Universitat de Lleida

Aspectos bioquímicos del bloqueo de la maduración de peras ‘Conference’ tratadas con 1-metilciclopropeno (1-MCP) y aplicación de sistemas de control

María de los Ángeles Chiriboga Varea

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Universitat de Lleida
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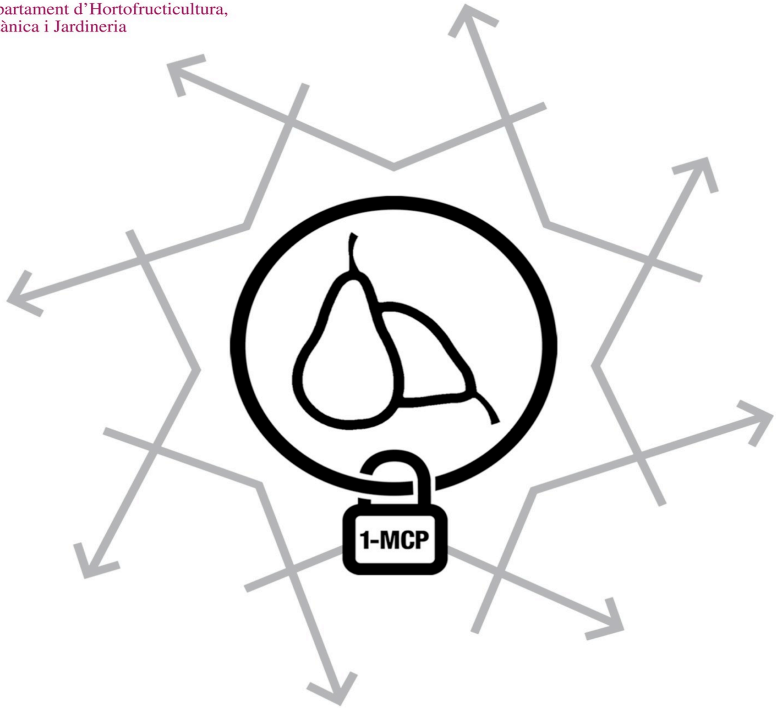

Aspectos bioquímicos del bloqueo de la maduración de peras 'Conference' tratadas con 1-metilciclopropeno (1-MCP) y aplicación de sistemas de control

Tesis doctoral

María Ángeles Chiriboga Varea

Lleida, noviembre 2012

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Escola Tècnica Superior d'Enginyeria Agrària
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Aspectos bioquímicos del bloqueo de la maduración de peras 'Conference' tratadas con 1-metilciclopropeno (1-MCP) y aplicación de sistemas de control

Memoria presentada por:
María de los Ángeles Chiriboga Varea
Para optar al grado de Doctor Ingeniero Agrónomo

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Lleida, noviembre 2012

La presente memoria de Tesis Doctoral que lleva por título **'Aspectos bioquímicos del bloqueo de la maduración de peras 'Conference' tratadas con 1-metilciclopropeno (1-MCP) y aplicación de sistemas de control'** es presentada por **María de los Ángeles Chiriboga Varea**, estudiante del Departamento de Hortofruticultura Botánica y Jardinería de la Universidad de Lleida, para poder optar al grado de Doctora. La parte experimental se ha realizado en los laboratorios de Bioquímica y Fisiología del Área de Postcosecha del Instituto de Investigación y Tecnología Agroalimentaria (IRTA) de Lleida, bajo la dirección de la **Dra. Inmaculada Recasens Guinjuan** y el **Dr. Christian Larrigaudière.** Ambos autorizan la presentación de la memoria de Tesis ya que reúne las condiciones necesarias para su defensa.

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María de los Ángeles Chiriboga Varea
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Lleida, noviembre 2012

A mi padre

por darme todo sin esperar nada a cambio,

por disfrutar y sufrir conmigo,

por enseñarme a vivir.

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Index

RESUMEN - RESUM - SUMMARY **1**

Resumen 3

Resum 5

Summary 7

INTRODUCCIÓN GENERAL **11**

ANTECEDENTES **15**

PARTE 1 17

Aplicación poscosecha de 1-metilciclopropeno en peras .....

PARTE 2 43

Últimos avances en la aplicación del 1-metilciclopropeno (1-MCP) en peras .....

OBJETIVOS - OBJECTIVES **61**

Objetivos 63

Objectives 65

PLAN DE TRABAJO **67**

RESULTADOS **75**

CAPÍTULO 1 77

Responsiveness of 'Conference' pears to 1-methylcyclopropene (1-MCP): the role of harvest date, orchard location and year .....

CAPÍTULO 2 93

Cold-induced changes in ACC metabolism determine softening recovery in 1-MCP treated 'Conference' pears .....

CAPÍTULO 3 113
Antioxidant potential of 'Conference' pears during cold storage and shelf life in response to 1-methylcyclopropene ...
CAPÍTULO 4 133
How to prevent ripening blockage in 1-MCP treated 'Conference' pears .....
CAPÍTULO 5 151
Effect of 1-MCP and exogenous ethylene on fruit ripening and ACC metabolism in 'Conference' pears .....

DISCUSIÓN GENERAL – GENERAL DISCUSSION 165

Discusión general 167
General discussion 177

CONCLUSIONES GENERALES – GENERAL CONCLUSIONS 185

Conclusiones generales 187
General conclusions 189

RESUMEN - RESUM - SUMMARY

RESUMEN

El objetivo de esta tesis fue investigar como el 1-metilciclopropeno (1-MCP) afecta a la conservación y a la calidad de peras de la variedad 'Conference'. El tratamiento con 1-MCP es muy eficaz para reducir la maduración y senescencia pero su aplicación puede resultar en una inhibición permanente de la maduración ('evergreen'). Este bloqueo de la maduración se asocia con una alta concentración de 1-MCP (600 nL L^{-1}), mientras que una concentración más baja (300 nL L^{-1}) da resultados muy variables.

El efecto del 1-MCP y específicamente, el comportamiento 'evergreen' se ve influenciado principalmente por la madurez a la cosecha y especialmente, por la capacidad de la fruta para producir etileno en el momento del tratamiento. El campo y las variaciones propias del año de cultivo también se relacionan con las diferencias en la madurez fisiológica. La combinación del índice de Streif y la producción de etileno a la cosecha pueden ser buenos indicadores para determinar la madurez fisiológica del fruto. Los frutos tratados con 1-MCP deben tener un índice Streif inferior a 0.8 y una producción de etileno inicial por encima de 0.23 µL kg^{-1} h^{-1} a la cosecha para poder madurar adecuadamente durante la vida útil, tras un periodo de conservación frigorífica.

El comportamiento de la maduración y la eficacia del tratamiento 1-MCP en pera 'Conference' están determinados por parámetros fisiológicos relacionados principalmente con el metabolismo del etileno. La regulación fisiológica del metabolismo del fruto durante la conservación en cámara frigorífica, la madurez inicial en cosecha y en menor grado las condiciones del campo son los factores más importantes. El efecto que produce el 1-MCP se debe probablemente a la actividad de la enzima ACC sintasa (ACS). La capacidad de reanudar la maduración está relacionada con el grado de inhibición de esta enzima durante la conservación en frío y la reactivación de la misma durante la vida útil.

El tratamiento con 1-MCP puede retrasar la maduración y prolongar la vida útil de las peras no sólo por la inhibición de la biosíntesis de etileno, sino también a través de un mejor mantenimiento de los sistemas antioxidantes de la fruta. La medida en que el 1-MCP puede tener un efecto positivo sobre el metabolismo antioxidante depende también de la madurez a la cosecha y del campo.

Finalmente, esta tesis propone un sistema que permite la prevención del comportamiento 'evergreen'. La combinación de una alta dosis de 1-MCP y etileno exógeno aplicado al mismo tiempo, inmediatamente después de la cosecha, permite un considerable retraso del proceso de maduración sin bloquearlo por completo y dando lugar a una homogeneización de la maduración entre los frutos de diferentes fechas de cosecha. La eficacia de este tratamiento combinado, es muy evidente cuando se aplica a frutos inmaduros y no se observó un efecto adicional en frutos con una madurez avanzada en los que la maduración no siempre queda bloqueada por el tratamiento. La base fisiológica del efecto del tratamiento

combinado no está del todo dilucidada pero está claramente relacionada con el retraso del inicio de la producción de etileno endógeno durante la vida útil.

RESUM

L'objectiu d'aquesta Tesi ha estat investigar com l' 1-metilciclopropè (1-MCP) afecta a les peres 'Conference'. El tractament amb 1-MCP es molt eficaç per retrasar la maduració i la senescència, però pot arribar a provocar una inhibició permanent de la maduració ('evergreen'). Aquest bloqueig de la maduració va associat amb una alta concentració d'1-MCP (600 nL L^{-1}), mentre que una concentració més baixa (300 nL L^{-1}) pot donar resultats molt variables.

L'efecte d'1-MCP i més específicament, el comportament 'evergreen' estan influenciats principalment per la maduresa de collita i especialment per la capacitat de la fruita per produir etilè en el moment del tractament. Els factors inherents al camp i les variacions entre anys també es relacionen amb les diferències de maduresa fisiològica del fruit a la collita. La combinació de l'índex de Streif i la producció d'etilè al moment de la collita semblen bons indicadors per determinar la maduresa fisiològica del fruit. Els fruits tractats amb 1-MCP necessiten tenir un índex Streif inferior a 0.8 i una producció d'etilè inicial per sobre de 0.23 µL kg^{-1} h^{-1} a la collita per poder madurar adequadament durant la vida útil, després de la conservació frigorífica.

El comportament de la maduració i l'eficàcia del tractament amb 1-MCP en pera 'Conference' venen determinats per paràmetres fisiològics relacionats principalment amb el metabolisme de l'etilè. Les regulacions fisiològiques que tenen lloc durant la conservació en fred, la maduresa inicial del fruit i en menor grau el camp son els factors més importants. L'efecte del tractament amb 1-MCP és produeix possiblement a través del enzim ACC sintasa (ACS). La capacitat de reprendre la maduració ve determinada pel grau d'inhibició d'ACS durant l'emmagatzematge en fred i la reactivació d'aquest enzim durant la vida útil.

El tractament amb 1-MCP pot retrasar la maduració i allargar la vida útil d'emmagatzematge de les peres, no només per la inhibició de la biosíntesi d'etilè, sinó també a través d'un millor manteniment dels sistemes antioxidants de la fruita. La mesura en que l'1-MCP pot tenir un efecte positiu sobre el metabolisme antioxidant depen del estat de maduresa a la collita i del camp.

Finalment, aquesta tesi proposa un sistema que evita la conducta 'evergreen'. La combinació d'una alta dosi d'1-MCP i etilè aplicats simultàniament e immediatament després de la collita, permeta conseguir un considerable retard de la maduració, sense bloquejar-la completament,a la vegada que donalloc a una homogeneïtzació de la maduració entre els fruits de diverses dates de collita. L'eficàcia d'aquest tractament combinates fa evident en el fruitsinmadurs i no té efecte addicional en fruits mes madurs en els que la maduració no sempre queda bloquejada. La base fisiològica de l'efecte del tractament combinat encara no es coneix en total certesa però esta clarament relacionada amb el retard de l'inici de la producció d'etilè endògen durant la vida útil.

SUMMARY

The aim of this thesis was to investigate how 1-methylcyclopropene (1-MCP) affects 'Conference' pears. The treatment with 1-MCP was very effective to reduce ripening and senescence but can result in a permanent inhibition of ripening. This ripening blockage was associated with a high concentration of 1-MCP (600 nL L^{-1}) whereas a lower concentration (300 nL L^{-1}) gave very variable results.

The effect of 1-MCP and more specifically, the 'evergreen' behavior were mainly influenced by the harvest maturity and especially by the ability of the fruit to produce ethylene at the moment of treatment. Orchard and year-to-year variations were also mainly related to differences in the physiological maturity of the fruit at harvest. Combining the Streif index and the ethylene production at harvest seem promising to determine the physiological maturity of the fruit at harvest. Fruit treated with 1-MCP need to have a Streif index less than 0.8 and an initial ethylene production above 0.23 µL kg^{-1} h^{-1} at harvest to be able to ripen properly during shelf life.

The softening behavior and the effectiveness of 1-MCP treatment in 'Conference' pear are determined by physiological parameters related principally with the ethylene metabolism. The physiological regulations taking place during cold acclimation, regulations that are greatly affected by the initial maturity of the fruit and to a lesser extent by orchard location seem to be most important in this regard. The effect of the 1-MCP treatment is probably through the enzyme ACC synthase (ACS) and more specifically the ability to resume ripening is determined by the extent of the inhibition of ACS during cold storage and the reactivation of the enzyme during shelf life.

1-MCP treatment may limit ripening and extend storage life of pears not only by inhibiting ethylene biosynthesis but also through better maintenance of the fruit's antioxidant system. The extent to which 1-MCP may have a positive effect on the antioxidant metabolism depended on the fruit maturity at harvest and the orchard location.

Finally, this thesis proposes a system that permits the prevention of the evergreen behavior. The combination of a high dose of 1-MCP and ethylene applied simultaneously immediately after harvest permitted a considerable delay of the ripening process without completely blocking it and resulted in a homogenization of ripening between fruit from different harvest dates. The efficacy of this treatment was clear in the less mature fruit and no additional effect was observed in more mature fruit where generally ripening is not blocked. The exact physiological basis behind the positive effect is not yet clear but it was clearly related to a shortening in the delay period to initiate ethylene production during shelf life.

Abreviaturas

1-MCP	1-methylcyclopropene 1-metilciclopropeno
AC	Atmósfera controlada
ACC	1-aminocyclopropane-1-carboxylic acid Ácido 1-aminociclopropano-1-carboxílico
ACO	ACC oxidase ACC oxidasa
ACS	ACC synthase ACC sintasa
AFS	α-farneseno sintetasa
ANOVA	Analysis of variance
AOS	Active Oxygen Species Especies activas de oxígeno
AsA	Ascorbic acid Ácido ascórbico
CA	Controlled atmosphere
CAT	Catalase Catalasa, EC 1.11.1.6
CTH	Compuestos trieno conjugados
CTIFL	Centre Technique Interprofessionnel des Fruits et Légumes
Dafb	Days after full bloom
DTT	Dithiothreitol
EC0	Electrical conductivity
ECT	Total electrical conductivity
EG	Endoglucanasas
EL	Electrolyte leakage
ETH	Ethylene
FID	Flame ionization detector
FW	Fresh weight

ABREVIATURAS

GLM	General lineal Model
HPLC	High-pressure liquid chromatography
LSD	Least Significant Differences
MACC	malonyl-ACC
	malonil-ACC
NBT	Nitrobluetetrazolium
PAL	Fenilalanina amonioliasa
PG	Poligalacturonasas
POX	Peroxidase
	Peroxidasa, EC 1.11.1.7
PPO	Polifenoloxidasa
PVP	Polyvinylpyrrolidone
RA	Regular atmosphere
RNA	Ribonucleic acid
	Ácido ribonucleico
ROS	Reactive oxygen species
s.d.	Standard deviation
SAM	S-adenosyl-l-methionine
SOD	Superoxide dismutase
	Superoxido dismutasa, EC 1.15.1.1
SSC	Soluble solids content
TA	Titratable acidity

INTRODUCCIÓN GENERAL

De la superficie total de fruta fresca (sin incluir cítricos) en Catalunya en el año 2010, la pera es la segunda especie más extensa con un 27%, siendo la variedad 'Conference' la más cultivada. En general, las frutas son una parte importante en la dieta humana ya que proporcionan muchos nutrientes esenciales tales como vitaminas, minerales, hidratos de carbono complejos, fibra y antioxidantes.

La demanda del mercado de exportación exige hoy en día frutas de alta calidad, inocuas y sin productos químicos cuestionados. Por ello, se hace especial énfasis en preservar la calidad de los frutos mediante el uso de tecnologías no agresivas para la salud humana y respetuosas con el medio ambiente. La combinación de una cosecha realizada en el momento óptimo y una adecuada conservación de los frutos, así como el uso de ciertos tratamientos en postcosecha, son algunas de las herramientas que se utilizan para lograr dicho objetivo. Estas prácticas permiten reducir la tasa de respiración y la producción del etileno a la vez que mantienen la calidad de los frutos, procesos que son necesarios para la maduración de la fruta.

En los últimos años se han descubierto agentes muy efectivos que actúan sobre estos procesos. Uno de estos compuestos, el 1-metilciclopropeno (1-MCP) ha demostrado ser particularmente efectivo en inhibir la acción del etileno y controlar la maduración y la senescencia. A su vez permite conservar la fruta por más tiempo y ofrece beneficios en regiones donde no es fácil conservar en atmósfera controlada siendo una alternativa para mantener la calidad, reduciendo los costos y los imprevistos asociados a este tipo de conservación.

Cabe mencionar que la efectividad del 1-MCP depende de la especie y del cultivar. En peras por ejemplo, el 1-MCP permite retrasar la maduración, pero en algunos casos este tratamiento impide que el fruto madure durante su vida comercial (bloqueo de maduración) lo que dificulta la flexibilidad de la comercialización.

Los trabajos presentados en esta tesis pretenden investigar cómo el 1-MCP actúa en pera 'Conference' analizando los cambios de calidad asociados a este tratamiento, pero también su base fisiológica a través del estudio del metabolismo del etileno y del metabolismo antioxidante. Además pretende proponer un sistema que permita superar el bloqueo de maduración asociado con este tratamiento. La presente tesis, incluye como antecedentes una primera revisión bibliográfica de la aplicación del 1-MCP en peras que incluye referencias hasta el año 2006 y una segunda con referencias más actualizadas. A continuación, consta de cinco capítulos que describen todos los resultados obtenidos.

ANTECEDENTES

PARTE 1
APLICACIÓN POSCOSECHA DE 1-METILCICLOPROPENO EN PERAS

Maria-Angeles Chiriboga, Yolanda Soria, Christian Larrigaudière, Inmaculada Recasens

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Resumen

En esta revisión se han recopilado la mayoría de los trabajos publicados hasta el momento sobre la aplicación poscosecha de 1-metilciclopropeno (1-MCP) en peras (*Pyrus communis* L.). El 1-MCP actúa como antagonista del etileno, ocupando los lugares de unión de esta hormona con sus receptores. En consecuencia, el 1-MCP retrasa la maduración en los frutos climatéricos y las respuestas fisiológicas etileno-dependientes incluyendo el ablandamiento de la pulpa, la producción de volátiles y la pérdida de color verde. Si bien, este compuesto no afecta al contenido de sólidos solubles, el efecto sobre la acidez es variable dependiendo de diferentes factores. A nivel fisiológico el tratamiento inhibe el metabolismo del ácido 1-aminociclopropano-1-carboxilico (ACC) y también aumenta el potencial antioxidante de la pera mediante un incremento de la actividad de la enzima peroxidasa. Como consecuencia de ello, el 1-MCP tiene un efecto beneficioso sobre la incidencia de desórdenes fisiológicos. Al igual que en otros frutos el tratamiento con 1-MCP inhibe el escaldado superficial y también desórdenes relacionados con la senescencia, como el corazón pardo ó la descomposición interna. El principal problema que pueden tener las peras tratadas con 1-MCP es que en ocasiones no recobran su capacidad para madurar después de la conservación frigorífica. En esos casos permanecen siempre verdes y no alcanzan la madurez óptima de consumo. Se están estudiando las condiciones necesarias para recuperar la capacidad de madurar, incluyendo tratamientos térmicos o aplicaciones exógenas de etileno. Algunos de los resultados se han descrito ya en la bibliografía, pero se precisan todavía más estudios en algunas variedades antes de su uso a nivel comercial.

Summary

Postharvest application of 1-methylcyclopropene on Pears

The main aim of this review was to describe the current knowledge of the application of 1-methylcyclopropene (1-MCP) in pears (*Pyrus communis L.*). The 1-MCP is an inhibitor of ethylene action that links at the receptor level. In this way, the 1-MCP delays ripening in climateric fruits and blocks all the ethylene dependent processes especially firmness loss and inhibits the aroma production in the treated pears. The treatment does not cause changes in sugar levels but may limit acid loss depending on different factors. At a physiological level, the treatment inhibits the metabolism of 1-aminocyclopropane-1-carboxylic acid (ACC) and also increases the total antioxidant potential of the pear mainly through an important increase of peroxidase activity. As a consequence, the 1-MCP treatment has significant effects on physiological disorders incidence. As in others fruits, the treatment clearly inhibits scald disorder but also some senescent-related disorders in pear such as internal breakdown and brown heart. The main problem that the pears treated with 1-MCP may suffer was found in their inability, for some reasons that are still inexplainable, to recover their ripening potential after storage. In this condition the fruits remain 'evergreen' and do not reach their optimal commercial ripeness. Recently, some studies have been carried out to find the conditions to recover this ripening ability in 'evergreen' pears. Post-storage ripening settings including the use of thermal treatments or exogenous ethylene have been tested. First interesting results are described in the literature but complementary experiments are needed in different cultivars before using 1-MCP in commercial stores.

1. INTRODUCCIÓN

El compuesto 1-metilciclopropeno (1-MCP) puede considerarse como una nueva herramienta añadida a la lista de opciones existentes para prolongar la vida poscosecha y mantener la calidad de algunas frutas y hortalizas (Blankenship y Dole, 2003). El producto comercial contiene un 0.14 % de ingrediente activo y está registrado con el nombre de EthylBloc®, de la empresa Floralife S.A., para productos hortícolas ornamentales y SmartFresh™, de Agrofresh Inc., Rohm and Haas, para los productos comestibles. Su uso está autorizado en los Estados Unidos, desde 1999.

Desde el punto de vista físico-químico, el 1-MCP es un compuesto sintético de estructura cíclica que se encuentra en forma de gas en condiciones normales de presión y temperatura. Está pensado para retrasar la maduración de los frutos climatéricos: actúa como antagonista del etileno al inhibir su mecanismo de acción, ocupando los lugares de unión de esta hormona con sus receptores. Debido a que la afinidad del 1-MCP con los receptores es aproximadamente 10 veces mayor que la del propio etileno (Blankenship y Dole, 2003), este compuesto es efectivo a dosis extremadamente bajas, del orden de nL L^{-1}. Hacen falta concentraciones de etileno de 100 µL L^{-1} o superiores para competir de manera eficaz con el 1-MCP por los receptores. Gracias a este modo de acción, el 1-MCP bloquea eficazmente los efectos del etileno tanto endógeno como exógeno.

Sin embargo, se ha visto que el 1-MCP no tiene la misma eficacia para reducir la acción del etileno y retrasar la maduración en diferentes tipos de frutos (Blankenship y Dole, 2003). No sólo la especie sino también la variedad junto con la concentración aplicada, influye notablemente en la respuesta del 1-MCP, tal como se ha demostrado en diferentes variedades de manzanas (Fan y col., 1999; Rupasinghe y col., 2000; Watkins y col., 2000; Mir y col., 2001; DeEll y col., 2002; Pre-Aymard y col., 2003) y peras (Baritelle y col., 2001; Argenta y col., 2003; Kubo y col., 2003; Calvo, 2004; Ekman y col., 2004; Trinchero y col., 2004).

La aplicación de 1-MCP en manzanas se ha investigado ampliamente y se usa ya de forma comercial desde hace algunos años en muchos países. En España ha sido autorizada su aplicación en algunas variedades de manzana en el año 2007. En peras, hoy en día se están realizando numerosas investigaciones sobre el efecto que produce en diferentes variedades tanto de verano como de otoño-invierno. En esta especie son menos los países donde está autorizado su uso. En Argentina y México se registró en el año 2001, en Estados Unidos y Sudáfrica en 2002 y en Australia en 2004. En China también está autorizado para peras asiáticas o nashis.

En peras, una calidad óptima de consumo se caracteriza por una textura mantecosa, un cambio de color apropiado y un sabor característico, asociado al contenido de azúcares, ácidos y a la producción de volátiles (Kappel y col., 1995; Ma y

col., 2000). La pera es un fruto climatérico y su proceso de maduración está regulado por el etileno. Una inhibición de la biosíntesis de esta hormona o de su mecanismo de acción supone ralentizar el proceso de maduración y aumentar la vida útil después de la cosecha (Argenta y col., 2003). La mayoría de las variedades de invierno requieren un periodo de frío para inducir la capacidad normal de maduración, por tanto necesitan la exposición a bajas temperaturas para estimular la producción de etileno endógeno y la subsiguiente maduración (Blankenship y Richardson, 1985).

Estudios recientes han demostrado que la aplicación poscosecha de 1-MCP retrasa considerablemente la maduración en muchas variedades de pera, incluyendo algunas peras de verano como 'Bartlett' (Baritelle y col., 2001; Ekman y col., 2004; Trinchero y col., 2004) y 'Williams' (Calvo, 2003; 2004) y otras de otoño e invierno tales como 'La France' (Hiwasa y col., 2003; Kubo y col., 2003); 'd´Anjou' (Baritelle y col., 2001; Argenta y col., 2003); 'Passe-Crassane' (Lelièvre y col., 1997) y 'Conference' (Eccher-Zerbini y col., 2005; Rizzolo y col., 2005). Las variedades de pera europeas en general se clasifican dentro de las que presentan una buena respuesta a la aplicación poscosecha de concentraciones relativamente bajas de 1-MCP.

2. FACTORES QUE AFECTAN LA APLICACIÓN DE1-MCP

2.1 Concentración, temperatura y duración del tratamiento

La concentración de 1-MCP requerida para saturar los sitios de unión de los receptores del etileno así como la persistencia del tratamiento difiere mucho entre las especies vegetales. La dosis mínima requerida es de 2.5 nL L^{-1} en clavel (Sisler y col., 1996), mientras que en manzanas son eficaces las concentraciones a partir de 600 nL L^{-1}. En parte, esta diferencia de comportamiento entre especies puede ser debida a una distinta afinidad por los receptores o a la síntesis de nuevos receptores en los tejidos en crecimiento (Sisler y Serek, 2003).

Asimismo, las concentraciones efectivas de 1-MCP varían ampliamente con respecto al tiempo, temperatura y método de aplicación, existiendo una estrecha relación entre todos estos factores. Para una determinada dosis, la eficacia es menor al disminuir la temperatura y la duración del tratamiento. Existe la hipótesis de que las bajas temperaturas pueden reducir la afinidad del 1-MCP con los receptores del etileno. La temperatura también afecta a la síntesis de nuevos receptores, siendo ésta mayor a temperaturas elevadas.

Las peras son notablemente más sensibles a la exposición de 1-MCP que las manzanas (Blankenship y Dole, 2003; Crouch, 2003; Watkins y Miller, 2005). Por ello, en peras se han ensayado diferentes dosis aunque en general más bajas

que las utilizadas en manzanas. Mattheis y col. (2000) encontraron que la inhibición de la maduración en peras mediante una sola aplicación de 1-MCP después de la cosecha, depende de la concentración dentro de un rango de 10 a 1000 nL L^{-1}. Los resultados indican sin embargo que la eficacia del tratamiento depende de la variedad, al igual que en manzanas (Watkins y col., 2000).

Las temperaturas más adecuadas para la aplicación de este compuesto también son variables, desde muy bajas (entre -0.5 y 2 ºC) hasta temperaturas ambientales (18-20 ºC). La duración puede ir desde 10-12 horas hasta 20-24 horas, dependiendo de la dosis y la temperatura del tratamiento. Cuando la dosis y/o la temperatura son bajas, es necesario aplicar un tratamiento más prolongado y viceversa (comunicación personal AgroFresh).

Lafer (2005) demostró que una dosis de 125 nL L^{-1} de 1-MCP, aplicado a 2 ºC durante 24 horas en peras 'Williams', no reduce las pérdidas debidas a pardeamiento interno durante el almacenamiento en atmósfera controlada, mientras que en 'Packham's Triumph', variedad con una mayor capacidad de conservación, sí reduce de forma significativa este desorden fisiológico. Dosis superiores de 625 nL L^{-1} no representan ninguna ventaja respecto a dosis más bajas para controlar alteraciones fisiológicas y podredumbres. En el estudio realizado por Calvo y Sozzi (2004) con peras 'Red Clapps' tratadas con 200 nL L^{-1} a 0-1 ºC durante 24 horas demostraron que la combinación del tratamiento con 1-MCP más la conservación en frío reduce significativamente los daños fisiológicos y el ablandamiento sin cambiar los atributos del sabor, evitando por tanto los problemas asociados a la sobremaduración. Ekman y col. en el año 2004 también reportaron que una exposición al 1-MCP entre 200 y 400 nL L^{-1} aplicada a 0 ºC durante 12 horas puede ser beneficiosa ya que reduce los desórdenes fisiológicos y retrasa la maduración en peras 'Bartlett' durante el periodo de vida útil, después de la conservación. Resultados semejantes obtuvieron Trinchero y col. (2004) aplicando 400 nL L^{-1} de 1-MCP a una temperatura de 20 ºC durante 10 horas en peras 'Bartlett', mostrando una inhibición temporal de la producción de etileno, un retraso del climaterio y también del ablandamiento y degradación de los tejidos.

2.2 Estado de madurez y momento de la aplicación

Cuando se aplica 1-MCP debe considerarse el estado de desarrollo del fruto. El estado de madurez en el momento de la cosecha es uno de los parámetros clave para la eficacia del 1-MCP durante la conservación frigorífica y la vida útil (Blankenship y Richardson, 1985). Los mejores resultados en general se han obtenido cuando los frutos se cosechan y se tratan antes del pico climatérico o al comienzo del mismo. Sin embargo, en peras el 1-MCP es capaz de suprimir la producción del etileno y el ablandamiento incluso cuando se aplica después de haber iniciado la maduración, aunque en ese caso se reduce la capacidad de respuesta al 1-MCP (Hiwasa y col., 2003).

La concentración requerida para inhibir el proceso de maduración depende por tanto del estado de madurez de los frutos en el momento del tratamiento (Sisler y Serek, 1997; Watkins y col., 2000). Calvo (2004) demostró en peras 'Williams' recolectadas en dos estados de madurez diferentes que la efectividad del 1-MCP decrece cuanto más avanzado es el estado de desarrollo; no obstante, los frutos cosechados tardíamente presentan una calidad superior al testigo. En ese estudio se ve que una dosis de 200 nL L^{-1} es efectiva aún para conservaciones prolongadas cuando los frutos son recolectados en el momento óptimo, mientras que para cosechas tardías se necesitan dosis más altas (400 y 500 nL L^{-1}) para mantener la firmeza, la acidez y el color verde hasta 150 días. Resultados parecidos obtuvo Lafer (2005) en peras 'Williams', 'Bosc' y 'Packham's Triumph' tratadas con 1-MCP. Para una misma dosis de tratamiento, las peras recolectadas una semana después de la fecha óptima perdieron más firmeza que aquellas cosechadas en su estado óptimo de madurez.

La importancia del tiempo que transcurre desde la cosecha hasta el tratamiento con 1-MCP varía con la especie. Generalmente, cuando más perecedero es el cultivo, más rápidamente debe ser aplicado después de la cosecha. En peras, si el tratamiento se hace antes de la conservación frigorífica, se recomienda tratar como máximo a los 7 días de la recolección, dependiendo de la variedad (comunicación personal, AgroFresh).

En peras, el tratamiento con 1-MCP también puede realizarse después de un periodo de frigoconservación. Sin embargo en este caso, se requieren dosis superiores a las dosis ensayadas en las aplicaciones previas a la conservación frigorífica. Así, un tratamiento con 400 nL L^{-1} de 1-MCP en peras 'Bartlett' aplicado después de 30 días de frigoconservación es incapaz de disminuir el etileno inducido por el propio frío y retrasar el proceso de ablandamiento (Trinchero y col., 2004). En 'Passe-Crassane' la maduración puede inhibirse con 4000 nL L^{-1} de 1-MCP cuando se aplica después de 27 días a 0º C (Lelièvre y col., 1997). En peras 'La France' se han ensayado concentraciones muy elevadas de 1-MCP, entre 10 y 100 µL L^{-1}, cuando la maduración debida a la exposición al frío ya se había iniciado (Kubo y col., 2003). Con 10 µL L^{-1} después de la conservación, se consiguió mantener la firmeza óptima de consumo durante un largo periodo de tiempo y se evitó el desarrollo de la descomposición interna y del escaldado de senescencia.

Por otro lado, también se han ensayado aplicaciones múltiples de 1-MCP a bajas concentraciones para superar el bloqueo de la maduración que produce en algunas ocasiones una sola aplicación con una concentración elevada. Una segunda aplicación de 1-MCP en peras 'Williams' después de 30 ó 60 días de conservación frigorífica produce una leve respuesta cuando las peras se tratan con 100 nL L^{-1} a la cosecha, pero no cuando se tratan con dosis más bajas (10 nL L^{-1}). Peras conservadas durante 75 días en frío y tratadas en la cosecha con 100 nL L^{-1} de 1-MCP y 600 nL L^{-1} después de 60 días de frigoconservación, alcanzan la calidad de consumo 2 días más tarde que las tratadas únicamente con 100 nL L^{-1} en la cosecha ó 4 días antes que las tratadas con 600 nL L^{-1} también en cosecha (Calvo, 2001b).

Resultados semejantes obtuvieron Trinchero y col. (2004), observando que aplicaciones entre 0.4 y 1.6 µL L^{-1} de 1-MCP a los 30 ó 60 días después de la conservación no modifican de manera significativa los índices de madurez. Ekman y col. (2004) comprobaron que una reaplicación con dosis entre 200 y 400 nL L^{-1} de 1-MCP después de 4 semanas de conservación mantiene los frutos más verdes y firmes en contraste con la reaplicación a las 6 semanas. Según estos autores, los resultados sugieren que en frutos que han iniciado su proceso de maduración una reaplicación con 1-MCP es poco eficaz.

3. RESPUESTAS FISIOLÓGICAS DEL TRATAMIENTO CON 1-MCP

3.1 Metabolismo del etileno y tasa de respiración

El tratamiento con 1-MCP inhibe la producción de etileno en los frutos climatéricos, de manera que se retrasan los procesos de ablandamiento y senescencia asociados a esta hormona. Sin embargo, la inhibición no persiste indefinidamente (Fan y col., 1999; Jeong y col., 2002; Dong y col., 2002; Ergun y col., 2005). Después de un periodo de tiempo se reinicia la producción de etileno dependiendo de varios factores entre ellos la dosis de 1-MCP aplicada, el estado de madurez y el tiempo y condiciones de conservación de los frutos.

Después de un período de tiempo que varía según la especie y la variedad, los tejidos vegetales tratados con 1-MCP recuperan parcialmente la sensibilidad al etileno. Como la unión o vinculación del 1-MCP a los receptores del etileno es en principio irreversible (Sisler y col., 1996), el reinicio de la maduración se da probablemente por la síntesis de nuevos receptores durante este periodo. La concentración de 1-MCP utilizada, la duración de la conservación (Watkins y col., 2000) y la madurez de los frutos en el momento del tratamiento (Calvo, 2004) influyen en el tiempo que necesitan los frutos para reanudar el proceso de maduración. Sin embargo, en algunos ensayos con peras se ha visto que los frutos tratados con 1-MCP pierden la habilidad para madurar normalmente (Crouch, 2003; Chen y Spotts, 2005; Bai y col., 2006) quedando bloqueado este proceso.

En algunos frutos, y concretamente en peras, la inhibición de la producción de etileno por una aplicación de 1-MCP va paralela a una menor expresión de los genes que codifican las dos enzimas claves de la ruta biosintética del etileno: ACC (ácido 1-aminociclopropano-1-carboxílico) sintasa y ACC oxidasa (Lelièvre y col., 1997).

