13.5 mm and 11.5 +13.5 mm treatments in Girona 2015. These treatments showed a higher Brevis® fruit thinning efficacy. That is, average fruit coloration increased according to the thinning effect induced by Brevis® in Girona 2015 (Table 7).

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

No.	Fruit size of application (mm)	Yield > 60% blush area					
		Girona 2015		Girona 2016		Mollerussa 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).

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

3.2. Chlorophyll fluorescence

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

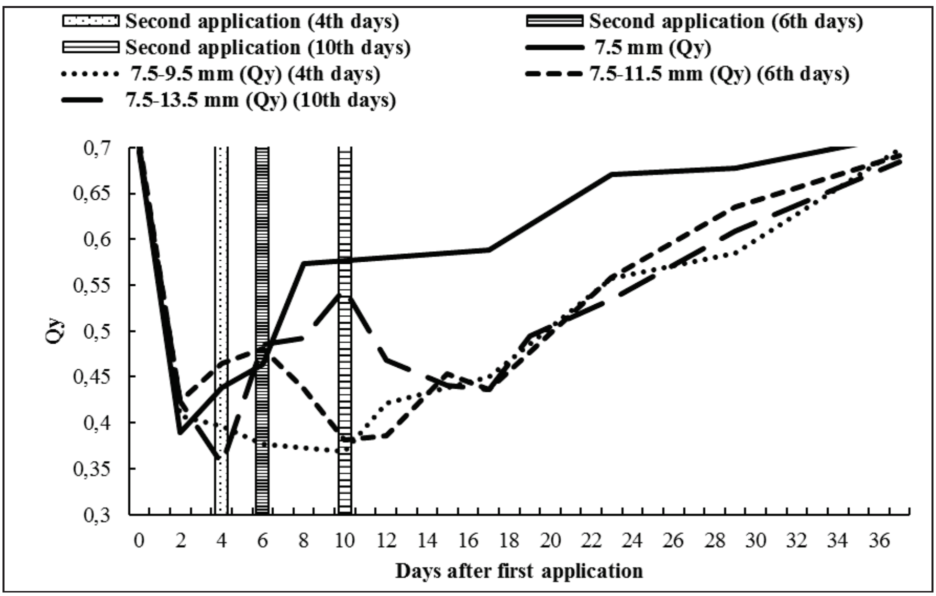

Figure 1: Effect of application of the photosystem II (PSII) inhibitor Brevis with one and two applications on chlorophyll fluorescence (Qy) in leaves of 'Gala' apple in Mollerussa 2016. The 7.5 mm application was on 26 April, the 9.5 mm application on 30 April, the 11.5 mm application on 2 May, and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

3.3. Night temperature

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

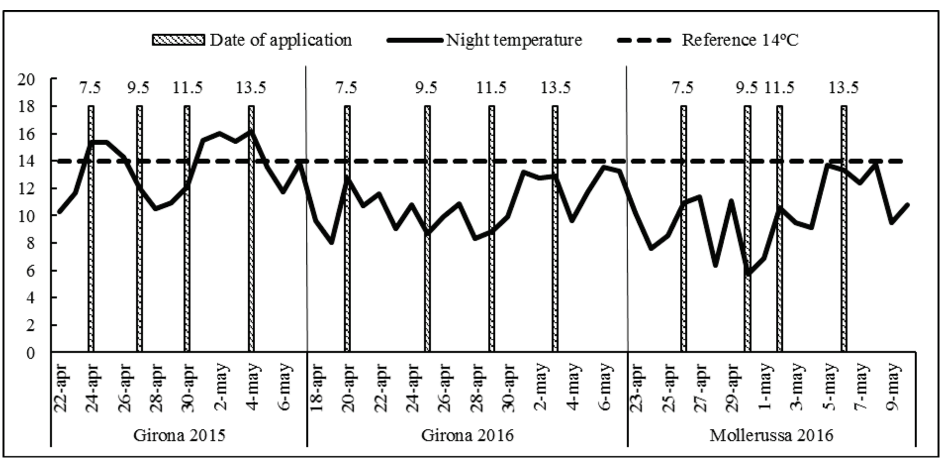

Figure 2: Average night temperatures and periods of king fruit diameter (mm) in apple trees over three trials.

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

In the single application treatments, when average night temperature was higher than 14ºC in the period of 0-4 days after Brevis® application, Brevis® efficacy was between 30% and 45%. However, when average night temperature was lower than 14ºC, Brevis® efficacy was less than 20% (Figure 3). In the double application treatments, when average night temperature 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% (Figure 3).

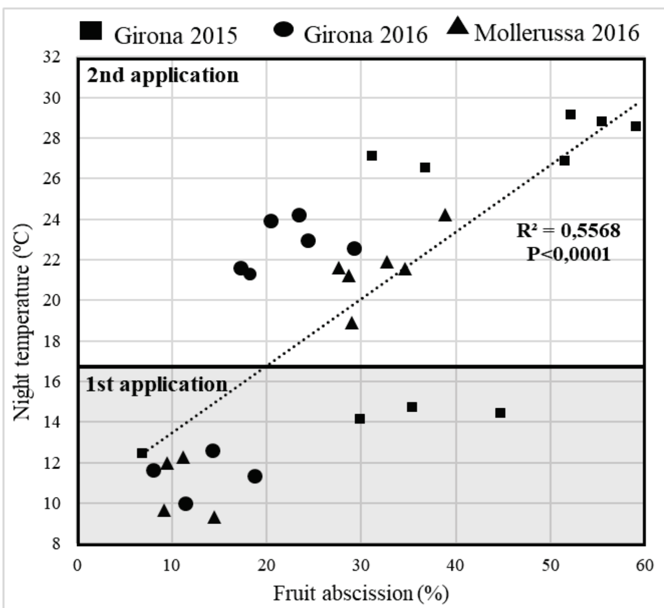

Figure 3: Scatter plot showing the relationship between night temperature (0/4) and fruit abscission (%) for 'Gala' (2015–2016). Night temperature for the single application treatments was calculated as the average night temperature (21:00 to 7:00) between the day of application and 4 days after application. 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.

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

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

According to the manufacturer, an interval of 5-10 days between applications is recommendable for Brevis®. In Girona 2015, the number of days between sprays in the double application treatment ranged between 3 and 10 days. Thinning efficacy was higher when 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 average night temperatures in the days immediately after spraying coinciding with greater Brevis® efficacy.

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

Capítulo V.

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

Gonzalez, L., G. Àvila, J. Carbó, J. Bonany, S. Alegre, E. Torres, B. Martin, I. Recasens and L. Asin "Hail nets do not affect the efficacy of metamitron for chemical thinning of apple trees."
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Abstract

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

Keywords

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

1. Introduction

Thinning can be used in fruit tree to improve overall profitability, which depends on yield, fruit size distribution and fruit quality (color, firmness, sugar, acidity, etc.). Return bloom is another factor that is influenced by fruit load. For all these reasons, fruit thinning is essential for high yields and quality in apple orchards (Byers et al., 1990b; Link, 2000).

Brevis® (metamitron 15% SG) is a chemical thinning agent which has been available for use in apple and pear in Spain since 2015. It inhibits photosynthesis and is different from other thinning products. Metamitron disrupts photosynthesis by blocking electron transfer between the primary and secondary quinones of PSII (McArtney et al., 2012). It can be applied at 165 to 330 g active ingredient (ai) per hectare in one or two applications, depending on the cultivar. The response to metamitron is affected by sunlight (Robinson et al., 2016), with a reduction in carbohydrates production as a result of lower photosynthesis under shade enhancing fruit drop. Kviklys and Robinson (2010) conducted a greenhouse study with potted trees and found that low temperatures and high light resulted in less thinning, while high temperatures (especially at night) and low light resulted in more thinning. They demonstrated that temperature and sunlight affected thinning, supporting the role of carbohydrates in the growth of fruitlets (Lakso, 2011).

Byers et al. (1985) reported that shading 16 to 26 days after full bloom, induced fruit drop in 'Starkrimson' apple, demonstrating a relationship between light and carbohydrate production. Shading decreases net CO_2 assimilation and reduces the amount of carbohydrates available for young fruitlets (Grappadelli et al., 1994), but the response is dependent on the cultivar (Mathieu et al., 2016). In other experiments, Greene and Groome (2010) showed that thinning agents such as carbaryl and naphthaleneacetic acid after shading does not modify their effect, but that when the chemicals are applied before shading the effect of thinning is greater. Research on shading has helped to clarify the impact of photosynthesis on fruit abscission (Kockerols *et al.*, 2008; Mathieu et al., 2016).

Hailstorms are common in Spain's apple production zone, causing significant damage. Currently, anti-hail nets are frequently used by Spanish growers. However, netting reduces the incidence of photosynthetically active radiation (PAR) above the trees (Ordonez et al., 2016). This situation, led us to consider the effects of anti-hail nets on the efficacy of metamitron. The objective of this study was to evaluate the effect of the anti-hail netting and metamitron on thinning efficacy, yield, fruit quality and chlorophyll fluorescence in three apple cultivars.

2. Materials and methods

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

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

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

Meteorological data were collected from the weather station of the official meteorological service of Catalonia, located 50 m in the Tallada d'Emporda orchard of the IRTA experimental agricultural station of Mas Badia. Night temperature was calculated as the average temperature recorded by the weather station between 20:00 and 07:00 h. Average temperature was calculated as the average temperature recorded by the weather station between 00:00 and 24:00 h.

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

2.1.1. Experiments 1 and 2

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

2.1.2. Experiments 3 and 4

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

2.2. Photosynthetically active radiation (PAR)

PAR was measured in an experimental orchard (IRTA-Mollerussa) in 2008 using an SS1-UM-1.05 Sun Scan ceptometer (Delta-T Devices Ltd, Cambridge, UK) with a 64-sensor photodiode linearly sorted in a 100 cm sword. The measurements were taken from other anti-hail net experiments (unpublished data). PAR was measured outside the nets (full light) and under black, green or white nets, 1.10 m above the ground. PAR was measured in spring under full sun at regular intervals, between 1200 and 1500 h.

2.3. Data collection and statistical analyses

The total number of flower clusters per tree was counted at bud break (BBCH 61-65), before the treatments were applied. At harvest, the number of fruits per tree was recorded. Fruit set was calculated as 100*(No. fruit/No. flower clusters per tree).

Fruit were harvested with in a single pick during the commercial harvest for each tree. Fruit weight, total fruit yield (kg per tree) and number of fruit per tree were measured with a commercial apple sorting and packing line (Calinda, Caustier Ibérica, S.A. with Aweta Technology).

First class fruit were >70 mm. Fruit size distribution, based on fruit diameter categories of >70 mm, was determined for each tree. Fruit size and coloration were measured with the commercial sorting machine.

Chlorophyll fluorescence was measured in Experiments 1 and 2, for ‘Galaxy’ and ‘Fuji’. Measurements were made on three recently fully-expanded leaves per control tree (6 leaves per block and 24 leaves per treatment), under full daylight in the shaded of the tree part between 1000 and 1600 h and at a height of 1-1.5 m. The measurements were taken once per week until values stabilized at 90% of the control.

QY (quantum yield) was measured with a handheld portable fluorimeter (FluorPen FP100, Photon Systems Instruments, Czech Republic) to provide an indication of the effect of the chemical on the maximum potential quantum yield efficiency of PSII.