Trinchero y col. (2004) observaron una clara inhibición de la producción de etileno en peras 'Bartlett' tratadas con 400 nL L^{-1} de 1-MCP respecto a los frutos control. Estos resultados coinciden con los obtenidos por Bai y col. (2006) en

peras 'd'Anjou' tratadas con una concentración de 300 nL L^{-1}, mientras que con dosis inferiores la inhibición es menor. En peras 'La France' se ha demostrado que una aplicación con una dosis elevada (20 µL L^{-1}) inhibe la producción de etileno en los frutos en estado preclimatérico y la reduce en aquellos que han empezado su proceso de maduración (Hiwasa y col., 2003). Otros autores, en cambio, han comprobado que en peras tratadas con una dosis de 10 nL L^{-1} (Argenta y col., 2003) ó de 500 nL L^{-1} de 1-MCP (Ekman y col., 2004), la producción de etileno no se inhibe totalmente aunque se retrasa varios meses el pico climatérico.

La inhibición de la producción de etileno depende de la dosis aplicada de 1-MCP, pero también depende del tiempo y de las condiciones de conservación. Según Rizzolo y col. (2005) la disminución de la producción de etileno en peras 'Conference' es mayor con 50 nL L^{-1} de 1-MCP que con 25 nL L^{-1}; y también se reduce en mayor grado en atmósfera controlada que en frío normal. Las mayores diferencias se observan para periodos de conservación prolongados.

El incremento en la respiración durante la maduración de los frutos climatéricos ha sido clásicamente asociado con el incremento del etileno. En general, el compuesto 1-MCP reduce la tasa de respiración en peras, pero no la inhibe totalmente (Argenta y col., 2003; Ekman y col., 2004; Trinchero y col., 2004). El efecto no es proporcional a la dosis aplicada, ya que a partir de una dosis efectiva, la reducción de la respiración no es mayor aunque se aumente la dosis (Argenta y col., 2003).

3.2 Metabolismo oxidante

Las condiciones ambientales durante la conservación frigorífica, tanto en frío normal como en atmósfera controlada, pueden inducir la acumulación de especies activas de oxígeno ('Active Oxygen Species' o AOS) y particularmente de peróxido de hidrógeno (Larrigaudière y col., 2001). La presencia de peróxido de hidrógeno en los tejidos puede ocasionar una degradación no enzimática de la membrana que acompaña a la aparición de desórdenes fisiológicos y a la senescencia (Miller, 1986).

El tratamiento con 1-MCP también induce importantes cambios en el metabolismo antioxidante de las peras durante la conservación frigorífica. Larrigaudière y col. (2004) demostraron que peras 'Blanquilla' tratadas con 100 nL L^{-1} de 1-MCP tenían niveles más bajos de peróxido de hidrógeno y ascorbato así como una capacidad antioxidante enzimática más elevada debida a una mayor actividad de las enzimas catalasa, peroxidasa y ascorbato peroxidasa a lo largo de la conservación frigorífica. Estos resultados suponen una evidencia de que el beneficio del 1-MCP en el retraso de la maduración y la senescencia no se debe, exclusivamente, a su efecto sobre inhibición del etileno sino también de manera indirecta a su acción sobre el potencial antioxidante del fruto.

4. EFECTO DEL 1-MCP SOBRE LOS PARÁMETROS DE CALIDAD

4.1 Evolución de la firmeza

La firmeza de la pera va disminuyendo durante los meses de conservación frigorífica, hasta alcanzar valores de 10 N o inferiores en frutos completamente maduros (firmeza medida por el método de Magness Taylor con un pistón de 8 mm de diámetro). Para una buena estimación organoléptica por parte del consumidor, la firmeza en peras no debería ser menor de 20 N en el momento del consumo. Minimizar el ablandamiento de la pulpa después de la cosecha, tanto durante el almacenamiento en frío como en la posterior vida útil o vida comercial, es la clave para mantener una buena calidad. El ablandamiento de la pulpa es etileno-dependiente, en consecuencia el 1-MCP puede retrasar ese proceso.

El descenso de la firmeza durante la conservación y vida útil depende de la variedad y del estado de madurez en cosecha. Después de la frigoconservación, el ablandamiento de los frutos se acelera debido a que la producción del etileno aumenta rápidamente a temperatura ambiente. La aplicación de 1-MCP antes de la conservación retarda el ablandamiento también durante la vida útil y mantiene los frutos con una firmeza comercial durante varios días adicionales con respecto a los frutos no tratados. Sin embargo, si la aplicación se realiza cuando los frutos ya han iniciado la maduración, el efecto sobre la retención de la firmeza es mucho menor (Hiwasa y col., 2003).

La pérdida de firmeza, ocasionada por el ablandamiento de la pared celular, corre paralela a la producción de etileno. En peras, el efecto del 1-MCP sobre el ablandamiento se asocia con un descenso de la actividad de la enzima β-galactosidasa y con un efecto diferencial sobre la expresión de los genes que la codifican (Mwaniki y col., 2005), una reducción de la actividad glicosidasa (Trinchero y col., 2004) y una reducción del RNAm de los genes PG1 y PG2 de las poligalacturonasas pero no de los genes EG1 y EG2 de las endoglucanasas (Hiwasa y col., 2003). Según estos autores, los resultados sugieren que la expresión de los dos genes PG está regulada por el etileno mientras que la expresión de los EG es etileno-independiente.

Diversos trabajos evidencian la eficacia del tratamiento con 1-MCP para mantener la firmeza en las peras, tanto después de largos periodos de conservación frigorífica como después de unos días a temperatura ambiente. Los resultados difieren según las variedades estudiadas y la dosis de 1-MCP aplicada. Según Trinchero y col. (2004), peras 'Bartlett' tratadas con 1-MCP y mantenidas de 6 días a 20 ºC son 75 N más firmes que las no tratadas. En la variedad 'Blanquilla', las peras tratadas con 100 nL L^{-1} mantienen una firmeza por encima de 49 N después de 5 meses de conservación frigorífica (Larrigaudière y col., 2004). Ensayos con peras 'Williams', han dado resultados variables según la fecha de cosecha y la dosis aplicada (Calvo, 2003). Este autor no observó diferencias significativas después de 60 días de

conservación en la firmeza de las peras procedentes de cosechas tempranas y tratadas dentro de un rango de 100 a 600 nL L^{-1}. En peras de cosechas tardías, la aplicación de 100 nL L^{-1} no fue suficiente para mantener niveles de firmeza aceptables siendo necesarias aplicaciones por encima de 200 nL L^{-1}. En ensayos posteriores se ha demostrado que la firmeza mantiene unos valores por encima de 20 N a los 180 días de conservación más 7 días de vida útil, independientemente de la dosis aplicada (200, 400 o 500 nL L^{-1} de 1-MCP), siempre que los frutos se recolecten en un estado de madurez óptimo. En cambio, si la cosecha es tardía se necesitan dosis mayores. El efecto del tratamiento también depende del periodo de vida útil después de la frigoconservación, de manera que se requieren dosis mayores para mantener una firmeza por encima de 20 N si los frutos permanecen 14 días a temperatura ambiente (Calvo, 2004).

En general se observa un efecto sinérgico entre el tratamiento con 1-MCP y la conservación en atmósfera controlada en el mantenimiento de la firmeza, aunque el comportamiento es variable en distintas variedades de pera (Eccher-Zerbini y col., 2005). El efecto es especialmente evidente después de periodos prolongados de vida útil (14 o 22 días) observándose diferencias significativas entre la atmósfera controlada y el frío normal (Rizzolo y col., 2005).

4.2 Producción de volátiles

El etileno regula la producción de componentes volátiles que contribuyen al aroma de los frutos climatéricos. El compuesto 1-MCP al reducir la producción de etileno, reduce la capacidad de producir estos volátiles. La duración de la respuesta viene determinada por las condiciones del tratamiento y particularmente por el estado de madurez de los frutos en el momento de la aplicación. Sin embargo, el efecto no es irreversible sino que revierte cuando se inicia de nuevo la síntesis de etileno en los frutos (Mattheis y col., 2000).

En peras 'd'Anjou' se ha demostrado que el tratamiento con 1-MCP retrasa y reduce la producción de esteres totales obteniéndose el máximo de estos compuestos a los 8 meses de conservación frigorífica, a diferencia de los frutos no tratados en los que la producción máxima se da a los 4 meses. La producción total de alcoholes y aldehídos no se retarda aunque sí disminuye. La producción de ácido acético también es menor entre los 2 y los 6 meses de conservación en los frutos tratados, pero no se presentan diferencias respecto al control, a los 8 meses (Argenta y col., 2003). Pese a esta reducción y retraso en la producción de volátiles, cuando los frutos tratados con 1-MCP empiezan a madurar la producción de volátiles es similar a la de los frutos control. Así en el ensayo anterior se observó que una aplicación de etileno después de la conservación estimuló la producción de algunos compuestos volátiles en los frutos tratados con 1-MCP, de manera que respondieron de igual forma a la aplicación exógena de etileno que los no tratados.

Eccher-Zerbini y col. (2005) demostraron también que en peras 'Conference' y 'Abbé Fetel' el tratamiento con 1-MCP tiene un efecto significativo en la reducción del total de volátiles. Los frutos tratados con 50 nL L^{-1} de 1-MCP redujeron la producción de volátiles tales como acetaldehído, metil acetato, metanol, etanol, butil acetato, butanol y etil butanoato. Estos compuestos generalmente se producen en altas cantidades en los frutos maduros y contribuyen al aroma característico de la pera madura.

El efecto del 1-MCP sobre la producción de volátiles y la calidad sensorial depende del tiempo de conservación y de la dosis aplicada. Rizzolo y col. (2005) comprobaron que la producción de volátiles en peras 'Conference' es mayor en los frutos control que en los tratados con 25 nL L^{-1} y en estos últimos mayor que en los tratados con 50 nL L^{-1}. La dosis más elevada, comparada con el control, provoca una menor producción de ésteres de acetato, etil butanoato, etanol, propanol, butanol, acetaldehído y propanal y una mayor producción de 3-metilbutil 2-metilbutanoato. Respecto a la calidad sensorial, los frutos control y los tratados con 25 nL L^{-1} alcanzan una calidad organoléptica óptima a las 14 semanas de almacenamiento, siendo más firmes, jugosos y aromáticos, mientras que los frutos tratados con 50 nL L^{-1} alcanzan estas características a las 22 semanas, cuando el resto de los frutos entra ya en una fase de deterioro.

4.3 Evolución del color

El compuesto 1-MCP reduce o retrasa la pérdida de color verde en diferentes frutas y hortalizas. Para muchos productos, especialmente vegetales de hoja y algunas variedades de manzana y también en algunas de peras, el mantenimiento del color verde es deseable en el mercado y el amarillamiento es considerado como un signo de senescencia (Watkins, 2006). Sin embargo, para otros frutos la pérdida de clorofila y el desenmascaramiento o la nueva síntesis de pigmentos coloreados es un aspecto esencial de la maduración (Kays, 1997). Por tanto, el éxito del uso del 1-MCP requiere un retraso, pero no una inhibición irreversible de los procesos que involucran el metabolismo de la pigmentación (Watkins, 2006).

Se ha comprobado que la aplicación de 1-MCP reduce la pérdida de color verde en peras 'Williams' tanto en conservación frigorífica como durante su posterior vida útil (Calvo, 2004). Los frutos tratados con 1-MCP presentan un valor del tono (ángulo Hue) mayor de 105º, por tanto mantienen el color verde hasta un período de 60 días de conservación más 7 días de vida útil, siempre que la cosecha se realice en el momento óptimo y hasta los 30 días cuando la cosecha es más tardía. Para un periodo de vida útil más prolongado (14 días) el color varía hacia a un tono amarillento (Hue menor de 100º) en ambas cosechas.

Ekman y col. (2004) comprobaron asimismo que la aplicación de 1-MCP influye en la evolución del color en la variedad de peras 'Bartlett'. Los frutos tratados tardan más días en alcanzar un tono amarillo (ángulo Hue igual o menor de 102º) que los frutos control. El efecto es variable respecto al tiempo de conservación y a la dosis aplicada. Estos autores ensayando aplicaciones de 500 y 1000 nL L^{-1}, obtuvieron diferencias estadísticamente significativas después de 6 o 12 semanas de conservación a -1 ºC, mientras que a partir de las 18 semanas no se apreció ninguna diferencia respecto al control.

Isidoro y Almeida (2006) también encontraron diferencias en el color respecto a la dosis de 1-MCP aplicada en peras 'Rocha'. En este estudio, los frutos tratados con 500 o 1000 nL L^{-1} mantuvieron el color verde después de 120 días a 0 ºC más 7 días a 20 ºC, mientras que en los tratados con sólo 100 nL L^{-1} y en los no tratados el color amarillo se manifestó rápidamente durante el periodo de vida útil.

En la evolución del color además de la duración de la frigoconservación, influye también el régimen de almacenamiento. En peras 'Conference', después 22 semanas de conservación en frío normal más 7 días a 20 ºC el efecto del 1-MCP sobre el color aparece en los frutos tratados con 50 nL L^{-1}, que presentan valores más elevados del tono (ángulo Hue) que los tratados con 25 nL L^{-1} y que los frutos sin tratar. En cambio, en atmósfera controlada, aunque durante todo el periodo de conservación los frutos tratados se mantienen más verdes que los control, a las 22 semanas no se detectan diferencias entre dosis (Eccher-Zerbini y col., 2005).

4.4 Contenido de ácidos orgánicos, azúcares y componentes nutricionales

El efecto del 1-MCP en la acidez titulable es variable, ya que muchas variedades se ven afectadas por el tratamiento pero otras no. Argenta y col. (2003) comprobaron en peras 'd'Anjou' que los valores de acidez titulable son superiores en los frutos tratados con 1-MCP que en los controles. Resultados similares obtuvo Lafer (2005) en un estudio efectuado con las variedades 'Williams', 'Bosc' y 'Packham's Triumph'. También Isidoro y Almeida (2006) observaron en pera 'Rocha' después de 120 días a 0 ºC más 7 días a 20 ºC que en los frutos tratados con dosis elevadas (500 o 1000 nL L^{-1}) la acidez se mantiene constante y superior a la de los frutos no tratados o tratados con dosis más bajas (100 nL L^{-1}). Con las dosis elevadas de 1-MCP se mantiene aproximadamente un valor de acidez de 1.4 g/L hasta los 14 días de vida útil, mientras que el resto de los frutos, a los 7 días ya presentan valores inferiores. Por otro lado, diversos estudios señalan que el tratamiento con 1-MCP no proporciona un efecto adicional (Trinchero y col., 2004) o consistente (Calvo, 2004) sobre la acidez en peras.

En cuanto al contenido de sólidos solubles en los frutos tratados con 1-MCP se podría esperar que fuera mayor que en aquellos no tratados, debido a la reducción que este compuesto provoca en la tasa de respiración. Sin embargo, se ha observado que el tratamiento puede aumentar, disminuir o no afectar al contenido de sólidos solubles, dependiendo de la especie, la variedad y las condiciones de conservación (Watkins, 2006). Algunos autores han demostrado que no existe efecto del tratamiento con 1-MCP en la concentración de sólidos solubles en las variedades de pera 'Beurré d'Anjou' (Calvo, 2003), 'Bosc' (Lafer, 2005), 'Packham's Triumph' (Calvo, 2003; Lafer, 2005), 'Bartlett' (Trinchero y col., 2004), 'Red Clapps' (después de al menos 45 días de conservación) (Calvo y Sozzi, 2004) y 'Williams' (Calvo, 2003; Calvo y Sozzi, 2004; Lafer, 2005). En otras variedades como 'Blanquilla' (Larrigaudière y col., 2004) y 'Rocha' (Isidoro y Almeida, 2006) se ha detectado un contenido de sólidos solubles inferior en las peras tratadas.

El efecto de la aplicación de 1-MCP sobre la calidad nutricional ha sido poco estudiada (Watkins, 2006). Sin embargo en peras 'Blanquilla' tratadas con 1-MCP se ha visto que el contenido de ácido ascórbico (vitamina C) permanece a unos niveles más bajos que en los frutos sin tratar. Probablemente los niveles de ácido ascórbico decrecen en los frutos tratados debido a que aumenta la actividad de la enzima ascorbato peroxidasa que promueve la oxidación de este compuesto a dehidroascorbato (Larrigaudière y col., 2004).

4.5 Pérdida de peso

Los resultados obtenidos en varios trabajos demuestran que el efecto del 1-MCP sobre la pérdida de peso durante la conservación no está del todo claro, ya que no son coincidentes para las distintas especies e incluso para las diferentes variedades de una misma especie. Según Calvo (2001a y 2001b), el 1-MCP no tiene efecto sobre la pérdida de peso en las variedades de pera 'Williams' y 'Beurré d'Anjou'. En 'Packham's Triumph', los resultados son variables entre autores; según Calvo (2001b) el tratamiento con 1-MCP no afecta mientras que según Moggia y col. (2001) reduce la pérdida de peso en esta variedad.

En otros casos se ha comprobado que el efecto del 1-MCP sobre la pérdida de peso es dependiente del estado de madurez de los frutos en el momento de la aplicación del producto. Así, en un estudio realizado por Calvo (2004) con la variedad 'Williams', se vio que las peras cosechadas en el momento óptimo y tratadas con 1-MCP presentaban una menor deshidratación durante 150 días de conservación frigorífica. Sin embargo, en las peras cosechadas tardíamente, el tratamiento con 1-MCP no afectaba de manera significativa a la pérdida de peso.

Por otro lado, contradiciendo los resultados anteriores, Mitcham y col. (2001) afirman que peras tratadas con 1-MCP y conservadas en atmósfera normal tienen un mayor riesgo de pérdida de humedad y por consiguiente de pérdida de peso,

que las conservadas en atmósfera controlada. Mattheis y col. (2000) sostienen que este mayor riesgo de deshidratación en los frutos tratados con 1-MCP se debe a una menor cantidad de componentes cuticulares lipídicos en la piel del fruto después del tratamiento. Como estos componentes lipídicos contribuyen a reducir la pérdida de humedad, el riesgo de deshidratación aumenta. Sin embargo, ambas referencias coinciden en que mediante un manejo adecuado de la humedad, este efecto puede contrarrestarse.

5. EFECTO DEL 1-MCP SOBRE LOS DESÓRDENES FISIOLÓGICOS, LOS DAÑOS MECÁNICOS Y LAS PODREDUMBRES

La pera es una fruta sensible a las alteraciones o desórdenes fisiológicos de piel y pulpa que se desarrollan durante la conservación frigorífica. Estos desórdenes provocan una pérdida parcial o total del valor comercial de la fruta. Los desórdenes externos más habituales en pera son el escaldado superficial (en las variedades sensibles) y los daños físicos o mecánicos (daños por impacto, compresión y vibración). Entre los desórdenes internos destacan el corazón pardo ('brown heart'), la descomposición interna ('internal breakdown'), el pardeamiento interno ("internal browning") y los desórdenes asociados a la senescencia que afectan tanto a la piel como a la pulpa. Resulta deseable por tanto, el desarrollo de protocolos para el uso comercial del 1-MCP en peras para prevenir estas alteraciones.

5.1 Escaldado superficial

Diversos estudios realizados en diferentes variedades de pera indican que el tratamiento con 1-MCP reduce la incidencia y severidad del escaldado superficial, demostrando que puede ser un sustituto efectivo de los antiescaldantes difenilamina y etoxiquina. El 1-MCP ha resultado muy efectivo para prevenir el escaldado superficial en peras 'Beurré d'Anjou' y 'Packham's Triumph' (Calvo, 2003). Por ejemplo, dosis de 200 nL L^{-1} en 'Beurré d'Anjou' y de 400 nL L^{-1} en 'Packham's Triumph' inhiben totalmente el desarrollo de escaldado después de 270 días de conservación más 14 días de vida útil. Otros autores afirman sin embargo que el escaldado superficial se reduce gracias a la aplicación de 1-MCP, pero no se controla totalmente (Argenta y col., 2003; Lafer, 2005). Rizzolo y col. (2005) indican que después de 22 semanas de conservación, tanto en frío normal como en atmósfera controlada el tratamiento con dosis bajas de 1-MCP (25 o 50 nL L^{-1}) no resulta eficaz para reducir el escaldado superficial en peras 'Conference'. Eccher-Zerbini y col. (2005) afirman que el 1-MCP no puede sustituir a la atmósfera controlada en el control de esta alteración, pero sí reforzar su efecto. El 1-MCP parece ser más efectivo en las variedades que tienen tasas de producción de etileno más bajas.

Es necesario tener en cuenta que un control total del escaldado superficial mediante la aplicación de 1-MCP podría inhibir por completo el proceso de maduración de las peras dependiendo de la dosis aplicada. Ekman y col. (2004) comprobaron que un tratamiento con dosis elevadas de 1-MCP (1000 nL L^{-1}) evita la manifestación de escaldado superficial en 'Bartlett' pero no permite que los frutos maduren normalmente después de la conservación frigorífica. Bai y col. (2006) observaron que el escaldado superficial en peras 'd'Anjou' no se controla con dosis de 25 nL L^{-1} o inferiores, mientras que utilizando dosis de 300 nL L^{-1} disminuye la incidencia de escaldado, pero las peras se mantienen duras, con una firmeza superior a 27 N y no alcanzan la calidad de consumo. Dosis intermedias de 50 nL L^{-1} permiten alcanzar la madurez de consumo a la vez que reducir de forma substancial, aunque no controlar totalmente el escaldado.

El origen bioquímico del escaldado superficial ha sido relacionado con la acumulación de α-farnaseno y de compuestos trieno conjugados (CTH) en la piel de los frutos durante la conservación a bajas temperaturas. Los CTH son resultado de la oxidación *in vivo* del α-farnaseno y son considerados como los agentes causales del escaldado superficial (Rowan y col., 1995; Whitaker y col., 1997). Isidoro y Almeida (2006) examinando el efecto del tratamiento con 1-MCP en los niveles de α-farnaseno y CTH en peras 'Rocha', encontraron que dosis de 500 y 1000 nL L^{-1} inhiben el desarrollo de escaldado superficial porque reducen la acumulación de estos compuestos durante la conservación. Resultados similares se han obtenido en peras 'd'Anjou' tratadas con 300 nL L^{-1} (Gapper y col., 2006).

En peras 'Conference' tratadas con 25 o 50 nL L^{-1} de 1-MCP y conservadas hasta 14 semanas en frío normal, los contenidos de α-farnaseno disminuyen por efecto del tratamiento, pero para periodos más largos (22-25 semanas) no se aprecian prácticamente diferencias respecto a los frutos control (Eccher-Zerbini y col., 2005; Rizzolo y col., 2005). Los diferentes compuestos trieno conjugados evolucionan de forma distinta. La concentración de los compuestos CT258 durante las primeras semanas de conservación es más elevada en los frutos tratados y va disminuyendo de manera que al final de la conservación la concentración es mayor en los frutos control. En cambio, los valores de los compuestos CT281 en los frutos tratados se mantuvieron siempre por debajo de los detectados en los frutos control.

Se ha comprobado en peras 'd'Anjou' (Gapper y col., 2006) que la aplicación de 1-MCP inhibe la expresión de *PcAFS1*, gen que codifica la enzima α-farnaseno sintetasa (AFS) reduciendo la síntesis y oxidación de α-farnaseno y por tanto la acumulación de CTH y retrasando el desarrollo de escaldado superficial. Un mecanismo similar se ha identificado en manzanas 'Law Rome' (Pechous y col., 2005), de donde se deduce que la regulación de la actividad y expresión de AFS es una herramienta para el control del escaldado superficial en estos frutos.

5.2 Daños mecánicos

El ablandamiento de las peras debido a la maduración incrementa su susceptibilidad a los daños físicos; los frutos parcial o totalmente maduros son mucho más susceptibles a las magulladuras por vibración e impacto. Según Calvo y Sozzi (2004), la aplicación de 200 nL L^{-1} de 1-MCP en peras 'Red Clapps' puede proporcionar una mayor flexibilidad y resistencia para resistir los posibles daños físicos ocasionados en las diferentes operaciones comerciales como la clasificación, el envasado y el transporte. Como el tratamiento con 1-MCP retrasa la senescencia, también reduce las alteraciones propias de este estado del fruto, por ejemplo el escaldado de senescencia en peras 'd'Anjou' (Calvo, 2003), 'Abbé Fétel' (Eccher-Zerbini y col., 2005), 'Bon Chretien' y 'Packham's Triumph' (Crouch, 2003) y en peras 'La France' disminuye el pardeamiento interno debido a la senescencia (Kubo y col., 2003).

5.3 Desórdenes internos

La aplicación de 1-MCP se ha mostrado eficaz en el control de alteraciones internas del fruto, aunque en muchos casos el control de estas alteraciones depende de la variedad y de la dosis de 1-MCP. Según Argenta y col. (2003) en peras 'd'Anjou' el tratamiento con una dosis de 100 nL L^{-1} es suficiente para controlar el pardeamiento interno y el escaldado de senescencia después de 6 u 8 meses de conservación frigorífica. En peras 'Red Clapp's' una dosis de 100 nL L^{-1} no es suficiente para disminuir la incidencia de descomposición interna, mientras que aumentando la dosis a 200 nL L^{-1} se reduce la manifestación de los síntomas durante un periodo de 14 días a 20 ºC después de 60 días a –0.5 ºC (Calvo y Sozzi, 2004).

Asimismo, Ekman y col. (2004) han evidenciado una reducción de la descomposición interna en peras 'Bartlett' tratadas con 1-MCP. Después de 18 semanas de conservación a –1 ºC más 8 días a 20 ºC, en los frutos tratados con una dosis de 500 nL L^{-1} la incidencia de esta alteración se reduce al 10% frente a los no tratados que presentan una incidencia igual o superior al 50 %. Después de 24 semanas en los frutos tratados con 500 nL L^{-1} la incidencia de la alteración aumenta al 50 % mientras que en los tratados con una dosis de 1000 nL L^{-1} no se observa ningún fruto afectado.

Lafer (2005) después de estudiar el efecto del tratamiento con 1-MCP en distintas variedades de pera indica que la incidencia de alteraciones asociadas a una recolección tardía, como el pardeamiento interno, el corazón pardo o la presencia de cavidades, se reducen significativamente con el tratamiento de 1-MCP, aunque depende del año evaluado, y de que la variedad sea más sensible ('Williams' y 'Bosc') o menos ('Packham's Triumph').

5.4 Podredumbres

El tratamiento con 1-MCP también puede modificar el desarrollo de podredumbres, aunque las respuestas son muy variables entre especies y condiciones de aplicación. Las podredumbres originadas por los hongos *Penicillium expansum*, *Alternaria spp.* y *Botritis cinerera* son las más habituales en peras después de prolongados períodos de conservación frigorífica (Lafer, 2005). La capacidad del 1-MCP para reducir estas podredumbres varía considerablemente según la variedad y el estado de madurez del fruto. Argenta y col. (2003) comprobaron que el tratamiento con 230 nL L^{-1} de 1-MCP disminuye la incidencia de podredumbres, al igual que lo observado en los desórdenes fisiológicos, pero de manera significativa únicamente en 'Williams' y 'Packham's Triumph'. También los frutos de una cosecha tardía presentan más podredumbres por hongos en comparación con los de la cosecha óptima o más temprana.

Se conoce poco acerca de cómo afecta el tratamiento con 1-MCP sobre las infecciones por patógenos. Según Saftner y col. (2003) 1-MCP puede reducir las podredumbres en manzanas gracias a que el tratamiento mantiene la firmeza y por tanto aumenta la resistencia a la infección. En melocotón la resistencia en los frutos tratados se ha correlacionado con una mayor actividad de las enzimas fenilalanina amonioliasa (PAL), polifenoloxidasa (PPO) y peroxidasa (POD) (Liu y col., 2005). En fresas, un incremento de susceptibilidad a las podredumbres en los frutos tratados con elevadas concentraciones de 1-MCP puede estar asociado con una baja actividad de la PAL (Jiang y col., 2001). En principio, el retraso de la maduración asociado a la reducción de la producción de etileno puede aumentar la resistencia a la infección (Watkins, 2006). Sin embargo, se necesitan pequeñas cantidades de etileno endógeno para mantener los niveles básicos de resistencia a los estreses patológicos y medioambientales debido al efecto de esta hormona sobre la regulación de los genes de defensa que actúan contra el estrés (Marcos y col., 2005).

6. BLOQUEO DE LA MADURACIÓN EN PERAS TRATADAS CON 1-MCP

Dependiendo del producto hortícola al que se aplique el compuesto 1-MCP, puede ser deseable que la respuesta persista indefinidamente (como en las hortalizas de hoja) o bien que remita (como es el caso de los frutos climatéricos), permitiendo que pasado un cierto tiempo después del tratamiento los frutos maduren correctamente y alcancen las características organolépticas deseadas por el consumidor (Watkins, 2006).

A diferencia de las manzanas, la calidad óptima de consumo en muchas de las variedades de pera se alcanza cuando los frutos están blandos a la vez que se mantienen jugosos. La aplicación comercial de 1-MCP en variedades europeas es potencialmente problemático ya que en varios ensayos experimentales se observa que las peras tratadas con 1-MCP no

llegan a recobrar su habilidad para madurar, y el efecto residual del tratamiento no se disipa fácilmente después de un período de conservación en frío ya que las peras mantienen una firmeza elevada y el color característico del estado inmaduro (Argenta y col., 2003; Ekman y col., 2004; Trinchero y col., 2004; Chen y Spotts, 2005).

Las peras 'Bartlett' y 'd'Anjou,' si no se tratan con 1-MCP, normalmente maduran después de la conservación frigorífica a lo largo de un periodo de vida útil de 5 y 7 días respectivamente y alcanzan su calidad de consumo con una firmeza de la pulpa entre 14 y 23 N (Chen y col., 2003). Sin embargo aplicando una dosis de 1-MCP igual o superior a 30 nL L^{-1} en peras 'Bartlett', éstas pierden completamente su capacidad de madurar, sin alcanzar la firmeza óptima de consumo después de 3-5 meses de frigoconservación (Chen y Spotts, 2005). Bai y col. (2006) en un ensayo realizado en 2003 también encontraron que aplicando una dosis de 300 nL L^{-1} en peras 'Bartlett' y 'd'Anjou', los frutos no maduran normalmente después de la conservación.

La respuesta al tratamiento y la posterior capacidad para madurar correctamente después de la conservación en frío depende de la variedad, de la dosis aplicada y del tiempo que las peras permanecen en la cámara frigorífica. En peras 'Williams' una concentración de 10 nL L^{-1} de 1-MCP no es suficiente para inhibir la maduración mientras que las dosis más altas (100, 200, 400 y 600 nL L^{-1}) aunque tampoco llegan a inhibirla, la retrasan (Calvo, 2003). Cuando se usan las dosis de 400 y 600 nL L^{-1} y las peras se conservan por un período de 90 días se necesitan más de 20 días a temperatura ambiente para adquirir la calidad de consumo. Estas mismas dosis (400 y 600 nL L^{-1}) inhiben la capacidad normal de maduración en peras 'Beurré d'Anjou' y 'Packham's Triumph' cuando se conservan 90 y 150 días en frío. Aplicando la dosis de 400 nL L^{-1} se necesitan 15 días a 20 ºC para adquirir una calidad comestible, después de 210 días de conservación frigorífica en 'Beurré d'Anjou' o 270 días en 'Packham's Triumph'. Con una dosis de 600 nL L^{-1}, 'Beurré d'Anjou' requiere 11 días para madurar después de 270 días de frigoconservación mientras que 'Packham's Triumph' no llega a madurar normalmente.

Según un estudio de Crouch (2003) con dosis de 300 a 1000 nL L^{-1} en las variedades 'Bon Chretien' y 'Packham's Triumph', las peras tratadas permanecen más firmes que las no tratadas (69 N y 40 N respectivamente) a la vez que tampoco se produce un cambio de color después de 7 días a 15 ºC, permaneciendo verdes mientras que los testigos maduran normalmente. Los frutos tratados sólo cambian a un color amarillo después de un periodo de 3 semanas a 15 ºC. Sin embargo, las peras de las cosechas más tardías recuperan mejor la maduración durante el almacenamiento a 15 ºC que las de las cosechas más tempranas. Estos resultados demuestran que la respuesta al tratamiento con 1-MCP en peras es también dependiente del estado de maduración de los frutos en el momento de la aplicación del 1-MCP.

Todos estos resultados sugieren que el 1-MCP tiene un efecto residual prolongado en pera y/o hay un lapso de tiempo antes de que los niveles de etileno permitan una respiración normal en los frutos y puedan darse los procesos propios de

la maduración. Considerando que 1-MCP se une irreversiblemente a los sitios de unión de los receptores del etileno (Sisler y col., 1996), una aplicación exógena o la propia síntesis de etileno no pueden superar este efecto hasta que se generen nuevos receptores (Crouch, 2003).

Existe un debate acerca de las principales causas fisiológicas que llevan a recobrar la habilidad para madurar en peras tratadas con 1-MCP. La síntesis de nuevos receptores y, probablemente, la disociación del 1-MCP de los sitios de unión de los receptores del etileno después de períodos largos de tiempo son las dos hipótesis que pueden explicar la reanudación del proceso de maduración (Blankenship y Dole, 2003). En cualquier caso, las condiciones de la aplicación de 1-MCP (concentración, temperatura, y duración del tratamiento) deberían permitir que las peras maduren normalmente después de un tiempo de conservación, para alcanzar los parámetros óptimos de comercialización y consumo.

Para evitar el problema del bloqueo de la maduración en peras debido al tratamiento con 1-MCP, Crouch (2003) propone utilizar dosis más bajas o reducir el tiempo de aplicación mientras que Bai y col. (2006) en principio sugieren el uso de tratamientos de acondicionamiento térmico después de la conservación frigorífica, para no modificar la dosis de 300 nL L^{-1} que se aplica a nivel comercial.

Bai y col. (2006) identificaron que la mejor combinación de acondicionamiento en peras 'Bartlett' tratadas con 300 nL L^{-1} de 1-MCP es de 10 días a 20 ºC o 20 días a 10 ºC, si los frutos han permanecido 2 meses en frío normal o 4 meses en atmósfera controlada, y de 10 días a 15 ºC, para conservaciones de 4 meses en frío normal y 6 meses de atmósfera controlada. Con esta combinación tiempo-temperatura, las peras mantienen una firmeza de la pulpa por encima de 45 N y pueden ser transportadas y distribuidas sin riesgo de daños mecánicos. Los frutos maduran durante los días siguientes a 20 ºC hasta alcanzar una firmeza inferior a 27 N (calidad comestible) en aproximadamente 7 días más tarde que los controles. Sin embargo, en este mismo ensayo se determinó que en peras 'd'Anjou' tratadas con 300 nL L^{-1} de 1-MCP ninguna combinación de pre-acondicionamiento de 10-20 ºC durante 5-20 días permite alcanzar un ablandamiento o madurez de consumo. Las peras de esta variedad sólo maduran reduciendo la dosis aplicada, necesitando un periodo de vida útil de aproximadamente 7 días cuando la dosis es inferior a 25 nL L^{-1} y de 21 días para tratamientos con 50 nL L^{-1} de 1-MCP.

En vista de los estudios anteriores, se hace necesario continuar las investigaciones sobre los efectos concretos que el 1-MCP provoca en las distintas variedades de pera y ajustar las condiciones del tratamiento en cuanto a dosis, temperatura, tiempo y momento de aplicación. Asimismo se requiere ensayar distintos sistemas para evitar que se altere la capacidad de madurar de los frutos después de un periodo de conservación frigorífica y alcanzar los valores de firmeza, color, aromas y ausencia de alteraciones fisiológicas que permitan una buena comercialización.

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Bibliografía

Argenta, L. C.; Fan, X. T.; Mattheis, J. P. 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit. *Journal of Agricultural and Food Chemistry,* 51, 3858-3864.

Bai, J.; Mattheis, J. P.; Reed, N. 2006. Re-initiating softening ability of 1-methylcyclopropene-treated 'Bartlett' and 'd'Anjou' pears after regular air or controlled atmosphere storage. *Journal of Horticultural Science & Biotechnology,* 81, 959-964.

Baritelle, A. L.; Hyde, G. M.; Fellman, J. K.; Varith, J. 2001. Using 1-MCP to inhibit the influence of ripening on impact properties of pear and apple tissue. *Postharvest Biology and Technology,* 23, 153-160.

Blankenship, S. M.; Dole, J. M. 2003. 1-methylcyclopropene: a review. *Postharvest Biology and Technology,* 28, 1-25.

Blankenship, S. M.; Richardson, D. G. 1985. Development of ethylene biosynthesis and ethylene-induced ripening in danjou pears during the cold requirement for ripening. *Journal of the American Society for Horticultural Science,* 110, 520-523.

Calvo, G. 2001a. Efecto del 1-MCP sobre la madurez y control de escaldadura en peras cv. Beurré D'anjou y Packham's Triumph. Instituto Nacional de Tecnología Agropecuaria. Alto Valle, Río Negro, Argentina. Pp. 53.

Calvo, G. 2001b. Efecto del 1-metilciclopropeno (1-MCP) en peras cv. 'Williams' cosechadas con dos estados estados de madurez. Instituto Nacional de Tecnología Agropecuaria. Alto Valle, Río Negro, Argentina. Pp. 33.

Calvo, G. 2003. Effect of 1-methylcyclopropene (1-MCP) on pear maturity and quality. *Acta Horticulturae,* 628, 203-211.

Calvo, G. 2004. Efecto del 1-metilciclopropeno (1-MCP) en peras cv. 'Williams' cosechadas con dos estados estados de madurez. *Revista de Investigaciones Agropecuarias,* 33, 3-26.

Calvo, G.; Sozzi, G. O. 2004. Improvement of postharvest storage quality of 'Red Clapp's' pears by treatment with 1-methylcyclopropene at low temperature. *Journal of Horticultural Science and Biotechnology,* 79, 930-934.

Chen, P. M.; Spotts, R. 2005. Changes in ripening behaviors of 1-MCP treated d'Anjou pears after storage. *International Journal of Fruit Science,* 5, 3-18.

Chen, P. M.; Varga, D. M.; Seavert, C. F. 2003. Developing a value-added fresh-cut 'd'Anjou' pear product. *HortTechnology,* 13, 314-320.

Crouch, I. 2003. 1-methylcyclopropene (SmartFresh™) as an alternative to modified atmosphere and controlled atmosphere storage of apples and pears. *Acta Horticulturae,* 600, 433-439.

DeEll, J. R.; Murr, D. P.; Porteous, M. D.; Rupasinghe, H. P. V. 2002. Influence of temperature and duration of 1-methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biology and Technology,* 24, 349-353.

Dong, L.; Lurie, S.; Zhou, H. W. 2002. Effect of 1-methylcyclopropene on ripening of 'Canino' apricots and 'Royal Zee' plums. *Postharvest Biology and Technology,* 24, 135-145.

Eccher-Zerbini, P.; Cambiaghi, P.; Grassi, M.; Rizzolo, A. 2005. The effect of 1-MCP on the quality of 'Conference' and 'Abbe Fetel' pears. *Acta Horticulturae,* 671, 397-403.

Ekman, J. H.; Clayton, M.; Biasi, W. V.; Mitcham, E. J. 2004. Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears. *Postharvest Biology and Technology,* 31, 127-136.

Ergun, M.; Sargent, S. A.; Fox, A. J.; Crane, J. H.; Huber, D. J. 2005. Ripening and quality responses of mamey sapote fruit to postharvest wax and 1-methylcyclopropene treatments. *Postharvest Biology and Technology,* 36, 127-134.

Fan, X. T.; Blankenship, S.; Mattheis, J. P. 1999. 1-methylcyclopropene inhibits apple ripening. *Journal of the American Society for Horticultural Science,* 124, 690-695.

Gapper, N. E.; Bai, J. H.; Whitaker, B. D. 2006. Inhibition of ethylene-induced α-farnesene synthase gene *Pc*AFS1 expression in 'd'Anjou' pears with 1-MCP reduces synthesis and oxidation of α-farnesene and delays development of superficial scald. *Postharvest Biology and Technology,* 41, 225-233.

Hiwasa, K.; Kinugasa, Y.; Amano, S.; Hashimoto, A.; Nakano, R.; Inaba, A.; Kubo, Y. 2003. Ethylene is required for both the initiation and progression of softening in pear (*Pyrus communis* L.) fruit. *Journal of Experimental Botany,* 54, 771-779.

Isidoro, N.; Almeida, D. P. F. 2006. Alpha-farnesene, conjugated trienols, and superficial scald in 'Rocha' pear as affected by 1-methylcyclopropene and diphenylarnine. *Postharvest Biology and Technology,* 42, 49-56.

Jeong, J.; Huber, D. J.; Sargent, S. A. 2002. Influence of 1-methylcyclopropene (1-MCP) on ripening and cell-wall matrix polysaccharides of avocado (Persea americana) fruit. *Postharvest Biology and Technology,* 25, 241-256.

Jiang, Y.; Joyce, D. C.; Terry, L. A. 2001. 1-methylcyclopropene treatment affects strawberry fruit decay. *Postharvest Biology and Technology,* 23, 227-232.

Kappel, F.; Fisherfleming, R.; Hogue, E. J. 1995. Ideal pear sensory attributes and fruit characteristics. *HortScience,* 30, 988-993.

Kays, S. J. 1997. Postharvest physiology of perishable plan products. Pp. 568.Athens, GA, Exon Press.

Kubo, Y.; Hiwasa, K.; Owino, W. O.; Nakano, R.; Inaba, A. 2003. Influence of time and concentration of 1-MCP application on the shelf life of pear 'La France' fruit. *HortScience,* 38, 1414-1416.

Lafer, G. 2005. Effects of 1-MCP treatments on fruit quality and storability of different pear varieties. *Acta Horticulturae,* 682, 1227-1231.

Larrigaudière, C.; Lentheric, I.; Pintó, E.; Vendrell, M. 2001. Short-term effects of air and controlled atmosphere storage on antioxidant metabolism in 'Conference' pears. *Journal of Plant Physiology,* 158, 1015-1022.

Larrigaudière, C.; Vilaplana, R.; Soria, Y.; Recasens, I. 2004. Oxidative behaviourof 'Blanquilla' pears treated with 1-methylcyclopropene during cold storage. *Journal of the Science of Food and Agriculture,* 84, 1871-1877.

Lelièvre, J. M.; Tichit, L.; Dao, P.; Fillion, L.; Nam, Y. W.; Pech, J. C.; Latché, A. 1997. Effects of chilling on the expression of ethylene biosynthetic genes in Passe-Crassane pear (*Pyrus communis* L.) fruits. *Plant Molecular Biology,* 33, 847-855.

Liu, H.; Jiang, W.; Zhou, L.; Wang, B.; Luo, Y. 2005. The effects of 1-methylcyclopropene on peach fruit (*Prunus persica* L. cv. Jiubao) ripening and disease resistance. *International Journal of Food Science and Technology,* 40, 1-7.

Ma, S.; Chen, P. M.; Mielke, E. A. 2000. Storage life and ripening behavior of 'Cascade' pears as influenced by harvest maturity and storage temperature. *Journal of the American Pomological Society,* 54, 138-147.

Marcos, J. F.; Gonzalez, C. L.; Zacarias, L. 2005. Involvement of ethylene biosynthesis and perception in the susceptibility of citrus fruits to *Penicillium digitatum* infection and the accumulation of defence-related mRNAs. *Journal of Experimental Botany,* 56, 2183-2193.

Mattheis, J.; Fan, X.; Argenta, L. 2000. Manipulation of 'Bartlett' pear fruit ripening with 1-MCP. In: Septiembre 4-9, Anonymous. Pp. 263-265.

Miller, A. R. 1986. Oxidation of cell-wall polysaccharides by hydrogen peroxide:a potential mechanism for cell-wall breakdown in plants. *Biochemical and Biophysical Research Communications,* 141, 238-244.

Mir, N. A.; Curell, E.; Khan, N.; Whitaker, M.; Beaudry, R. M. 2001. Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. *Journal of the American Society for Horticultural Science,* 126, 618-624.

Mitcham, E. J.; Mattheis, J. P.; Bower, J.; Biasi, B.; Clayton, M. 2001. Responses of European Pears to 1-MCP. *Perishables Handling Quarterly,* 108, 16-19.

Moggia, C. E.; Pereira, M.; Yuri, J. A. 2001. Efectividad de aplicaciones de Smartfresh (1-MCP) en peras Packham's Triumph. *Revista Fruticola,* 22, 83-87.

Mwaniki, M. W.; Mathooko, F. M.; Matsuzaki, M.; Hiwasa, K.; Tateishi, A.; Ushijima, K.; Nakano, R.; Inaba, A.; Kubo, Y. 2005. Expression characteristics of seven members of the beta-galactosidase gene family in 'La France' pear (Pyrus communis L.) fruit during growth and their regulation by 1-methylcyclopropene during postharvest ripening. *Postharvest Biology and Technology,* 36, 253-263.

Pechous, S. W.; Watkins, C. B.; Whitaker, B. D. 2005. Expression of α-farnesene synthase gene AFS1 in relation to levels of α-farnesene and conjugated trienols in peel tissue of scald-susceptible 'Law Rome' and scald-resistant 'Idared' apple fruit. *Postharvest Biology and Technology,* 35, 125-132.

Pre-Aymard, C.; Weksler, A.; Lurie, S. 2003. Responses of 'Anna', a rapidly ripening summer apple, to 1-methylcyclopropene. *Postharvest Biology and Technology.,* 26, 163-170.

Rizzolo, A.; Cambiaghi, P.; Grassi, M.; Zerbini, P. E. 2005. Influence of 1-methylcyclopropene and storage atmosphere on changes in volatile compounds and fruit quality of 'Conference' pears. *Journal of Agricultural and Food Chemistry,* 53, 9781-9789.

Rowan, D. D.; Allen, J. M.; Fiedler, S.; Spicer, J. A.; Brimble, M. A. 1995. Identification of conjugated triene oxidation products of α-farnesene and conjugated trienols in apple skin. *Journal of Agricultural and Food Chemistry,* 43, 2040-2054.

Rupasinghe, H. P. V.; Murr, D. P.; Paliyath, G.; Skog, L. 2000. Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. *Journal of Horticultural Science and Biotechnology,* 75, 271-276.

Saftner, R. A.; Abbott, J. A.; Conway, W. S.; Barden, C. L. 2003. Effects of 1-methylcyclopropene and heat treatments on ripening and postharvest decay in 'Golden Delicious' apples. *Journal of the American Society for Horticultural Science,* 128, 120-127.

Sisler, E. C.; Dupille, E.; Serek, M. 1996. Effects of 1-methylcyclopropene and methylcyclopropene on ethylene binding and ethylene action on cut carnation. *Plant Growth Regulation,* 18, 79-86.

Sisler, E. C.; Serek, M. 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiologia Plantarum,* 100, 577-582.

Sisler, E. C.; Serek, M. 2003. Compounds interacting with the ethylene receptor in plants. *Plant Biology,* 5, 473-480.

Soria, Y.; Recasens, I. 1997. Escaldado superficial en manzanas. *Informacion Tecnica Economica Agraria - Producción Vegetal,* 93, 49-64

Trinchero, G. D.; Sozzi, G. O.; Covatta, F.; Fraschina, A. A. 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of `Bartlett` pears. *Postharvest Biology and Technology,* 32, 193-204.

Watkins, C. B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances,* 24, 389-409.

Watkins, C. B.; Miller, W. B. 2005. 1-methylcyclopropene (1-MCP) based technologies for storage and shelf-lifeextension. *Acta Horticulturae,* 687, 201-207.

Watkins, C. B.; Nock, J. F.; Whitaker, B. D. 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biology and Technology,* 19, 17-32.

Whitaker, B.; Solomos, T.; Harrison, P. A. 1997. Quantification of α-farnesene and its conjugated trienol oxidation products from apple peel by C18-HPLC with UV detection. *Journal of Agricultural and Food Chemistry,* 45, 760-765.

PARTE 2
ÚLTIMOS AVANCES EN LA APLICACIÓN DEL 1-METILCICLOPROPENO (1-MCP) EN PERAS

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Resumen

Esta segunda revisión recoge todos los artículos publicados sobre la aplicación postcosecha en pera de 1-metilciclopropeno (1-MCP) desde el año 2007 hasta la actualidad. Hoy por hoy, el 1-MCP se aplica comercialmente en algunas variedades de pera mientras que en otras, aún se continúa investigando debido a la complejidad de la respuesta al tratamiento. Una variedad de factores de precosecha pueden afectar la respuesta de las peras a la aplicación del 1-MCP como son el estado de madurez y las condiciones en que se efectúa el tratamiento y otros aspectos como el sistema de enfriamiento y la naturaleza del envase también pueden ser importantes. Dependiendo de la especie y el cultivar, el tratamiento con 1-MCP puede tener una gran variedad de respuestas fisiológicas y bioquímicas ya que puede afectar la respiración, la producción de etileno, el metabolismo oxidativo, y otros parámetros de calidad, como el color, la firmeza, la acidez ó los azúcares. Puede también afectar a la aparición de desórdenes fisiológicos y patologías. Esta revisión pretende recoger lo que se conoce acerca de los usos tecnológicos para el uso del 1-MCP y describe las discrepancias entre las distintas publicaciones, así cómo las áreas que requieren mayor estudio.

Summary

This second review collects all the published papers related to the postharvest application of 1-methylcyclopropene (1-MCP) on pear since 2007 to the present. Currently, 1-MCP is commercially applied in some varieties while in others, investigations still continue due to the complexity of the fruit response to the treatment. A variety of preharvest factors can affect the application of 1-MCP in pears, including fruit maturity, treatment conditions but also the cooling method and the bin material play an important role. Depending on the species treated, 1-MCP may have a variety of physiological and biochemical responses and differing effects on respiration, ethylene production, oxidative metabolism, and other quality changes like color, softening, disorders and diseases, acidity and sugars. This review compiles what is known about the technological uses of 1-MCP, defines where discrepancies exist between reports, and defines areas requiring further study.

1. INTRODUCCIÓN

El 1-metilciclopropeno (1-MCP) es una nueva herramienta que actualmente permite alargar la conservación y la vida útil de una gran variedad de frutas, manteniendo los estándares de calidad en poscosecha. Las investigaciones acerca de la aplicación de este producto en diferentes especies han sido muy numerosas en los años posteriores a su descubrimiento (Blankenship y Dole, 2003; Watkins, 2006; Schotsmans y col., 2009; Sozzi y Beaudry, 2007). En lo que respecta a peras se han publicado también numerosos trabajos que fueron recogidos en su día por Chiriboga y col. (2008) y en los últimos años todavía se sigue investigando en la aplicación de este producto en diferentes variedades. En España, en el año 2009 se registró el 1-MCP en peras con ciertos usos restringidos para el control del escaldado y el mantenimiento de la de firmeza (BOE, 10/08/09). En el año 2011 se aprobó el uso a nivel comercial (comunicación personal Agrofresh) para las variedades 'Blanquilla', 'Ercolini', 'Limonera' y 'Williams-Bartlett' con recomendaciones específicas para cada variedad. En otras variedades como 'Conference' ó 'Alejandrina' todavía se sigue investigando ampliamente debido a la complejidad de las respuestas al tratamiento con 1-MCP.

La presente revisión bibliográfica pretende ser una actualización de todas las publicaciones realizadas acerca de la aplicación del 1-MCP en pera en los últimos años. De esta manera, constituye una continuación de la revisión bibliográfica publicada anteriormente que incluía todas las publicaciones realizadas en este tema hasta el año 2006 (Chiriboga y col., 2008).

2. FACTORES QUE AFECTAN LA APLICACIÓN DE 1-MCP

Según Sozzi y Beaudry (2007) los factores pre y pos cosecha que afectan la respuesta al tratamiento de 1-MCP son numerosos. Se incluyen factores como el genotipo (especies y variedades), las condiciones ambientales, las prácticas culturalesen el campo, la fecha de cosecha (estado fisiológico del fruto), las condiciones de aplicación, la susceptibilidad de la fruta a las alteraciones y las condiciones del manejo en poscosecha. En este apartado se han considerado los factores más importantes que afectan la aplicación del 1-MCP en peras.

2.1 Concentración, temperatura y duración del tratamiento

Las peras, en general, son muy sensibles a la exposición al 1-MCP y la eficacia del tratamiento depende de la variedad. Por ejemplo, la variedad 'd'Anjou' es mucho más sensible que 'Bartlett' o 'Red Clapp's' (Trinchero y col., 2004; Ekman y col., 2004; Chen y Spotts, 2005; Calvo y Sozzi, 2004), por ello, se precisa aplicar una concentración adecuada a cada variedad.

En general en peras 'Bartlett', Villalobos-Acuña y col., (2011a) recomiendan una dosis mínima de 300 nL L^{-1} de 1-MCP para lograr retrasar la maduración e inhibir la producción del etileno. La respuesta al 1-MCP depende de la temperatura durante el tratamiento y durante el periodo de conservación. En todos los casos el 1-MCP inhibe la maduración durante un tiempo, pero las peras tratadas a una temperatura de 0 °C recuperan más rápidamente sus parámetros de maduración, tales como la producción de etileno, el ablandamiento y la pérdida de color verde, que las tratadas a 20 °C.

Otro factor importante es la duración del tratamiento. Se ha observado que si se realiza el tratamiento a 20 ºC no hay diferencias entre mantenerlo durante 12 ó 24 horas, mientras que a 0 °C, el efecto es mucho mayor cuando los frutos se tratan los frutos durante 24 horas en lugar de 12 horas (Villalobos-Acuña y col., 2011a).

En otras variedades de pera, las concentraciones recomendadas pueden ser diferentes. Por ejemplo en la variedad 'Spadona', una dosis de 200 nL L^{-1} de 1-MCP durante 20 horas a 20 °C es suficiente para inhibir la maduración (Gamrasni y col., 2010). En la variedad 'Patharnakh', también a 20 ºC, se precisan concentraciones más altas (500 -1000 nL L^{-1}) y una menor duración del tratamiento (4 horas) para retener la firmeza y la pérdida de peso (Mahajan y col., 2010). Sin embargo en peras 'Kikusu' y 'Akemizu', el tratamiento con 1000 nL L^{-1} 1-MCP requiere de 12 horas a 25 °C para inhibir la tasa de respiración y retrasar el pico de etileno (Li y Wang, 2009; Li y col., 2010)

2.2 Estado de madurez y momento de aplicación

En los últimos años, muchos estudios se han enfocado en estudiar el efecto que produce el estado de madurez en cosecha, sobre la respuesta al 1-MCP. Todavía este efecto no está muy claro. Por ejemplo, en peras 'Bartlett' cosechadas en cuatro diferentes estados de madurez, la aplicación de 1-MCP retrasa la maduración en todos los casos, (producción de etileno y respiración y desarrollo del color amarillo). Sin embargo, la recuperación de la maduración después de la conservación depende de la madurez a la cosecha y del año (Villalobos-Acuña y col., 2011b). Un estado de madurez avanzado hace posible una maduración más rápida después de la conservación ya que va acompañada de una mayor estimulación en la producción de etileno.

Gamrasni y col. (2010) confirman la importancia que puede tener la madurez a la cosecha en peras de la variedad 'Spadona' cosechadas en cuatro diferentes estados de madurez. Estos autores demuestran que la efectividad del tratamiento 1-MCP depende de la etapa climatérica del fruto en el momento de la aplicación. El tratamiento inhibe la maduración tanto en peras tempranas como tardías cuando éste se aplica inmediatamente después de la cosecha, pero estos frutos posteriormente no maduran después de 6 meses de conservación en frío y 2 semanas de vida comercial. Sin embargo cuando el tratamiento se aplica 7 días después de la cosecha manteniéndolos a 20 °C, estos frutos se encuentran en las primeras etapas del climaterio al momento del tratamiento y maduran, alcanzando la calidad óptima de consumo tras 6 meses de conservación y 2 semanas de vida comercial. Una aplicación 12 días después de la cosecha (en la mitad del pico climatérico) permite tener una firmeza inferior a los controles cuando se sacan de la cámara frigorífica. El conjunto de estos resultados muestran una vez más que el estado climatérico en que se encuentra el fruto en el momento del tratamiento es una de las razones que provoca las variaciones en la respuesta al 1-MCP (Zhang y col., 2009).

En algunos casos el momento del tratamiento es más importante que la temperatura a la que éste se realiza. Villalobos-Acuña y col. (2011) mostraron que peras 'Bartlett' tratadas 1 día después de la cosecha a diferentes temperaturas (0, 5, 10, 15 y 20 °C) presentaban valores más elevados del tono (color más verde) que los controles después de la conservación. Sin embargo, los frutos tratados 3, 5 y 7 días después de la cosecha a las mismas temperaturas, no mostraban diferencias ni en firmeza ni en color con los frutos no tratados.

2.3 Sistema de enfriamiento

La respuesta al tratamiento con 1-MCP también depende del sistema de pre-enfriamiento que se realiza después de la cosecha. En peras 'Bartlett' Calvo y Sozzi (2009) demostraron que utilizando una sistema de aire forzado, el tratamiento con 1-MCP inhibía la firmeza después de 150 días de conservación, mientras que en los frutos pre-enfriados con hidrocooling, el 1-MCP no producía ningún efecto.

En este estudio las peras enfriadas con hidrocooling, tratadas con 500 nL L^{-1} de 1-MCP y almacenadas en envases de madera, maduraron después de 7 días a 20 °C (firmeza ≈ 20 N) mientras que la fruta conservada en envases de plástico retrasaron su maduración y alcanzaron esta firmeza a los 21 días a 20 °C. Según estos autores, la naturaleza del envase puede absorber parte del compuesto 1-MCP y afectar la eficacia del tratamiento, especialmente si son cajas de madera húmedas y si se utilizan concentraciones relativamente bajas de 1-MCP. Vallejo y Beaudry (2006) observaron a su vez que la madera humedecida reduce la eficacia del compuesto 1-MCP, debido a que puede proporcionar un número indeterminado de sitios no específicos de absorción para el 1-MCP, aunque este mecanismo no está del todo explicado.

3. RESPUESTA FISIOLÓGICA AL TRATAMIENTO CON 1-MCP

3.1 Metabolismo del etileno y tasa de respiración

En todos los estudios que se han realizado en pera en los últimos años, se confirma que el 1-MCP bloquea no sólo los receptores sino también la producción auto catalítica de etileno (Gamrasni y col., 2010; Yazdani y col., 2011; Li y Wang, 2009; Villalobos-Acuña y col., 2011b; MacLean y col., 2007; Yamane y col., 2007; Li y col., 2010). No esta claro todavía el sitio exacto en la ruta de biosíntesis de etileno en donde el 1-MCP interviene pero es probable que sea en una de las dos enzimas claves de esta ruta, la ACC sintasa (ACS) o la ACC oxidasa (ACO).

Gamrasni y col. (2010) en peras 'Spadona' observaron una disminución de la actividad de la ACO y de la expresión de de los genes *PcACS1b y PcACO1* debida el tratamiento con 1-MCP. En peras 'Bartlett' tratadas con 1-MCP, Villalobos-Acuña y col. (2011) también comprobaron la inhibición de la actividad de las enzimas ACS y ACO acompañada por una disminución de la expresión de los genes *PcACO1, PcACS4 y PcACS5* que son inducidos por la conservación en frío.

3.2 Receptores del etileno

En la última década, ha habido muchos avances en conocimiento sobre cómo el 1-MCP regula la expresión de los receptores de etileno en muchas especies de plantas. Los genes de algunos receptores se activan en los tejidos que producen etileno, como en los frutos maduros y están regulados por la sobreexpresión del etileno (Tatsuki, 2010). Se han identificados varios genes de receptores del etileno en pera (*PcETR1, PcETR5 y PcERS1*). En peras 'Kikusui' tratadas con 1-MCP inmediatamente después de la cosecha, la expresión de *PpETR3* aumenta entre los 0 y 9 días a 25 °C después del tratamiento, mientras que la expresión de *PpERS2* queda inhibida entre los 6 y 15 días a 25 °C (Li y col., 2010). Por el contario, en los frutos no tratados la expresión de *PpETR3* disminuye durante los primeros 6 días a 25 °C. Estos resultados sugieren que la unión del 1-MCP al receptor *PpERS2* altera directamente la regulación de la transcripción de este receptor, inhibiendo así la capacidad de regulación del etileno. Estos resultados son también consistentes con el patrón de expresión del receptor *PpERS1* que observaron en pera 'Red d'Anjou' (MacLean y col. 2007). En este caso, en los frutos tratados con 1-MCP, la expresión del receptor *PpERS1* así como la tasa de producción de etileno disminuyó durante una semana a temperatura ambiente.

3.3 Metabolismo oxidativo

El tratamiento con 1-MCP inhibe la acumulación de peróxido de hidrógeno (H_2O_2), como lo observado en peras 'Suli' (Dong y col., 2011) durante 24 días a 20 °C. La acumulación de H_2O_2 en las plantas es a menudo considerada como un indicador de estrés oxidativo durante la maduración de la fruta (Quan y col., 2008). En consecuencia, el tratamiento con 1-MCP parece reducir el daño de la membrana celular y de esta manera retrasa la maduración y senescencia de la fruta.

Larrigaudière y col. (2004) observaron que el tratamiento con 1-MCP promueve las actividades de las enzimas antioxidantes en pera 'Blanquilla'. Algo similar se observa en pera cv 'Kikusui' (Li y col., 2010) tratada con 1000 nL L^{-1}de 1-MCP. Los frutos tratados presentan actividades más altas de las enzimas SOD, CAT y APX después de 12 días a 20 °C. No se conoce con certeza cómo el tratamiento 1-MCP causa este incremento en el sistema antioxidante, sin embargo, algunos autores sugieren que puede ser debido a la capacidad del 1-MCP para inhibir la generación de radicales libres normalmente presentes en la respiración climatérica, a través de mecanismos todavía desconocidos (MacLean y col., 2003).

Por el contrario, en peras asiáticas (*Pyrus serotina* cv. KS_6) se han observado actividades de las enzimas CAT y POX inferiores en la fruta tratada con 1-MCP, mientras que la actividad de la SOD no se ve afectada (Yazdani y col., 2011). Es posible que en este caso el 1-MCP reduzca la necesidad de los tejidos de activar los procesos enzimáticos antioxidantes (Shaham y col., 2003).

El tratamiento 1-MCP en algún caso también se ha asociado con menores niveles de ácido ascórbico (Larrigaudière y col., 2004), mientras que en otros casos como en pera 'Rocha', el tratamiento no afecta los niveles de ácido ascórbico ni de glutatión (Silva y col., 2010), lo que permite mantener la capacidad antioxidante de la fruta durante los 8 meses en conservación.

4. EFECTO EN LA CALIDAD

4.1 Evolución de la firmeza

En general, el objetivo del tratamiento con 1-MCP es lograr un mantenimiento de la firmeza durante la conservación en cámara frigorífica y durante su manipulación, a la vez que permitir una calidad óptima para el consumo en el momento de la comercialización.

Se ha demostrado que una dosis de 1000 nL L^{-1} de 1-MCP en peras de la variedad 'Akemizu' (Li y Wang, 2009) y de la variedad, 'Patharnakh' (Mahajan y col., 2010), retrasa la pérdida de firmeza tanto durante la conservación a 4 °C como durante la vida comercial a 25 °C. Li y col. (2010) sugieren que el mantenimiento de la firmeza de la fruta por el 1-MCP en peras 'Kikusu' se debe a la inhibición de las enzimas que degradan la pectina.

El retraso en el pérdida de firmeza depende a su vez de muchos otros factores tales como el sistema de enfriamiento (Calvo y Sozzi, 2009), la dosis o la variedad. Por ejemplo en peras 'Rocha' una dosis de 500 nL L^{-1} de 1-MCP no es suficiente para retener la firmeza después 8 meses de almacenamiento en atmósfera controlada (Silva y col., 2010), mientras que en peras 'Bartlett' tratadas con 300 nL L^{-1} de 1-MCP, la perdida de firmeza se reduce considerablemente incluso después de largos períodos de almacenamiento (Villalobos-Acuña y col., 2011b). En algunos casos esta retención de la firmeza se mantiene aún después de varios días a 20 °C.

En peras de la variedad 'Spadona', cosechadas en diferentes estados de madurez, el tratamiento con 300 nL L^{-1} de 1-MCP logra mantener la firmeza en todos los frutos durante la conservación, pero después esa fruta no madura suficiente y no se vuelve blanda, mantiendo valores superiores a 37 N aún tras 2 semanas a 20 °C, (Gamrasni y col., 2010)

4.2 Producción de volátiles y calidad sensorial

El tratamiento con 1-MCP puede actuar sobre la producción de volátiles. Se ha visto en peras tratadas de la variedad 'Ya' conservadas durante 40 días en frío y 21 días a 20 °C, que el tratamiento retrasa la aparición de sabores desagradables debidos al acetaldehído y etanol, mientras que el contenido de acetato de etilo y hexanal se incrementa lentamente y el hexanol se mantiene estable durante este periodo (Kou y col., 2012). El efecto del tratamiento sobre estos compuestos puede atribuirse a que estas peras presentan una menor respiración y menor producción de etileno.

En otro estudio realizado en peras 'Packham's Triumph' tratadas con 200 nL L^{-1} de 1-MCP, las condiciones de conservación y la fecha de cosecha, afectaron también la producción de compuestos volátiles. Después de 2 meses de conservación en frío, el 1-MCP redujo la producción de compuestos volátiles aromáticos, pero al cabo de 6 meses estas peras recuperaron su capacidad para producir volátiles con olor activo, adquiriendo un mejor sabor y siendo las preferidas por los paneles de catadores (Moya-León y col., 2006). Esta preferencia no es debida únicamente a los volátiles sino a su capacidad para desarrollar una textura más suave, una dulzura superior y un mejor aroma durante el período de conservación. En cualquier caso, las peras tratadas y recolectadas en una fecha tardía produjeron mayores cantidades de compuestos volátiles que las cosechadas en la fecha comercial.

4.3 Evolución del color

El tratamiento 1-MCP afecta al cambio de color durante la maduración. Villalobos-Acuña y col. (2011) demostraron que el cambio de color depende del momento de la aplicación del 1-MCP. Peras 'Bartlett' tratadas inmediatamente después de la cosecha, a cualquier temperatura (0 a 20 °C) retienen el color verde (valores más elevados del tono) mientras que al tratarlas días después (3, 5 y 7 días) de la cosecha, a estas mismas temperaturas, el color cambia igual que en las peras no tratadas. En el mismo estudio, se observa que la temperatura a la que se efectúa el tratamiento puede hacer variar la respuesta, tratar a 0 °C induce un mayor cambio de color en la fruta después de 45 días de conservación que si se trata a 20 °C.

La concentración aplicada también puede afectar el cambio de color. En peras 'Bartlett', una concentración de 300 nL L^{-1} de 1-MCP no impide un cambio de color mientras que con 600 nL L^{-1} de 1-MCP, se mantienen verdes después 60, 90 o 120 días de almacenamiento en frío y 7 días a 20 ºC, además, se evidencia una disociación entre el cambio de color de la piel y el ablandamiento de la pulpa después de largos períodos de conservación (Calvo y Sozzi, 2009).

4.4 Contenido de ácidos orgánicos, azúcares y componentes nutricionales

El contenido de sólidos solubles también puede verse afectado por el tratamiento 1-MCP en función de la variedad. Así, en pera 'Akemizu', los frutos tratados tienen valores más altos de sólidos solubles tanto a 4 °C como a 25 °C, después de 48 y 8 días respectivamente, mientras que en la variedad 'Suli' no se ve ningún efecto del tratamiento 1-MCP en el contenido de sólidos solubles tanto en frutos conservados en frío o temperatura ambiente (Dong y col., 2011).

Por otro lado, en peras 'Patharnakh' el efecto del tratamiento depende de los días de conservación a 0 °C. El contenido de sólidos solubles se incrementa hasta los 75 días en los frutos tratados y posteriormente baja de forma gradual (Mahajan y col., 2010). Curiosamente en este estudio los frutos tratados con dosis altas de 1-MCP (1000 nL L^{-1}) presentan un mayor contenido tanto en sólidos solubles y también en azúcares totales en comparación con las dosis más bajas. El incremento en sólidos solubles durante los primeros meses de conservación puede deberse a la hidrólisis del almidón en azúcares, aunque después ya no signifique un aumento adicional de azúcares en los meses posteriores, ya que los azúcares junto con otros ácidos orgánicos son los sustratos para la respiración (Wills y col., 1980). El 1-MCP en general retiene la acidez al igual que la firmeza, ya que retrasa el proceso de maduración tal se observa en peras 'Akemizu' (Li y Wang, 2009) y 'Patharnakh' (Mahajan y col., 2010).

4.5 Pérdida de peso

El efecto del 1-MCP en el peso, se traduce en una menor pérdida durante la conservación y vida comercial lo cual puede atribuirse a la menor tasa de respiración y también a una menor transpiración debida al mantenimiento de la estructura de los tejidos de los frutos. En peras cv 'Patharnakh' tratadas con 500, 750 y 1000 nL L^{-1}con 1-MCP, se observa que la pérdida de peso es menor conforme se aumenta la dosis aplicada (Mahajan y col., 2010).

5. EFECTO EN DESÓRDENES FISIOLÓGICOS Y DAÑOS MECÁNICOS

5.1 Escaldado superficial

La inhibición del escaldado superficial por el tratamiento con 1-MCP se ha demostrado ampliamente en pera. Según varios autores, el control del 1-MCP se asocia a una menor acumulación de α-farneseno y de sus productos de oxidación (compuestos trieno conjugados). El 1-MCP actúa probablemente limitando los niveles de α-farneseno y de sus productos de oxidación (Bai y col., 2009; Li y Wang, 2009; Yazdani y col., 2011). Sin embargo, en algunos casos, el tratamiento con 1-MCP puede limitar la incidencia de escaldado sin prevenir completamente la acumulación de compuestos trieno conjugados (Hui y col., 2011).

Gapper y col. (2006) relacionan la presencia de escaldado en peras 'd'Anjou' tratadas con 300 nL L^{-1} con un incremento en la concentración de etileno interno así como en los niveles de transcripción de *AFS1*. De este modo, el 1-MCP provoca una disminución de la producción de los compuestos trieno conjugados a través de la inhibición de la expresión de *AFS1* dependiente del etileno (Gapper y col., 2006).

Otros autores relacionan el control del escaldado superficial por el 1-MCP en pera japonesa 'Akemizu' con el aumento de la actividad LOX y de las concentraciones de malondialdehído (Li y Wang, 2009). En ese estudio el tratamiento con 1-MCP inhibe completamente el escaldado cuando se conservan las peras en frío a 4 °C y reduce su incidencia cuando los frutos están a 20 °C.

5.2 Daños mecánicos

El 1-MCP reduce la susceptibilidad de los frutos a las magulladuras por vibración e impacto (Calvo y Sozzi, 2004), lo que se traduce en una mayor resistencia durante la clasificación, envasado y transporte. Según Gamrasni y col. (2010) la aplicación de 200 nL L^{-1} de 1-MCP es suficiente para mejorar la apariencia de peras 'Spadona' cosechadas en diferentes estados de madurez, debido a la menor fricción y presencia de magulladuras en los frutos tratados. La eficacia del 1-MCP radica en prevenir la acción entre las enzimas oxidativas y sus sustratos y así mantener la integridad de membrana celular.