Each experiment was analyzed individually because the nets were different colours and average PAR values were different. The cultivars and application doses were also different. Statistical analyses were performed in SAS 9.3 (SAS Institute Inc., 2009). Means were separated with the general linear model using Duncan’s multiple range tests at $P<0.05$ by one-way or factorial analysis of variance (Proc GLM), considering netting and chemical application as the main factors.

3. Results

3.1. Photosynthetically active radiation (PAR)

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

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

	12:00 h	13:00 h	14:00 h	15:00 h
Control	1576 (100%)	1770 (100%)	1901 (100%)	1900 (100%)
White	1402 (89%)	1657 (94%)	1767 (93%)	1746 (92%)
Green	1335 (85%)	1536 (87%)	1646 (87%)	1611 (85%)
Black	1248 (80%)	1377 (78%)	1540 (81%)	1521 (80%)

3.2. Temperatures

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

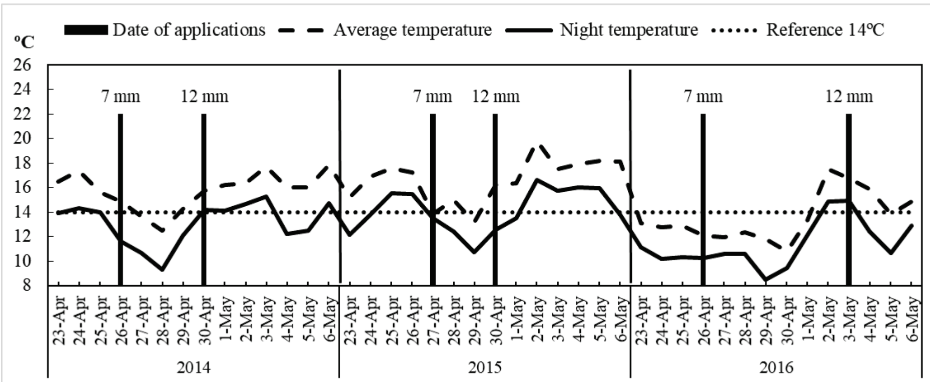

Figure 1: Average temperatures, average night temperatures and periods of king fruit diameter in apple trees over three years in Girona, Spain.

3.3. Growth, yield and fruit quality

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

There were mixed effects of thinning on fruit production and fruit set (Table 2). In the 2014 experiments with 'Gala' and 'Fuji', metamitron decreased fruit production and fruit set compared with the controls, with a greater response with the double applications in the first experiment. In the 2015 experiment with 'Fuji' and the 2016 experiment with 'Pink Lady' only the double applications decreased fruit production and fruit set (Table 2). Netting had only a small effect on fruit production and fruit set, with netting increasing these parameters compared with the control no net plots only in the 2014 experiment with 'Gala' (Table 2). There was no significant ($P > 0.05$) interaction between thinning and netting on fruit production and fruit set in any of the experiments (data not presented).

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

	'Gala' 2014		'Fuji' 2014		'Fuji' 2015		'Pink Lady' 2016	
Treatment	**No. of fruit per tree**	**Fruit set**	**No. of fruit per tree**	**Fruit set**	**No. of fruit per tree**	**Fruit set**	**No. of fruit per tree**	**Fruit set**
Control	415 a	132 a	379 a	134 a	360 a	173 a	214 a	128 a
165 g/ha							181 ab	109 ab
248 g/ha	307 b	96 b	221 b	76 b	328 a	162 a	171 ab	102 abc
330 g/ha					302 a	150 a		
165+165 g/ha							162 b	94 bc
248+248 g/ha	156 c	50 c	170 b	59 b	211 b	103 b	132 b	77 c
330+330 g/ha					219 b	107 b		
Anti-hail net								
With	295 a	92 a	254 a	94a	285 a	135 a	173 a	95 a
Without (Control)	242 b	78 b	260 a	84a	283 a	144 a	171 a	109 a

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

There were mixed effects of thinning on total yield and average fruit fresh weight (Table 3). The double applications of metamitron decreased yields compared with the control in the first two experiments, whereas there was no significant (*P* > 0.05) of the chemical in the last two experiments. The single applications of the chemical increase average fruit weight compared with the control in the first and second experiments (two out of two cases), while the double applications of the chemical increased average fruit fresh weight in five out of six cases in all the experiments (Table 3). There was no significant (*P* > 0.05) effect of netting on total yield and average fruit weight, and no interaction between thinning and netting (data not presented).

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

	'Gala' 2014		'Fuji' 2014		'Fuji' 2015		'Pink Lady' 2016	
Treatment	**Yield (kg/tree)**	**Fruit weight (g)**	**Yield (kg/tree)**	**Fruit weight (g)**	**Yield (kg/tree)**	**Fruit weight (g)**	**Yield (kg/tree)**	**Fruit weight (g)**
Control	47 a	113 b	38 a	101 c	31 a	87 b	26 a	125 b
165 g/ha							25 a	136 ab
248 g/ha	39 a	125 b	33 ab	149 b	30 a	90 b	24 a	142 ab
330 g/ha					29 a	98 b		
165+165 g/ha							23 a	144 ab
248+248 g/ha	26 b	168 a	29 b	177 a	28 a	138 a	20 a	156 a
330+330 g/ha					29 a	134 a		
Anti-hail net								
With	38 a	135 a	33 a	142 a	29 a	106 a	23 a	139 a
Without (Control)	33 a	143 a	35 a	146 a	30 a	112 a	23 a	142 a

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

There were significant ($P > 0.05$) negative relationships between fruit weight and the number fruit per tree in the experiments. There was a negative relationship between fruit weight and the number of fruit per tree (Figure 2). Fruit weight decreased as fruit production increased.

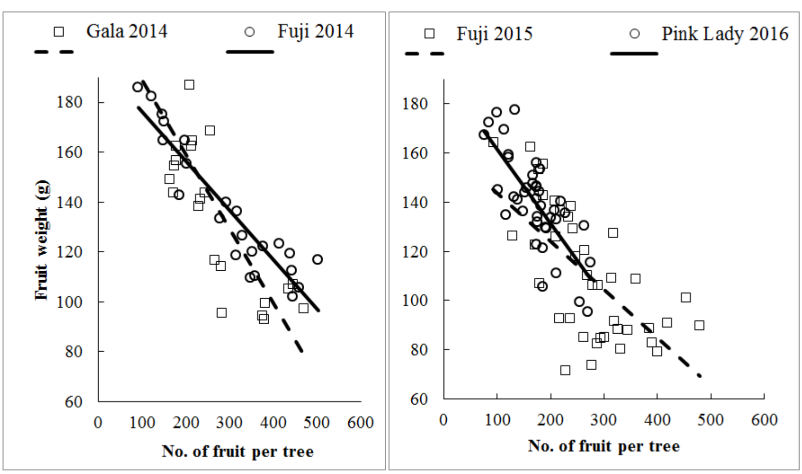

Figure 2: Relationships between fruit weight (g) and the number of fruit per tree in apple in Girona, Spain. Each symbol represents the average fruit weight per tree and number of fruit per tree. For 'Gala' 2014 y=-0.30*No. fruit+218 (R^2=0.71, $P > 0.001$), 'Fuji' 2014 y=-0.20* No. fruit+196 (R^2=0.86,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

The effect of thinning on the yield of premium fruit and the percentage of total yield that included fruit greater than 70 mm varied in the different experiments (Table 4). The double applications increased premium yield compared with the controls in all experiments except the last, while the single applications increased premium yield only in the second experiment. Thinning increased the percentage of total yield in the larger fruit category in seven out of twelve cases (Table 4). There was no significant (*P* > 0.05) effect of netting and no interaction between thinning and netting on premium fruit production in any of the experiments (data not presented).

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

	Yield >70 mm							
	'Gala' 2014		'Fuji' 2014		'Fuji' 2015		'Pink Lady' 2016	
Treatment	**Percent of total**	**kg of total**	**Percent of total**	**kg of total**	**Percent of total**	**kg of total**	**Percent of total**	**kg of total**
Control	19 c	9 b	8c	3b	4 b	1 b	14 b	4 a
165 g/ha							18 b	5 a
248 g/ha	33 b	14 b	59 b	20 a	6 b	2 b	27 ab	6 a
330 g/ha					9 b	3 b		
165+165 g/ha							25 ab	5 a
248+248 g/ha	80 a	20 a	78 a	23 a	47 a	12 a	46 a	9 a
330+330 g/ha					43 a	13 a		
Anti-hail net								
With	45 a	15 a	50 a	16 a	20 a	5 a	24 a	5 a
Without (Control)	54 a	16 a	52 a	17 a	24 a	7 a	28 a	6 a

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

Thinning had no significant (*P* > 0.05) effect on the yield of fruit that were highly coloured (60% of fruit surface coloured) (Table 5). There was no consistent effect of thinning on the percentage of yield that had coloured fruit. The double applications of metamitron increased the percentage of coloured fruit compared with the control in the first and third experiments, while none of the applications had an effect in the second experiment (Table 5). Netting had at best a small effect on fruit colour development (Table 5), while there was no significant (*P* > 0.05) interaction between thinning and netting (data not presented).

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

	Yield > 60% blush area					
	'Gala' 2014		'Fuji' 2014		'Fuji' 2015	
Treatment	**Percent of total**	**kg of total**	**Percent of total**	**kg of total**	**Percent of total**	**kg of total**
Control	23 b	11 a	18 a	7 a	10 c	2 a
248 g/ha	31 b	11 a	26 a	9 a	13 bc	3 a
330 g/ha					13 bc	2 a
248+248 g/ha	50 a	12 a	29 a	8 a	23 a	4 a
330+330 g/ha					17 b	3 a
Anti-hail net						
With	39 a	13 a	21 a	6 b	14 a	2 b
Without (Control)	39 a	11 a	27 a	9 a	17 a	3 a

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

3.4. Chlorophyll fluorescence

The netted and un-netted control trees tended to have similar values of quantum yield (QY) over the experiments (Figs. 3 and 4). In contrast, the trees sprayed with the thinning agent had lower values for most of the experiments, and recovered fully or almost fully after about 35 or 40 days. The maximum inhibition was three days after first application in any of the experiments. The maximum inhibition was maintained for a longer period (11 days) in all strategies with double applications than with single applications (3 days) (Figs. 3 and 4).