5.3 Desórdenes internos

Se ha reportado que el tratamiento con 1-MCP puede controlar también las alteraciones internas en peras. Un tratamiento con 300 nL L^{-1} de 1-MCP puede controlar la aparición de descomposición interna en peras 'Bartlett' cosechadas a diferentes estados de madurez y conservadas por 180 días a -1 °C (Villalobos-Acuña y col., 2011b). En otro estudio (DeEll y Ehsani-Moghaddam, 2011), el tratamiento de 1-MCP realizado después de 1, 3 ó 7 días a 3 °C después de la cosecha, se ve que reduce substancialmente el escaldado blando y la descomposición interna, alteraciones ligadas a procesos de senescencia, aunque el tratamiento realizado a los 7 días, proporciona un menor control de los desórdenes comparado con los otros dos tratamientos.

5.4 Podredumbres

Se ha demostrado que los frutos tratados con 1-MCP son menos susceptibles al desarrollo de podredumbres. Según Spotts y col. (2007) una dosis con 300 nL L^{-1} de 1-MCP' reduce la pudrición causada por *Neofabraea* spp. y *Phacidiopycnis piri* en peras 'd'Anjou', conservadas 4, 6 y 8 meses a -1 °C. La pudrición por moho gris del peciolo (Stem end gray mold) también se controla por el tratamiento incluso con una dosis más baja de 100 nL L^{-1} y así mismo, la podredumbre blanca (Snow-mold rot) se reduce con un tratamiento de 30 nL L^{-1} de 1-MCP. Estos autores sugieren que el 1-MCP es capaz de inhibir las enzimas que degradan las paredes celulares como las poligalacturonasas secretadas por los patógenos y de esta manera controlar las patogénesis. Sin embargo Akagi y Stotz (2007) observan que el 1-MCP tiene poco efecto sobre *Botrytis cinerea* en peras 'd'Anjou' y 'Bartlett' tratadas con 300 nL L^{-1}. Los resultados de este estudio

demuestran que el tratamiento tiene un impacto mucho mayor en el ablandamiento de la pera que en la presencia o ausencia del gen *Bcpg1* de la poligalacturonasa de *Botrytis cinerea,* responsable de la virulencia de esta cepa.

6. BLOQUEO DE LA MADURACIÓN EN PERAS TRATADAS CON 1-MCP

El tratamiento con 1-MCP en peras ha demostrado ser más complejo que en otros frutos, debido en parte a la alta sensibilidad del fruto al tratamiento y al hecho de que en algunas ocasiones, las peras quedan excesivamente firmes y verdes y pierden su capacidad de madurar (Chen y Spotts, 2005; Calvo, 2003; Bai y col., 2006). Es así el caso de peras 'Conference' y 'Blanquilla', en las que el tratamiento con 300 nL L^{-1} de 1-MCP bloquea completamente el proceso de maduración durante los 120 días de conservación y después de 7 días a 20 ºC (Chiriboga y col., 2010). En peras de la variedad 'Spadona' incluso una menor dosis (200 nL L^{-1} de 1-MCP) también provoca que los frutos permanezcan verdes y no alcancen la firmeza óptima para que ser comestibles aún después de 2 semanas a 20 °C (Gamrasni y col., 2010). En un estudio en peras 'Bartlett' tratadas con 500 nL L^{-1} de 1-MCP, los frutos tardaron 21 días a temperatura ambiente en alcanzar valores óptimos de firmeza de consumo entre 14 y 23 N (Calvo y Sozzi, 2009).

En algunas variedades de peras, todavía no está claro cuál es la mejor combinación de madurez a la cosecha, concentración de 1-MCP, condiciones de aplicación y tiempo de almacenamiento, que permita controlar de manera adecuada el ablandamiento y el desarrollo de trastornos fisiológicos y que a la vez permita madurar a la fruta dentro de un período razonable de vida comercial. En variedades como 'Ercolini', 'Limonera' y 'Williams-Bartlett' la aplicación del tratamiento de 1-MCP acompañada de recomendaciones específicas para cada variedad permiten que la fruta madure entre 7 y 14 días (comunicación personal Agrofresh). En 'Blanquilla' por ejemplo, es necesario acortar el período de conservación y realizar un tratamiento térmico al final de la misma de 5 ó 10 días a 15 °C para que la fruta madure y alcance la calidad de consumo (Chiriboga y col., 2010). Sin embargo en el caso de peras 'Conference' ningún tratamiento térmico permite restaurar el proceso de maduración en los frutos tratados con 1-MCP (Chiriboga y col., 2010), por lo que se necesitan más estudios antes de una posible aplicación comercial del tratamiento 1-MCP para esta variedad.

Bibliografía

Akagi, A.; Stotz, H. U. 2007. Effects of Pathogen Polygalacturonase, Ethylene, and Firmness on Interactions Between Pear Fruits and Botrytis cinerea. Plant Disease, 91, 1337- 1344.

Bai, J.; Mattheis, J. P.; Reed, N. 2006. Re-initiating softening ability of 1-methylcyclopropene-treated 'Bartlett' and 'd'Anjou' pears after regular air or controlled atmosphere storage. Journal of Horticultural Science & Biotechnology, 81, 959-964.

Bai, J. H.; Yin, X. H.; Whitaker, B. D.; Deschuytter, K.; Chen, P. M. 2009. Combination of 1-methylcyclopropene and ethoxyquin to control superficial scald of 'Anjou' pears. HortTechnology 19, 521-525.

Blankenship, S. M.; Dole, J. M. 2003. 1-methylcyclopropene: a review. Postharvest Biology and Technology, 28, 1-25.

Calvo, G. 2003. Effect of 1-methylcyclopropene (1-MCP) on pear maturity and quality. Acta Horticulturae, 628, 203-211.

Calvo, G.; Sozzi, G. O. 2004. Improvement of postharvest storage quality of 'Red Clapp's' pears by treatment with 1-methylcyclopropene at low temperature. Journal of Horticultural Science and Biotechnology, 79, 930-934.

Calvo, G.; Sozzi, G. O. 2009. Effectiveness of 1-MCP treatments on 'Bartlett' pears as influenced by the cooling method and the bin material. 51, 49-55.

Chen, P. M.; Spotts, R. 2005. Changes in ripening behaviors of 1-MCP treated d'Anjou pears after storage. International Journal of Fruit Science, 5, 3-18.

Chiriboga, M. A.; Schotsmans, W. C.; Larrigaudière, C. 2010. Comparative study of techniques to restore the ripening process in 1-MCP treated 'Blanquilla' and 'Conference' pears. Acta Horticulturae, 858, 149-154.

Chiriboga, M. A.; Soria, Y.; Larrigaudiere, C.; Recasens, I. 2008. Postharvest application of 1-methylcyclopropene on pears. Información Técnica Económica Agraria, 104, 12-30.

DeEll, J.; Ehsani-Moghaddam, B. 2011. Timing of postharvest 1-methylcyclopropene treatment affects Bartlett pear quality after storage. Canadian Journal of Plant Science, 91, 853- 858.

Dong, Y.; Liu, L. Q.; Guan, J. F. 2011. Effects of 1-methylcyclopropene on NO Content, NOS Activity, and H_2O_2 Content in Postharvest Suli Pears. Agricultural Sciences in China, 10, 797-804.

Ekman, J. H.; Clayton, M.; Biasi, W. V.; Mitcham, E. J. 2004. Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears. Postharvest Biology and Technology, 31, 127-136.

El-Sharkawy, I.; Jones, B.; Li, Z. G.; Lelievre, J. M.; Pech, J. C.; Latche, A. 2003. Isolation and characterization of four ethylene perception elements and their expression during ripening in pears (Pyrus communis L.) with/without cold requirement. Journal of Experimental Botany, 54, 1615-1625.

Gamrasni, D.; Ben-Arie, R.; Goldway, M. 2010. 1-methylcyclopropene (1-MCP) application to Spadona pears at different stages of ripening to maximize fruit quality after storage. Postharvest Biology and Technology, 58, 104-112.

Gapper, N. E.; Bai, J. H.; Whitaker, B. D. 2006. Inhibition of ethylene-induced α-farnesene synthase gene *PcAFS1* expression in 'd'Anjou' pears with 1-MCP reduces synthesis and oxidation of α-farnesene and delays development of superficial scald. Postharvest Biology and Technology, 41, 225-233.

Hui, W.; Niu, R. X.; Song, Y. Q.; Li, D. Y. 2011. Inhibitory Effects of 1-MCP and DPA on Superficial Scald of Dangshansuli Pear. Agricultural Sciences in China, 10, 1638-1645.

Kou, X.; Liu, X.; Li, J.; Xiao, H.; Wang, J. 2012. Effects of ripening, 1-methylcyclopropene and ultra-high-pressure pasteurisation on the change of volatiles in Chinese pear cultivars. Journal of the Science of Food and Agriculture, 92, 177-183.

Larrigaudière, C.; Vilaplana, R.; Soria, Y.; Recasens, I. 2004. Oxidative behavior of 'Blanquilla' pears treated with 1-methylcyclopropene during cold storage. Journal of the Science of Food and Agriculture, 84, 1871-1877.

Li, Z. Q.; Qiao, Y. S.; Tong, Z. G.; Zhou, J.; Zhang, Z. 2010. Effect of ethylene and 1-MCP on post-harvest physiology and on expression of the ethylene receptor genes *PpETR3* and *PpERS2* in pear (Pyrus pyrifolia Nakai 'Kikusui') fruit. Journal of Horticultural Science & Biotechnology, 85, 71-77.

Li, Z. Q.; Wang, L. J. 2009. Effect of 1-methylcyclopropene on ripening and superficial scald of Japanese pear (Pyrus pyrifolia Nakai, cv. Akemizu) fruit at two temperatures. Food Science and Technology Research, 15, 483-490.

MacLean, D. D.; Murr, D. P.; DeEll, J. R. 2003. A modified total oxyradical scavenging capacity assay for antioxidants in plant tissues. Postharvest Biology and Technology, 29, 183-194.

MacLean, D. D.; Murr, D. P.; DeEll, J. R.; Mackay, A. B.; Kupferman, E. M. 2007. Inhibition of PAL, CHS, and ERS1 in 'Red d'Anjou' pear (Pyrus communis L.) by 1-MCP. Postharvest Biology and Technology, 45, 46-55.

Mahajan, B. V. C.; Singh, K.; Dhillon, W. S. 2010. Effect of 1-methylcyclopropene (1-MCP) on storage life and quality of pear fruits. Journal of Food Science and Technology-Mysore, 47, 351-354.

Moya-León, M. A.; Vergara, M.; Bravo, C.; Montes, M. E.; Moggia, C. 2006. 1-MCP treatment preserves aroma quality of 'Packham's Triumph' pears during long-term storage. Postharvest Biology and Technology, 42, 185-197.

Quan, L. J.; Zhang, B.; Shi, W. W.; Li, H. Y. 2008. Hydrogen peroxide in plants: A versatile molecule of the reactive oxygen species network. Journal of Integrative Plant Biology, 50, 2-18.

Schotsmans, W. C.; Prange, R. K.; Binder, B. M. 2009. 1-methylycloropene (1-MCP): Mode of action and applications in postharvest horticulture. Horticultural Reviews, 35, 263-313.

Shaham, Z.; Lers, A.; Lurie, S. 2003. Effect of heat or 1-methylcyclopropene on antioxidative enzyme activities and antioxidants in apples in relation to superficial scald development. Journal of the American Society for Horticultural Science, 128, 761-766.

Silva, F. J. P.; Gomes, M. H.; Fidalgo, F.; Rodrigues, J. A.; Almeida, D. P. F. 2010. Antioxidant properties and fruit quality during long-term storage of 'Rocha' pear: Effects of maturity and storage conditions. Journal of Food Quality, 33, 1-20.

Sozzi, G. O.; Beaudry, R. M. 2007. Current perspectives on the use of 1-methylcyclopropene in tree fruit crops: an international survey. 3, 1- 16.

Spotts, R. A.; Sholberg, P. L.; Randall, P.; Serdani, M.; Chen, P. M. 2007. Effects of 1-MCP and hexanal on decay of d'Anjou pear fruit in long-term cold storage. Postharvest Biology and Technology, 44, 101-106.

Tatsuki, M. 2010. Ethylene Biosynthesis and Perception in Fruit. Journal of the Japanese Society for Horticultural Science, 79, 315-326.

Trinchero, G. D.; Sozzi, G. O.; Covatta, F.; Fraschina, A. A. 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of 'Bartlett' pears. Postharvest Biology and Technology, 32, 193-204.

Vallejo, F.; Beaudry, R. M. 2006. Depletion of 1-MCP by 'non-target' materials from fruit storage facilities. Postharvest Biology and Technology, 40, 177-182.

Villalobos-Acuña, M.; Biasi, W. V.; Mitcham, E. J.; Holcroft, D. 2011a. Fruit temperature and ethylene modulate 1-MCP response in 'Bartlett' pears. Postharvest Biology and Technology, 60, 17-23.

Villalobos-Acuña, M. G.; Biasi, W. V.; Flores, S.; Jiang, C. Z.; Reid, M. S.; Willits, N. H.; Mitcham, E. J. 2011b. Effect of maturity and cold storage on ethylene biosynthesis and ripening in 'Bartlett' pears treated after harvest with 1-MCP. Postharvest Biology and Technology, 59, 1-9.

Watkins, C. B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnology Advances, 24, 389-409.

Wills, R. B. H.; Bembridge, P. A.; Scott, K. J. 1980. Use of flesh firmness and other objective tests to determine consumer acceptability of Delicious apples. Australian Journal of Experimental Agriculture, 20, 252-256.

Yamane, M.; Abe, D.; Yasui, S.; Yokotani, N.; Kimata, W.; Ushijima, K.; Nakano, R.; Kubo, Y.; Inaba, A. 2007. Differential expression of ethylene biosynthetic genes in climacteric and non-climacteric Chinese pear fruit. Postharvest Biology and Technology, 44, 220-227.

Yazdani, N.; Arzani, K.; Mostofi, Y.; Shekarchi, M. 2011. Alpha-Farnesene and antioxidative enzyme systems in Asian pear (Pyrus serotina Rehd.) fruit. Postharvest Biology and Technology, 59, 227-231.

Zhang, Z. K.; Huber, D. J.; Hurr, B. M.; Rao, J. P. 2009. Delay of tomato fruit ripening in response to 1-methylcyclopropene is influenced by internal ethylene levels. Postharvest Biology and Technology, 54, 1-8.

OBJETIVOS - OBJECTIVES

Objetivo general

El objetivo general de la presente tesis doctoral es determinar las bases fisiológicas y bioquímicas del bloqueo de la maduración en peras de la variedad 'Conference' tras la aplicación del compuesto 1-meticiclopropeno (1-MCP).

Objetivos específicos

En base a dicho objetivo general, se plantearon los siguientes objetivos específicos, cada uno destacado en los diferentes capítulos de la tesis:

1) Determinar el efecto del tratamiento con 1-MCP en la calidad durante la conservación en frío y la vida comercial y establecer si existe una relación entre el bloqueo de la maduración tras el tratamiento y los factores de campo, fecha de recolección y campaña. (Capitulo 1)
2) Analizar el efecto del tratamiento con 1-MCP en el metabolismo del etileno y metabolismo antioxidante en relación al factor campo y fecha de recolección. (Capitulo 2 y 3)
3) Evaluar un método para prevenir el bloqueo de la maduración después del tratamiento con 1-MCP mediante la aplicación de etileno exógeno. (Capitulo 4)
4) Determinar el efecto de un tratamiento con 1-MCP y etileno exógeno para reanudar el proceso de maduración en frutos tratados con 1-MCP y analizar el efecto de este tratamiento combinado sobre el metabolismo del etileno. (Capitulo 5)

General objective

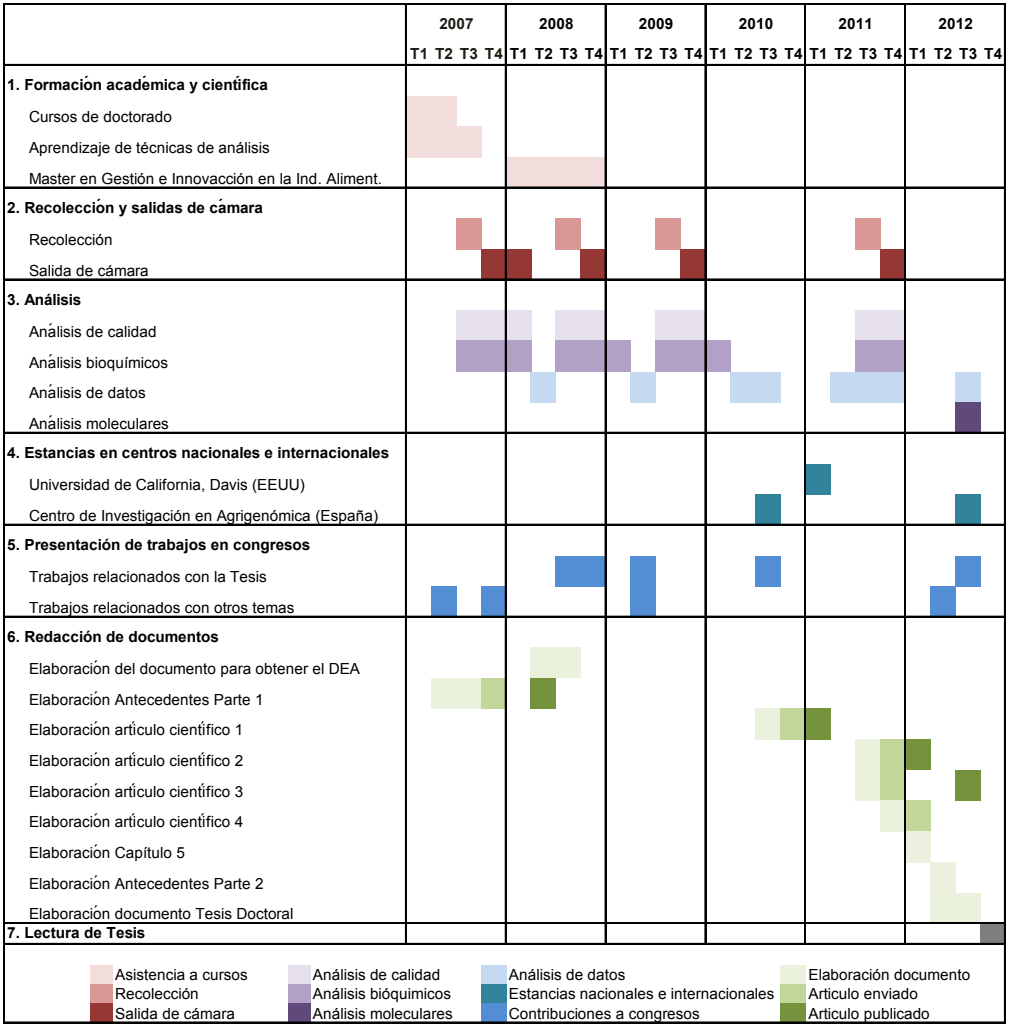
The general objective was to determine the physiological and biochemical basis of the ripening blockage in 'Conference' pears after the application of 1-methylcyclopropene (1-MCP).

Specific objectives

1) Determine the effect of 1-MCP treatment on quality during cold storage and shelf life as well as the relation between the ripening blockage and the growing location, harvest date and year. (Chapter 1)
2) Analyze the effect of 1-MCP treatment on ethylene metabolism and antioxidant metabolism in relation to growing location and harvest date. (Chapter 2 and 3)
3) Evaluate how to prevent ripening blockage after 1-MCP treatment through the application of exogenous ethylene. (Chapter 4)
4) Determine the effect of simultaneous 1-MCP and exogenous ethylene treatment on ripening and the ACC metabolism. (Chapter 5)

PLAN DE TRABAJO

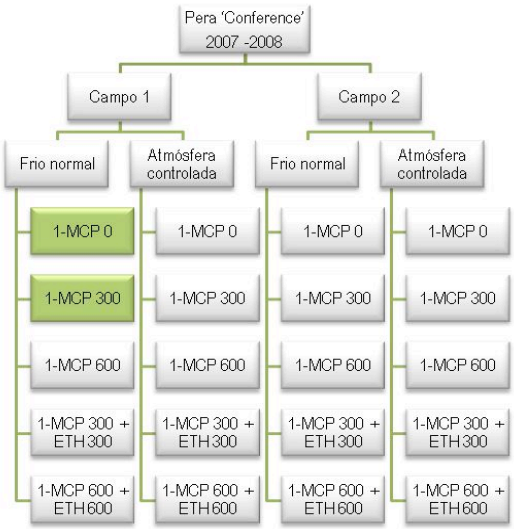
Cronograma de las distintas actividades realizadas a lo largo de la presente Tesis y durante las campañas frutícolas: 2007-2008, 2008-2009 y 2009-2010.



Campaña 2007-2008

Esta primera campaña fue de exploración en cuanto al tratamiento 1-MCP y su efecto en diferentes aspectos de calidad, fisiológicos y bioquímicos. Se realizó varios tratamientos combinados con 1-MCP y etileno exógeno como sistemas de control.

1-MCP 0 = Frutos sin tratar
1-MCP 300 = tratamiento con 300 nL L^{-1} de 1-metilciclopropeno
1-MCP 600 = tratamiento con 600 nL L^{-1} de 1-metilciclopropeno
ETH 300 = tratamiento con 300 nL L^{-1} de etileno exógeno
ETH 600 = tratamiento con 600 nL L^{-1} de etileno exógeno



Las determinaciones analíticas que se realizaron fueron los siguientes:

En el momento de cosecha:

- Parámetros estándar de calidad: firmeza de la pulpa, contenido de sólidos solubles, acidez titulable y color de la epidermis.
- Parámetros estándar de madurez: índice almidón y producción de etileno.

Después del almacenamiento:

- Parámetros estándar de calidad: firmeza de la pulpa y color de la epidermis.
- Parámetros estándar de madurez: producción de etileno.
- Análisis bioquímicos (para los lotes en verde):
  - Metabolismo de etileno: ACC, MACC, ACS, ACO
  - Metabolismo antioxidante: SOD, CAT , POX, H_2O_2, AsA

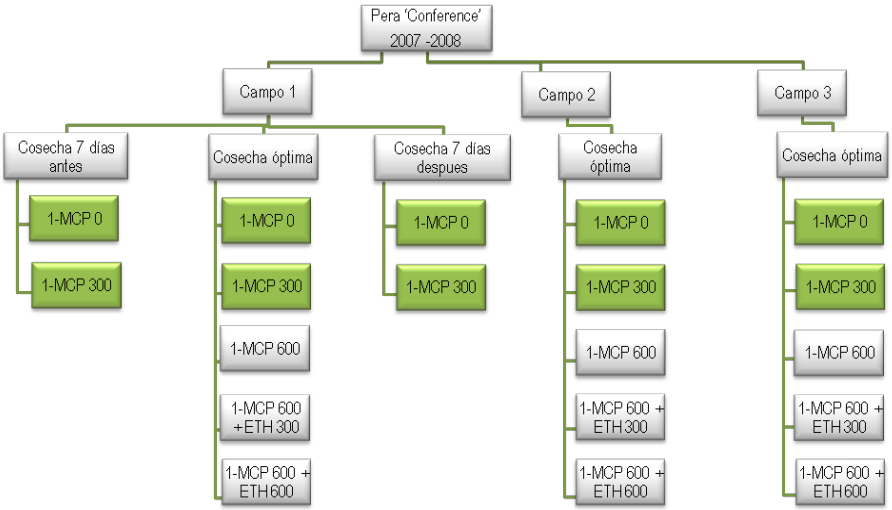
Durante la vida comercial:

- Parámetros estándar de calidad: firmeza de la pulpa y color de la epidermis.
- Parámetros estándar de madurez: producción etileno.

Los resultados aquí obtenidos constan en el Capítulo 1 (aspectos de calidad), Capítulo 3 (metabolismo antioxidante), Capítulo 4 (tratamientos con etileno exógeno).

Campaña 2008-2009

La segunda campaña se enfocó en determinar el efecto de la fecha de recolección y del campo en el tratamiento 1-MCP en aspectos de calidad, fisiológicos y bioquímicos. Se realizó varios tratamientos combinados con 1-MCP y etileno exógeno como sistemas de control.



Las determinaciones analíticas que se realizaron fueron los siguientes:

En el momento de cosecha:

- Parámetros estándar de calidad: firmeza de la pulpa, contenido desólidos solubles, acidez titulable y color de la epidermis.
- Parámetros estándar de madurez: índice almidón y producción de etileno.

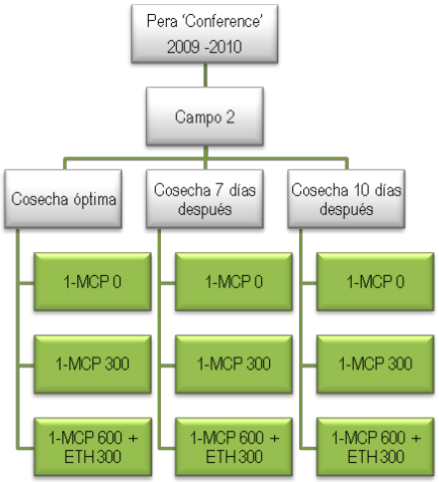
Después del almacenamiento y vida comercial:

- Parámetros estándar de calidad: firmeza de la pulpa y color de la epidermis.
- Parámetros estándar de madurez: producción de etileno.
- Análisis bioquímicos (para los lotes en verde):
    - Metabolismo del etileno: ACC, MACC, ACS, ACO
    - Metabolismo antioxidante: SOD, CAT , POX, H_2O_2
- Conductimetría de las membranas

Los resultados aquí obtenidos constan en el Capítulo 1 (aspectos de calidad), Capítulo 2 (metabolismo del etileno), Capítulo 3 (metabolismo antioxidante), Capítulo 4 (tratamientos de etileno exógeno).

Campaña 2009-2010

La tercera campaña se enfocó en el comportamiento bioquímico en la fruta tratada con 1-MCP y etileno exógeno durante la conservación y vida comercial y como está afectado por la fecha de cosecha.



Las determinaciones analíticas que se hicieron fueron los siguientes:

En el momento de cosecha:

- Parámetros estándar de calidad: firmeza de la pulpa, contenido desólidos solubles, acidez titulable y color de la epidermis.
- Parámetros estándar de madurez: índice almidón y producción etileno.

Después del almacenamiento y vida comercial:

- Parámetros estándar de calidad: firmeza de la pulpa, y color de la epidermis.
- Parámetros estándar de madurez: producción etileno.
- Análisis bioquímicos:
    - Metabolismo de etileno: ACC, MACC, ACS, ACO

Los resultados de esos experimentos se pueden encontrar en el Capítulo 2 y Capítulo 5.

RESULTADOS

CAPÍTULO 1
RESPONSIVENESS OF 'CONFERENCE' PEARS TO 1-METHYLCYCLOPROPENE (1-MCP): THE ROLE OF HARVEST DATE, ORCHARD LOCATION AND YEAR

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ABSTRACT

BACKGROUND: In some pear varieties like 'Conference', 1-MCP treatment often impairs the ripening process indefinitely and the pears remain 'evergreen'. To better understand this behavior, the influence of the harvest date, orchard location and year on the effectiveness of 1-MCP treatment was investigated.

RESULTS: Pear softening was inhibited by 1-MCP treatment and the effectiveness of the treatment depended on harvest date, orchard location and the year. Differences in the rate of softening in 1-MCP treated pears depended mainly on the fruit physiological maturity at the moment of 1-MCP treatment. Accordingly, the combination of the Streif index and ethylene production at harvest appeared to be able to predict the evergreen behavior. Treated pears with a low Streif index (< 0.8) and high ethylene production at harvest (≥ 0.23 µL kg^{-1} h^{-1}) maintain significantly high firmness but did soften during shelf life reaching acceptable eating quality.

CONCLUSION: The evergreen behavior was mainly influenced by the initial fruit maturity and especially by the ability of the fruit to produce ethylene at the moment of treatment. More mature fruit were able to overcome the inhibition by 1-MCP and the solution to prevent the evergreen behavior therefore lies in the adequate determination of harvest maturity.

Key words: Conference, 1-methylcyclopropene, evergreen, ripening, ethylene

1. INTRODUCTION

'Conference', the major pear cultivar grown in Spain, is a summer pear that does not require postharvest chilling for satisfactory ripening. This cultivar is usually stored several months at low temperatures to preserve the harvest quality, especially firmness and color, after cold storage. This cultivar is very tasty and has a typical buttery texture when reaching optimal ripeness; fruit between 30 to 10 N flesh firmness are considered ready-to-eat (Agrofresh, personal communication).

The commercial application of the ethylene action inhibitor, 1-methylcyclopropene (1-MCP) has been evaluated for its ability to delay ripening and extend the storage life in several fruits. (Sisler and Serek, 2003). Most of the commercial application of 1-MCP has occurred for apple and have become widely available to the apple industry worldwide (Watkins, 2006). Apples generally respond well to 1-MCP application, showing inhibition of ethylene production and respiration rates and the associated maintenance of firmness and other quality aspects both during and after cold storage (Fan et al., 1999; Watkins et al., 2000; Rupasinghe et al., 2000).

In contrast, 1-MCP treatment is more complicated in pears, partly because of their high sensitivity to the treatment as well as the fact that they do need to soften considerably before being ready-to-eat. In several cultivars, the residual effect of the treatment does not dissipate during a reasonable period of shelf life following cold storage, thereby preventing ripening (Argenta et al., 2003; Ekman et al., 2004; Trinchero et al., 2004).

The successful application of 1-MCP strongly depends on many factors, among which the applied concentration, the storage time and the maturity stage at the moment of treatment are the most important. In 'Conference' pears, concentrations of 600 nL L^{-1} 1-MCP always result in the evergreen behavior with a complete blockage of softening (Chiriboga et al., 2011), whereas lower concentrations (300 nL L^{-1} 1-MCP) resulted in only some pears being able to soften. Such heterogeneity in the capacity of the pears to ripen following 1-MCP treatment is amongst the main reasons underlying the limited use of 1-MCP in 'Conference' pears and hence requires further investigation.

The maturity of the fruit at the moment of the 1-MCP treatment has a crucial influence on the effect of the treatment (Watkins, 2006) and it may be affected by the harvest date, orchard location and the temperature during the growing season. Calvo (2004) applied 1-MCP on Williams pears at two different harvest maturities based on firmness (81 and 69 N) and concluded that the effectiveness of 1-MCP to maintain firmness decreased with the more advanced stage of maturity (lower firmness). For the optimum harvest maturity (81 N), fruit treated with 200 nL L^{-1} 1-MCP developed adequate edible firmness after 15 days at shelf life, while fruit from the late harvest (69 N) softened faster and required only 10 days to be

ready-to-eat. Similar results were found by Lafer et al. (2005) in 'Williams', 'Bosc' and 'Packham's Triumph' pears treated with 1-MCP. For the same dose, treated pears harvested one week after the optimum date lost more firmness than those harvested at the optimal date.

The location where the fruit are grown has also been shown to affect the responsiveness of the fruit to 1-MCP treatment. For instance, Agar et al. (1999) concluded that differences in ripening behavior and response to ripening inhibitors might occur in fruit of the same cultivar grown in different environments. They found that 'Bartlett' pears from growing locations with cooler preharvest temperatures and/or from later harvests within a growing location had higher ethylene production rates during ripening, indicating a difference in their ability to ripen.

Ripening of pears is induced by a threshold concentration of internal ethylene (Chen and Mellenthin, 1981), for this reason, the presence of ethylene in the fruit at the moment of the 1-MCP treatment could affect the response of the pears to the treatment. Gamrasni et al. (2010) applied 200 nL L^{-1} 1-MCP to 'Spadona' pears at four stages of ripeness as determined by ethylene production. The treatment with 1-MCP immediately after harvest inhibited pear softening during storage and shelf life, irrespective of the harvest date, but recovery of sensory attributes during shelf life was faster for fruit from the later harvest date. The inhibition decreased when the treatment was delayed and harvested fruit was conditioned at 20 °C. Under this condition, pears began to produce ethylene and the climacteric rise took place. When treated early during the climacteric rise, softening was still inhibited whereas for mid-climacteric fruit it was not.

To better understand the evergreen phenomena in 1-MCP treated 'Conference' pears, the influence of harvest date, growing location and year was investigated during storage and shelf life.

2. MATERIALS AND METHODS

2.1 Fruit material

'Conference' pears (*Pyrus communis*) were harvested in 2008 and 2009 at three harvest dates in Lleida, located in the northeast of Spain. In 2008, fruit were collected from three orchards (1, 2, 3), 7 days before the commercial harvest date (Early) corresponding to 138 days after full bloom (dafb), in the middle of the commercial harvest window (Mid, 144 dafb) and 7 days later (Late, 151 dafb).

In 2009, fruit were harvested at two different dates, the middle of the commercial harvest window (Mid, 145 dafb) and 7 days later (Late, 152 dafb) but only from orchard 2. Both seasons, fruit were harvested at random and placed directly in cold storage (-0.5 °C, 90% RH) after harvest.

Groups of 30 fruit (randomly selected from 3 different boxes) were used for assessment of firmness, color, soluble solids content, titratable acidity and starch index immediately after harvest. In addition, 3 replicates of 2 pears were used to determine ethylene production at 20 °C, 1 day after harvest. Fruit were analysed for firmness and color during shelf life at 20 ºC in 2008 at 0, 3, 7 and 11 days after removal from cold storage. In 2009, the shelf life assessment period was extended to 15 days at 20 °C and the firmness was assessed.

2.2 Experimental set up

In both years, 1-MCP was applied the day after harvest on previously cooled fruit and using Smartfresh™ (Agrofresh Inc, Rohm and Haas, Spring House, PA, USA) containing 0.14% 1-MCP as active ingredient. For the treatment, the fruit was covered with a 1 m^3 plastic bag and a treatment device consisting of a little battery powered ventilator over a small plastic flask containing the reaction mixture was placed inside. The fruit were treated with 0 and 300 nL L^{-1} 1-MCP during 24 hours at -0.5 ºC.

The following day (after 24 h), the plastic bag was opened and the cold room well aired. The fruit was stored at - 0.5 ºC in air (RA) for 30, 60, 90, and 105 days.

2.3 Measurements

Firmness (N) was determined on opposite peeled sides of the fruit using a penetrometer (Effegi, Milan, Italy), fitted with an 8 mm Magness Taylor probe.

Color (Hue angle, °) was determined at two points on the equator of each fruit with a portable tri-stimulus colorimeter (Chroma Meter CR-200, Minolta, Osaka, Japan) with a CIE D65 illuminant, an 8-mm aperture diameter and a 0° viewing angle. The results were reported using the L* a* b* color space. The hue angle was calculated with the formula arctg b*/a*.

Ethylene production (µL kg^{-1} h^{-1}) was measured in an acclimatized chamber at 20 ºC. The pears were placed in 1.5 L flasks continuously ventilated with humidified air at a flow rate of 1.5 L h^{-1}. One day later, gas samples (1 mL) were taken

from the headspace and injected into a gas chromatograph (Agilent Technologies 6890, Wilmington, Germany) fitted with a FID detector and an alumina column 80/100 (2 m x 1/8 x 2.1 mm, Teknokroma, Barcelona, Spain).

Soluble solids content (SSC, °Brix) was determined using a digital refractometer (Atago, Tokyo, Japan) by measuring the refractive index of juice from 5 pear slices. Titratable acidity (TA, g malic acid L^{-1}) was determined with 10 mL of pulp juice diluted in 10 mL of water and 0.1% phenolphthalein, which was titrated with 0.1 M NaOH to a pH of 8.2.

The starch index was determined by comparing the staining pattern when pears halved at their widest diameter were dipped in a I_2KI solution with the EUROFRU (Ctifl, France) reference chart using a scale of 1 (fully stained, all starch) to 10 (no staining, no starch).

The Streif Index was also calculated at harvest, as firmness*SSC^{-1}*starch $index^{-1}$.

2.4 Statistical analysis

The experiment was set up as a completely randomized design and the data analysis was performed using the Statistical Analysis System (SAS version 9.1, SAS Institute, Inc, 1992, Cary, NC, USA). The analysis of variance (ANOVA) and analysis of treatment effects was done using PROC GLM, using data during shelf life. Mean comparisons were performed using Tukey's LSD test at $p \leq 0.05$.

3. RESULTS

3.1 Effect of 1-MCP treatment as influenced by the interaction between orchard and harvest date

In 2008 at harvest, there were no significant differences for most of the quality parameters between fruit from the Early and the Mid harvest for all the orchards except for firmness in orchard 2. The Late harvested fruit were significantly more mature (lower firmness, lower Streif index and higher ethylene production, Table 1).

Control fruit from the Early and Mid harvest, despite having a similar maturity at the moment of harvest, behaved differently during storage (Table 2). After 30+7 days of storage, Early harvest fruit remained significantly firmer than Mid

and Late fruit, and this trend was especially noticeable in fruit from orchard 3. Later, all the control fruit exhibited a similar firmness loss independent of orchard location and initial harvest date.

Table 1: Maturity parameters at harvest of 'Conference' pears. The fruit were picked at three (2008) and two (2009) different harvest dates (Early, Mid, Late). Different lowercase letters in columns and uppercase letters in rows indicate significant differences ($p \leq 0.05$) between harvest dates and orchards, respectively.

		2008						2009	
Quality Parameters	**Harvest**	**Orchard 1**		**Orchard 2**		**Orchard 3**		**Orchard 2**	
Firmness (N)	Early	74.9	aA	74.6	aA	68.8	aB	-	
	Mid	74.2	aA	69.6	bB	71.8	aB	51.6	a
	Late	62.3	bB	66.2	cA	62.1	bB	52.8	a
Color (Hue º)	Early	98.8	bB	103.5	aA	99.4	bB	-	
	Mid	101.3	aAB	100.2	bB	102.6	aA	115.8	a
	Late	100.7	abA	98.9	bB	101.3	abA	115.3	a
SSC (º Brix)	Early	12.7	aA	12.7	aA	12.5	bA	-	
	Mid	12.5	aA	12.4	aA	12.4	bA	13.1	a
	Late	13.3	aB	12.9	aB	15.1	aA	12.8	a
TA (g malic acid L^{-1})	Early	2.6	aA	2.3	aA	1.6	abB	-	
	Mid	1.9	bA	2.0	abA	1.9	aA	2.1	a
	Late	2.2	abA	1.6	bB	1.3	bC	2.1	a
Starch Index (1-10)	Early	4.7	bB	4.8	aB	6.7	bA	-	
	Mid	4.5	bB	5.0	aB	6.6	bA	3.0	b
	Late	7.3	aB	5.7	aC	8.6	aA	5.1	a
Streif Index	Early	1.3	aAB	1.4	aA	1.0	aB	-	
	Mid	1.3	aA	1.1	abB	0.9	aC	1.4	a
	Late	0.7	bB	0.9	bA	0.5	bC	0.8	a
C_2H_4 production (µL kg^{-1} h^{-1})	Early	0.03	cC	0.07	bA	0.05	bB	-	
	Mid	0.17	bA	0.08	bB	0.05	bC	0.16	b
	Late	0.23	aA	0.18	aB	0.13	aC	0.56	a

Table 2: Fruit firmness (N) after 7 days at shelf life conditions (20 ºC) for 'Conference' pears harvested at three harvest dates in 2008. Fruit were treated with 0 nL L^{-1} 1-MCP (Control) or 300 nL L^{-1} 1-MCP (Treated) and removed at different time-intervals from cold storage. Overall LSD value for comparison between all factors including removal periods, harvest dates, orchard locations and 1-MCP treatment was $LSD_{0.05}$= 2.5 ($p \leq 0.001$)

Removal periods + 7 days at 20°C	Harvest	Control			Treated		
		Orchard			Orchard		
		1	2	3	1	2	3
30+7 $LSD_{0.05}$= 2.4 $p \leq 0.001$	Early	24.0	24.7	47.0	63.9	67.2	64.4
	Mid	9.7	10.2	13.5	59.8	63.2	64.9
	Late	14.4	10.0	12.6	58.6	60.8	56.5
60+7 $LSD_{0.05}$= 1.9 $p \leq 0.001$	Early	10.4	8.1	13.4	65.0	66.3	64.5
	Mid	8.3	7.9	9.5	54.9	57.0	59.7
	Late	9.8	8.4	11.2	52.6	61.7	57.1
90+7 $LSD_{0.05}$= 2.4 $p \leq 0.001$	Early	10.4	8.3	10.0	60.5	63.6	62.8
	Mid	10.0	8.5	9.5	52.7	60.6	65.0
	Late	10.2	9.2	12.7	53.0	56.1	55.2
105+7 $LSD_{0.05}$= 3.0 $p \leq 0.001$	Early	9.1	8.6	8.6	59.4	63.7	62.4
	Mid	8.4	9.3	9.0	52.7	63.2	61.1
	Late	6.1	6.0	14.1	44.6	60.6	55.2

Upon removal, 1-MCP-treated fruit maintained similar firmness than at harvest (Table 1 and 2). Later, there was slight softening as the period in cold storage lengthened and even though significant differences were seen between the harvest dates, these were relatively small when compared to differences between control and treated fruit.

After long-term storage (Figure 1), control fruit from all harvest dates softened fast reaching the minimal level of accepted eating quality (10 N) after 7 days of storage at 20ºC. Pear softening was greatly inhibited by 1-MCP and treated fruit did not soften during a 7-day shelf life period independent of the harvest date. Nonetheless, late-harvest fruit from orchard 1 exhibited significant firmness loss immediately after removal from cold storage, but still far less than the softening observed in control fruit. Statistical analysis of the data (Table 3) showed that although all the factors were important for firmness loss, the variability was mainly explained by the treatment effect, followed by the shelf life duration, harvest date and to a lesser extent the orchard location.

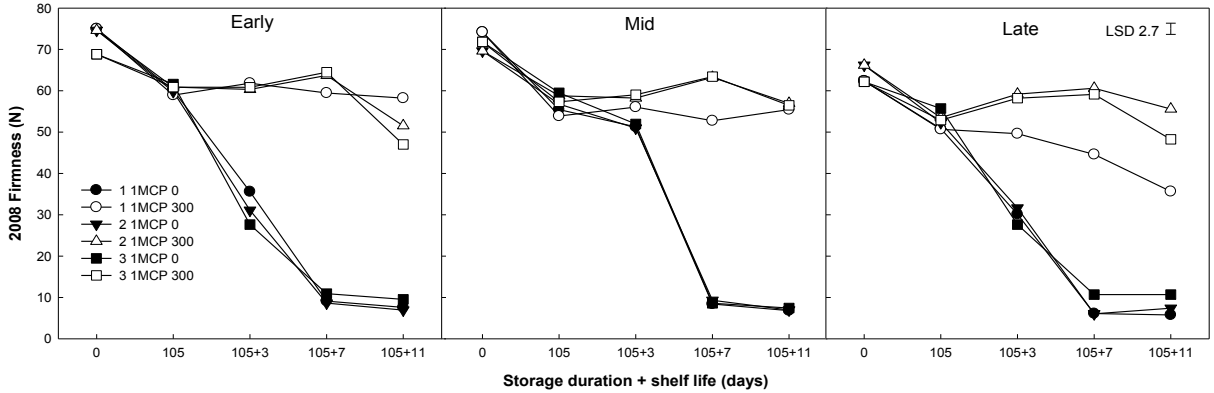

Figure 1: Softening during cold storage and shelf life of 'Conference' pears harvested from 3 orchards (1, 2, 3) at 3 harvest dates (Early, Mid and Late) in 2008. Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300). Each point represents the mean of a sample of 30 fruit and the bar represents the LSD value for the interaction between factors.

Table 3: F-value and *p*-level from ANOVA results for firmness and color in 3 orchards and 3 harvest dates during shelf life at 20°C in 2008. The variables studied were: days of shelf life (Days), harvest date (Harvest), orchard location (Orchard) and 1-MCP treatment (Treatment). Up to double interactions between factors are shown.

	Firmness		Color	
Source of variation	**F**	***p*-value**	**F**	***p*-value**
Days	2919.31	< 0.001	916.82	< 0.001
Harvest	245.68	< 0.001	154.15	< 0.001
Orchard	62.59	< 0.001	247.43	< 0.001
Treatment	15355.97	< 0.001	875.8	< 0.001
Days x Harvest	49.80	< 0.001	16.36	< 0.001
Days x Orchard	11.28	< 0.001	2.24	0.037
Harvest x Orchard	38.43	< 0.001	6.22	< 0.001
Days x Treatment	2532.27	< 0.001	62.19	< 0.001
Harvest x Treatment	44.46	< 0.001	11.71	< 0.001
Orchard x Treatment	45.63	< 0.001	9.19	< 0.001

Fruit color changes were in agreement with the results of firmness and were mainly affected by 1-MCP treatment and the duration of shelf life (Figure 2). Nonetheless, color differences between orchards were more noticeable than those between harvest dates. Hue angle (Figure 2) decreased mainly during shelf life but not as dramatically as firmness and was more pronounced in control fruit resulting in a visual color difference between treated and control fruit (Figure 2). Although all the factors contributed noticeably to the observed differences in color change (Table 3), the variability was mainly explained by the shelf life duration, followed by the treatment, orchard location and to a lesser extent the harvest date.

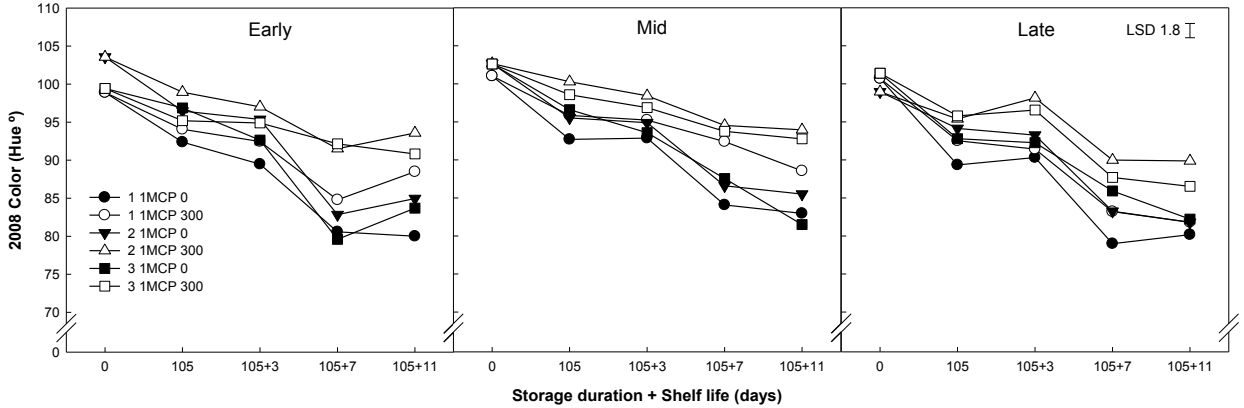

Figure 2: Color (hue angle) changes during cold storage and shelf life of 'Conference' pears harvested from 3 orchards (1, 2, 3) at 3 harvest dates (Early, Mid and Late) in 2008. Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300). The vertical bars represent the 95% confidence interval surrounding the mean. Each point represents the mean of a sample of 30 fruit and the bar represents the LSD value for the interaction between factors.

3.2 Effect of 1-MCP treatment as influenced by the interaction between year and harvest date

Even though pears from orchard 2 were harvested at a similar dafb in two consecutive years, the maturity of the fruit differed drastically in the Mid and Late harvest (Table 1). In 2009, firmness was lower but fruit were greener (higher hue angle) and had lower starch index when compared to fruit from 2008, especially for the Mid harvest. The Streif index showed a consistent decrease during on-tree maturation for both years. In contrast, a sharper increase in ethylene production from the Mid to the Late harvest was observed in 2009 when compared to 2008.

The control fruit from all the harvest dates reached the lower limit of eating quality (10 N) after cold storage and 7 days at 20 °C. In contrast, the 1-MCP treated fruit showed no significant loss of firmness during this time. After this period, the effect of the 1-MCP treatment was different between years, in 2008 (Figure 1) softening of treated fruit was clearly inhibited after 11 days at 20 °C while in 2009 (Figure 3) treated fruit started to soften already after 7 days at 20 °C.

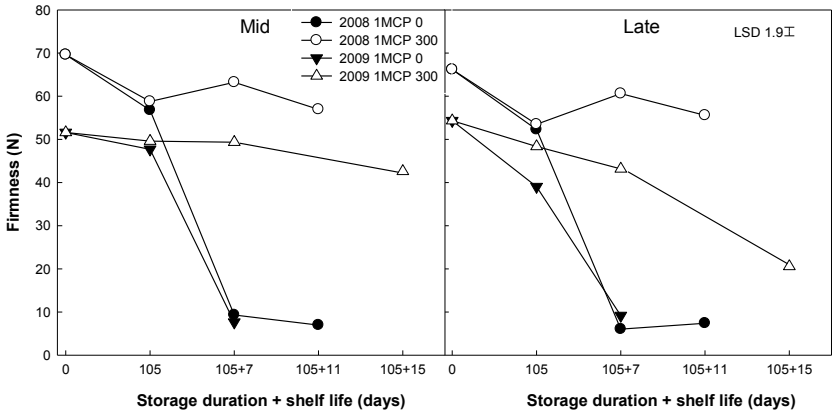

Figure 3: Softening during cold storage and shelf life of 'Conference' pears harvested from one orchard (2) in 2008 and 2009 at 2 harvest dates (Mid, Late). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1}1-MCP (1-MCP 300). Each point represents the mean of a sample of 30 fruit and the bar represents the LSD value for the interaction between factors.

Statistical analysis of the data (Table 4) revealed that all factors accounted for the observed variability in firmness values. However the variability appeared to be mainly explained by the treatment and shelf life duration. In this particular case, year-to-year differences in firmness values were more important than differences between harvest dates.

Table 4. F-value and p-level from ANOVA results for firmness in 2008 and 2009 in orchard 2 during shelf life at 20°C, studying days of shelf life (Days; 0 and 7 days), harvest date (Harvest; Mid and Late), 1-MCP treatment (Treatment with 0 or 300 nL L^{-1} 1-MCP) and year (Year; two consecutive years). Up to double interactions between factors are shown.

	Firmness	
Source of variation	**F**	***p*-value**
Days at shelf life	3224.24	< 0.001
Harvest	129.56	< 0.001
Treatment	4460.60	< 0.001
Year	512.24	< 0.001
Days x Harvest	4.44	0.036
Days x Treatment	3776.77	< 0.001
Harvest x Treatment	13.38	< 0.001
Days x Year	0.20	0.658
Harvest x Year	0.25	0.618
Treatment x Year	121.89	< 0.001

4. DISCUSSION

This study confirms that 1-MCP treatment applied immediately after harvest can lead to permanent inhibition of softening in 'Conference' pears as was reported by Chiriboga et al. (2011). Some treated fruit remained firm after 3.5 months of cold storage and 11 days at 20 ºC and did not reach the firmness of consumption. In contrast, untreated pears exhibited significant softening and yellowing during storage regardless of the year or orchard location.

The effect of 1-MCP treatment on 'Conference' pears was influenced by the harvest date, the year as well as the orchard location. When applied to less mature fruit, 1-MCP treatment resulted in a complete inhibition of fruit ripening. Fruit harvested before or around the commercial harvest date (Mid) in both experimental years remained firm after treatment with 1-MCP and lost their ability to soften even after several days at 20 ºC. In contrast, 1-MCP applied at more advanced stages of maturity slowed down the softening process without completely blocking it.

In the first year (2008), fruit from the Late harvest from orchard 1 did soften after 11 days at 20 °C, as did fruit from the Late harvest from orchard 2 in 2009. Generally, delaying the harvest date within the commercial picking period resulted in a

greater loss of firmness thereby confirming that if the 1-MCP treatment is done when fruit have started to ripen, the effect on firmness retention is much lower (Hiwasa et al., 2003; Calvo, 2004a; Lafer, 2005).

Orchard location may act on the fruit sensitivity to 1-MCP treatment. In this study, fruit softening of fruit from different orchard locations was similar for control fruit but varied considerably for 1-MCP treated fruit, especially when harvested at more advanced maturity. Climate and soil factors determine in part the fruit physiology and especially the ability of the fruit to regulate endogenous ethylene production which can result in differences in ripening behavior and response to 1-MCP treatment for fruit grown in different environments (Villalobos-Acuña and Mitcham, 2008). Nevertheless, the difference in the softening behavior between fruit from different orchards could in this study be explained by differences in the initial physiological maturity as reflected by dissimilar firmness and ethylene production rates at harvest for different orchard at the same harvest date.

A year effect was also observed in this study when comparing fruit from the same orchard (2) over two consecutive years. In this case, fruit collected at similar harvest dates (dafb) were not at the same physiological maturity and were therefore differently affected by the 1-MCP treatment. Fruit was more mature in 2009 at a specific dafb, it had a lower starch index, but also lower firmness and higher ethylene production at harvest, both indicating more advanced maturity. In 2009 1-MCP treated pears had a higher capacity to restore ripening and fruit softened more during shelf life. This is in agreement with the results obtained by Chen et al. (1997) who showed that 'Red d'Anjou' pears harvested with firmness lower than 53.4 N exhibited some ripening after 1 month of storage, while the fruit harvested at higher firmness (53.3 - 62.3 N) did not ripen normally. Concomitant to this, ethylene production rates were double in 2009, especially for the Late harvest when compared to 2008, corroborating the more advanced physiological maturity of the fruit in 2009 at the moment of treatment which in turn resulted in 1-MCP treated pears with higher capacity to restore ripening.

Differences in the softening behavior of 1-MCP treated fruit were mainly explained by differences in the initial physiological maturity. A clear definition of maturity at harvest is then needed to eliminate the orchard and year related variability. In this study and as commonly assessed commercially, the middle of the commercial window was set at 144-145 dafb. However, it is clear that harvesting at the same dafb does not ensure equal physiological maturity and that individual quality parameters are not able to consistently describe this physiological maturity nor can they be used to predict the evergreen behavior in 'Conference' pear. Indeed, this physiological maturity clearly depends on orchard location, cultural practices, climatic changes, growing year, etc.

Some authors (Hoehn et al., 1996; Johnson and Luton, 1996) have suggested the use of the Streif index (Streif, 1996) to determine optimal harvest date for long term storage of apples and pears. In this study, treated fruit with a Streif index of 0.7 at harvest and an initial ethylene production of 0.23 $\mu L\ kg^{-1}\ h^{-1}$ significantly softened during shelf life, whereas fruit with

a lower Streif index (0.5) but also lower ethylene production (0.13 μL kg^{-1} h^{-1}) remained firm. The Streif index, alone, was not sufficient to explain the tendency to the evergreen behavior when fruit were treated with 1-MCP. To optimize the prediction, the harvest index needs to include a parameter representative of the physiological maturity of the fruit such as the initial rates of ethylene production. Although the Streif index and initial ethylene production can be used to explain both the orchard and the year-to-year effects on softening of control fruit, only the initial ethylene production rate appeared to be able to predict the evergreen behavior. We can hypothesize that the level of ethylene produced by the pears (≥ 0.23 μL kg^{-1} h^{-1}) was high enough to occupy enough receptors at the time of treatment preventing them to be blocked by 1-MCP. Consequently normal ethylene production and ripening during shelf life after storage could be initiated as well as degradation of the receptor protein, thus preventing negative feedback control and enabling the recovery of ethylene production (Gamrasni et al., 2010). This result definitively shows that the determining factor that characterizes the evergreen behavior is the fruit physiological maturity and thus ethylene. The ability to ripen depends on the presence of critical levels of ethylene at the moment of treatment.

5. CONCLUSIONS

The effect of 1-MCP on 'Conference' pears and more specifically, the evergreen behavior were mainly influenced by the harvest maturity and especially by the ability of the fruit to produce ethylene at the moment of treatment. Orchard and year-to-year variations were also mainly related to differences in the physiological maturity of the fruit at harvest. Determining the optimal maturity with an index representing the physiological maturity of the fruit at harvest would be a solution to ensure the ripening recovery in 1-MCP treated fruit. The level of ethylene at the moment of the 1-MCP treatment could be the key factor to determine if the fruit can ripen during shelf life or not.

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References

Agar, I. T.; Biasi, W. V.; Mitcham, E. J. 1999. Exogenous ethylene accelerates ripening responses in Bartlett pears regardless of maturity or growing region. Postharvest Biology and Technology, 17, 67-78.

Argenta, L. C.; Fan, X. T.; Mattheis, J. P. 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit. Journal of Agricultural and Food Chemistry, 51, 3858-3864.

Calvo, G. 2004a. Aplicación de etileno a peras 'Bartlett', 'Beurre d'Anjou' y 'Packhams Triumph'previamente tratadas con 1-metilciclopropeno (1-MCP). Technical report. INTA. Alto Valle, Río Negro, Argentina. Pp. 1-18.

Calvo, G. 2004b. Efecto del 1-metilciclopropeno (1-MCP) en peras cv. 'Williams' cosechadas con dos estados estados de madurez. Revista de Investigaciones Agropecuarias, 33, 3-26.

Chen, P. M.; Mellenthin, W. M. 1981. Effects of harvest date on ripening capacity and postharvest life of 'd'Anjou' pears. Journal of the American Society for Horticultural Science, 106, 38-42.

Chen, P. M.; Varga, D. M.; Facteau, T. J. 1997. Promotion of ripening of 'Gebhard' red 'd'Anjou' pears by treatment with ethylene. Postharvest Biology and Technology, 12, 213-220.

Chiriboga, M. A.; Schotsmans, W. C.; Larrigaudiere, C.; Dupille, E.; Recasens, I. 2011. How to prevent ripening blockage in 1-MCP-treated 'Conference' pears. Journal of the Science of Food and Agriculture, 91, 1781-1788.

Curry, E. A. 2008. Regulating Ripening of 'Bartlett' Pears Using Preharvest Plus Postharvest Aminoethoxyvinylglycine (AVG). The Open Horticulture Journal, 1, 21-25.

Ekman, J. H.; Clayton, M.; Biasi, W. V.; Mitcham, E. J. 2004. Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears. Postharvest Biology and Technology, 31, 127-136.

Fan, X.; Mattheis, J. P.; Blankenship, S. 1999. Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. Journal of Agricultural and Food Chemistry, 47, 3063-3068.

Gamrasni, D.; Ben-Arie, R.; Goldway, M. 2010. 1-methylcyclopropene (1-MCP) application to Spadona pears at different stages of ripening to maximize fruit quality after storage. Postharvest Biology and Technology, 58, 104-112.

Hiwasa, K.; Kinugasa, Y.; Amano, S.; Hashimoto, A.; Nakano, R.; Inaba, A.; Kubo, Y. 2003. Ethylene is required for both the initiation and progression of softening in pear (Pyrus communis L.) fruit. Journal of Experimental Botany, 54, 771-779.

Hoehn, E.; Datwyler, D.; Gasser, F. (1996). Maturity indices to predict optimun harvest date for the storage of Conference pears in Switzerland. In: De Jager, A., Johnson, D., Hoehn, E. (Eds.). Determination and prediction of optimal harvest dates of apples and pears. Proceedings of the Working Group COST 94: The Postharvest Treatment of Fruit and Vegetables. Lofthus, Norway. Pp. 149-156.

Johnson, D. S.; Luton, M. T. (1996). Maturity indices to predict optimun harvest date for the storage of Conference pears in the UK. In: De Jager, A., Johnson, D., Hoehn, E. (Eds.). Determination and prediction of optimal harvest dates of apples and pears. Proceedings of the Working Group COST 94: The Postharvest Treatment of Fruit and Vegetables. Lofthus, Norway. Pp. 133-148.

Lafer, G. 2005. Effects of 1-MCP treatments on fruit quality and storability of different pear varieties. Acta Horticulturae, 682, 1227-1231.

Rupasinghe, H. P. V.; Murr, D. P.; Paliyath, G.; Skog, L. 2000. Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. Journal of Horticultural Science and Biotechnology, 75, 271-276.

Sisler, E. C.; Serek, M. 2003. Compounds interacting with the ethylene receptor in plants. Plant Biology, 5, 473-480.

Streif, J. 1996. Optimun harvest date for different apple cultivars in the Bodense area. In: Proceedings of a Meeting of the Working Group on optimun harvest date (COST 94). Pp. 15-20.

Trinchero, G. D.; Sozzi, G. O.; Covatta, F.; Fraschina, A. A. 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of `Bartlett` pears. Postharvest Biology and Technology, 32, 193-204.

Villalobos-Acuña, M.; Mitcham, E. J. 2008. Ripening of European pears: The chilling dilemma. Postharvest Biology and Technology, 49, 187-200.

Watkins, C. B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnology Advances, 24, 389-409.

Watkins, C. B.; Nock, J. F.; Whitaker, B. D. 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biology and Technology, 19, 17-32.

CAPÍTULO 2
COLD-INDUCED CHANGES IN ACC METABOLISM DETERMINE SOFTENING RECOVERY IN 1-MCP TREATED 'CONFERENCE' PEARS

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ABSTRACT

To better understand physiological response to cold stress and physiological changes triggered by 1-MCP treatment in relation to softening recovery, 'Conference' pears were harvested in three orchards, at three maturities and treated with 300 nL L^{-1} 1-MCP for 24 h. Changes in 1-aminocyclopropane carboxylic acid (ACC) and malonyl-ACC (MACC) levels, ACC synthase (ACS) and ACC oxidase (ACO) activity were followed during cold storage and 2 weeks of further ripening at 20 °C. Cold storage induced significant changes in ACC metabolism, which depended on the maturity of the fruit at harvest and to a lesser extent on the orchard location. In control fruit, ripening behavior upon removal from cold storage directly relied on the amount of ACC accumulated during cold storage as well as on harvest maturity-dependent increases in ACO and the capacity to convert ACC to MACC. Similar to control fruit, the increase of ACC levels during cold storage determined the further softening behavior of 1-MCP treated fruit. However, in 1-MCP treated fruit, malonylation had a more limited role and the capacity of the fruit to recover softening was directly related to the inhibition of ACO and mainly to the residual ACS activity, which was maintained after 1-MCP treatment during cold storage.

1. INTRODUCTION

In climacteric fruits such as 'Conference' pear, the regulation of ethylene biosynthesis is autocatalytic and mediated by the binding of ethylene to an ethylene receptor (Barry and Giovannoni, 2007). Without ethylene, the signal transduction cascade is negatively regulated by the receptors which actively block the ethylene pathways (Kevany et al., 2007). When ethylene binds, these receptors become inactive and the physiological responses occur. 1-methylcyclopropene (1-MCP) also binds to these receptors and activates them resulting in a permanent inhibition of ethylene perception and thereby a prevention of ethylene dependent responses (Sisler and Blankenship, 1996; Sisler and Serek, 1997).

Among the ethylene-modulated responses, the most important is ethylene production itself, which is inhibited at high 1-MCP doses in pears (Hiwasa et al., 2003; Trinchero et al., 2004; Bai et al., 2006). At lower doses, like 10 nL L^{-1} in 'd'Anjou' (Argenta et al., 2003) or as high as 500 nL L^{-1} 1-MCP in 'Bartlett' pear (Ekman et al., 2004), 1-MCP does not completely inhibit ethylene production but rather delays the climacteric peak. Additionally, the fruit treated with 1-MCP partially recover their sensitivity to ethylene during shelf life (Fan et al., 1999; Jeong et al., 2002). The time required for fruit to resume the ripening process (starting with ethylene production) depends on the 1-MCP concentration applied, the length of storage (Watkins, 2000) the species and/or cultivar (Watkins, 2006), the timing of 1-MCP treatment (DeEll and Ehsani-Moghaddam, 2011) and the maturity of the fruit at the time of treatment (Calvo and Sozzi, 2004). Nevertheless, some pears treated with 1-MCP never recover their ability to ripen (Chen and Spotts, 2005; Chiriboga et al., 2011) and the fruit remain firm and green. Although this may be desirable in some commodities like leafy vegetables, in the case of pears, fruit ripening is needed to reach the organoleptic characteristics demanded by consumers (Villalobos-Acuña and Mitcham, 2008).

There is only a limited amount of research elucidating the effects of 1-MCP treatment in 'Conference' pears. In general, 1-MCP treatment (10 to 50 nL L^{-1}) at harvest is effective in retarding ripening. However, the effect is not totally uniform, with a small percentage of treated fruit reaching their climacteric peak, losing green color and firmness. With higher doses (> 300 nL L^{-1}), ripening can be delayed more effectively, but sometimes the fruit lose their ability to ripen completely and remain firm and green even after shelf life as described with the term 'evergreen' (Chiriboga et al., 2011).

Although the inhibition of ethylene production by 1-MCP has been well described, the exact site in the ethylene biosynthesis pathway where 1-MCP plays a role is not yet clearly defined. Down-regulation of ACO expression and/or activity by 1-MCP has already been described in various species, whereas down-regulation of ACS expression and activity has been found in apple (Defilippi et al., 2005; Dal Cin et al., 2006), avocado (Owino et al., 2002) and banana (Zhang et al.,

2006). In peach, ACS activity was not affected by 1-MCP treatment (Mathooko et al., 2001) whereas in pears, chilling induced accumulation of ACS and ACO transcripts happened at reduced rates during storage after 1-MCP treatment (Villalobos-Acuña et al., 2011).

The biochemical basis of the ripening recovery process in 1-MCP treated pear is poorly understood. This process strongly depends on the specific behavior of the cultivar to chilling temperatures. Low temperatures induced the synthesis of ACS and ACO in 'd'Anjou' (Blankenship and Richardson, 1985), 'Bosc' (Sfakiotakis and Dilley, 1974) and 'Passe Crassane' (Lelièvre et al., 1997), all so called winter-pears. How do other cultivars behave and how is the ACC metabolism regulated by low temperature and 1-MCP treatment ?. To answer this question the present study describes how the ACC metabolism is regulated during cold stress and subsequent shelf life and in response to 1-MCP in 'Conference' pears. It further relates the physiology to the observed softening recovery in control and 1-MCP treated fruit, and further assesses the relationship between the physiological mechanisms and two factors that undoubtedly affect the response to 1-MCP treatment; more specifically orchard location and harvest maturity.

2. MATERIALS AND METHODS

2.1 Fruit Material and Experimental design

In a first experiment, pears (*Pyrus communis* cv. Conference) were harvested at the optimal commercial harvest date in Lleida (Spain) from three different orchards (O_1, O_2 and O_3). In a second experiment, fruit were harvested from orchard 2 (O_2) in the middle of the commercial harvest window (H_0), 7 days (H_7) and 10 days later (H_{10}). For both experiments, firmness was measured and fruit flesh samples were taken immediately after harvest and after different storage periods up to 105 days as well as during subsequent shelf life at 20 ºC. At each removal time, fruit flesh samples were immediately frozen in liquid N_2 and stored at -80 ºC until analysis.

The experiment was set up using a completely randomised design and the data were analyzed by analysis of variance using SAS statistical software (version 9.1, SAS Institute Inc., USA). Tukey's least significant differences values (LSD; $p = 0.05$) were calculated for mean separation.

2.2 Treatments

Immediately after harvest, fruit were placed in cold storage (-0.5 °C) for one day and then treated with 0 or 300 nL L^{-1} 1-MCP during 24 hours at -0.5 ºC using the product Smartfresh™ (Agrofresh Inc., Rohm and Haas, Spring House, PA, USA) and following the company recommendations. Briefly, the 1-MCP treatment was carried out as described in Chiriboga et al. (2011), covering the boxes with a 1 m^3 plastic bag and using the device provided by Agrofresh Inc. After the treatment, the bag was opened and the chamber thoroughly aerated.

2.3 Analytical

Determination of harvest maturity and softening recovery

The harvest maturity of 30 randomly selected fruit from each orchard and harvest date was determined using standard protocols. Firmness was determined with a manual penetrometer (Effegi, Milan, Italy) fitted with an 8 mm Magness Taylor probe. Starch index was determined by iodine staining using the radial EUROFRU reference chart (CTIFL, France). Ethylene production was determined on 3 replicates of 2 pears by gas chromatography as described below.

Softening recovery was determined by analyzing the difference (%) between the firmness of 30 fruits (randomly selected out of 3 boxes) immediately after removal from cold storage (time 105 in the figures) and after 7 and 15 days of shelf life at 20ºC. Data were expressed as % of firmness loss from the firmness immediately after removal.

Analysis of ethylene production

Ethylene production (µL kg^{-1} h^{-1}) was measured in an acclimatized chamber at 20 ºC. The pears were placed in 1.5 L respiration flasks continuously ventilated with humidified air at a flow rate of 1.5 L h^{-1}. Samples (1 ml) of effluent air from the flasks, were taken using a syringe and injected into a gas chromatograph (Agilent Technologies 6890, Wilmington, Germany) fitted with a FID detector and an alumina column F1 80/100 (2 m x 1/8 x 2.1, Tecknokroma, Barcelona, Spain). The injector and detector were kept at 120 and 180 °C, respectively.

Extraction and analysis of ACC and MACC levels:

ACC and MACC levels were analysed from 6 individual fruit per sample extracting 2 g of frozen flesh tissue with 30 ml of 80% ethanol. The ACC concentration was assayed according to the method of Lizada and Yang (1979) with minor modifications as described by Vilaplana et al. (2007) and expressed as nmol ACC g^{-1} fresh weight (FW). MACC was measured by analyzing the ACC content of a hydrolyzed extract as described by Hoffman et al. (1982). Data were expressed as nmol ACC g^{-1} FW.

Extraction and analysis of ACS and ACO enzyme activity:

ACS and ACO activity were determined using 10 g of frozen flesh tissue from 4 individual fruit per sample. For ACS activity, the frozen tissue was homogenized with 10 ml of buffer containing 200 mM Tricine buffer at pH 8.5, 10 mM dithiothreitol (DTT), 20 µM pyridoxal phosphate and 2% (w/v) polyvinylpyrrolidone (PVP). The homogenized tissue was then filtered through two layers of miracloth and centrifuged at 18,000 x g for 20 min at 4 ºC. Subsequently, a 2.5 ml aliquot was loaded into a Sephadex G-25 column (PD 10, Pharmacia, Madrid, Spain), previously equilibrated with 5 mM Tricine buffer (pH 8), 1 mM DTT and 2 µM pyridoxal 5-phosphate. The enzyme was eluted with 3.5 ml of the same buffer and 1.5 ml was incubated for 2 hours at 25 ºC with 200 mM Tricine buffer at pH 8 and 100 µM of S-adenosyl-L-methionine (SAM). The reaction was then stopped with 100 mM $HgCl_2$ and 1 ml of the product was mixed and stirred with 100 µl of NaOCl and saturated NaOH (2:1 v/v). After two minutes, a 1 ml headspace gas sample was injected into a gas chromatograph and the results were expressed as nmol C_2H_4 g^{-1} h^{-1}.