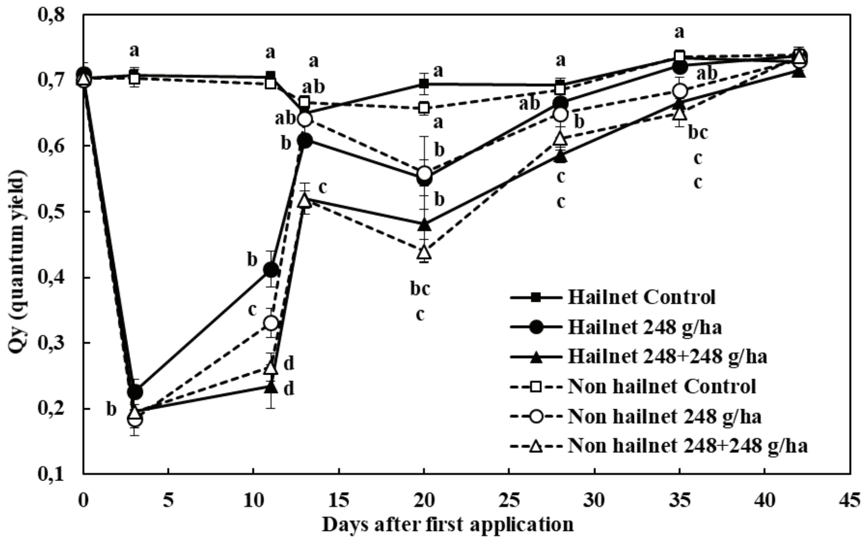

Figure 3: Effect of thinning and netting on quantum yield (QY) of chlorophyll fluorescence applied in leaves of 'Gala' apple in Girona, Spain. Metamitron was applied on 26 April and 30 April. Vertical bars indicate standard error of the means; n = 24. Means at the same time followed by different letters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

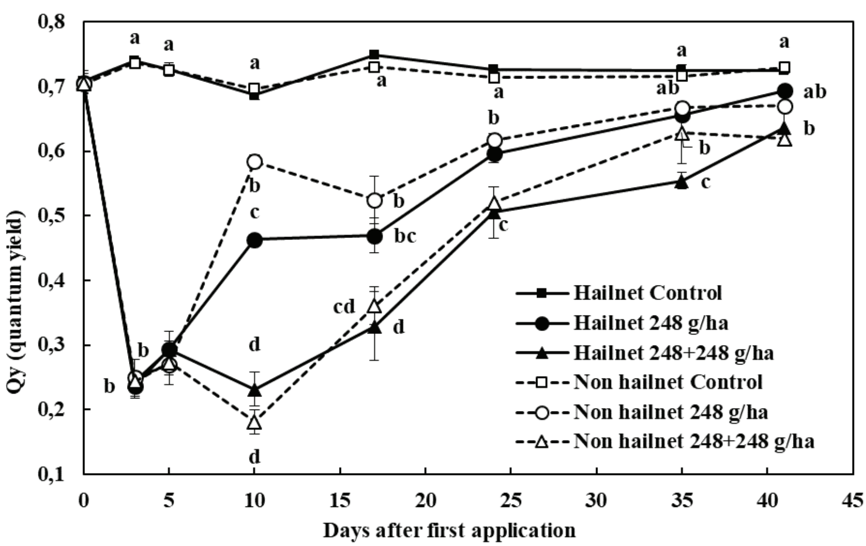

Figure 4: Effect of thinning and netting on quantum yield (QY) of chlorophyll fluorescence applied in leaves of 'Fuji' apple in Girona, Spain. Metamitron was applied on 26 April and 30 April. Vertical bars indicate standard error of the means; n = 24. Means at the same time followed by different letters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

4. Discussion

Netting can modify plant water status, light interception, photosynthesis and carbohydrate accumulation in crop plants (Mupambi *et al.*, 2018). The response of the leaves and the canopy to light is the major factor affecting carbohydrate production in apple trees (Lakso, 1994). For a whole canopy, as well as total light, the distribution of light between direct and diffuse components may be important since many leaves may be dependent on diffuse light (Lakso, 1994). At Girona, the PAR was reduced by 19%-22% by black nets, by 13%-15% by green nets and 6%-11% by white nets. These values were similar to those reported by Dussi *et al.* (2005), Iglesias and Alegre (2006), Blanke (2009) and Ordonez et al. (2016). While this would not affect leaf photosynthesis on sunny summer days, photosynthesis under the nets would be reduced in autumn, spring or on overcast days (Widmer, 2001).

Many authors have reported that temperature plays an important role in chemical thinning (Kviklys and Robinson, 2010; Lakso et al., 2006; Li and Cheng, 2011; Lordan et al., 2019; Parra-Quezada et al., 2005; Pretorius et al., 2011). Typically warm nights increase respiration during the night, decrease overall carbon balance of the tree (Costa et al., 2018), and increase the efficacy of thinning. Metamitron was generally more effective after the second application under warmer weather. Byers (2003) indicate that the efficacy of a chemical depended on the diameter of the fruit, application dose, cultivar and weather.

Double applications of metamitron tended to reduce fruit set and number of fruit per tree more than single applications as shown previously (Dorigoni and Lezzer, 2007; Stern, 2014). Single application typically reduce fruit set compared with control untried, again concurring with earlier studies (Deckers et al., 2010; Dorigoni and Lezzer, 2007; Lafer, 2010; Reginato et al., 2017).

In 2014, thinning was associated with lower yields whereas these were no effect in 2015 and 2016. McArtney et al. (2012) reported that thinning decreased yields in ‘Gala’ in New Zealand. Average fruit weight trended to increase with thinning as shown by Brunner (2014), Maas and Meland (2016) and McArtney et al. (1996). These authors reported a negative relationship between average fruit weight and the number of fruit per tree. Fruit size moved to the larger category as the number fruit per tree decrease, as shown by Bergh (1990), Dorigoni and Lezzer (2007), Lafer (2010) and Mathieu et al. (2016).

Netting generally had no effect on production, fruit size or fruit quality as demonstrated by Iglesias and Alegre (2006) and Ordonez et al. (2016). Netting also had no effect in the response to the thinning agent. Shade provided by nets has been shown to reduce net CO_2 assimilation, increase vegetative growth and, therefore, reduce yield and fruit size in apple (Amarante *et al.*, 2011; Amarante *et al.*, 2007; Middleton and McWaters, 2002; Romo-Chacón *et al.*, 2007). Moreover, there was a fewer and smaller cell in the fruit of apple under net, leading to a lower fruit size count (Amarante et al., 2011).

Metamitron disrupted the photosynthetic apparatus for 41 to 43 days after he chemical was applied, although this differs from some published studies in which shorter inhibition periods have been reported (McArtney et al., 2012; Stern, 2014, 2015). Quantum yield decreased rapidly for the first three days, and the maximum inhibition of QY values were recorded for three to ten days after the treatment, depending on the number of applications. These results concur with earlier observations by Brunner (2014) and McArtney et al. (2012).

5. Conclusions

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

Discusión general y conclusiones

1. Discusión general

El exceso de flores y frutos es un fenómeno habitual en los árboles frutales como el manzano, en el caso de no hacer ninguna actuación al respecto. Ello condiciona tanto la calidad del fruto como la inducción floral, afectando a la floración de retorno de la campaña posterior. Dicha situación posiciona el aclareo como una de las prácticas más importantes en el cultivo del manzano, ya que determinará la rentabilidad, la productividad, la calidad del fruto y la floración de retorno. Por todo ello, el aclareo de fruta es una etapa esencial en la producción comercial de manzanas de calidad (Byers, 2003).

Mediante la presente tesis se ha estudiado la eficacia de aclareo de Brevis® y determinado algunos factores que afectan a su eficacia y comportamiento, con el objeto de posicionarlo en un programa de aclareo químico en manzano. El aclareo químico está condicionado por diversos y múltiples factores que pueden interactuar entre sí, los más importantes son la variedad, el tamaño de fruto, las dosis de aplicación, el número de aplicaciones y las condiciones climáticas.

Los estudios realizados han pretendido evaluar y responder dichos condicionantes.

1. Variedad: si bien es conocido que existen diferencias en la susceptibilidad de las diferentes variedades de manzano al aclareo frutos, en los Capítulos I, III y V se ha evaluado la eficacia de Brevis® en diferentes variedades (‘Fuji’, ‘Gala’ y ‘Pink Lady’) para comprobar si también existían dichas diferencias.

2. Tamaño del fruto: en este sentido se evaluó la susceptibilidad del fruto realizando aplicaciones desde 6 mm hasta 21 mm de tamaño de fruto central. De esta manera se pudo evaluar en que tamaños de fruto se obtuvo eficacia de aclareo y se posicionó el tamaño de máxima eficacia (Capítulo I).

3. Dosis de aplicación: se realizaron aplicaciones únicas desde 1,1 kg/ha hasta 4,4 kg/ha (el doble de la dosis que establece el registro) para comprender la relación dosis efecto de Brevis® (Capítulo III).

4. Número de aplicaciones: otra de las cuestiones importantes en el aclareo es el número de aplicaciones que se pueden realizar (1vs2) y su separación en el tiempo. A partir de dicha hipótesis se evaluó el efecto de una y dos aplicaciones en diferentes variedades de manzano (Capítulo V). Además, en ‘Gala’ se realizaron diferentes combinaciones de una y dos aplicaciones en diferentes momentos y se evaluó la importancia de realizar las

aplicaciones juntas o separadas (Capítulo IV).

5. Modelización de la fluorescencia: se estudió y modelizó la evolución del Qy causada por Brevis® (Capítulos I, III y V).
6. Condiciones meteorológicas: mediante un total de 28 estrategias se ha podido evaluar, modelizar y validar a nivel local los factores climáticos clave antes y después de la aplicación que explican la eficiencia anual de Brevis® (Capítulos II). Del mismo modo se ha podido analizar el efecto de reducción de la radiación mediante el empleo de mallas antigranizo (Capítulo V).

1.1. Efecto de aclareo y beneficios de la reducción de la carga

En el manzano los frutos, hojas, brotes, tallos, raíces y otros órganos de las plantas compiten entre sí por los asimilados y los hidratos de carbono producidos en la fotosíntesis. Una reducción en la disponibilidad de carbohidratos, bien sea tanto una reducción de la fotosíntesis por una menor radiación PAR, una mayor tasa de respiración o una inhibición de la fotosíntesis provocada por productos químicos que la inhiben, provoca competencia entre los órganos mencionados anteriormente, si bien existen diferentes estrategias de adaptación a las situaciones de estrés, en el caso del manzano, la estrategia es la abscisión de frutos (Byers et al., 1990b; Byers et al., 1985; Lafer, 2010). Los primeros trabajos que demostraron que los inhibidores de la fotosíntesis tenían efecto de aclareo fueron realizados por Byers et al. (1984), (1985) y DelValle (1983). Posteriormente Byers et al. (1990b) concluyó que la aplicación de inhibidores químicos de la fotosíntesis inducía la abscisión de frutos en el manzano.

En todos los capítulos de la tesis las aplicaciones de Brevis® mostraron una reducción del cuajado, el número de frutos por árbol y frutos por cm^2. Dicha situación provocó que, en prácticamente todos los ensayos en los que hubo eficacias significativas de aclareo respecto el testigo, se viera reducida la producción, coincidiendo con los resultados obtenidos por McArtney et al. (2012). Además, resultados obtenidos por McArtney et al. (1996), Brunner (2014) y Maas and Meland (2016) mostraron que el peso, el color y el calibre del fruto se veían beneficiados al reducirse la carga del árbol, lo que se traduce en un mayor valor económico de la producción. Dichos resultados también se pueden observar en el capítulo IV de la tesis, en el que una reducción de la producción y el número de fotos estuvo relacionada linealmente con un incremento del peso, color y calibre del fruto.

Por otro lado, hay diferencias entre variedades en relación a la reducción de la carga como respuesta a la aplicación de productos de aclareo químico (Ferree, 1996; Rosa *et al.*, 2017). Este factor varietal también se ha constatado en el caso de Brevis®, de modo que se han observado diferencias significativas entre variedades en todos los parámetros productivos y de eficacia. 'Gala' se ha mostrado como una variedad más sensible al aclareo que 'Fuji' mediante aplicaciones de Brevis®. Estas diferencias también fueron observadas por Brunner (2014), quien indicó que Golden es más sensible al aclareo con metamitrona que 'Fuji'.