For ACO activity, the sample was homogenized in 20 ml of buffer containing 0.1 M Tris-HCl at pH 7.4, 10% glycerol, 30 mM Na-ascorbate, 5 mM DTT and 1% (w/v) PVP. The homogenate was filtered through two layers of miracloth and centrifuged at 16,000 x g for 20 min at 4 ºC. A 2.5 ml aliquot was loaded into a Sephadex G-25 column (PD 10, Pharmacia, Madrid, Spain), previously equilibrated with 20 mM Tris-HCl buffer at pH 7.4, 10% glycerol, 3 mM Na-ascorbate and 1mM DTT. The enzyme was eluted with 3.5 ml of the same buffer and 500 µl of enzyme extract was then mixed with 10 µM $FeSO_4$, 3 mM sodium bicarbonate and 50 µM ACC. The mixture was aired and incubated for 20 min at 26 ºC, after which a 1 ml headspace gas sample was injected into a gas chromatograph and the results were expressed as nmol C_2H_4 g^{-1} h^{-1}.

3. RESULTS AND DISCUSSION

For a storage strategy to be successfully implemented, all fruit should behave similar during storage, commercial life and in response to 1-MCP treatment. Unfortunately, current research shows that this may be difficult to achieve since important variability to 1-MCP treatment has been reported in various fruits (Mir and Beaudry, 2001; DeEll et al., 2002; Blankenship and Dole, 2003).

3.1 Changes in ACC metabolism in relation to orchard location

Initial maturity and softening recovery

Except for the starch index, which was higher in orchard 3 (fruit more mature), only slight differences in firmness and initial ethylene rates were found between orchards (Table 1). 1-MCP treated fruit from the three different orchards did not soften even after 15 days of shelf life at 20 ºC (Table 2, maximum 3.1 % firmness loss) while control fruit did soften considerably (more than 85 % firmness loss).

Table 1: Maturity indices at harvest in relation to orchard location (O_1, O_2 and O_3) and harvest date (H_0, H_7, H_{10}). Values represent the means of 30 fruit. Different letters in the same row indicate significant differences between orchards or harvest dates ($p<0.05$)

	Orchard			**Harvest**		
Maturity indices	**O_1**	**O_2**	**O_3**	**H_0**	**H_7**	**H_{10}**
Firmness (N)	74.2 a	69.6 b	71.8 b	51.6 a	52.8 a	54.3 a
Starch (1-10)	4.5 b	5.0 b	6.6 a	2.7 c	4.9 b	6.6 a
C_2H_4 (µL kg^{-1} h^{-1})	0.17 a	0.08 b	0.05 b	0.16 b	0.56 a	0.58a

Table 2: Softening recovery (% firmness loss) in 1-MCP treated fruit compared to controls. Fruit were stored 105 days in air and % firmness loss was determined both in control and treated fruit after 7 or 15 days of shelf life at 20 ºC. Recovery was expressed in % of firmness v.s fruit directly after removal from cold storage.

		Softening recovery (% firmness loss)					
		Orchard			**Harvest**		
Days at 20ºC	**1-MCP (nL L^{-1})**	**O_1**	**O_2**	**O_3**	**H_0**	**H_7**	**H_{10}**
7	0	84.8	89.9	85.5	84.0	81.3	76.5
	300	2.1	-7.6	-10.6	0.5	5.2	10.8
15	0	87.7	92.4	87.5	--	--	--
	300	-2.9	3.1	1.5	7.0	46.1	57.5

Physiological changes during cold storage

During cold storage, ACC levels sharply increased in control fruit in an orchard dependent manner (Figure 1A). Changes in ACC levels were not associated to a reduced conversion of ACC to MACC (Figure 1B) or to changes in ACS activity (Figure 1C), but rather to the changes in ACO activity (Figure 1D) during cold storage.

These results for 'Conference' pears are similar to those in 'Red d´Anjou' pears (Chen et al., 1997) which also exhibited this specific increase of ACO activity but different to results with 'Passe Crassane' pears (Lelièvre et al., 1997) where the increase of ACO activity was paralleled by an increase in ACS activity. The opposite behavior has been observed in apples, with an increase in ACS but not in ACO activity (Tian et al., 2002). All these results show that the activation of the ACC metabolism during cold stress directly depends on the cultivar and/or species investigated.

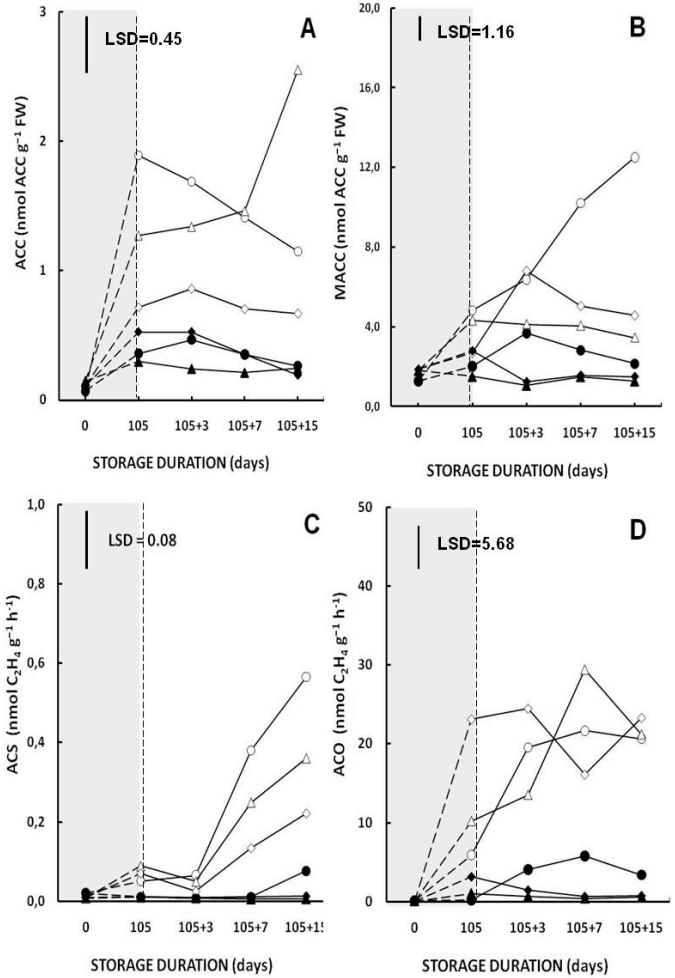

Figure 1: Changes in ACC levels (A), MACC levels (B), ACS activity (C) and ACO activity (D) during cold storage (grey area) and shelf life at 20 ºC in relation to orchard location. Open symbols = non-treated fruit; filled symbols = 1-MCP treated fruit. (○,●): Orchard 1; (Δ,▲): Orchard 2; (◇,◆): Orchard 3. Vertical bar represents LSD value (p=0.05).

The activation of the ACC metabolism was orchard dependent with significant differences in ACC accumulation and ACO activity between orchards during cold stress. The orchard that exhibited the higher ACO activity (orchard 3) also presented the lower levels of ACC, likely because ACC was converted to ethylene during cold storage.

Our observations show an activation of ACO before ACS in cold storage, which is consistent with the results of Lelièvre et al. (1997) in 'Passe Crassane' pears. We can therefore speculate that, as observed in 'Passe Crassane', ACO activity in 'Conference' may be induced by either ethylene or chilling whereas ACS depends on both chilling and ethylene. Other studies are needed to further define the molecular basis of this kind of regulation in 'Conference' pear. Those studies will have to determine if like in 'Passe Crassane' the regulation is defined at the gene expression level.

Despite significant inhibition of ACS and ACO activity during cold storage, 1-MCP treated fruit exhibited slight increases in ACC levels (3 to 5 fold when compared to harvest) regardless of orchard location. Only small changes were found for MACC levels, which generally remained at the same levels compared to harvest.

Physiological changes upon removal

After a one-day lag period, ethylene production (Figure 2) sharply increased in control fruit for the three orchards reaching a maximum value of 20 to 30 µl kg^{-1} h^{-1} after 6 to 7 days of shelf life. 1-MCP treatment significantly inhibited ethylene production in all orchards even though some capacity to produce ethylene was observed for orchards 1 and 3.

Significant differences between orchards were found for the ACC accumulation pattern in control fruit upon removal (Figure 1A). ACC levels in orchard 3 remained stable but an increase was observed in orchard 2 as well as a decrease in orchard 1. In orchard 1, ACC accumulation was inversely related to the changes in MACC levels (Figure 1B) with MACC accumulation accompanied by a reduction of ACC levels.

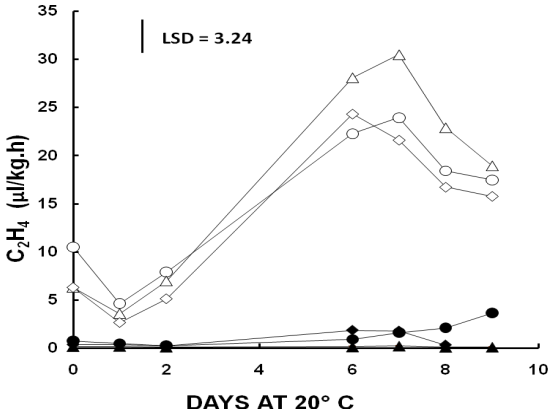

Figure 2: Ethylene production in Conference pears during shelf life at 20ºC, after removal from cold storage (105 days) in relation to orchard location. Open symbols = non-treated fruit; filled symbols = 1-MCP treated fruit. (○,●): Orchard 1; (Δ,▲): Orchard 2; (◇,◆): Orchard 3. Vertical bar represents LSD value (p=0.05).

ACS activity (Figure 1C) during shelf life followed a similar pattern as ACC accumulation did during cold storage. After a 3-day lag period, a sharp increase in ACS activity was found for orchard 1, followed by orchard 2 and to a lesser extent by orchard 3. Such behavior shows the relationship that exists between the metabolic changes that occur during cold stress (the increase in ACC levels) and further metabolic changes (increase in ACS activity) during shelf life.

In contrast to ACS, no clear pattern was found for ACO upon removal from cold storage. 1-MCP treated fruit exhibited a slight decrease in ACC levels during shelf life (Figure 1A). This decrease was not related to a burst in ethylene production and malonylation like in control fruit but rather to the complete inhibition of ACS activity and to some residual ACO activity. The down regulation of both ACS and ACO activity by 1-MCP has also been shown in 'Passe Crassane' (Lelièvre et al., 1997) and in other fruits (Dal Cin et al., 2006; Owino et al., 2006) but not in 'Bartlett' pears treated with 1-MCP, where ACS and ACO activity increased during shelf life (Villalobos-Acuña et al., 2011).

Generally, the initial differences observed between orchards in control fruit, mainly for ACC and ACO during cold stress and ACS during shelf life, were not apparent in fruit treated with 1-MCP. This indicates that1-MCP treatment resulted in a harmonization of samples, inhibiting ethylene production regardless of the growing location and impairing softening during shelf life. This is not always the case as seen in papaya (Moya-León et al., 2004), where treatment with 1-MCP blocked ethylene perception but did not stop the softening process.

3.2 Changes in ACC metabolism in relation to harvest maturity

Initial maturity and ripening recovery

Even though initial firmness was the same, significant differences were found in initial ethylene production and starch index between harvest dates (Table 1) especially in H_{10} and H_7 compared to H_0. Consequently, significant softening recovery was observed in fruit harvested later (H_{10} and H_7) which exhibited about 50 % firmness loss after 15 days of shelf life (Table 2).

Physiological changes during cold storage

In control fruit, a sharp accumulation of ACC was observed during cold storage especially in the fruit harvested later (Figure 3A). This increase was promoted by a slight increase of ACS activity (Figure 3C) but also limited by a sharp increase of ACO activity in the more mature fruit (Figure 3D) and by considerable accumulation of MACC in H_{10} (Figure 3B). This last increase in MACC shows that the malonylation process may be a key element in regulating the burst of ACC levels during cold stress. MACC accumulation clearly depended on the harvest date and is likely related to the higher activity of the enzyme malonyltransferase, which may be induced during on-tree maturation. As in control fruit, ACC levels significantly increased in 1-MCP treated fruit during cold storage. However in this case, the increase was likely related to the complete inhibition of ACO activity as well as a reduced capacity of the fruit to convert ACC to MACC compared with control fruit.

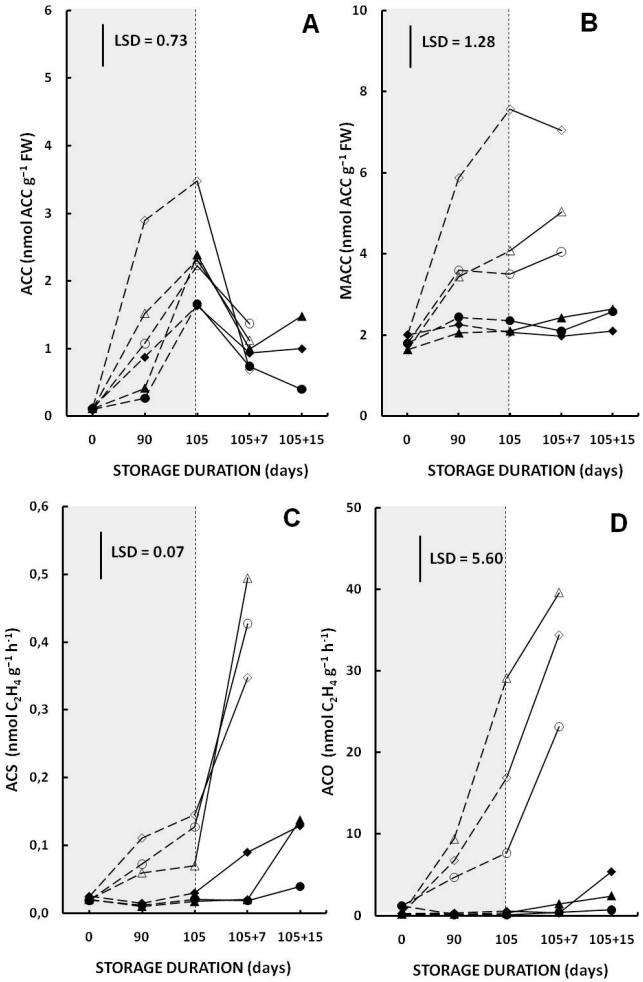

Figure 3: Changes in ACC levels (A), MACC levels (B), ACS activity (C) and ACO activity (D) during cold storage (grey area) and shelf life at 20 ºC in relation to harvest maturity. Open symbols = non-treated fruit; filled symbols = 1-MCP treated. (○,●): Commercial harvest date (H_0); (Δ,▲): Commercial harvest date + 7 days (H_7); (◇,◆): Commercial harvest date + 10 days (H_{10}). Vertical bar represents LSD value (p=0.05).

Physiological changes upon removal

Ethylene production in control fruit increased immediately after removal (Figure 4), and reached a maximum within 2 days of shelf life in the case of H_{10} fruit and 5 to 6 days for fruit harvested earlier (H_0 and H_7).

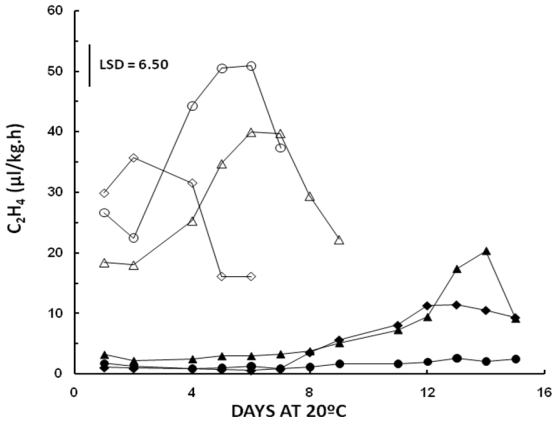

Figure 4: Ethylene production in Conference pears during shelf life at 20ºC, after removal from cold storage (105 days) in relation to harvest maturity. Open symbols = non-treated fruit; filled symbols = 1-MCP treated fruit. (○,●): Commercial harvest date (H_0); (Δ,▲): Commercial harvest date + 7 days (H_7); (◇,◆): Commercial harvest date + 10 days (H_{10}). Vertical bar represents LSD value (p=0.05).

ACS and ACO activity also increased markedly but with small differences between harvest dates. Despite the lower ACO activity, fruit picked earlier (H_0) produced more ethylene during shelf life. These results not only suggest that ACS and ACO activities were never limiting during shelf life, but also that in the more mature fruit, ethylene production may be limited by other parameters (i.e. softening and over-ripening) rather than the ACC metabolism.

The treatment with 1-MCP did not completely inhibit ethylene production during shelf life but delayed the climacteric peak to 12 days and 14 days for H_{10} and H_7 respectively and to 25 days for H_0 sample (data not shown). When compared to controls, a 2 to 5-fold decrease in maximal ethylene production was observed in 1-MCP-treated fruit.

All samples (control and treated) exhibited significant decreases in ACC levels upon removal (Figure 3A). In control fruit, the decline in ACC was related to an increased activity of ACO (Figure 3D) and consequently to the burst of ethylene production observed upon removal (Figure 4). In this case, ACC malonylation and ACS activity (Figure 3C) were not

involved. In contrast, the reduced ethylene production in 1-MCP treated fruit was associated with significant inhibitions of ACS, ACO and MACC levels during cold storage. Considering that the capacity of the fruit to convert ACC to MACC remained inhibited during shelf life, the decrease of ACC levels in 1-MCP treated fruit appears to be mainly related to the antagonist action of ACS and ACO.

Collectively these results show that malonyltransferase is also a key element in the regulation of the ACC metabolism in 1-MCP treated fruit during and after cold stress. In 'Conference' pears, 1-MCP treatment caused an important inhibition of the ability to malonylate ACC. In contrast, in 'Golden Smoothee' apples, 1-MCP treatment did not impair and even promoted the malonylation both in pulp and skin (Vilaplana et al., 2007). Therefore, these results show that the mechanism of response of the fruit to 1-MCP treatment depends on the species. More studies are needed to exactly define the effect that 1-MCP has on malonyltransferase in different species. These studies would be of interest to better understand the role that this enzyme plays in the response of the fruit to cold stress and on the effectiveness of the 1-MCP treatment.

3.3 Physiological basis of the softening recovery

Recovery of softening in 1-MCP treated pears directly depends on the harvest maturity. This softening recovery is determined by the ethylene levels at the moment of treatment but may also be the result of a downstream regulation of ethylene perception and changes in ethylene receptor turnover during cold storage (Villalobos-Acuña et al., 2011).

Although we cannot discard that the greater softening recoveries observed in this work were related, at least in part, to higher ethylene levels at harvest in H_7 and H_{10}, our data are also consistent with the hypothesis proposed by Villalobos-Acuña et al. (2011). Indeed, even though changes in ethylene production during shelf life strictly depend on the activation of the ACC metabolism at warmer temperatures, the initiation of the recovery process certainly takes place during cold storage.

To better understand the key factors involved in softening recovery, we established in Table 3 the relationship between the residual ACS and ACO activity in 1-MCP treated fruit just after removal and the capacity of the fruit to soften after 15 days of shelf life at 20ºC. Softening recovery clearly depended on the intensity by which ACS was inhibited by 1-MCP treatment during cold stress. In case of important inhibition of ACS activity during cold storage (less than 15% of residual activity for O_1, O_2, O_3 and H_0), the fruit remained evergreen and did not soften. In contrast, less inhibition of ACS during cold storage (25% and 58% of residual activity for H_7 and H_{10}, respectively) led to significant softening during shelf life. The inhibition of ACO by 1-MCP during cold stress (1% and 3.2% of residual activity for H_7 and H_{10}, respectively) did not play an important role.

Table 3: Relationship between residual ACS and ACO activity (%) in 1-MCP treated fruit at removal from cold storage and softening recovery (% firmness loss) in 1-MCP treated fruit after 15 days of shelf life compared to the control immediately after removal from cold storage (105 days). O_1, O_2 and O_3: fruit from different orchards; H_0, H_7 and H_{10}: fruit from different harvest dates.

Sample	Residual ACS activity (%)	Residual ACO activity (%)	Softening recovery (% Firmness loss)
O_1	14.6	3.7	-2.9
O_2	11.2	10.1	3.1
O_3	15.3	13.7	1.5
H_0	11.1	1.0	7.0
H_7	25.3	1.0	46.1
H_{10}	58.0	3.2	57.5

In conclusion, the softening behavior during shelf life and the effectiveness of 1-MCP treatment in 'Conference' pear are determined by the physiological regulations taking place during cold acclimation, regulations that are greatly affected by the initial maturity of the fruit and to a lesser extent by orchard location.

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References

Argenta, L. C.; Fan, X. T.; Mattheis, J. P. 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit. Journal of Agricultural and Food Chemistry, 51, 3858-3864.

Bai, J.; Mattheis, J. P.; Reed, N. 2006. Re-initiating softening ability of 1-methylcyclopropene-treated 'Bartlett' and 'd'Anjou' pears after regular air or controlled atmosphere storage. Journal of Horticultural Science & Biotechnology, 81, 959-964.

Barry, C. S.; Giovannoni, J. J. 2007. Ethylene and fruit ripening. Journal of Plant Growth Regulation, 26, 143-159.

Blankenship, S. M.; Dole, J. M. 2003. 1-methylcyclopropene: a review. Postharvest Biology and Technology, 28, 1-25.

Blankenship, S. M.; Richardson, D. G. 1985. Development of ethylene biosynthesis and ethylene-induced ripening in danjou pears during the cold requirement for ripening. Journal of the American Society for Horticultural Science, 110, 520-523.

Calvo, G.; Sozzi, G. O. 2004. Improvement of postharvest storage quality of 'Red Clapp's' pears by treatment with 1-methylcyclopropene at low temperature. Journal of Horticultural Science and Biotechnology, 79, 930-934.

Chen, P. M.; Spotts, R. 2005. Changes in ripening behaviors of 1-MCP treated d'Anjou pears after storage. International Journal of Fruit Science, 5, 3-18.

Chiriboga, M. A.; Schotsmans, W. C.; Larrigaudiere, C.; Dupille, E.; Recasens, I. 2011. How to prevent ripening blockage in 1-MCP-treated 'Conference' pears. Journal of the Science of Food and Agriculture, 91, 1781-1788.

Dal Cin, V.; Rizzini, F. M.; Botton, A.; Tonutti, P. 2006. The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit. Postharvest Biology and Technology, 42, 125-133.

DeEll, J.; Ehsani-Moghaddam, B. 2011. Timing of postharvest 1-methylcyclopropene treatment affects Bartlett pear quality after storage. Canadian Journal of Plant Science, 91, 853- 858.

DeEll, J. R.; Murr, D. P.; Porteous, M. D.; Rupasinghe, H. P. V. 2002. Influence of temperature and duration of 1-methylcyclopropene (1-MCP) treatment on apple quality. Postharvest Biology and Technology, 24, 349-353.

Defilippi, B. G.; Dandekar, A. M.; Kader, A. A. 2005. Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. Journal of Agricultural and Food Chemistry, 53, 3133-3141.

Ekman, J. H.; Clayton, M.; Biasi, W. V.; Mitcham, E. J. 2004. Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears. Postharvest Biology and Technology, 31, 127-136.

Fan, X. T.; Blankenship, S.; Mattheis, J. P. 1999. 1-methylcyclopropene inhibits apple ripening. Journal of the American Society for Horticultural Science, 124, 690-695.

Hiwasa, K.; Kinugasa, Y.; Amano, S.; Hashimoto, A.; Nakano, R.; Inaba, A.; Kubo, Y. 2003. Ethylene is required for both the initiation and progression of softening in pear (Pyrus communis L.) fruit. Journal of Experimental Botany, 54, 771-779.

Hoffman, N. E.; Yang, S. F.; McKeon, T. 1982. Identification of 1-(malonylamino)cyclopropane-1-carboxylic acid as a major conjugate of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor in higher-plants. Biochemical and Biophysical Research Communications, 104, 765-770.

Jeong, J.; Huber, D. J.; Sargent, S. A. 2002. Influence of 1-methylcyclopropene (1-MCP) on ripening and cell-wall matrix polysaccharides of avocado (Persea americana) fruit. Postharvest Biology and Technology, 25, 241-256.

Kevany, B. M.; Tieman, D. M.; Taylor, M. G.; Cin, V. D.; Klee, H. J. 2007. Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant Journal, 51, 458-467.

Lelièvre, J. M.; Tichit, L.; Dao, P.; Fillion, L.; Nam, Y. W.; Pech, J. C.; Latché, A. 1997. Effects of chilling on the expression of ethylene biosynthetic genes in Passe-Crassane pear (Pyrus communis L.) fruits. Plant Molecular Biology, 33, 847-855.

Lizada, M. C. C.; Yang, S. F. 1979. A simple and sensitive assay for 1-amino cyclopropane-l-carboxylic acid. Analytical Biochemistry, 100, 145.

Mathooko, F. M.; Tsunashima, Y.; Owino, W. O.; Kubo, Y.; Inaba, A. 2001. Regulation of genes encoding ethylene biosynthetic enzymes in peach (Prunus persica L.) fruit by carbon dioxide and 1-methylcyclopropene. Postharvest Biology and Technology, 21, 265-281.

Mir, N. A.; Beaudry, R. M. 2001. Use of 1-MCP to reduce the requirement for refrigeration in the storage of apple fruit. Acta Horticulturae, 553, 577-580.

Moya-León, M. A.; Moya, M.; Herrera, R. 2004. Ripening of mountain papaya (Vasconcellea pubescens) and ethylene dependence of some ripening events. Postharvest Biology and Technology, 34, 211-218.

Owino, W. O.; Manabe, Y.; Mathooko, F. M.; Kubo, Y.; Inaba, A. 2006. Regulatory mechanisms of ethylene biosynthesis in response to various stimuli during maturation and ripening in fig fruit (Ficus carica L.). Plant Physiology and Biochemistry, 44, 335-342.

Owino, W. O.; Nakano, R.; Kubo, Y.; Inaba, A. 2002. Differential regulation of genes encoding ethylene biosynthesis enzymes and ethylene response sensor ortholog during ripening and in response to wounding in avocados. Journal of the American Society for Horticultural Science, 127, 520-527.

Sfakiotakis, E. M.; Dilley, D. R. 1974. Induction of ethylene production in 'Bosc' pears by postharvest cold stress. HortScience, 9, 336-338.

Sisler, E. C.; Blankenship, S. M. (1996). Methods of counteracting an ethylene response in plants. Pp. 518, 988. U.S. Patent 5.

Sisler, E. C.; Serek, M. 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. Physiologia Plantarum, 100, 577-582.

Tian, M. S.; Prakash, S.; Zhang, N.; Ross, G. S. 2002. Chilling-induced ethylene biosynthesis in Braeburn apples. Plant Growth Regulation, 38, 249-257.

Trinchero, G. D.; Sozzi, G. O.; Covatta, F.; Fraschina, A. A. 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of `Bartlett` pears. Postharvest Biology and Technology, 32, 193-204.

Vilaplana, R.; Soria, Y.; Valentines, M. C.; Larrigaudière, C. 2007. Specific response of apple skin and pulp tissues to cold stress and 1-MCP treatment. Postharvest Biology and Technology, 43, 215-220.

Villalobos-Acuña, M.; Mitcham, E. J. 2008. Ripening of European pears: The chilling dilemma. Postharvest Biology and Technology, 49, 187-200.

Villalobos-Acuña, M. G.; Biasi, W. V.; Flores, S.; Jiang, C. Z.; Reid, M. S.; Willits, N. H.; Mitcham, E. J. 2011. Effect of maturity and cold storage on ethylene biosynthesis and ripening in 'Bartlett' pears treated after harvest with 1-MCP. Postharvest Biology and Technology, 59, 1-9.

Watkins, C. B. 2000. Responses of horticultural commodities to high carbon dioxide as related to modified atmosphere packaging. HortTechnology, 10, 501-506.

Watkins, C. B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnology Advances, 24, 389-409.

Zhang, M. J.; Jiang, Y. M.; Jiang, W. B.; Liu, X. J. 2006. Regulation of ethylene synthesis of harvested banana fruit by 1-methylcyclopropene. Food Technology and Biotechnology, 44, 111-115.

CAPÍTULO 3
ANTIOXIDANT POTENTIAL OF 'CONFERENCE' PEARS DURING COLD STORAGE AND SHELF LIFE IN RESPONSE TO 1-METHYLCYCLOPROPENE

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ABSTRACT

Antioxidant metabolism and changes in membrane permeability were studied during cold storage and shelf life in 'Conference' pears. The fruit were harvested at different harvest dates and growing locations and treated with 300 nL L^{-1} of 1-methylcyclopropene (1-MCP). 1-MCP-treated fruit exhibited higher superoxide dismutase (SOD) and catalase (CAT) activity, together with higher ascorbic acid levels during cold storage. During shelf life, the 1-MCP treatment reduced the electrolyte leakage and improved the capacity of the fruit to remove reactive oxygen species by increasing SOD, CAT and peroxidase (POX) activities. The effect of 1-MCP treatment on the antioxidant metabolism differed between orchards but not between harvest dates.

Keywords: ascorbic acid, catalase, electrolyte leakage, peroxidase, superoxide dismutase, 1-MCP.

1. INTRODUCTION

Pears are climacteric fruit exhibiting a rise in ethylene production and respiration rates during ripening, with substantial variability among different cultivars (Paul et al., 2012). To control ripening and extend the storage life of pears, fruit are stored at low temperatures immediately after harvest with or without controlled atmosphere conditions. Another alternative is to treat the pears with the ethylene inhibitor 1-methylcyclopropene (1-MCP) which has been intensively studied over the last decades (Watkins, 2006). 1-MCP blocks ethylene receptors and thus inhibits ethylene action (Sisler and Serek, 1997), affects fruit ripening and improves the postharvest quality of climacteric fruits (Blankenship, 2003). The biochemical changes that this treatment has on the ethylene metabolism in 'Conference' pears both during cold storage and during shelf life were recently elucidated (Chiriboga et al., 2012) as also was the effect of 1-MCP on fruit quality including firmness (Chiriboga et al., 2011, in press).

Cold storage in regular or controlled atmosphere conditions is known to be stressful to a harvested fruit and may induce the accumulation of reactive oxygen species (ROS) and particularly superoxide radical ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) (Imahori et al., 2008; Larrigaudière et al., 2001; Sala and Lafuente, 2004). Increased accumulation of ROS may cause lipid peroxidation and membrane disintegration accelerating the onset of senescence and the development of certain physiological disorders (Miller, 1986). Nevertheless, plants have different defence mechanisms, enzyme-mediated or not, to counteract the effects of ROS. The enzymatic antioxidant system involves a wide range of enzymes including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX), all of which play an important role in the formation and degradation of H_2O_2. Within the cell, SOD is the first line of defence against free radicals and higher activity in fruits has been related to a greater resistance to stress and longer commercial life (Wang and Jiao, 2001; Mondal et al., 2004). POX is less specific than SOD and is related to a great number of biochemical and physiological processes, which may undergo both quantitative and qualitative changes during fruit development. A decrease in the activity of these two enzymes has previously been linked to the appearance of internal browning in pears (Lentheric et al., 2003; Larrigaudière et al., 2004). Among the non-enzymatic antioxidants, ascorbic acid (AsA) plays an important role in the detoxification of ROS (Foyer et al., 1991). Loss of AsA is directly related to the initiation and development of internal browning in 'Conference' pears (Franck et al., 2003; Eccher-Zerbini et al., 2002). Whereas the effect of cold storage on antioxidant metabolism in certain fruit is well known (Larrigaudière et al., 2001; Rapisarda et al., 2008) few studies are available describing changes in the antioxidant potential in pears treated with 1-MCP.

1-MCP has been associated with low accumulation of ROS during cold storage and shelf life in 'Granny Smith' apples (Sabban-Amin et al., 2011) and also with the promotion of SOD and/or POX activities in pears (Fu et al., 2007;

Larrigaudière et al., 2004) and peaches (Liu et al., 2005). In 'Blanquilla' pears, 1-MCP treatment at a dose rate of 100 nL L^{-1} reduced the levels of H_2O_2 and AsA because the fruit exhibited higher enzymatic antioxidant potential, especially CAT, POX and ascorbate peroxidase during cold storage (Larrigaudière et al., 2004). In contrast, 1-MCP delayed the loss of AsA in some fruits like peaches. In 'Golden Smoothee' apples, 1-MCP treatment has been associated with low levels of AsA during storage (Vilaplana et al., 2006) and with an increased activity of ascorbate peroxidase, thereby promoting the oxidation of AsA to dehydroascorbate.

To date, no information is available on the changes in antioxidant compounds or antioxidant metabolism in 'Conference' during cold storage and shelf life in response to 1-MCP treatments. Accordingly, the objective of the present study was to determine the effects of 1-MCP treatment on the antioxidant metabolism and membrane disintegration in 'Conference' pears during cold storage and shelf life as well as to assess any possible influence of the harvest date and growing location.

2. MATERIALS AND METHODS

2.1 Fruit material

'Conference' pears (*Pyrus communis*) were harvested in commercial orchards in Lleida, (Northeast, Spain). In 2007, fruit were collected from one orchard (O_1) at the optimal harvest date for long term storage according to local recommendations and stored in air at -0.5 °C and 90% RH. Four replicates of 4 fruit each were removed from storage every 15 days up to 105 days for determination of H_2O_2 and AsA levels as well as SOD, CAT and POX activity.

In 2008, two different experiments were carried out. In the first experiment, fruit were harvested from the same set of trees from the same orchard (O_1), at three different harvest dates: 7 days before the optimal harvest date (H_{-7}), at the optimal harvest date (H_0) and 7 days after the optimal harvest date (H_{+7}). In the second experiment, pears were harvested at the optimal harvest date from three different orchards (O_1, O_2 and O_3). For both experiments, fruit were removed from cold storage after 105 days and 4 individual fruit per sample were analysed after 0, 3, 7 and 11 days of shelf life at 20 °C. The analysis included determination of H_2O_2 levels, electrolyte leakage as well as SOD, CAT and POX activity.

2.2 Experimental set up

In both years, 1-MCP was applied the day after harvest on previously cooled fruit using Smartfresh™ (Agrofresh Inc.) containing 0.14 % 1-MCP as active ingredient. For the treatment, fruit were covered with a 1 m^3 plastic bag and a treatment device consisting of a little battery powered ventilator over a small plastic flask containing the reaction mixture was placed inside. The fruit were treated with 0 and 300 nL L^{-1} 1-MCP during 24 hours at - 0.5 ºC. The following day (after 24 h), the plastic bag was opened and the cold room well aired.

2.3 Measurements

Determination of hydrogen peroxide (H_2O_2)

To determine H_2O_2 levels, 15 g of fresh pulp tissue were homogenized in 20 mL of 5% trichloroacetic acid, filtered through two layers of Miracloth (Textil Planas Oliverassa, Manresa, Spain) and centrifuged at 20,000 x g for 15 min at 4 ºC. H_2O_2 content was determined using the Bioxytech H_2O_2-560 (OXIS International Inc., Portland, OR USA) colourimetric assay following the manufacturer's instructions. The assay is based on the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) by hydrogen peroxide under acidic conditions. Ferric ions bind with the indicator dye xylenol orange to form a stable coloured complex that is measured at 560 nm. H_2O_2 content was expressed as mg kg^{-1} of fresh weight (FW).

Determination of electrolyte leakage

Electrolyte leakage (EL) measurements were done as described by Larrigaudière et al. (2004) with minor modifications. Fresh pulp tissue (2 g) was cut into 8 mm diameter disks and placed in 40 mL 0.4 M mannitol. The flasks were shaken for 5 hours at 95 rpm and the electrical conductivity of the sample solution (EC_0) after removing the disks was measured with a microdigital conductivity meter (Testo Instruments, Premia de Mar, Spain; model Testo 240). The disks were then frozen in liquid N_2 and grounded to a fine powder and placed again into the sample solution. After stirring, the homogenized tissue was filtered through two layers of miracloth and the total electrical conductivity of the sample solution was determined (ECT). Ion leakage (%) was calculated as (EC_0 /ECT)×100.