1.2. Tamaño del fruto en el momento de aplicación

La aparición de nuevas materias activas hace necesario evaluar su comportamiento en diferentes condiciones de aplicación o cultivo y buscar nuevos enfoques que ayuden a mejorar su eficacia. El momento de aplicación de los productos de aclareo tiene una importancia significativa en su eficacia (Buban, 2000; Mathieu et al., 2016). En este sentido, definir en qué momentos resulta efectivo un producto y cuándo se produce la máxima susceptibilidad del fruto en base al tamaño es un factor clave dentro de los programas de aclareo, ya que condicionará el momento en que se posicionarán las aplicaciones. Esta circunstancia tiene una mayor relevancia cuando la materia permite realizar varias aplicaciones seriadas, como es el caso de Brevis®

El registro de Brevis® en España permite aplicaciones de los 8 a los 16 mm de diámetro del fruto central. No obstante, los resultados obtenidos en la presente tesis mostraron eficacia en tamaños de fruto superiores al que marca el registro de Brevis®, ampliando la ventana de aplicación hasta los 19 mm. Este rango posiciona a Brevis® en un momento de desarrollo del fruto más avanzado que el resto de productos que hay en el mercado, permitiendo prolongar los programas de aclareo químico más allá de los 14 mm. Los resultados obtenidos coinciden con trabajos previos realizados en los que Brevis® mostró eficacia en aplicaciones realizadas entre los 8 y los 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). Además, la máxima susceptibilidad del fruto se obtuvo en el rango de diámetros de 11,5-14 mm, coincidiendo con los resultados obtenidos por Brunner (2014). Sin embargo, este resultado difiere del obtenido por Reginato et al. (2017) quienes observaron la máxima eficacia de Brevis® en 16 mm.

1.3. Clima

Uno de los factores clave son las condiciones climáticas en el periodo de aplicación de productos de aclareo (Byers, 2003; Lakso et al., 2006; Lordan et al., 2019). En el capítulo II se estudiaron todos los factores climáticos que podían tener una influencia en la eficacia de Brevis®. Se analizaron las temperaturas (media, diurna y nocturna), la radiación, humedad relativa, el viento (medio diario, diurno y nocturno) y la evapotranspiración (PM-ETo). Se realizó un primer análisis PLS para seleccionar aquellos factores que tuvieran importancia en la eficacia de Brevis®. Dicho análisis seleccionó como parámetros más importantes para explicar la eficacia de Brevis® a las temperaturas (media, diurna y nocturna), el viento (medio diario, diurno y nocturno) y la evapotranspiración (PM-ETo). Además, se realizó un análisis de multiregresión en el que se analizaron los parámetros seleccionados) y el periodo en el que afectaban a la eficacia de Brevis®. En el análisis se estudiaron los 8 días antes y después de la aplicación en los que se agruparon todos los periodos de 3 y 5 días. Los resultados obtenidos mostraron en orden descendiente de importancia, que Brevis® mostró mayor sensibilidad a la temperatura nocturna y a la temperatura media diaria, seguidas de la temperatura diurna, PM-ETO, el viento nocturno y, finalmente, el viento medio diario. Además, los modelos indicaron que las temperaturas y el viento eran importantes antes y después de las aplicaciones de Brevis®, y la PM-ETo tenía más importancia después de la aplicación.

Todos los modelos de temperatura mostraron mejores coeficientes de determinación que el resto de parámetros evaluados. Confirmaron que tanto la temperatura de 5 días antes como la de 3 días después de la aplicación tenían importancia, y explicaban un alto porcentaje de la eficacia de Brevis®, corroborando los resultados obtenidos por Parra-Quezada et al. (2005). En este sentido, las temperaturas nocturnas tenían una mayor importancia para predecir la eficacia de Brevis® cuando las comparamos con las diurnas. Dichos resultados son similares a los obtenidos por Costa et al. (2018), Stern (2014) and Robinson et al. (2016) quienes han sugerido que a medida que aumenta la temperatura nocturna, aumenta la eficacia del aclareo. Además, las temperaturas nocturnas disminuyen el balance de carbohidratos, ya que incrementa la respiración (Costa et al., 2018), y si a dicha situación se le suma la reducción de la inhibición provocada por Brevis®, el árbol entra en situación de déficit del balance global de carbohidratos, provocando que los carbohidratos restantes se dirijan a los brotes ocasionando la caída de los frutos más pequeños (ADAMA, New Zealand 2017).

Los modelos del viento y la PM_ETo fueron los que mostraron una relación más baja a la hora de predecir la eficacia de Brevis®. Los modelos del viento medio y nocturno seleccionaron la venta de 5 días comprendidos entre el día antes de la aplicación hasta 3 días después. Además, los resultados mostraron que el viento nocturno tenía más importancia que el diurno en la predicción de eficacia de Brevis®. La PM-ETo es un parámetro calculado a partir de la temperatura, la humedad relativa, la radiación y el viento, siendo la radiación y la temperatura los parámetros con más peso. Los resultados mostraron que en condiciones de radiación y humedad constantes la temperatura pasa a ser el factor más importante de la fórmula. En este sentido, la PM-ETo siguió un comportamiento igual al de la temperatura, pero con un coeficiente de determinación inferior.

Por último, se realizó un análisis global con todos los parámetros seleccionados anteriormente en todos los modelos, con la intención de corroborar qué parámetros tenían más peso en la eficacia de Brevis®. Los resultados obtenidos mostraron que el modelo con el coeficiente de determinación más alto seleccionó, como parámetros más importantes y que explican mejor la eficacia de Brevis®, la temperatura media de 5 días antes y la temperatura nocturna de 3 días después de la aplicación. Sin embargo, dicho modelo mostraba diferencias mínimas con el modelo de temperatura nocturna y el de temperatura media.

Dichos resultados se corroboran en todos los capítulos IV y V de la tesis, en los que se mostró de manera gráfica como las estrategias en las que Brevis® mostraba mayor eficacia coincidían con aquellas estrategias en las que las temperaturas se incrementaba después de la aplicación de Brevis®, coincidiendo con los resultados obtenidos por Kviklys and Robinson (2010). Además, Stern (2014) propuso que Brevis® era más efectivo en países cálidos que en los fríos, porque las altas temperaturas nocturnas incrementaban la respiración en un periodo de aplicación. Estos resultados concuerdan con los obtenidos en la presente tesis. En los capítulos I y III en los que se comparó la eficacia de Brevis® en diferentes años, se pudo corroborar que la eficacia era mayor aquellos años en que las temperaturas nocturnas eran altas.

En último lugar el análisis PLS indicó que la radiación y la humedad relativa no influían en el grado de aclareo de Brevis® en condiciones españolas. Sin embargo, dichos resultados no son coincidentes con los obtenidos con la humedad por Robinson et al. (2016). Ellos concluyeron que la humedad jugaba un papel inportante en el momento de la aplicación de productos. Cotradiciendo los resultados obtenidos con la radiacion en la presente tesis, diferentes autores concluyeron en distintas condiciones y con diferentes productos que periodos nublados o con poca luz favorecía tanto la abscisión de frutos y se incrementaba el efecto de aclareo (Byers et al., 1985; Grappadelli et al., 1994; Greene and Groome, 2010; Lakso, 2011). No obstante, trabajos previos realizados por González (no publicados) concluyeron que se incrementó la eficacia de Brevis® si se cubrían los árboles con mallas de sombreo durante 4 días seguidos (reducción de un 60% de la radiación). Sin embargo, estas condiciones de reducción de radiación (4 días al 60%) no son habituales en las zonas productoras de Lleida y Girona, ya que solo se produjeron en un tratamiento de los 30 que se introdujeron en el modelo, y por ello la radiación no resultó significativa en condiciones naturales, según se concluyó en capítulo II.

1.4. Efecto de la dosis

Otro de los factores clave en la aplicación de los productos de aclareo es conocer la correlación entre la dosis y la eficacia. De acuerdo con el registro de Brevis® en España, la dosis máxima de aplicación es de 2,2kg/ha, sin embargo, en el capítulo III se analizaron los posibles efectos de la dosis realizando aplicaciones al doble de la dosis de registro (4.4kg/ha). Hasta la fecha solo hay un trabajo publicado por Mathieu et al. (2016) en el que se estudie el efecto de la dosis de Brevis®. Sus resultados indicaban una correlación lineal entre la dosis y la eficacia, y remarcaban la importancia de tal resultado ya que éste difiere en cierta medida de los obtenidos con los biorreguladores, en los que la correlación resulta menos evidente (Mathieu et al., 2016). No obstante, en dicho trabajo solo se analizó la relación dosis efecto y no se profundizó más en los parámetros productivos y de calidad del fruto.

Los resultados obtenidos en la presente tesis mostraron que Brevis® presentó, tanto en ‘Gala’ como en ‘Fuji’, una relación lineal entre la dosis y la eficacia, evaluada como cuajado, número de frutos árbol y número de frutos por cm^2. Además, el hecho de que se redujera la carga penalizó la producción (kg/árbol), como ya se ha mencionado en trabajos previos realizados por McArtney et al. (2012). La reducción en la producción y el número de frutos árbol ocasionó una correlación positiva entre la dosis y el peso, el color y el calibre del fruto, por lo tanto, aunque la producción se penalizó en las dosis más altas, fue de más calidad puesto que se incrementó el peso, el calibre y el color. Cabe destacar que en el capítulo se analizaron otros factores que podían tener influencia en la eficacia de Brevis®, como es el caso del año y variedad. Dichos factores eran significativos, pero en ningún caso se observaron interacciones significativas, lo que indica que el comportamiento de Brevis® independientemente de la eficacia del año y la sensibilidad de la variedad siempre mostró la relación dosis efecto.

1.5. Importancia del número de aplicaciones (1 vs 2) y su separación en la eficacia de Brevis®

En la presente tesis se evaluaron el efecto de realizar 1 y 2 aplicaciones, en diferentes estados fenológicos del fruto (entre 7,5 y 13 mm) y el efecto de la separación en número de días entre la primera y la segunda aplicación (Capítulo IV). De esta manera se pudo analizar aplicaciones juntas y separadas en el tiempo en las que existía diferentes condiciones climáticas de la aplicación. Los resultados en el capítulo IV mostraron que existía un efecto aditivo de la aplicación cuando existían condiciones climáticas optimas. Asimismo, cuando las condiciones climáticas eran favorables, en las dos aplicaciones se obtenía un incremento en la reducción de frutos en comparación con aquellas en que solo había condiciones óptimas en una aplicación. Además, aquellas estrategias dobles en que las condiciones no eran favorables en ninguna aplicación no mostraban un efecto significativo de aclareo. Resultados similares en el cuajado fuero mencionados por Stopar (2017), quien concluyó que el cuajado final era principalmente la suma de las eficacias de las dos aplicaciones realizadas. Los resultados obtenidos evidencian que el hecho de realizar las aplicaciones juntas o separadas no es un factor que tenga importancia en la eficacia de Brevis®, ya que se obtuvo buen efecto en los dos tipos de estrategias siempre que las condiciones climáticas fueron favorables a la aplicación. Este resultado contradice al registro de Brevis®, en el que se recomienda no realizar la segunda aplicación hasta pasados 5 días de la primera. En este sentido, si las condiciones son favorables pasados 2 días de la primera aplicación es recomendable realizar la segunda, en lugar de esperar 5 días y que las condiciones sean desfavorables.