Analysis of enzymes involved in the antioxidant metabolism

Total peroxidase (POX, EC 1.11.1.7) extraction was carried out using the protocol of Lurie et al. (1997). Fresh pulp tissue (10 g) was homogenized in 10 mL phosphate buffer (0.1 M pH 6) with 0.5 mM cysteine. The extract was filtered through two layers of Miracloth and centrifuged at 30,000 x g for 15 min at 4 ºC. A 2.5 mL sample of the supernatant was then loaded into a Sephadex G-25 column (PD 10; Pharmacia, Madrid, Spain) that had previously been equilibrated with 10 mL phosphate buffer (pH 6). The enzyme was eluted with 3.5 mL of the same buffer. The enzyme activity was measured by the method described by Lurie et al. (1997) following the colour changes of the extract at 470 nm after adding 10 mM guaiacol and 10 mM H_2O_2.

For the extraction of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6), 20 g of fresh pulp tissue were homogenized in 60 mL 0.1 M potassium phosphate buffer (pH 7.8), 2mM DTT.5% (w/v) polyvinylpolypyrrolidone, 0.1 mM EDTA and 1.25 mM polyethylene glycol (Bailly et al., 1996). The homogenate was filtered through two layers of Miracloth and centrifuged at 20,000 x g for 15 min at 4 ºC and then a 2.5 mL aliquot was loaded into a Sephadex G-25 column (PD 10; Pharmacia, Madrid, Spain) equilibrated with 10 mL 0.1 M phosphate buffer (pH 7.8). The enzymes were eluted with 3.5 mL of the same buffer. The resulting supernatant was used as an enzyme extract to determine enzyme activity. SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) using the method described by Giannopolitis and Ries (1977). One unit of SOD was considered to be the amount of enzyme required to inhibit NBT reduction by 50%. CAT activity was measured by the Clairbone method (1985) following the disappearance of H_2O_2 at 240 nm.

Determination of total ascorbic acid content

Total ascorbic acid was extracted following the Brubaker et al. (1985) protocol mixing 50 g of fresh pulp tissue with 10 g of a solution of 10% (w/v) metaphosphoric acid and 5% 2-3-mercaptopropanol and twice-distilled water to a total weight of 100 g. The homogenate was centrifuged at 20,000 x g for 25 min and the supernatant was made up to 200 mL with distilled water. The resulting solution was then analyzed with an Applied Biosystem HPLC (Foster City, CA, USA) using an LC18 (25 cm × 4.6 mm × 5 µm) column (Supelco Ltd. Bellefonte, PA, USA). Elution was isocratic and absorbance was recorded with a UV/VIS detector at 254 nm. Changes in ascorbic acid content were only measured during cold storage.

2.4 Statistical analysis

The experiment was set up as a completely randomized design and the data analysis was performed using the Statistical Analysis System (SAS version 9.1, SAS Institute, Inc, 1992, Cary, NC, USA). The analysis of variance (ANOVA) and analysis of treatment effects was done using PROC GLM, using data during shelf life and/or cold storage. Mean comparisons were performed using Tukey's LSD test at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of 1-MCP treatment during cold storage

Changes in the antioxidant metabolism of 1-MCP treated or control fruit were investigated during cold storage in 2007. All fruit, treated or not, showed a fast decrease in H_2O_2 levels during the first two weeks of cold storage (Fig. 1A), with values being 3-fold lower if compared to values at harvest. Changes in H_2O_2 levels were not affected by the 1-MCP treatment ($p=0.785$), and these levels remained relatively constant from 15 days onwards and up to 75 days of cold storage. After this period, H_2O_2 content showed a slight, yet not significant increase. This behaviour is in disparity to the results observed in 'Blanquilla' pears (Larrigaudière et al., 2004) where H_2O_2 levels increased between 1 and 2 months of cold storage and decreased slowly thereafter. In 'Blanquilla', H_2O_2 levels were also higher in control than in 1-MCP treated fruit thereby suggesting a beneficial role of this treatment on H_2O_2 accumulation during cold stress (Larrigaudière et al., 2004). Our results do not support this hypothesis or at least suggest that H_2O_2 metabolism was differently regulated by 1-MCP in 'Conference' pears.

To clarify the latter idea, we further analysed the changes of the enzymes involved in H_2O_2 turn-over. Generally, the activity of all the enzymes decreased during the first two weeks of storage and remained relatively stable for the rest of the storage period. For instance, SOD and CAT activities clearly decreased during cold storage in 'Conference' pears whereas they both increased in 'Blanquilla' (Larrigaudière et al., 2004). Significant differences were encountered between 1-MCP treated or control fruit for SOD (Fig. 1B) and CAT (Fig. 1C) activities, but not for POX activity (Fig. 1D) throughout storage. Similar trends were reported earlier for 'Blanquilla' pears (Larrigaudière et al., 2004) and 'Yali' pears (Fu et al., 2007) as well as for apples (Vilaplana et al., 2006), where 1-MCP treatment resulted in fruit with higher SOD and CAT activities if compared to untreated fruit. In 'Granny Smith' apples, 1-MCP treatment caused an important induction of *Mn*SOD expression levels after 8 weeks in cold storage (Sabban-Amin et al., 2011). In the later study, the authors hypothesize on

the possible involvement of *MnSOD* as a singlet oxygen scavenger (Zubini et al., 2007). Besides, Zubini et al. (2007) also showed, contrary to SOD and to our results, a low CAT expression levels in the 1-MCP-treated apples throughout the storage period. Other authors (Yazdani et al., 2011) reported lower POX and CAT activities in 1-MCP treated Asian pears especially after 40 days of cold storage.

In the present study, the observed higher SOD and CAT activities were most probably related to the fruit's ability to maintain these enzyme activities during the early stages of cold storage (i.e. first two weeks) and hence highlights the importance that the ethylene-regulated pathways may have on the fruit physiology at the beginning of cold storage as reported earlier (Chiriboga et al., 2012). In addition, this result is also consistent with the big difference in softening observed between 1-MCP treated and control fruit during the first weeks of cold storage (Chiriboga et al., in press).

A similar pattern to that described earlier was observed for AsA content (Fig. 1E) with a sharp decrease during the first 30-45 days of cold storage after which the AsA content remained stable. Similar results were also found in previous experiments with 'Conference' pears (Eccher-Zerbini et al., 2002; Larrigaudière et al., 2001) and 'Golden Smoothee' apples (Vilaplana et al., 2006), with the decrease of AsA being related to cold-mediated oxidative stress and to the development of physiological disorders (Veltman et al., 1999; Pintó et al., 2001). In the results presented herein, significant differences were found between treated and control fruit ($p<0.001$) with higher AsA levels in 1-MCP treated fruit throughout the storage duration (Fig. 1E). Accordingly, 1-MCP treatments did not reduce the AsA loss but rather delayed this loss in peach (Liu et al., 2005). This said, our results are in disagreement with those found in 'Blanquilla' where AsA levels increased during cold storage and remained lower in fruit treated with 1-MCP (Larrigaudière et al., 2004). The difference between our results in 'Conference' and those reported for 'Blanquilla' pears may be related to varietal differences since the regulation of the enzymes involved in AsA metabolism, or generally antioxidant-related compounds, differ between species but also between varieties (Kalt, 2005). These discrepancies may also explain the different sensitivity to develop internal disorders observed between 'Blanquilla' and 'Conference' pears.

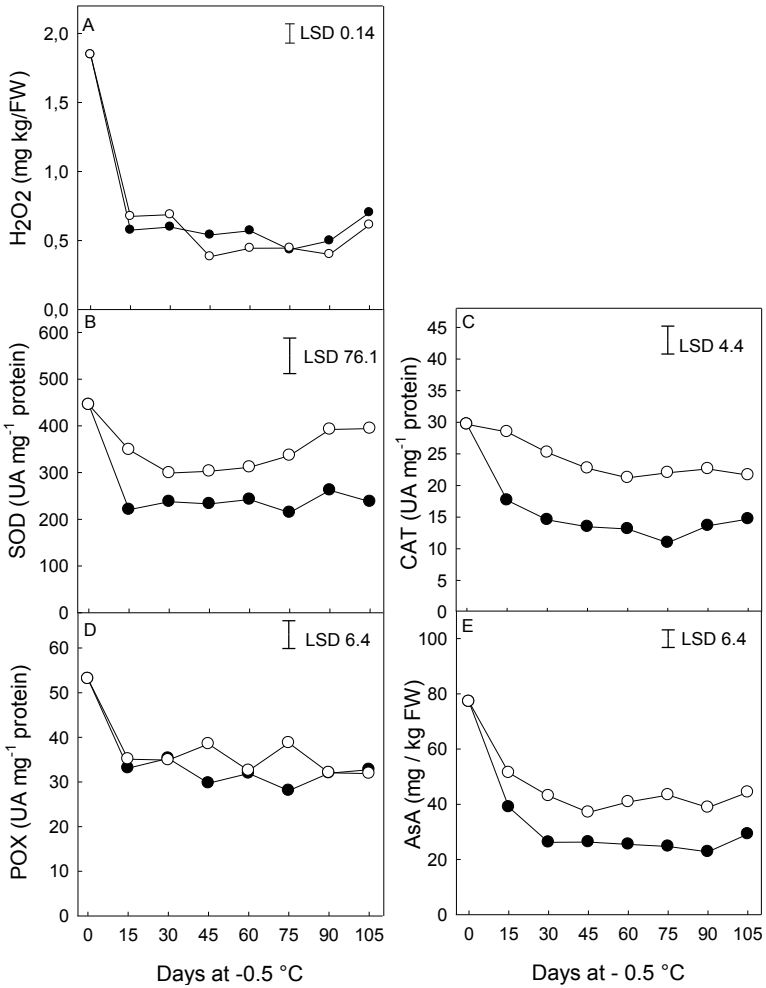

Figure 1: Changes in H_2O_2 levels (A), SOD (B) CAT (C) and POX (D) activity and AsA (E) during cold storage of 'Conference' pears harvested at the optimal harvest date in 2007. Control fruit (●) or fruit treated with 1-MCP (○). The vertical bar represents the LSD value ($p \leq 0.05$).

3.2 Maturity and orchard differences in fruit antioxidant metabolism

Immediately after harvest, fruit from different maturities or harvested from different orchards had similar H_2O_2 values (Table 1). In contrast, electrolyte leakage was lower in fruit from orchard 3 (Table 1). Fruit from earlier harvests (H_{-7} and H_0) had somehow slightly higher EL if compared to fruit harvested later. SOD, CAT and POX activities only differed slightly among fruit from different maturities, being SOD and CAT higher in fruit of advanced maturity and POX lower in fruit from the optimum harvest date (H_0) (Table 1). The marginal differences found herein between harvest dates concur with the small differences in the fruit physiological maturity found between the different harvest dates in earlier studies with the same fruit (Chiriboga et al., 2012). In contrast, other authors (Lentheric et al., 1999) found that SOD and CAT activities in 'Conference' pears declined during on-tree maturation, whereas a similar trend was found for POX activity. In agreement with our results, Masia (1998) found maximum SOD activity in 'Golden Smoothee' apples at commercial harvest if compared to fruit from earlier or later harvests. Discrepancies between our results and those reported earlier (Larrigaudière et al., 2001) may be due to different agroclimatic conditions that may affect the fruit physiology and development prior to harvest.

Table 1: H_2O_2 levels, EL and SOD, CAT and POX activity at harvest in relation to harvest date (H_{-7}, H_0 and H_{+7}) and orchard location (O_1, O_2 and O_3) in 'Conference' pears harvested in 2008. Different letters in rows indicate significant differences between orchards or harvest dates ($p \leq 0.05$).

	HARVEST			**ORCHARD**		
	H_{-7}	**H_0**	**H_{+7}**	**O_1**	**O_2**	**O_3**
H_2O_2 (mg / kg FW)	0.82 a	0.84 a	0.75 a	0.84 a	0.80 a	0.74 a
EL (%)	44.6 a	48.1 a	36.4 b	48.1 a	46.4 a	36.1 b
SOD (Ua mg^{-1} prot)	356.4 ab	322.2 b	396.5 a	322.2 a	230.6 b	248.2 b
CAT (Ua mg^{-1} prot)	21.3 b	27.0 ab	30.3 a	27.0 b	36.6 a	33.6 ab
POX (Ua mg^{-1} prot)	69.8 a	44.7 b	73.7 a	44.7 b	70.5 a	70.9 a

Enzyme activities in fruit from different orchards were also noticeably different (Table 1). Fruit from orchard 1 had higher SOD but lower CAT and POX activities than fruit harvested from other orchards. Differences in the metabolism of 'Conference' pears from different orchards are not unusual (Chiriboga et al., 2012) and may be linked, as suggested earlier, to the different agroclimatic conditions.

3.3 Effect of 1-MCP treatment during shelf life

The aim of this part of our work was to determine the effect that the 1-MCP treatment may have on the antioxidant metabolism and membrane disintegration during shelf life and this in relation to two factors that undoubtedly affect the response to the 1-MCP treatment; the orchard location and harvest maturity. A similar approach focussed on ACC metabolism and firmness loss was recently reported (Chiriboga et al., 2012, in press).

In general, H_2O_2 levels decreased for both treated and control fruit between 3 and 7 days at 20 °C and slightly increased thereafter (Fig. 2A). 1-MCP-treated fruit from the mid harvest behaved differently and H_2O_2 rose up to day 7 of shelf life at 20 ºC.

EL was affected by the harvest date as well as by the treatment and length of shelf life (Fig. 2B). A significant increase in EL values was observed in control but not in 1-MCP treated fruit immediately after removal from cold storage and during 7 days at 20 °C. The higher EL values in control fruit were in accordance with the loss of firmness observed by Chiriboga et al. (2012) and highlight the initiation of fruit senescence in control or untreated fruit. These findings, as well as the relatively stable antioxidant enzymes activities during late stages of storage and/or storage under shelf life conditions is consistent with previous studies in other pear varieties (Larrigaudière et al., 2001; 2004), or different species (Vilaplana et al., 2006). For instance, in 'Blanquilla' pears (Larrigaudière et al., 2004), as well as in other senescent fruit, EL values may be as high as > 80% yet antioxidant enzymes activities may remain fairly unchanged (Larrigaudière et al., 2004). Whether this may be the result of some late-stage catabolic enzymes protecting these antioxidant enzymes or other catabolic processes should be further investigated.

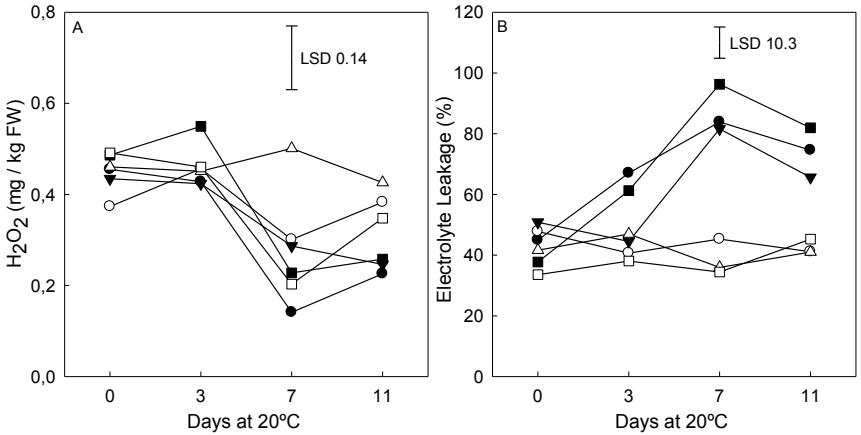

Figure 2: Changes in H_2O_2 levels (A) and electrolyte leakage (B) during shelf life at 20 ºC, after 105 days cold storage, in fruit from different harvest dates in 2008. Commercial harvest date - 7 days (●,○); Commercial harvest date (▲ ,Δ); Commercial harvest date + 7 days (■,□) . Filled symbols = Control fruit; Open symbols = 1-MCP treated fruit. Vertical bars represent LSD values ($p \leq 0.05$).

EL is commonly considered to be a measure of cell membrane deterioration where an increase in EL values is generally associated with alterations in membrane permeability during storage (Larrigaudière et al., 2004) and/or membrane lipid peroxidation by ROS within the fruit tissue (Thompson, 1998). Our results support previous studies in which 1-MCP treatment was associated with lower increase of EL during storage and shelf life (Larrigaudière et al., 2004; Sabban-Amin et al., 2011) and may account, at least in part, for the higher firmness observed in 1-MCP treated fruit during shelf life.

A similar pattern to that described above for fruit from different harvests was also found for EL changes in treated or control fruit from different orchards (Fig. 3). However, H_2O_2 levels were, in this case, higher in 1-MCP treated from all the orchards.

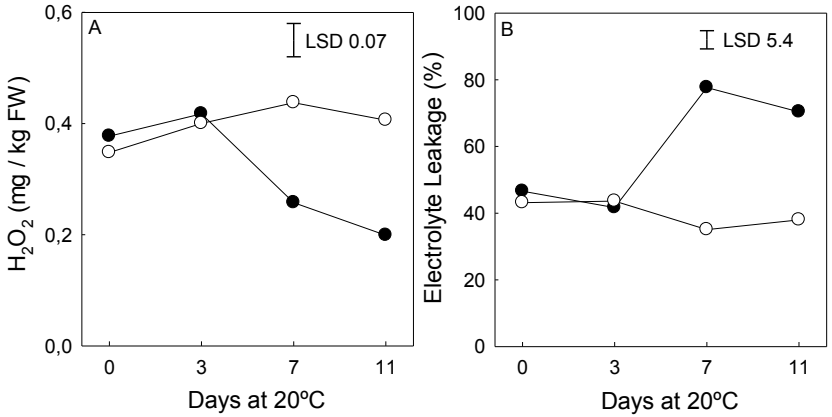

Figure 3: Changes in H_2O_2 levels (A) and electrolyte leakage (B) during shelf life at 20 ºC after 105 days cold storage, in fruit harvested from different orchards in 2008. Since no significant differences were observed, values represent the means of three different orchards (O_1, O_2, O_3). Control fruit (●) or fruit treated with 1-MCP (○). Vertical bars represents LSD values ($p \leq 0.05$).

Changes in the enzymatic antioxidant potential of 'Conference' pears differed between treatments and orchards but not between harvests (Fig. 4 and 5). Accordingly, since no significant differences were observed between harvest dates the results depicted in Fig. 4 represent the mean values over different harvests. SOD activity (Fig. 4A) decreased gradually during shelf life with minor differences between treatments. In contrast, CAT activity (Fig. 4B) remained constant for both treatments but was significantly higher in 1-MCP treated fruit. This seems in contrast with findings in 'Granny Smith' apples where a positive correlation was found between the level of H_2O_2 and the transcription of anti-oxidant enzyme genes of CAT (Zubini et al., 2007). On the other hand, POX activity of control fruit decreased during shelf life while it remained fairly constant and higher in 1-MCP treated fruit (Fig. 4C). Changes in the antioxidant metabolism during shelf life have not been studied extensively but antioxidant enzymes usually decrease with the start of fruit senescence (Mondal et al., 2004; Li et al., 2010).

With this idea in mind, and based on our results, 1-MCP treatment in 'Conference' pears may limit fruit ripening not only by inhibiting ethylene biosynthesis, as extensively reported (Chiriboga et al., 2012) but also through better maintenance of the fruit's enzymatic antioxidant potential (*viz*. CAT and POX; Fig. 4).

This hypothesis was further confirmed when assessing changes of these enzymes in fruit from different orchards (Fig. 5). In this case, SOD and POX activities were better maintained in 1-MCP treated fruit from orchard 1 whereas no significant differences were found for CAT activity of treated or control fruit from this orchard. In fruit from the other orchards, CAT activity was significantly improved in 1-MCP treated fruit.

In summary, the results from this study show that treatment with 1-MCP in 'Conference' pears has an important role in improving the capacity of the fruit to remove ROS by increasing the activity of both enzymatic and non-enzymatic (*viz*. AsA) antioxidants during cold storage and shelf life. Nonetheless, the extent to which 1-MCP may have a positive effect on the antioxidant metabolism of 'Conference' pears clearly depended on the fruit maturity at harvest or the orchard location. Lower activities of these enzymes are generally associated with ripening and senescence and hence 1-MCP treatment may limit ripening and extend storage life of 'Conference' pears not only by inhibiting ethylene biosynthesis but also through better maintenance of the fruit's antioxidant system.

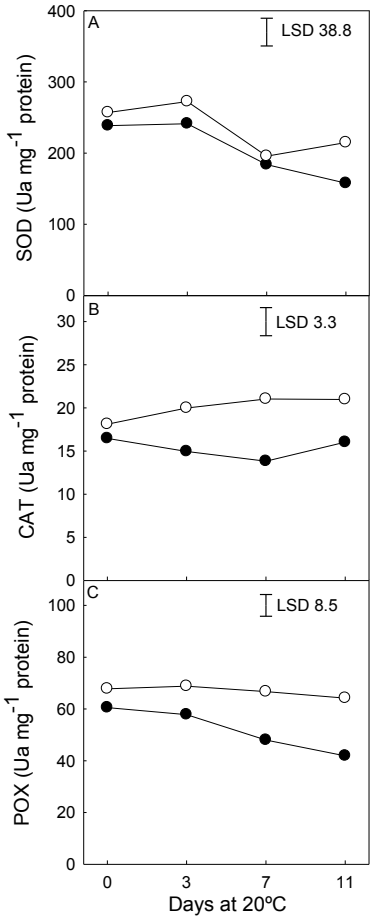

Figure 4: SOD (A), CAT (B) and POX (C) activity during shelf life at 20 ºC after 105 days cold storage, in fruit from different harvest dates during the 2008 season. Since no significant differences were observed between harvests, values represent the means of three harvest dates (H_{-7}, H_0, H_{+7}). Control fruit (●) or fruit treated with 1-MCP (○). Vertical bars represent LSD values ($p \leq 0.05$).

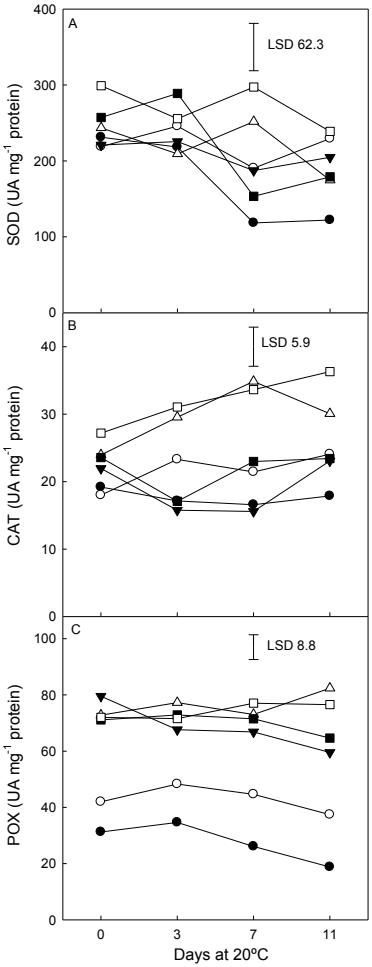

Figure 5: SOD (A), CAT (B) and POX (C) activity during shelf life at 20ºC after 105 days cold storage, in fruit harvested from different orchards during 2008. Orchard 1 (●,○); Orchard 2 (▲ ,Δ); Orchard 3 (■,□). Filled symbols = Control fruit; Open symbols = 1-MCP treated fruit. Vertical bars represent LSD values (p≤0.05).

Acknowledgements

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References

Bailly, C.; Benamar, A.; Corbineau, F.; Come, D. 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiologia Plantarum,* 97, 104-110.

Blankenship, S. M. 2003. Discovery and commercialization of 1-methylcyclopropene as an ethylene inhibitor. *Acta Horticulturae,* 628, 189-191.

Brubaker, G.; Müller-Mullot, W.; Southgate, D. 1985. Methods for determination of vitamins in food.Recommended by COST 91. Elsevier Applied Science, London.

Chiriboga, M. A.; Recasens, I.; Schotsmans, W. C.; Dupille, E.; Larrigaudiere, C. 2012. Cold-induced changes in ACC metabolism determine softening recovery in 1-MCP treated Conference pears. *Postharvest Biology and Technology,* 68, 78-85.

Chiriboga, M. A.; Schotsmans, W. C.; Larrigaudiere, C.; Dupille, E.; Recasens, I. 2011. How to prevent ripening blockage in 1-MCP-treated 'Conference' pears. *Journal of the Science of Food and Agriculture,* 91, 1781-1788.

Chiriboga, M. A.; Schotsmans, W. C.; Larrigaudiere, C.; Dupille, E.; Recasens, I. Responsiveness of 'Conference' pears to 1-Methylcyclopropene (1-MCP): The role of harvest date, orchard location and year *Journal of the Science of Food and Agriculture,* in press.

Clairbone, A. (1985). Catalase activity. In: R. Greenwald (ed). Handbook of Methods for Oxygen Radical Research. Pp. 283-284. CRC Press, Boca Raton, FL.

Eccher-Zerbini, P.; Rizzolo, A.; Brambilla, A.; Cambiaghi, P.; Grassi, M. 2002. Loss of ascorbic acid during storage of 'Conference' pears in relation to the appearance of brown heart. *Journal of the Science of Food and Agriculture,* 82, 1007-1013.

Foyer, C.; Lelandais, M.; Edwards, E.; Mullineaux, P. 1991. The role of ascorbate in plants, interactions with photosynthesis and regulatory significance. In: Active Oxygen/oxidative Stress and Plant Metabolism, Ed by Pell E and Steffen K. American Society of Plant Physiology, Rockville, MD. Pp. 131-144.

Franck, C.; Baetens, M.; Lammertyn, J.; Verboven, P.; Davey, M. W.; Nicolaï, B. M. 2003. Ascorbic acid concentration in cv. Conference pears during fruit development and postharvest storage. *Journal of Agricultural and Food Chemistry,* 51, 4757-4763.

Fu, L.; Cao, J.; Li, Q.; Lin, L.; Jiang, W. 2007. Effect of 1-methylcyclopropene on fruit quality and physiological disorders in 'Yali' pear (*Pyrus bretschneideri* Rehd.) during storage. *Food Science and Technology International,* 13, 49-54.

Giannopolitis, C. N.; Ries, S. K. 1977. Superoxide Dismutases I: Occurrence in higher plants. *Plant Physiology,* 59, 309-314.

Imahori, Y.; Takemura, M.; Bai, J. 2008. Chilling-induced oxidative stress and antioxidant responses in mume (Prunus mume) fruit during low temperature storage. *Postharvest Biology and Technology,* 49, 54-60.

Larrigaudière, C.; Lentheric, I.; Pintó, E.; Vendrell, M. 2001. Short-term effects of air and controlled atmosphere storage on antioxidant metabolism in 'Conference' pears. *Journal of Plant Physiology,* 158, 1015-1022.

Larrigaudière, C.; Vilaplana, R.; Soria, Y.; Recasens, I. 2004. Oxidative behaviour of 'Blanquilla' pears treated with 1-methylcyclopropene during cold storage. *Journal of the Science of Food and Agriculture,* 84, 1871-1877.

Lentheric, I.; Pintó, E.; Graell, J.; Larrigaudière, C. 2003. Effects of CO_2 pretreatment on oxidative metabolism and core-browning incidence in controlled atmosphere stored pears. *Journal of Horticultural Science and Biotechnology,* 78, 177-181.

Lentheric, I.; Pintó, E.; Vendrell, M.; Larrigaudiere, C. 1999. Harvest date affects the antioxidative systems in pear fruits. *Journal of Horticultural Science & Biotechnology,* 74, 791-795.

Li, Z. Q.; Qiao, Y. S.; Tong, Z. G.; Zhou, J.; Zhang, Z. 2010. Effect of ethylene and 1-MCP on post-harvest physiology and on expression of the ethylene receptor genes PpETR3 and PpERS2 in pear (Pyrus pyrifolia Nakai 'Kikusui') fruit. *Journal of Horticultural Science & Biotechnology,* 85, 71-77.

Liu, H.; Jiang, W.; Zhou, L.; Wang, B.; Luo, Y. 2005. The effects of 1-methylcyclopropene on peach fruit (*Prunus persica* L. cv. Jiubao) ripening and disease resistance. *International Journal of Food Science and Technology,* 40, 1-7.

Lurie, S.; Fallik, E.; Handros, A.; Shapira, R. 1997. The possible involvement of peroxidase in resistance to *Botrytis cinerea* in heat-treated tomato fruit. *Physiological and Molecular Plant Pathology,* 50, 141-149.

Masia, A. 1998. Superoxide dismutase and catalase activities in apple fruit during ripening and post-harvest and with special reference to ethylene. *Physiologia Plantarum,* 104, 668-672.

Miller, A. R. 1986. Oxidation of cell-wall polysaccharides by hydrogen peroxide: a potential mechanism for cell-wall breakdown in plants. *Biochemical and Biophysical Research Communications,* 141, 238-244.

Mondal, K.; Sharma, N. S.; Malhotra, S. P.; Dhawan, K.; Singh, R. 2004. Antioxidant systems in ripening tomato fruits. *Biologia Plantarum,* 48, 49-53.

Paul, V.; Pandey, R.; Srivastava, G. C. 2012. The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene-An overview. *Journal of Food Science and Technology-Mysore,* 49, 1-21.

Pintó, E.; Lentheric, I.; Vendrell, M.; Larrigaudière, C. 2001. Role of fermentative and antioxidant metabolisms in the induction of core browning in controlled-atmosphere stored pears. *Journal of the Science of Food and Agriculture,* 81, 364-370.

Rapisarda, P.; Bianco, M. L.; Pannuzzo, P.; Timpanaro, N. 2008. Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes [*Citrus sinensis* (L.) Osbeck]. *Postharvest Biology and Technology,* 49, 348-354.

Sabban-Amin, R.; Feygenberg, O.; Belausov, E.; Pesis, E. 2011. Low oxygen and 1-MCP pretreatments delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples. *Postharvest Biology and Technology,* 62, 295-304.

Sala, J. M.; Lafuente, M. T. 2004. Antioxidant enzymes activities and rindstaining in 'Navelina' oranges as affected by storage relative humidity and ethylene conditioning. *Postharvest Biology and Technology,* 31, 277-285.

Sisler, E. C.; Serek, M. 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiologia Plantarum,* 100, 577-582.

Thompson, A. K. 1998. Controlled atmosphere storage of fruits and vegetables. Pp. 288.Wallingford, UK, CAB International.

Veltman, R. H.; Sanders, M. G.; Persijn, S. T.; Peppelenbos, H. W.; Oosterhaven, J. 1999. Decreased ascorbic acid levels and brown core development in pears (*Pyrus communis* L-cv. Conference). *Physiologia Plantarum,* 107, 39-45.

Vilaplana, R.; Valentines, M. C.; Toivonen, P.; Larrigaudiere, C.2006. Antioxidant potential and peroxidative state of 'Golden Smoothee' apples treated with 1-methylcyclopropene. *Journal of the American Society for Horticultural Science,*131, 104-109.

Wang, S. Y.; Jiao, H. J. 2001. Changes in oxygen-scavenging systems and membrane lipid peroxidation during maturation and ripening in blackberry. *Journal of Agricultural and Food Chemistry,* 49, 1612-1619.

Watkins, C. B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances,* 24, 389-409.

Yazdani, N.; Arzani, K.; Mostofi, Y.; Shekarchi, M. 2011. Alpha-Farnesene and antioxidative enzyme systems in Asian pear (Pyrus serotina Rehd.) fruit. *Postharvest Biology and Technology,* 59, 227-231.

Zubini, P.; Baraldi, E.; De Santis, A.; Bertolini, P.; Mari, M. 2007. Expression of anti-oxidant enzyme genes in scald-resistant 'Belfort' and scald-susceptible 'Granny Smith' apples during cold storage. *Journal of Horticultural Science & Biotechnology* 82, 149-155.

CAPÍTULO 4
HOW TO PREVENT RIPENING BLOCKAGE IN 1-MCP TREATED 'CONFERENCE' PEARS

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ABSTRACT

BACKGROUND: Some European pear varieties treated with 1-MCP often remain 'evergreen', meaning that their ripening process is blocked and does not resume after removal from cold storage. In this work this was confirmed to also be the case in 'Conference' pears. To reverse the blockage of ripening 1-MCP treatments combined with external exogenous ethylene were tested.

RESULTS: The 1-MCP treatment in 'Conference' pears is very effective in delaying ripening and more specifically softening. The same 1-MCP concentration in different experimental years caused a different response. The higher dose of 1-MCP (600 nL L^{-1}) always resulted in irreversible blockage of ripening, whereas the behavior of fruit receiving a lower dose (300 nL L^{-1}) depended on the year, and this did not depend on maturity at harvest or storage conditions. Simultaneous exposure to 1-MCP and exogenous ethylene significantly affected fruit ripening allowing significant softening to occur but at a lower rate compared with control fruit.

CONCLUSION: The application of exogenous ethylene and 1-MCP simultaneously after harvest permitted restoration of the ripening process after storage in 'Conference' pears, extending the possibility of marketing and consumption.

1. INTRODUCTION

In the majority of European pears, the optimal quality for consumption is characterized by a buttery texture, an appropriate color and a characteristic taste associated with the content of sugars, acids and with the aroma production (Argenta et al., 2003; Kappel et al., 1995; Ma et al., 2000). As a climacteric fruit, the ripening process of pears is regulated by ethylene and an inhibition of the biosynthesis of this hormone or its action slows down ripening and increases shelf life (Argenta et al., 2003).

The organic compound 1-methylcyclopropene (1-MCP) was designed to delay ripening of climacteric fruits by competing for the binding site of ethylene with its receptors and in doing so inhibiting the activation of the ethylene signal transducing pathway (Blankenship and Dole, 2003). Postharvest application of 1-MCP delays or decreases softening in 'Bartlett' (Baritelle et al., 2001; Ekman et al., 2004; Trinchero et al., 2004), 'Williams' (Calvo, 2003; Lafer, 2005), 'La France' (Hiwasa et al., 2003; Kubo et al., 2003), 'd'Anjou' (Argenta et al., 2003; Baritelle et al., 2001) and 'Passe-Crassane' (Lelièvre et al., 1997). Additionally 1-MCP delays or decreases internal browning, color development, storage scald, respiration rate, ethylene production, and 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase activity in pear fruit (Baritelle et al., 2001; Argenta et al., 2003; Ekman et al., 2004; Trinchero et al., 2004).

There are only a few studies in 'Conference' pears. 1-MCP treatments at harvest between 10 and 100 nL L^{-1} were effective in retarding ripening in this cultivar (Eccher Zerbini et al., 2003). However, the effect of ripening was not totally uniform, with a small percentage of treated fruit reaching their climacteric peak, losing green color and softening prematurely (Cambiaghi et al., 2004). Repeating the 1-MCP treatment at low doses (25 and 50 nL L^{-1}) during storage did not resolve this problem (Rizzolo et al., 2005). With higher doses (300 nL L^{-1}), ripening can be delayed more effectively (Chiriboga et al., 2010), but sometimes the fruit lose their ability to ripen and remain firm and green even after shelf life (referred to in this article as evergreen behavior). Similar problems have occurred in other European pear varieties like 'd'Anjou' (Argenta et al., 2003), 'Bartlett' (Trinchero et al., 2004; Ekman et al., 2004; Chen and Spotts, 2005) and 'Blanquilla' (Chiriboga et al., 2010).