1.6. Efecto del sombreado ocasionado por las mallas antigranizo.

En el capítulo V se evaluó el posible efecto de la reducción de la radiación ocasionada por 3 tipos de mallas antigranizo (blanca, negra, y verde) en una y dos aplicaciones de Brevis® en tres variedades (Gala, ‘Fuji’ y ‘Pink Lady’). Si bien ya se ha documentado los efectos de las mallas y que modifican la situación hídrica, la intercepción de luz, la fotosíntesis y la acumulación de carbohidratos de la planta (Mupambi et al., 2018) no hay trabajos realizados sobre los posibles efectos del sombreado en la eficacia de los productos de aclareo.

Los resultados obtenidos mostraron diferencias en la reducción de la radiación de los tres tipos de mallas. La malla negra redujo entre un 19%-22%, seguida de la verde (13%-15%) y la blanca (6%-11%), como se ha mencionado en otros trabajos realizados por Dussi et al. (2005), Iglesias and Alegre (2006), Blanke (2009) and Ordonez et al. (2016). Sin embargo, cuando se evaluó el efecto de aclareo se pudo observar que no existían diferencias entre las estrategias con y sin malla en las diferentes variedades. Además, no se observaron diferencias en todos los valores productivos entre aquellas estrategias con y sin sombreo. Cabe destacar que en el capítulo se analizó la interacción entre el factor malla y tratamiento que en ningún caso fueron significativas, lo que indica que el comportamiento de Brevis®, independientemente del número de aplicaciones, la variedad y las dosis de aplicación, no se ve condicionado por el sombreado de las mallas antipiedra. Si bien es conocido que la reducción de la radiación tiene un efecto importante en la eficacia del aclareo, los resultados obtenidos muestran que reducciones de hasta el 22% de la radiación no intensifican la eficacia de aclareo de Brevis®. A pesar de ello y como se ha mencionado anteriormente, trabajos previos realizados por Gonzalez (no publicados) concluyeron que 4 días seguidos con una reducción del 60% de la radiación tenían un efecto importante en la eficacia de Brevis®.

1.7. La inhibición de la fluorescencia causada por Brevis®

Brevis® actúa inhibiendo la fotosíntesis, y uno de los enfoques más antiguos para evaluar la fotosíntesis es la medición de la fluorescencia. De esta manera, los cambios en la fluorescencia pueden utilizarse para evaluar y cuantificar diversas situaciones de estrés en las plantas. Kautsky and Hirsch (1931) fueron los primeros en informar de la relación significativa entre la fotosíntesis y la fluorescencia clorofílica. Asimismo, la fluorescencia se ha utilizado para evaluar la actividad de la fotosíntesis, en especial del fotosistema II (Fernandez et al., 1997; Krause and Weis, 1984). Por todo ello, en la presente tesis se ha evaluado la inhibición causada por Brevis® mediante el análisis de la fluorescencia clorofílica, en concreto del parámetro Qy (*Quantum yield*), con la intención de utilizarla como herramienta para gestionar la toma de decisiones del aclareo ocasionado por Brevis® (Capítulos I, III y V).

Los resultados obtenidos en la presente tesis han mostrado diferencias en los periodos de inhibición causada por Brevis® hasta el 90% de recuperación, que han variado desde 14 hasta 43 días dependiendo de la dosis de aplicación, la variedad y el número de aplicaciones. Estos resultados también se pueden observar en la bibliografía, McArtney et al. (2012) y Stern (2015) observaron que la inhibición causada por la metamitrona duraba entre 10 y 11 días, aunque el producto aún no se había reformulado como Brevis®. Costa et al. (2017) mostró que el periodo de inhibición varió en función de las condiciones antes y después la aplicación obteniendo periodos de inhibición que variaban de 10 a 18 días. Rosa (2016) en su tesis de final de master, en estrategias de 2 aplicaciones obtuvo periodos de inhibición de entre 29 y 37 días, dependiendo de las variedades analizadas.

Dicha variabilidad también se pudo observar en el periodo de máxima inhibición, que se produjo entre 3 y 10 días después de la aplicación. Posteriormente el árbol se recuperó lentamente. Dichos resultados difieren ligeramente de los obtenidos por diferentes autores (Brunner, 2014; McArtney et al., 2012; Rosa, 2016; Stern, 2015), quienes informaron de periodos de entre 2 y 6 días de inhibición máxima.

En el capítulo V se realizó un análisis de la inhibición de la fluorescencia provocada por Brevis® en el caso de realizar una y dos aplicaciones. Los resultados mostraron que el periodo de inhibición máxima se prolongaba después de la segunda aplicación, coincidiendo con los resultados obtenidos por Rosa (2016). En los capítulos I y III se modelizó la inhibición de una aplicación causada por Brevis® mediante un modelo farmacológico, utilizado una función biexponencial. Cuando se suministra un fármaco, durante un corto periodo de tiempo se llega a la concentración máxima del fármaco en sangre, posteriormente, el organismo va eliminando el fármaco lentamente hasta que desaparece. La inhibición causada por Brevis® se comporta de la misma manera; se aplica el producto y durante los días posteriores llega a la máxima inhibición, y posteriormente el árbol se recupera lentamente. Además, los modelos también permitieron realizar un análisis más profundo del área bajo la curva y del área diaria en las diferentes fases de la inhibición causada por Brevis®.

Los resultados obtenidos mostraron que tanto para el estudio de la dosis, como para el de momentos de aplicación en ‘Gala’ y ‘Fuji’, la función biexponencial mostró un alto grado de ajuste y los valores calculados se correlacionaron con los valores reales. En el estudio de la dosis, los parámetros estimados obtenidos mostraron una correlación lineal significativa con los parámetros productivos, de esta manera se pudo observar la relación dosis efecto. Sin embargo, en el estudio de la susceptibilidad del fruto, los parámetros estimados no se correlacionaron con los valores productivos. Cabe destacar que en el análisis de área en el estudio de la dosis también se observó la relación dosis-efecto, por lo que a medida que se aumentaba la dosis, el área bajo la curva era más pequeña y por lo tanto mostraba una inhibición mayor. No obstante, en el estudio de la susceptibilidad del fruto se pudo observar como a medida que se incrementaba el tamaño del fruto la inhibición causada por Brevis® era menor y no estaba relacionada con los parámetros de eficacia. En resumen, los resultados evidenciaron que el modelo biexponencial puede ser utilizado para predecir la eficacia de la aplicación y el grado de inhibición de Brevis® cuando se realizan aplicaciones en el mismo momento. Por el contrario, y puesto que la inhibición en los diferentes estados fenológicos es diferente, dichos modelos no pueden ser utilizados para predecir la eficacia y el grado de inhibición en los diferentes estados fenológicos del fruto.

Por último, y puesto que los modelos también mostraron diferencias entre variedades y momentos de aplicación, se decidió realizar un estudio de la inhibición en las diferentes hojas de los brotes en las dos variedades (Capitulo I). Los resultados mostraron que los valores de fluorescencia en el control fueron inferiores en 'Gala' que en 'Fuji'. Además, las estrategias control mostraron que en ambas variedades la sección vegetativa del brote mostraba valores inferiores de fluorescencia. Al evaluar la inhibición causada por Brevis® en las diferentes secciones del brote en 'Fuji' se observaron diferencias, siendo la sección vegetativa la que mostró mayor inhibición y la sección más próxima a la rama la que menor. Estas diferencias entre las secciones no se observaron en los brotes de 'Gala', que mostraron la misma inhibición en todo el brote. Además, los valores de fluorescencia después de la aplicación de Brevis® fueron muy inferiores en 'Gala' en comparación con 'Fuji'. Dicha situación podría explicar que 'Gala' es más sensible al aclareo con Brevis® que 'Fuji'. No obstante, es necesario profundizar más en el tema para conseguir resolver el motivo por el cual existen estas diferencias entre las hojas de las dos variedades.

2. Trabajos futuros

En la actualidad existen numerosos trabajos que han evaluado la eficacia de las aplicaciones de Brevis® a nivel de árbol, centrándose en gran medida en el cuajado final y/o en la calidad del fruto. A pesar de ello es necesario profundizar en aspectos complementarios para explicar y predecir la intensidad de aclareo, sería el caso de cuestiones sobre cómo el producto penetra en la hoja y qué nivel de estrés le ocasiona al árbol debido a la inhibición de la fotosíntesis que provoca. Por ello, futuros trabajos deberían ir dirigidos a evaluar el efecto de Brevis® en el intercambio de gases, los azucares no estructurales y en la actividad de los enzimas encargados de la respiración y la fotosíntesis. Estos enfoques podrían ser válidos para establecer modelos de predicción en base al efecto de Brevis® en fotosíntesis, respiración, balance de carbohidratos, etc. En este sentido es necesario caracterizar la respuesta de árbol al estrés ocasionado por las condiciones climáticas y su efecto a nivel fisiológico que permitan conocer las rutas y eventos metabólicos se producen i desencadenan la abscisión de los frutos. Estos permitirían trabajar en encontrar biomarcadores para evaluar la eficacia del producto y definir el momento de la segunda aplicación en caso de que fuera necesaria. Dichos Biomarcadores podrían ir dirigidos hacia caracterizar el modo de acción de Brevis® en al aparato fotosintético y definir las condiciones ideales de absorción.

Otro punto a tener en cuenta es el estudio de la Fluorescencia. Si bien los resultados obtenidos en la presente tesis han demostrado que no es una buena herramienta para predecir la eficacia de Brevis®, si que ha permitido caracterizar la eficacia del año. A pesar de ello es difícil obtener conclusiones en base a lo que explica la fluorescencia por si sola puesto que los valores varían dependiendo de las condiciones climáticas del día y la edad de la hoja, situación que hace necesario el evaluar un árbol sin tratar para reducir dicha variabilidad. Aun así, parece que fisiológicamente es posible predecir la eficacia, aunque es probable que, como se ha mencionado anteriormente, parametrizar la fotosíntesis o la respiración sea más exitoso que la fluorescencia. Para ello un modelo biexponencial similar a los empleados en la tesis puede ser una buena herramienta. Si bien los resultados mostrados con los modelos farmacológicos son una primera aproximación, es necesario conocer la inhibición causada en los diferentes estados fenológicos del fruto. Dichos resultados permitirían tener datos suficientes en cada fase de crecimiento del fruto y a partir de los parámetros estimados en el modelo en la fase de reducción se podría predecir la eficacia de la aplicación pocos días después de que se realice. El hecho de poder predecir la eficacia de la primera aplicación podría ser una herramienta de gran ayuda a la hora de la toma de decisiones para realizar la segunda. En esta línea, el estudio de la inhibición causada por Brevis® con sistemas de medición de la fotosíntesis y observar si su comportamiento sigue el mismo patrón que las mediciones de fluorescencia para poder modelizar y conocer mejor el comportamiento de la inhibición de Brevis®.

Por otro lado, también es necesario el estudio de las diferentes hojas que forman parte de la copa del árbol y su evolución en el tiempo. Además, es necesario conocer cómo evolucionan las hojas en las diferentes variedades. En el capítulo I se han formulado diferentes hipótesis que podrían resolver las diferencias entre la eficacia en las diferentes variedades y en los diferentes estados fenológicos del fruto. Además, permitiría regular la dosis de aplicación en cada fase del fruto y del tipo de hoja predominante con el objetivo de homogenizar la aplicación y la eficacia.

Adicionalmente, los resultados obtenidos también sugieren que es necesario profundizar más en el análisis de las condiciones climáticas que favorecen la aplicación de Brevis®. Es necesario realizar estudios de campo con el objetivo de intentar obtener datos climáticos en diferentes condiciones. El hecho de obtener un gran número de datos en diferentes condiciones climáticas permitiría alcanzar la suficiente variabilidad y determinar de forma más eficiente todas aquellas variables climáticas clave para el aclareo con Brevis®. Si bien en la presente tesis se ha trabajado en este sentido, los resultados obtenidos explican un 70% en de la eficacia en base a la climatología. Sin embargo, es posible que al aumentar el número de ensayos aparezcan nuevos parámetros climáticos que, junto con los parámetros fisiológicos, permitan mejorar las predicciones. Otra forma de enfocar futuros trabajos en determinar las condiciones climáticas clave en la eficacia de Brevis® serían los estudios en condiciones controladas (radiación, temperatura, humedad, etc.). Si bien en condiciones de campo o en cámaras climáticas se podrían modificar dichos parámetros y crear situaciones climáticas controladas. Un ejempló sería modificar la temperatura nocturna, situación que podría permitir establecer umbrales exactos de eficacia. De esta manera se pueden modificar diferentes factores climáticos y determinar su importancia en la eficacia de Brevis®.

La eficacia de Brevis® no es uniforme en el árbol, de modo que en la parte alta la eficacia es menor que en las partes bajas, hasta el punto que en algunas ocasiones se ha observado sobreaclareo en el tercio inferior, y un nivel de carga adecuado en el tercio superior. Estas diferencias sugieren que la radiación y consecuentemente el balance de carbohidratos es diferente en el árbol, lo que provoca que la eficacia de Brevis® varíe. A pesar de ello, apenas existen trabajos con aplicaciones dirigidas a diferentes partes del árbol. Desde un punto de vista práctico en el empleo de Brevis®, parece interesante profundizar en aplicaciones dirigidas en base al balance de carbohidratos según zonificación del árbol. Dichos estudios, aparte de conseguir una carga más equilibrada, también podrían reducir el coste de la aplicación.

Por último, también es necesario el estudio combinando todas las materias activas que hay en el mercado para el aclareo. Dichos estudios permitirían conocer sinergismos y antagonismos entre productos y así poder definir un programa de aclareo más eficaz con todas las materias activas que hay en el mercado. Actualmente en España se está trabajando en programas de aclareo en los que se combinan ANA, BA y Brevis®. Los resultados obtenidos parecen indicar que existe sinergismo entre la aplicación de BA seguida de la de Brevis®, situación que puede ser interesante porque potenciaría la eficacia de este programa. Además, es bien conocido que en algunas variedades no es recomendable el uso de BA con ANA ya que produce frutos pigmeos. Con todo esto es necesario el conocimiento de los efectos de las aplicaciones combinadas con el objetivo de mejorar los programas de aclareo en base a los productos que hay en el mercado y los modelos climáticos y fisiológicos.

3. Aplicación práctica de la tesis

Antes del registro de Brevis®, el aclareo químico en manzano se basaba y se basa en el empleo de diversas materias activas cuya eficacia varía en función de diversos factores. Uno de dichos factores es la variedad, de modo que en aquellas que presentan eficacias bajas con el empleo de los productos autorizados, el aclareo manual se realiza cada año con un requerimiento elevado de horas/hectárea, mientras que para aquellas variedades que presentan eficacias correctas en el aclareo químico, puede llegar a no ser necesario el aclareo manual, o éste es reducido. En el primer grupo tendríamos variedades como 'Gala' y 'Fuji', sobre las que se han centrado la mayor parte de los ensayos realizados en IRTA-E.E.Lleida e IRTA-E.E.A. Mas Badia.

Los resultados obtenidos en la presente tesis han resuelto cuestiones clave en un programa de aclareo de manzano basado en Brevis® para optimizarlo con el objetivo de alcanzar la carga óptima, reduciendo de esa manera las necesidades de aclareo manual. Las cuestiones claves que se han abordado han sido aquellos factores que afectan a la eficacia de Brevis® y que deben tenerse presentes en un programa de aclareo, como es el caso de condiciones meteorológicas, la variedad, el momento de aplicación, la dosis y el número de aplicaciones.

Aún así existen otros factores que se deben considerar a la hora de definir una estrategia de aclareo, y que no han sido abordados en esta tesis; es el caso del histórico de dificultad de aclareo. Es necesario evaluar nivel de floración y las condiciones climáticas durante el periodo de cuajado para definir una estrategia de aclareo adecuada. Es muy importante saber en qué situación y que carga potencial de partida presentan los árboles a la hora de plantear una estrategia de aclareo.

De los factores estudiados, y en referencia a las condiciones meteorológicas, las aplicaciones de Brevis® deberían situarse en aquellos momentos o periodos en que los que se dan condiciones de déficit de carbohidratos, para así provocar la caída de los frutos. Estas circunstancias suelen ocurrir cuando la capacidad fotosintética del árbol es reducida y/o el consumo de carbohidratos es elevado, lo que ocurre si la radiación se reduce a causa de días nublados y/o si las temperaturas nocturnas son elevadas.

En el período de aplicaciones de aclareo químico, y en las condiciones de cultivo de Lleida y de gran parte del Valle del Ebro, es habitual la entrada de frentes lluviosos, que pueden llegar muy debilitados y sin precipitación. Habitualmente esta situación viene acompañada de 1-2 días en que la radiación se reduce aproximadamente un 50% (13 MJ/m2). Los resultados obtenidos han podido constatar que las temperaturas medias y nocturnas altas desde 5 días antes de la aplicación hasta 3 días después favorecen el efecto de Brevis® y son consideradas el factor más importante. Trabajos realizados en condiciones de temperatura controlada han constatado que la temperatura nocturna juega un papel más relevante en la eficacia de Brevis® que la temperatura media. En este sentido se ha establecido como umbral óptimo de aplicación de Brevis® las noches con temperaturas superiores a 14ºC, por el contrario, las temperaturas nocturnas inferiores a 12ºC son consideradas situación de baja eficacia. Además, se ha de tener presente que la reducción de radiación también juega un papel importante cuando hay un periodo prolongado de 4 días, situación poco habitual en la zona donde se ha desarrollado la tesis.

En este sentido es importante destacar que en el periodo de aplicación de Brevis® se pueden producir tres situaciones que favorezcan la eficacia en el aclareo: la primera sería periodos de altas temperaturas nocturna antes de la aplicación, la segunda días con altas temperaturas nocturnas después de la aplicación y por último altas temperaturas antes y después de la aplicación. Estas situaciones maximizan la acción de Brevis®, puesto que la temperatura nocturna es el factor más importante, por lo que es recomendable situar alguna de las aplicaciones en estos periodos favorables. Se ha de tener presente que si las temperaturas altas van acompañadas de más de 3 días de baja radiación el efecto de la aplicación puede ser provocar sobreaclareo. Por el contrario, si las temperaturas son bajas y la radiación es baja la eficacia de Brevis® se ve reducida en función del número de días de reducción de radiación. La experiencia adquirida durante el transcurso de la tesis hace necesario que durante el periodo de aplicación de Brevis® se tenga especial cuidado a las predicciones climáticas para la toma de decisiones a la hora de realizar la aplicación.

Los resultados de la presente tesis evidenciaron que la eficacia de Brevis® varía según la variedad, por lo que las dosis deberían ser superiores en 'Fuji' que en 'Gala', y del mismo modo en 'Gala' deberían ser superiores a Golden. Este factor varietal en la eficacia de Brevis® es fundamental, ya que puede suponer doblar la dosis de aplicación en función de la variedad, pasando de una dosis de 1,1 kg/ha en Golden, como dosis de referencia, a valores de 1,65 y 2,2 kg/ha para 'Gala' y 'Fuji' respectivamente. Aún así es importante que estos valores de referencia deban ser modulados en función de otros factores como el histórico de la finca, las condiciones de cuajado, intensidad de floración, condiciones climáticas, momento de aplicación y numero de aplicaciones. Todos estos factores se han de tener presentes en el momento de aplicación y a partir de las observaciones realizadas a nivel de finca a la hora de decidir la estrategia a seguir y modular la dosis en función de la variedad. La dosis de aplicación tiene una relación directa con la eficacia de aclareo hasta 4,4 kg/ha (dosis doble de la permitida según registro), por lo que a medida que aumenta la dosis se obtiene una mayor eficacia de la aplicación. La respuesta de Brevis® al aumento de dosis implicaría que se puede ajustar la dosis de aplicación en función de la intensidad de aclareo que se pretende conseguir en cada parcela. Además, el hecho de que la eficacia tenga esta relación con la dosis hace que, en función de la variedad y las condiciones climáticas, se pueda modular la dosis de aplicación. Si bien se ha mencionado anteriormente las dosis de referencia en las diferentes variedades se ha de tener presente que, si en el momento de decidir la estrategia nos encontramos ante una finca de difícil aclareo a nivel histórico, alta floración y cuajado, baja susceptibilidad del fruto o mala climatología la estrategia más adecuada sería el subir la dosis de referencia. Por el contrario, ante una finca de fácil aclareo a nivel histórico, baja floración y cuajado, alta susceptibilidad del fruto y condiciones climáticas favorables lo más recomendable es no subir la dosis de referencia de la variedad. En este sentido si la estrategia a seguir es de dos aplicaciones es importante observar todos estos factores después de la primera aplicación y decidir la necesidad y la dosis de la segunda aplicación en función de estos condicionantes.

En el estudio de la sensibilidad del fruto se puede establecer que es sensible a las aplicaciones de Brevis® entre 7 y 19 mm de diámetro. Dicha situación amplia el rango de aplicación de Brevis® respecto los 8 y los 16 mm que establece el registro y amplía el número de días del periodo de aplicación. Aunque el margen de actuación de Brevis® permite que se detecte eficacia entre 7 y 19 mm, se ha podido constatar que la máxima sensibilidad del fruto se obtiene entre los 11,5 y los 14 mm. En una estrategia basada en Brevis® sería recomendable hacer dos aplicaciones cuando el tamaño del fruto esté entre 7 y 19 mm. A la hora de realizar la primera aplicación se podría esperar condiciones climáticas favorables en el periodo de 7 a 14 mm y si no se producen, se realizaría la primera aplicación en el momento de máxima sensibilidad de fruto (11,5-14mm), a partir de ese momento se podría esperar hasta los 19 mm buscando condiciones óptimas de aclareo y, si éstas no se producen, modular la dosis para incrementar el efecto de la segunda aplicación. Es importante destacar que las condiciones climáticas favorables son el factor más importante a la hora de aplicar Brevis®, por lo que si durante el periodo de aplicación de Brevis® se producen, es recomendable aplicar siempre que se esté en el rango de tamaños en los que presenta eficacia.

Los trabajos previos realizados y las recomendaciones de la etiqueta sugerían la doble aplicación de Brevis® como la estrategia a utilizar. Además, posicionaban la primera aplicación en 8 mm y la segunda en 12 mm separadas entre ellas entre 5 y 10 días. Los resultados obtenidos en la presente tesis coinciden en que la doble aplicación es la estrategia más correcta a seguir, ya que siempre se obtienen mejores resultados que en las aplicaciones únicas. Sin embargo, el número de días entre aplicaciones no es importante, puesto que son necesarias condiciones climáticas óptimas de aplicación. No obstante, es importante tener en cuenta que si la situación de partida es de baja floración y mal cuajado es posible que si la aplicación se realiza en condicione climáticas optimas no sea necesaria la segunda aplicación. Asimismo, cuando la situación es la inversa y los árboles presentan una carga potencial elevada, es necesario posicionar las aplicaciones en condiciones climáticas adecuadas, sin tener en cuenta el número de días entre ellas, para obtener un efecto de aclareo adecuado.

En conclusión, Brevis® resulta ser una herramienta muy versátil, pero a la vez técnica para el aclareo químico en manzano, permitiendo manejar la carga final del árbol en base a dosis, momento de aplicación y número de aplicaciones. Es probable que en un futuro próximo pueda monitorizarse el aclareo para definir el momento, la dosis y el número de aplicaciones óptimo, en base a la floración, al nivel inicial de cuajado, al seguimiento del crecimiento de los frutos, la evaluación del balance de carbohidratos y/o de la inhibición de la fotosíntesis, lo que facilitaría la toma de decisiones al técnico y al fruticultor.

4. Conclusiones

i. En todos los ensayos realizados en los que se evaluó la respuesta de aclareo de Brevis® se observaron reducciones significativas en el carga o número de frutos por árbol y en la producción. Mientras que el color, el calibre y el peso del fruto fueron superiores al reducirse la carga (Capítulos I, II, III, IV y V).

ii. Brevis® mostró eficacia en el aclareo en aplicaciones entre 7,5 y 19 mm del fruto central en estudios realizados en ‘Gala’ y ‘Fuji’, constatándose también que la máxima eficacia se registró en el rango de 11,5 a 14mm (Capítulo I).

iii. ‘Gala’ mostró mayor respuesta al aclareo con Brevis® que ‘Fuji’. Del mismo modo, la inhibición de la fotosíntesis causada por Brevis® fue mayor en ‘Gala’ que en ‘Fuji’ (Capítulos I y III).

iv. Los factores climáticos que afectan en mayor medida la eficacia de Brevis® son las temperaturas medias (24h) y nocturnas en los 5 días anteriores a la aplicación y los 3 días posteriores a la aplicación (Capitulo II).

v. Brevis® mostró una relación lineal entre dosis y todos los parámetros de eficacia en el aclareo, calidad (calibre, color y pesos del fruto) y fluorescencia evaluados en ‘Gala’ y ‘Fuji’ entre 1,1 y 4,4 kg/ha (Capitulo III).

vi. El modelo farmacológico a partir de la función biexponencial mostró un alto grado de ajuste y los valores calculados se correlacionaron con los valores reales. El modelo bioexponencial mostró un ajuste significativo para explicar la evolución de Qy, mostrando que el valor de máxima inhibición se produce entre los días 4 y 10 después de la aplicación. A pesar de ello el valor Qy por sí solo no es válido para modelizar la fluorescencia ya que es muy variable y es necesario utilizar la fluorescencia de un testigo sin tratar para calcular el porcentaje de Qy y reducir dicha variabilidad (Capítulo I y III).

vii. Los parámetros estudiados en la función biexponencial muestran relación directa entre eficacia e inhibición de fotosíntesis. Sin embargo, dichos parámetros actualmente solo pueden utilizarse para caracterizar la eficacia del año, ya que es necesario que el periodo de inhibición haya finalizado. Por lo tanto, el número de días para realizar la predicción es demasiado elevado para la toma de decisiones en el caso de realizar aplicaciones posteriores (Capítulo I y III).

viii. En el caso de un programa de aclareo basado en dos aplicaciones de Brevis® entre 7,5 mm y 13,5 mm, la eficacia del programa está definida por las condiciones climáticas que se dan en los dos momentos de aplicación, mientras que la separación entre las aplicaciones no afecta a la eficacia final (Capitulo IV).

ix. La reducción en la radiación ocasionada por las redes antigranizo no influyó en la eficacia de Brevis® en el aclareo, ni en la inhibición de la fluorescencia (Capítulo V).

BIBLIOGRFÍA

ADAMA, New Zealand 2017. Over 600 trials globally back innovative new apple thinner Brevis®.

Alegre, S., Bonany, J., Carbo, J., 2008. Optimizar la carga de los frutos mediante el aclareo para aumentar la rentabilidad del manzano. Vida rural 15, 46-52.

Alins Valls, G., 2009. Tècniques de maneig de pomera (*Malus domestica* Borkhausen) en agricultura ecològica. Universitat de Lleida.

Almanza, F.J., Elos, M., López, A., Valdez, A., 2000. Efecto del aclareo quimico y manual en inflorescencias y frutos de manzano. Agronomia Mesoamericana 11.

Amarante, C.V.T., Steffens, C.A., Argenta, L.C., 2011. Yield and fruit quality of 'Gala' and 'Fuji' apple trees protected by white anti-hail net. Sci. Hortic. 129, 79-85.

Amarante, C.V.T., Steffens, C.A., Mota, C.S., dos Santos, H.P., 2007. Radiation, photosynthesis, yield, and fruit quality of 'Royal 'Gala'' apples under hail protection nets. Pesqui. Agropecu. Bras. 42, 925-931.

Anthony, B., Serra, S., Musacchi, S., 2019. Optimizing Crop Load for New Apple Cultivar: "WA38". Agronomy 9, 107.

Bain, J.M., Robertson, R., 1951. The physiology of growth in apple fruits I. Cell size, cell number, and fruit development. Australian Journal of Biological Sciences 4, 75-91.

Bangerth, F., 2000. Abscission and thinning of young fruit and thier regulation by plant hormones and bioregulators. Plant Growth Regul. 31, 43-59.

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.

Belzunces, B., 2017. Atomic-scale study of pesticide interaction with soil mineral matter. Université Paul Sabatier-Toulouse III.

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

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

Blanke, M.M., 2009. The structure of coloured hail nets affects light transmission, light spectrum, phytochrome and apple fruit colouration., 817 ed. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 177-184.

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.

Buban, T., 2000. The use of benzyladenine in orchard fruit growing: a mini review. Plant Growth Regul. 32, 381-390.

Burkholder, C., McCown, M., 1941. Effect of scoring and of a-naphthyl acetic acid and amide spray upon fruit set and of the spray upon pre-harvest fruit drop, Proc. Amer. Soc. Hort. Sci, pp. 117-120.

Burnside, O., LeBaron, H.M., McFarland, J.E., 2008. The Triazine Herbicides: 50 Years Revolutionizing Agriculture. Elsevier.

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., Lyons, C., Barden, J., Young, R., 1986. Desiccating chemicals for bloom thinning of peach and photosynthetic inhibition for post-bloom thinning of apple and peach. Acta Horticulturae (Netherlands).

Byers, R., Lyons Jr, C., Del Valle, T., Barden, J., Young, R., 1984. Peach fruit abscission by shading and photosynthetic inhibition. Hortscience.

Byers, R.E., 1997. Effects of bloom-thinning chemicals on apple fruit set. Journal of tree fruit production 2, 13-31.

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

Byers, R.E., Barden, J.A., Carbaugh, D.H., 1990a. Thinning of spur 'Delicious' apples by shade, terbacil, carbaryl and Ethephon. J. Am. Soc. Hortic. Sci. 115, 9-13.

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

Byers, R.E., Carbaugh, D., 2002. Effects of thinning time on yield, fruit size, and return bloom of 'York'and 'Golden Delicious' apple trees. Journal of tree fruit production 3, 55-62.

Byers, R.E., Lyons, C.G., Yoder, K.S., Barden, J.A., Young, R.W., 1985. Peach and apple thinning by shading and photosynthetic inhibition. J. Horticult. Sci. 60, 465-472.

Catania, P., Inglese, P., Pipitone, F., Vallone, M., 2011. Assessment of the Wind Influence on Spray Application using an Artificial Vineyard. Eur. J. Hortic. Sci. 76, 102-108.

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.

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

Costa, G., Finetti, L., Civolani, S., Bernacchia, G., 2017. Evaluation of Brevis® as a thinning agent for 'Fuji', EUFRIN Thinning Working Group Symposia 1221, pp. 37-38.

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.

DelValle, T., Barden, J., Byers, R., 1985. Thinning of peaches by temporary inhibition of photosynthesis with terbacil. Journal of the American Society for Horticultural Science (USA).

DelValle, T.B.G., 1983. Influence of photosynthetic inhibitors on peach. Virginia Polytechnic Institute and State University.

Dennis, F.G., 2000. The history of fruit thinning. Plant Growth Regul. 31, 1-16.

Dini Viñoly, M.A., 2013. Manejo de la carga frutal en manzano del grupo 'Gala' con diferentes tratamientos químicos y manuales.

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

Dussi, M.C., Giardina, G., Sosa, D., Junyent, R.G., Zecca, A., Reeb, P.R., 2005. Shade nets effect on canopy light distribution and quality of fruit and spur leaf on apple cv. 'Fuji'. Spanish Journal of Agricultural Research 3, 253-260.

Fallahi, E., Greene, D.W., 2010. The Impact of Blossom and Postbloom Thinners on Fruit Set and Fruit Quality in Apples and Stone Fruits 884 ed. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 179-187.

FAO, 2018. Food and Agriculture Organization of the united nations. Accessed on april the 30 of 2019.

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.

Ferree, D.C., 1996. Performance of benzyladenine as a chemical thinner on eight apple cultivars. Journal of Tree Fruit Production 1, 33-50.

Ferree, D.C., Warrington, I.J., 2003. Apples: botany, production, and uses. CABI.

Fischer, G., 2000. Efecto de las condiciones en precosecha sobre la calidad poscosecha de los frutos. Rev. Comalfi 27, 39-50.

Funes, I., Aranda, X., Biel, C., Carbó, J., Camps, F., Molina, A.J., Herralde, F.d., Grau, B., Savé, R., 2016. Future climate change impacts on apple flowering date in a Mediterranean subbasin. Agricultural Water Management 164, 19-27.

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.

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

Grappadelli, L.C., Lakso, A.N., Flore, J.A., 1994. Early season patterns of carbohydrate partitioning in exposed and shaded apple branches. J. Am. Soc. Hortic. Sci. 119, 596-603.

Greene, D., Costa, G., 2013. Fruit thinning in pome-and stone-fruit: state of the art, EUFRIN Thinning Working Group Symposia 998, 998 ed. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 93-102.

Greene, D.W., 2002. Chemicals, timing, and environmental factors involved in thinner efficacy on apple. Hortscience 37, 477-481.

Greene, D.W., 2014. Use of Metamitron Alone and in Combination with 6-Benzyladenine for Thinning Apples, In: McArtney, S.J., Spann, T. (Eds.), XII International Symposium on Plant Bioregulators in Fruit Production. Int Soc Horticultural Science, Leuven 1, pp. 167-172.

Greene, D.W., Groome, P., 2010. Effect of Thinner and Time of Shading on Fruit Set of 'McIntosh' Apples., In: Costa, G. (Ed.), XI International Symposium on Plant Bioregulators in Fruit Production. Int Soc Horticultural Science, Leuven 1, pp. 505-510.

Greene, D.W., Krupa, J., Vezina, M., 2015. It is time to rediscover Amid-Thin. UMass Fruit Notes 80(2), 1-4.

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.

Hamacher, M., Blanke, M.M., Lenz, F., 1994. Diurnal CO2 fluctuations in an apple orchard. Environ. Exp. Bot. 34, 385-391.

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

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

Iwanami, H., Moriya-Tanaka, Y., Honda, C., Hanada, T., Wada, M., 2018. A model for representing the relationships among crop load, timing of thinning, flower bud formation, and fruit weight in apples. Sci. Hortic. 242, 181-187.

Iwanami, H., Moriya-Tanaka, Y., Honda, C., Wada, M., Moriya, S., Okada, K., Haji, T., Abe, K., 2012. Relationships among apple fruit abscission, source strength, and cultivar. Sci. Hortic. 146, 39-44.

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

Jones, K.M., Bound, S.A., Oakford, M.J., 1992. Identifying the optimum thinning time for red 'Fuji' apples. J. Horticult. Sci. 67, 685-694.

Jonkers, H., 1979. Biennial bearing in apple and pear: A literature survey. Sci. Hortic. 11, 303-317.

Kalcsits, L., Musacchi, S., Layne, D.R., Schmidt, T., Mupambi, G., Serra, S., Mendoza, M., Asteggiano, L., Jarolmasjed, S., Sankaran, S., Khot, L.R., Espinoza, C.Z., 2017. Above and below-ground environmental changes associated with the use of photoselective protective netting to reduce sunburn in apple. Agric. For. Meteorol. 237, 9-17.

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

Kockerols, K., Widmer, A., Golles, M., Bertschinger, L., Schwan, S., 2008. Apple crop thinning by tree shading. Agrarforschung 15, 258-263.

Kolarič, J., 2010. Abscission of young apple fruits (*Malus domestica* Borkh.): a review. Agricultura 7, 31-36.

Kon, T.M., Schupp, J.R., Yoder, K.S., Combs, L.D., Schupp, M.A., 2018. Comparison of Chemical Blossom Thinners Using 'Golden Delicious' and 'Gala' Pollen Tube Growth Models as Timing Aids. Hortscience 53, 1143-1151.

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., 2009. Effects of chemical thinning with metamitron on fruit set, yield and fruit quality of'Elstar', XI International Symposium on Plant Bioregulators in Fruit Production 884, pp. 531-536.

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., Goffinet, M., 2013. Apple fruit growth. New York Fruit Quarterly 21, 11-14.

Lakso, A., Goffinet, M., 2017. Advances in understanding apple fruit development, Achieving sustainable cultivation of apples. Burleigh Dodds Science Publishing, pp. 127-158.

Lakso, A.N., 1994. Apple, Handbook of environmental physiology of fruit crops. CRC Press, pp. 3-42.

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.

Lakso, A.N., Robinson, T.L., 2015. Decision Support for Apple Thinning Based on Carbon Balance Modeling, In: Bourgeois, G. (Ed.), IX International Symposium on Modelling in Fruit Research and Orchard Management. Int Soc Horticultural Science, Leuven 1, pp. 235-242.

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.

Lakso, A.N., Wunsche, J.N., Palmer, J.W., Grappadelli, L.C., 1999. Measurement and modeling of carbon balance of the apple tree. Hortscience 34, 1040-1047.

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.

Link, H., 2000. Significance of flower and fruit thinning on fruit quality. Plant Growth Regul. 31, 17-26.

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.

Lordan, J.A., L; Gonzalez, L; Àvila, G; Carbó, J, 2018. ¿Como podemos optimizar el aclareo químici en manzano? XXIII Jornada fruticola Mollerussa.

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.

MAPA, 2019. Fruta de Pepita. Campaña. 2018-19 Ministerio de Agricultura, Pesca y Alimentacion. BOLETÍN 5/2018-19 Mayo 2019.

Marini, R.P., 1996. Chemically Thinning SpurDelicious' Apples with Carbaryl, NAA, and Ethephon at Various Stages of Fruit Development. HortTechnology 6, 241-246.

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.

Mechant, E., 2011. Metamitron resistant Chenopodium album: Characterisation, detection and distribution in Belgian sugar beet. Ghent University.

Middleton, S., McWaters, A., 2002. Hail netting of apple orchards australian experience. Compact Fruit Tree 35, 51-55.

Milic, B., Magazin, N., Keserovic, Z., Doric, M., 2011. Flower thinning of apple cultivar Braeburn using ammonium and potassium thiosulfate - Short communication. Hortic. Sci. 38, 120-124.

Milić, B., Tarlanović, J., Keserović, Z., Zorić, L., Blagojević, B., Magazin, N., 2017. The Growth of Apple Central Fruits as Affected by Thinning with NAA, BA and Naphthenic Acids. Erwerbs-Obstbau 59, 185-193.

Mupambi, G., Anthony, B.M., Layne, D.R., Musacchi, S., Serra, S., Schmidt, T., Kalcsits, L.A., 2018. The influence of protective netting on tree physiology and fruit quality of apple: A review. Sci. Hortic. 236, 60-72.

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.

Olesen, T., Muldoon, S.J., 2009. Branch development in custard apple (cherimoya Annona cherimola Miller x sugar apple A. squamosa L.) in relation to tip-pruning and flowering, including effects on production. Trees-Struct. Funct. 23, 855-862.

Ordonez, V., Molina-Corral, F.J., Olivas-Dorantes, C.L., Jacobo-Cuellar, J.L., Gonzalez-Aguilar, G., Espino, M., Sepulveda, D., Olivas, G.I., 2016. Comparative study of the effects of black or white hail nets on the fruit quality of 'Golden Delicious' apples. Fruits 71, 229-238.

Ouma, G., Kunz, A., Blanke, M.M., 2005. Chemical and non-chemical thinning methods in apple (*Malus domestica* Borkh). Discovery and Innovation 17, 50-55.

Paltineanu, C., Chitu, E., 2006. An estimation model of the climatic rating in the 'Golden delicious' apple cultivar in the southern part of Romania, 707 ed. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 119-125.

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.

Parra-Quezada, R., Robinson, T., Osborne, J., Parra-Bujanda, L., 2008. Efecto de carga de fruto y déficit hídrico en la calidad y producción de manzana. Revista Chapingo. Serie horticultura 14, 49-54.

Parra-Quezada, R.A., Robinson, T.L., Osborne, J.L., Lakso, A.N., Parra-Bujanda, L.B., 2006. Temperature and chemical thinner affect fruit set, and growth of 'empire' apple, In: Webster, A.D., Ramirez, H. (Eds.), Proceedings of the Xth International Symposium on Plant Bioregulators in Fruit Production. Int Soc Horticultural Science, Leuven 1, pp. 327-336.

Peifer, L., Ottnad, S., Kunz, A., Damerow, L., Blanke, M., 2018. Effect of non-chemical crop load regulation on apple fruit quality, assessed by the DA-meter. Sci. Hortic. 233, 526-531.

Petri, J.L., Couto, M., Gabardo, G.C., Francescatto, P., Hawerroth, F.J., 2016. Metamitron replacing carbaryl in post bloom thinning of apple trees. Rev. Bras. Frutic. 38, 14.

Pirkner, M., Dicken, U., Tanny, J., 2014. Penman-Monteith approaches for estimating crop evapotranspiration in screenhouses - a case study with table-grape. Int. J. Biometeorol. 58, 725-737.

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., 1997. Raleo químico de manzanas. Revista Frutícola 18, 73-75.

Reginato, G., Osorio, H., Navarrete, J., 2014. A Single Metamitron Spray Thinned 'Brookfield ‘Gala’' Apples as Effectively as a Program of Treatments Based on NAA, Carbaryl and BA, In: Theron, K. (Ed.), X International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems. Int Soc Horticultural Science, Leuven 1, pp. 397-404.

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.

Roberts, J.A., Elliott, K.A., Gonzalez-Carranza, Z.H., 2002. Abscission, dehiscence, and other cell separation processes. Annual review of plant biology 53, 131-158.

Robinson, T., Hoying, S., Iungerman, K., Kviklys, D., 2010. Retain combined with NAA controls pre-harvest drop of McIntosh apples better than either chemical alone. New York Fruit Quart 18, 9-13.

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., Lakso, A., Greene, D., Hoying, S., 2013. Precision crop load management part 1. NY Fruit Quarterly 21, 3-9.

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., 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.

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.

Romo-Chacón, A., Orozco-Avitia, J.A., Gardea, A.A., Guerreo-Prieto, V., Soto-Parra, J., 2007. Hail Net Effect on Photosynthetic Rate and Fruit Color Development of Starkrimson Apple Trees. Journal-American Pomological Society 61, 174.

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

Rosa, N., Verjans, W., Oliveira, C., Bylemans, D., Remy, S., 2017. Comparison between 6-benzyladenine and metamitron as thinning agents in'Royal 'Gala'', 'Cripps Pink' and 'Red Delicious' apple cultivars, EUFRIN Thinning Working Group Symposia 1221, pp. 51-58.

Schröder, M., 2001. Bedeutung und Wirkungsweise chemischer Ausdünnungsmittel beim Apfel am Beispiel von Ammoniumthiosulfat, Benzyladenin und Harnstoff. Grauer.

Seehuber, C., Damerow, L., Kunz, A., Blanke, M., 2012. Selective mechanical thinning for crop load management (CLM) of fruit trees, X International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems 1058, pp. 405-410.

Serra, S., Leisso, R., Giordani, L., Kalcsits, L., Musacchi, S., 2016. Crop load influences fruit quality, nutritional balance, and return bloom in 'Honeycrisp' apple. Hortscience 51, 236-244.

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.

Stopar, M., 2006. Thinning of "Fuji" apple trees with ethephon, NAD and BA, alone and in combination. Journal of Fruit and Ornamental Plant Research 14, 39.

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

Stover, E., Greene, D., 2005. Environmental effects on the performance of foliar applied plant growth regulators: A review focusing on tree fruits. HortTechnology 15, 214-221.

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.

Torres, E.A., L., 2013. Jornada PGRs.

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

van Doorn, W.G., Stead, A.D., 1997. Abscission of flowers and floral parts. J. Exp. Bot. 48, 821-837.

Villeneuve, F., Ferre, G., 1988. [Apple. Chemical thinning: effect of bromacil and of terbacil (dates and rates on Smoothee); action mechanisms of bromacil, follow up of the future of fruits. 1987].[French]. Essais CTIFL (France). no. 9.

Widmer, A., 2001. Light intensity and fruit quality under hail protection nets, In: Palmer, J.W., Wunsche, J.N. (Eds.), Proceedings of the Seventh International Symposium on Orchard and Plantation Systems. International Society Horticultural Science, Leuven 1, pp. 421-426.

Williams, M.W., 1979. Chemical thinning of apples. Horticultural Reviews 1, 270-300.

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.