To avoid or reverse the evergreen behavior due to the 1-MCP treatment, several strategies have been investigated. One has been the application of a post-storage heat treatment in 'Blanquilla' and 'Conference' (Chiriboga et al., 2010). In 'Blanquilla', the heat treatments restored the ripening process in 1-MCP treated pears with the efficacy increasing with the days at 15 ºC. On the contrary, in 'Conference', the evergreen behavior was not reversed with the thermal treatments tested and the 1-MCP treated fruit remained green, excessively firm and were not commercially acceptable.

Exogenous ethylene has been used commercially to induce and accelerate the ripening of a large number of crops (Reid, 1992). In case of winter pears, like 'd'Anjou' (Wang et al., 1972; Facteau and Mielke, 1998) and 'Bartlett' (Puig et al., 1996; Mitcham et al., 2000; 2006; Chen et al., 1997), exogenous ethylene has proven successful to induce their ripening, with the dose needed depending on the treatment temperature (Mitcham et al., 2000; 2006) and on the duration of storage at chilling temperatures (-1 °C) (Puig et al., 1996).

With this in mind, several authors applied exogenous ethylene on 1-MCP treated pears leading to distinct results. Chen (2002) found no reversal of the effects of 1-MCP in 'Bartlett' pears after exogenous ethylene application; neither did Calvo (2004) who also studied 'd'Anjou' and 'Packham's Triumph' pears with the same conclusion. On the contrary, Manriquez (2002) did find that saturating levels of ethylene reversed the effects of 1-MCP for 'Packham's Triumph' pears, depending on the concentration and duration of storage.

The same kind of work is reported here in 'Conference' pears to demonstrate that exogenous ethylene can be used to reverse the blockage of ripening.

2. MATERIALS AND METHODS

2.1 Fruit material

'Conference' pears (*Pyrus communis*) were harvested at the optimal commercial harvest date for long-term storage from 2 different orchards. Fruits were selected based on size and absence of defects, and stored at -0.5 °C and 92% RH during 3 months in regular atmosphere (RA) or during 6 months in controlled atmosphere (CA, 2% O_2 + 1% CO_2) conditions according to the standard commercial practice.

2.2 Experimental set up

Experiment 1: Effects of 1-MCP treatment on fruit quality

This experiment was conducted during three consecutive years (2007-08, 2008-09, 2009-10) using different doses of 1-MCP. The first two years, the pears were treated with 0, 300 or 600 nL L^{-1} of 1-MCP and the third year only the treatments with 0 and 300 nL L^{-1} of 1-MCP were repeated. Immediately after harvest, fruits were stored at -0.5 ºC. After 1

day of storage, fruits were treated with 1-MCP using Smartfresh™ (Agrofresh Inc.) according to the manufacturer recommendations.

The fruit was covered with a 1 m^3 plastic bag and the treatment was done with a little ventilator and a small plastic flask containing the reaction mixture was placed inside. The reaction mixture contains 1-MCP as a powder (0.5 g for the 300 nL L^{-1} and 1 g for the 600 nL L^{-1}) and water at 30 ºC (ratio of 1-MCP: water = 1:5). After 24 hours, the plastic bag was opened and the entire cool room well aired.

Experiment 2: Effect of exogenous ethylene on the quality of 1-MCP treated pears.

This experiment was conducted during two consecutive years (2007-08, 2008-09). The fruit was treated using combinations of 1-MCP and exogenous ethylene. In the first year, four treatments were used. The first two treatments correspond to 1-MCP application with 300 and 600 nL L^{-1} 1-MCP. The other two are the combined treatments at the following doses: 300 nL L^{-1} 1-MCP + 300 nL L^{-1} C_2H_4 and 600 nL L^{-1} 1-MCP + 600 nL L^{-1} C_2H_4. The second year, the fruits were treated again with 300 and 600 nL L^{-1} 1-MCP and for the combined treatment with 600 nL L^{-1} 1-MCP + 300 nL L^{-1} C_2H_4 and 600 nL L^{-1} 1-MCP+ 600 nL L^{-1} C_2H_4.

In the combined treatment, 1-MCP as Smartfresh™ powder (0.5 g for the 300 nL L^{-1} and 1 g for the 600 nL L^{-1}) was put in a small plastic flask compatible with the ventilating device and sealed. Water was added through the seal using a syringe and the flask was placed inside the 1 m^3 plastic bag, containing the boxes with fruit, which was then sealed. Gaseous C_2H_4 was withdrawn with a syringe (0.3 ml for the 300 nL L^{-1} and 0.6 ml for the 600 nL L^{-1}) from a gas bottle with pure ethylene (98%) and injected into the plastic bag at the same moment as the flask containing 1-MCP was opened.

2.3 Measurements

Firmness was determined on opposite sides of the fruit after removing sections of skin, using a manual penetrometer (Effegi, Milan, Italy), fitted with an 8 mm Magness Taylor probe. Hue angle (H°) was measured using a chromameter (model CR-200, Minolta, Osaka, Japan) and reported using the L* a* b* color space. The hue angle was calculated with the formula arctg b*/a*.

Ethylene production was measured one day after harvest in an acclimatized chamber at 20 ºC. The day of harvest, three replicates of 2 pears were placed in 1.5 L flasks continuously ventilated with humidified air at a flow rate of 1.5 L h^{-1}. One day later, gas samples (1 ml) were taken from the headspace and injected into a gas chromatograph fitted with a FID

detector (Agilent Technologies 6890, Wilmington, Germany) and an alumina column 80/100 (2 m x 3 mm) (Teknokroma, Barcelona, Spain).

2.4 Statistical analysis

Data analysis was performed using the Statistical Analysis System (SAS version 9.1, SAS Institute, Inc, 1992, Cary, NC, USA). The analysis of variance (ANOVA) and analysis of treatment effects was done using PROC GLM, and non-significant treatment effects were averaged. Mean comparisons were performed using Tukey's LSD test at $P<0.05$.

3. RESULTS

The average of the two orchards is presented in these results since there were no significant treatment effects for orchard (data not shown).

3.1 Ripening indexes at harvest

All the fruits were harvested at the optimal harvest date for long-term storage as proven by their overall low ethylene production (Table 1) confirming their pre-climacteric state.

Table 3**:** Maturity parameters at harvest of 'Conference' pears harvested in 3 experimental years. Firmness and background color were assessed immediately after harvest and ethylene production after 1 day at 20 ºC. Data represent the mean (+/- s.d. 95%).

Year	Firmness (N)	Background color (Hue angle, º)	Ethylene production ($\mu L\ kg^{-1}\ h^{-1}$)
2007	64.0 ± 5.9	103.0 ± 7.3	0.28 ± 0.06
2008	72.2 ± 4.3	101.8 ± 2.1	0.11 ± 0.07
2009	51.7 ± 6.6	115.7 ± 2.3	0.29 ± 0.05

However, ethylene production, firmness and hue angle were different between experimental years (Table 1). The fruit from the second year had the highest firmness, lowest hue angle and lowest ethylene production and can be considered as slightly less mature than fruit from the first year, whereas the fruit from the third year was most mature with the lowest firmness and highest hue angle.

3.2 Effect of 1-MCP application on fruit quality during storage and shelf life

In 2007 and after 3 months of RA (Figure 1A) or 6 months of CA (Figure 1B) storage at - 0.5 ºC, the fruit had softened somewhat (4 – 7 N) but there were no significant differences in firmness between the control and 1-MCP treated fruit. A similar behavior was found in all experimental years with slightly more softening (13 – 16 N) in the second experimental year (2008) in both RA (Figure 1C) and CA (Figure 1D) storage.

There were important differences in the firmness of the fruit after shelf life when comparing the different treatments. In the first year, the control fruit (1-MCP 0) softened fast and reached values lower than 9.3 N and 10.2 N within 7 days at 20 ºC after RA (Figure 1A) and CA (Figure 1B) storage, respectively. In contrast, no softening was observed in the fruit treated with 600 nL L^{-1} 1-MCP within 7 days, and even though there was some softening between 7 and 12 days, these fruit remained firm even after 12 days at 20 ºC with values over 50 N in both storage conditions. In 2007, the pears treated with 300 nL L^{-1} 1-MCP did soften but more gradually compared with the control fruit and they reached eating quality (15 – 30 N) within 7 days at 20 ºC after RA storage and within 12 days at 20 ºC after CA storage with values of 28.8 N and 24.2 N, respectively.

In the second year, as in the previous year, control fruit softened fast and had reached the eating quality after 7 days at 20 ºC following RA (Figure 1C) and CA (Figure 1D) storage. On the other hand, fruit treated with 600 nL L^{-1}1-MCP did not soften, and neither did the fruit treated with 300 nL L^{-1}1-MCP. This is in contrast with the previous year when the fruit treated with 300 nL L^{-1} 1-MCP did soften during shelf life.

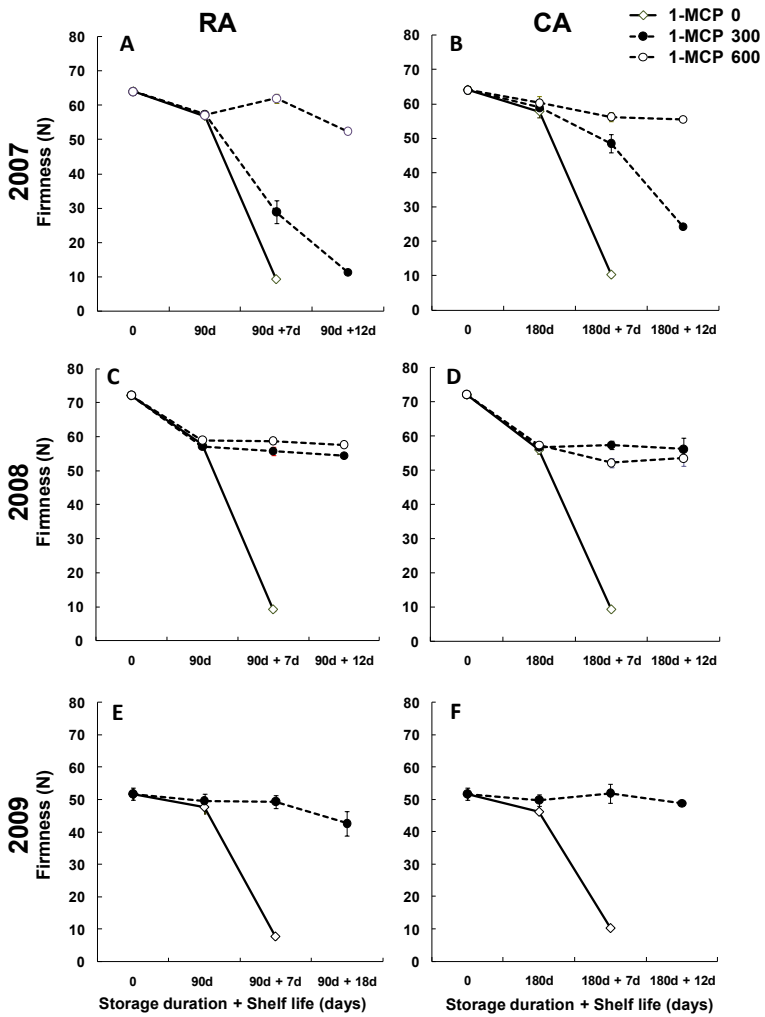

Figure 1. Softening during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life at 20 ºC in 3 experimental years (2007, 2008, 2009). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1} 1-MCP (1-MCP 600). The vertical bars represent the 95% confidence interval surrounding the mean.

In the third year, as in both previous years, control fruit exhibited a significant loss in firmness to 7.6 N for RA (Figure 1E) and 10.3 N for CA (Figure 1F) stored fruit after 7 days at 20 ºC. The treatment of 600 nL L^{-1} 1-MCP was not repeated since in the previous two years its blocking effect was proven. As in the second experimental year, 300 nL L^{-1} 1-MCP treated fruit remained significantly firmer than control fruit. However, when shelf life was prolonged past the 12 days used in previous years, the treated fruit stored in RA do appear to start softening.

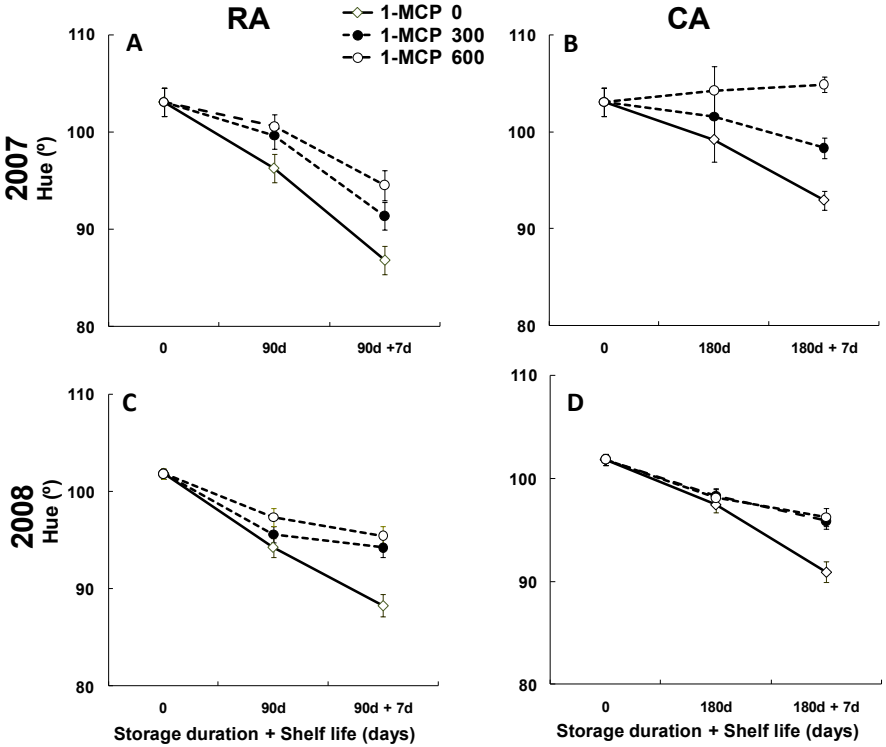

Figure 2. Skin color (hue) during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life at 20 ºC in 2 experimental years. Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1} 1-MCP (1-MCP 600). The vertical bars represent the 95% confidence interval surrounding the mean.

Hue angle also slightly decreased during storage in RA and CA (Figure 2) and there were small differences between control and 1-MCP treated fruit and between 1-MCP doses, with the higher doses resulting in greener fruit. In RA storage, the loss of green color was similar in both years whereas CA stored fruit behaved slightly different in both years. In 2007, 1-MCP treated fruit did not lose green color whereas in 2008 the hue angle decreased for all three treatments.

Comparing the evolution of color in the first and second year (Figure 2), the trend seen during storage continues at a slightly higher rate during shelf life. In general, control fruit (not treated with 1-MCP) exhibited significant yellowing during shelf life. At the same time, fruit treated with 1-MCP remained significantly greener, with the higher dose of 1-MCP resulting in less decrease of hue angle compared with the lower dose. Similar results were found for both storage conditions, with the exception being the CA fruit in the first year where no yellowing was seen in fruit treated with 600 nL L^{-1} 1-MCP and some yellowing occurred in fruit treated with 300 nL L^{-1} 1-MCP.

3.3 Effect of combined application of 1-MCP + ethylene on fruit quality during storage and shelf life

In the first year (Figure 3), no differences were found in softening between the treatments during storage. Although directly after removal from storage (0 days at 20 ºC) all the treatments started from similar fruit firmness around 57 N, significant differences in firmness were observed after 7 days at 20 ºC.

As mentioned before, the control fruit (1-MCP 0) softened fast and reached their eating quality within 7 days at 20 ºC. The fruit treated with 600 nL L^{-1} of 1-MCP showed no significant loss of firmness whereas the fruit treated with 300 nL L^{-1} 1-MCP and the combined treatments exhibited significant firmness loss even though firmness remained higher compared with the control fruit. Fruit treated with 300 nL L^{-1} of 1-MCP with or without 300 nL L^{-1} of C_2H_4 and stored in RA reached eating quality after 7 days of shelf life with values of 20.7 N and 28.8 N, respectively. Nevertheless, five days later, fruit had softened beyond eating quality. On the contrary, the fruit treated with 600 nL L^{-1} of 1-MCP + 600 nL L^{-1} C_2H_4, had not reached eating quality after 7 days of shelf life, it took five more days to reach this eating quality.

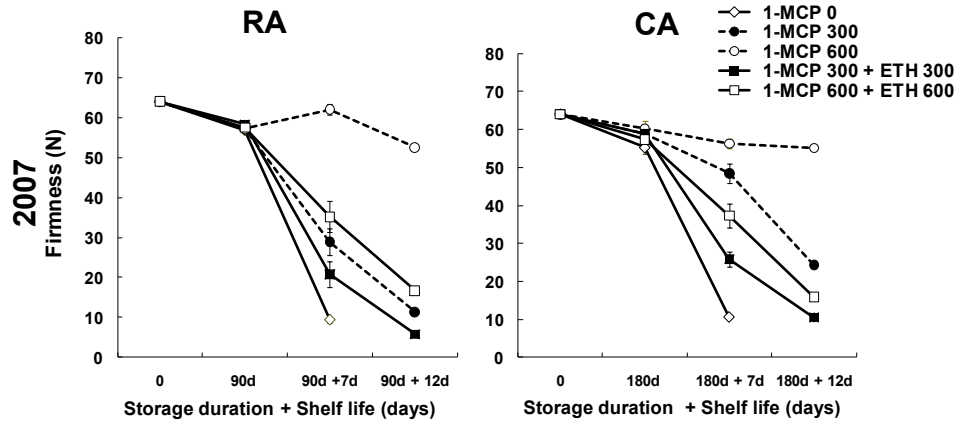

Figure 3. Softening during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life at 20 ºC in the first year (2007). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1} 1-MCP (1-MCP 600), fruit treated with 300 nL L^{-1} 1-MCP and 300 nL L^{-1} C_2H_4 (1-MCP 300 + ETH 300), fruit treated with 600 nL L^{-1} 1-MCP and 600 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 600). The vertical bars represent the 95% confidence interval surrounding the mean.

In the case of CA, softening of treated fruit was slower compared with RA and only fruit treated with 600 nL L^{-1} of 1-MCP with and without 600 nL L^{-1} C_2H_4 behaved exactly the same in both storage conditions. The fruit treated with 300 nL L^{-1} of 1-MCP and 300 nL L^{-1} of C_2H_4 still reached eating quality after 7 days of shelf life comparable to RA stored fruit but fruit treated with 300 nL L^{-1} of 1-MCP without ethylene did not and only reached eating quality after 12 days of shelf life and remained significantly firmer compared with the fruit treated with 600 nL L^{-1} of 1-MCP + 600 nL L^{-1} C_2H_4.

In the second year (Figure 4), the treatment with 300 nL L^{-1} of 1-MCP and 300 nL L^{-1} of C_2H_4 was replaced by a treatment with 600 nL L^{-1} of 1-MCP and 300 nL L^{-1} of C_2H_4. As in the first year, no differences were found between treatments during storage. The main changes in firmness were observed during shelf life, when also the differences between the fruit treated with 1-MCP alone and the fruit treated with 1-MCP and ethylene became more apparent. In this year the fruit stored in RA and in CA had the same behavior.

The softening of the pears was markedly inhibited in the fruit treated with only 1-MCP with commercially negligible loss of firmness for both doses and in both storage conditions. The fruit treated with 600 nL L^{-1} of 1-MCP + 600 nL L^{-1} C_2H_4 behaved like the control fruit in that both reached their eating quality within 7 days of shelf life. The fruit treated with 600 nL L^{-1} of 1-MCP and 300 nL L^{-1} C_2H_4 also softened but slower compared with the control fruit and was still within the

eating quality window after 7 days at 20 ºC and for CA fruit even after 12 days, whereas the fruit treated with 600 nL L^{-1} of 1-MCP and 600 nL L^{-1} C_2H_4 was already below the 15 N limit at that point.

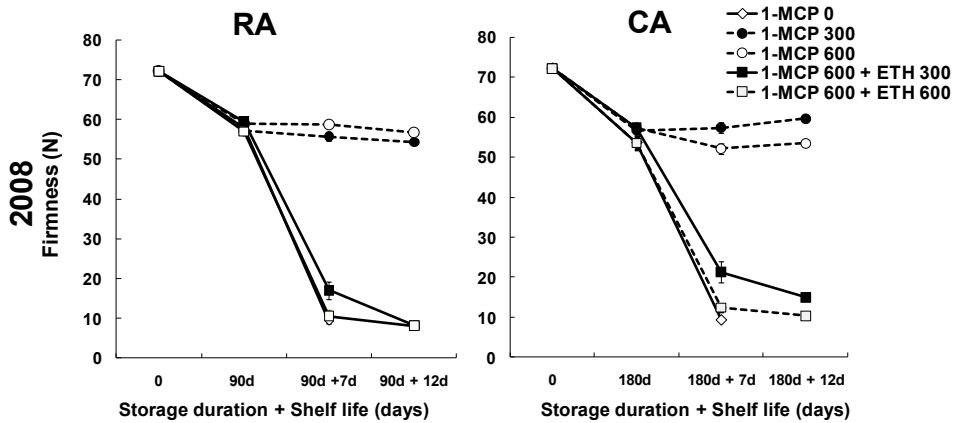

Figure 4. Softening during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life at 20 ºC in the second year (2008). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1} 1-MCP (1-MCP 600), fruit treated with 600 nL L^{-1} 1-MCP and 300 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 300), fruit treated with 600 nL L^{-1}1-MCP and 600 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 600). The vertical bars represent the 95% confidence interval surrounding the mean.

Differences in color could be noted between the two years. In the first year (Figure 5), control fruit stored in RA exhibited a higher decrease of hue angle compared with the other treatments. However, no distinction could be made between the different 1-MCP and C_2H_4 doses used. After CA storage, the control fruit had also lost significantly more of their green color. Additionally, significant differences were found between the treatment with only 600 nL L^{-1} of 1-MCP and the other treatments, where the former remained greener.

In the second year (Figure 6), all fruit lost hue at the same rate during storage and during shelf life. Two groups can be observed, those treated with only 1-MCP remaining significantly greener, and those not treated or treated with ethylene which became significantly more yellow.

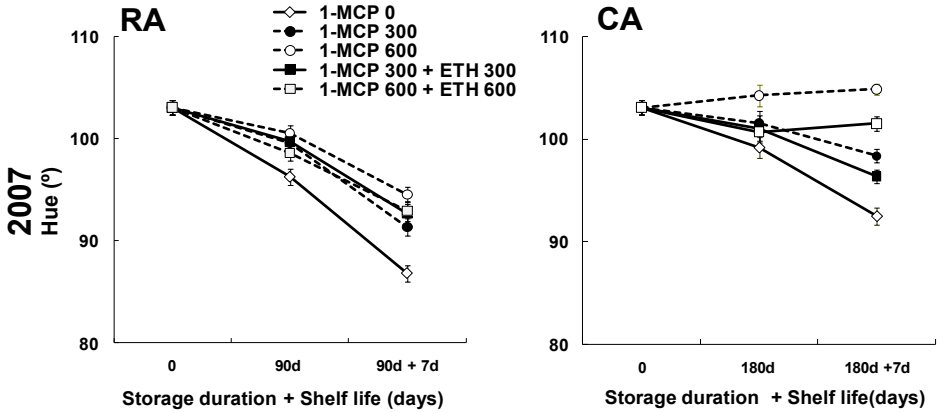

Figure 5. Skin color (hue) during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life in the first year (2007). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1} 1-MCP (1-MCP 600), fruit treated with 300 nL L^{-1} 1-MCP and 300 nL L^{-1} C_2H_4 (1-MCP 300 + ETH 300), fruit treated with 600 nL L^{-1} 1-MCP and 600 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 600). The vertical bars represent the 95% confidence interval surrounding the mean.

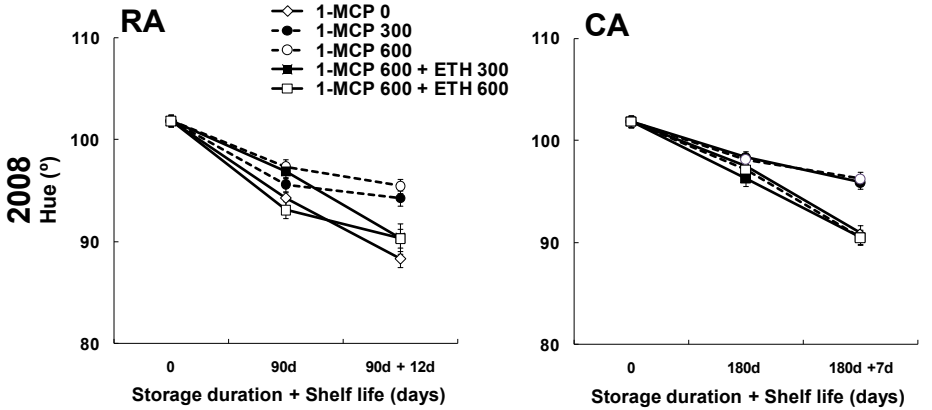

Figure 6. Skin color (hue) during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life at 20 ºC in the second year (2008). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 1-MCP nL L^{-1} (1-MCP 600), fruit treated with 600 nL L^{-1} 1-MCP and 300 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 300), fruit treated with 600 nL L^{-1} 1-MCP and 600 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 600). The vertical bars represent the 95% confidence interval surrounding the mean.

4. DISCUSSION

The results of this study show that 1-MCP treatment in 'Conference' pears is very effective in delaying ripening and more specifically softening. However, the same 1-MCP concentration in different experimental years caused a different response. The high concentration (600 nL L^{-1} of 1-MCP) gave the same result with a complete blockage of softening in both years, whereas the lower concentration (300 nL L^{-1} of 1-MCP) resulted in slower softening (as desired) the first year and no softening in the second and third. This blockage is not paralleled by a similar blockage in the background color changes which were slowed down but not completely stopped by 1-MCP, indicating a differential effect of 1-MCP on different ripening indicators (firmness, color). It is well known that the maturity at treatment strongly influences the effect of the 1-MCP treatment (Blankenship and Dole, 2003), but in this case, a difference in maturity could not explain this discrepancy. At harvest, the maturity parameters (firmness, color and ethylene production) were different between the years, the larger difference was found between the second and the third year with the first year in between. This suggests that maturity based on fruit firmness or on ethylene production are not appropriate indicators for reliable prediction of the response of pears to 1-MCP.

Therefore, the evergreen behavior does not seem to be linked exclusively with maturity at harvest and is likely due to another trigger process in this cultivar. The evergreen behavior suggests that 1-MCP has a long residual effect on pear and/or there is a period of time before ethylene levels are high enough to allow ripening recovery. Considering that 1-MCP binds irreversibly to the ethylene receptor (Sisler et al., 1996), the new synthesis of ethylene cannot be the key to reverse the evergreen behavior, not until new receptors are generated. Plant tissues have been shown to vary widely in their ability to regenerate new receptors (Blankenship and Dole, 2003).The difference in ripening behavior in 1-MCP treated 'Conference' pears might also be related to the abundance of ethylene receptors at the moment of the treatment and/or to the turnover of ethylene receptors during cold storage. Another possibility could be that the recovery of ripening capacity is produced by other proteins involved in the ethylene perception pathway (other than the ethylene receptor proteins), as well as related to changes in ethylene receptors turnover during storage (Blankenship, 2003).

Simultaneous exposure to 1-MCP and exogenous ethylene significantly affected fruit ripening. The application of exogenous ethylene allowed significant softening to occur but at a lower rate compared with control fruit. This contrasts with findings in 'Bartlett' pear treated with exogenous ethylene where the ethylene had no effect on softening (Trinchero et al., 2004). In 'd'Anjou' pears treated with 1-MCP, exogenous ethylene had some effect but only caused a minor increase in softening (maximum difference 8.9 N) (Argenta et al., 2003). In the results presented here, the differences between single 1-MCP and combined 1-MCP + C_2H_4 treatments were much more substantial, especially for the 600 nL L^{-1} treatments where the difference after 7 days was 27 N. There are several possible explanations for this discrepancy. First of all, we

have to consider that these results relate to three different cultivars that react differently to 1-MCP treatment. In 'Bartlett' pear, ripening and ethylene production were not blocked by 1-MCP but delayed (Trinchero et al., 2004), whereas in 'Conference' pear, ripening was completely inhibited. It stands to reason that this could also be the case when ethylene is added to the treatment. The second explanation might be found in the moment of treatment. Ethylene was applied 13 days after the 1-MCP treatment in the case of 'Bartlett' (Trinchero et al., 2004) and during shelf life after removal from storage in the case of 'd'Anjou' (Argenta et al., 2003) while in 'Conference' the treatment was done simultaneously with the 1-MCP treatment.

The effectiveness of the 1-MCP treatment is based on the fact that 1-MCP occupies the ethylene binding receptors (Jiang et al., 2004) and that this is irreversible. Subsequent treatment with ethylene cannot overcome this problem unless new receptors are formed or unless 1-MCP dissociates from the receptor binding sites. These are the main two hypotheses that could explain the recuperation of the ripening process (Blankenship and Dole, 2003). In this work, the application of 1-MCP and ethylene was done at the same time, thus making them compete for the same receptors resulting in some being irreversibly occupied by 1-MCP but others not. This would ensure that exogenous or endogenous ethylene during the storage or shelf life period would have receptors to bind to and set in motion the ripening process. Additionally, exogenous ethylene stimulates the activity of ACC synthase and ACC oxidase, resulting in endogenous production of ethylene and uniform ripening when the fruit is placed at 20 °C (Liu et al., 2005).

These results show the potential interest of the combined treatment of 1-MCP and ethylene in 'Conference' pears to prevent the 'evergreen' behavior and allow the fruit treated with 1-MCP to recover their ability to ripen. However, more work is needed. On one hand, although it seems that the 600 nL L^{-1} of 1-MCP and 300 nL L^{-1} of C_2H_4 combination is the most promising treatment, this needs to be repeated and verified. On the other hand, the combination of 600 nL L^{-1} of 1-MCP and 600 nL L^{-1} of C_2H_4 which was repeated over the two years did not give the same results which calls for more research.

5. CONCLUSIONS

The big challenge for the successful application of 1-MCP in 'Conference' pears is to delay ripening, maintaining the firmness and the green color without totally blocking this process. Considering our results, the combined treatment appears to be an interesting tool to counteract the evergreen behavior in 'Conference' pears. This treatment allows the pears to ripen in a reasonable period of time after storage extending the possibility of marketing and consumption. However, it appears to be very important to apply the exogenous ethylene and 1-MCP simultaneously.

Acknowledgments

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References

Argenta, L. C.; Fan, X.; Mattheis, J. P. 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit. Journal of Agricultural and Food Chemistry, 51, 3858-3864.

Baritelle, A. L.; Hyde, G. M.; Fellman, J. K.; Varith, J. 2001. Using 1-MCP to inhibit the influence of ripening on impact properties of pear and apple tissue. Postharvest Biology and Technology, 23, 153-160.

Blankenship, S. M. 2003. Discovery and commercialization of 1-methylcyclopropene as an ethylene inhibitor. Acta Horticulturae, 628, 189-191.

Blankenship, S. M.; Dole, J. M. 2003. 1-methylcyclopropene: a review. Postharvest Biology and Technology, 28, 1-25.

Calvo, G. 2003. Effect of 1-methylcyclopropene (1-MCP) on pear maturity and quality. Acta Horticulturae, 628, 203-211.

Calvo, G. 2004. Aplicación de etileno a peras 'Bartlett', 'Beurre d'Anjou' y 'Packhams Triumph'previamente tratadas con 1-metilciclopropeno (1-MCP). Technical report. INTA. Alto Valle, Río Negro, Argentina. Pp. 1-18.

Cambiaghi, P.; Eccher Zerbini, P.; Grassi, M.; Rizzolo, A. 2004. Problematiche nel dosaggio di1-MCP nelle pere. Atti VII Giornate Scientifiche SOI, Società Octicola Italiana: Firenze.

Chen, P. M. 2002. Pre-conditioning 'd'Anjou' pears in transit during early marketing season. Washington State University Tree Fruit Research and Extension Center.

Chen, P. M.; Spotts, R. 2005. Changes in ripening behaviors of 1-MCP treated d'Anjou pears after storage. International Journal of Fruit Science, 5, 3-18.

Chen, P. M.; Varga, D. M.; Facteau, T. J. 1997. Promotion of ripening of 'Gebhard' red 'd'Anjou' pears by treatment with ethylene. Postharvest Biology and Technology, 12, 213-220.

Chiriboga, M. A.; Schotsmans, W. C.; Larrigaudière, C. 2010. Comparative study of techniques to restore the ripening process in 1-MCP treated 'Blanquilla' and 'Conference' pears. Acta Horticulturae, 858, 149-154.

Eccher Zerbini, P.; Cambiaghi, P.; Grassi, M.; Rizzolo, A. 2003. Effect of 1-MCP on the quality of pears during storage. In: In Biology and Biotecnology of the Plant Hormone Ethylene III. Pp. 402 - 407. Eds.; IOS Press: Amsterdam: Vendrell M, Klee H,Pech JC, Romojaro F.

Ekman, J. H.; Clayton, M.; Biasi, W. V.; Mitcham, E. J. 2004. Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears. Postharvest Biology and Technology, 31, 127-136.

Facteau, T. J.; Mielke, E. A. 1998. Effect of harvest maturity and postharvest-prestorage ethylene treatment on the storage and ripen ability of d'Anjou pears. Acta Horticulturae, 475, 567-569.

Hiwasa, K.; Kinugasa, Y.; Amano, S.; Hashimoto, A.; Nakano, R.; Inaba, A.; Kubo, Y. 2003. Ethylene is required for both the initiation and progression of softening in pear (Pyrus communis L.) fruit. Journal of Experimental Botany, 54, 771-779.

Jiang, W.; Zhang, M.; He, J.; Zhou, L. 2004. Regulation of 1-MCP-treated banana fruit quality by exogenous ethylene and temperature. Food Science and Technology International, 10, 15-20.

Kappel, F.; Fisherfleming, R.; Hogue, E. J. 1995. Ideal pear sensory attributes and fruit characteristics. HortScience, 30, 988-993.

Kubo, Y.; Hiwasa, K.; Owino, W. O.; Nakano, R.; Inaba, A. 2003. Influence of time and concentration of 1-MCP application on the shelf life of pear 'La France' fruit. HortScience, 38, 1414-1416.

Lafer, G. 2005. Effects of 1-MCP treatments on fruit quality and storability of different pear varieties. Acta Horticulturae, 682, 1227-1231.

Lelièvre, J. M.; Tichit, L.; Dao, P.; Fillion, L.; Nam, Y. W.; Pech, J. C.; Latché, A. 1997. Effects of chilling on the expression of ethylene biosynthetic genes in Passe-Crassane pear (Pyrus communis L.) fruits. Plant Molecular Biology, 33, 847-855.

Liu, H.; Jiang, W.; Zhou, L.; Wang, B.; Luo, Y. 2005. The effects of 1-methylcyclopropene on peach fruit (Prunus persica L. cv. Jiubao) ripening and disease resistance. International Journal of Food Science and Technology, 40, 1-7.

Ma, S.; Chen, P. M.; Mielke, E. A. 2000. Storage life and ripening behavior of 'Cascade' pears as influenced by harvest maturity and storage temperature. Journal of the American Pomological Society, 54, 138-147.

Manriquez, D.; Defilippi, B.; Castillo, P.; Retamales, J. 2002. Ripening control of Packham´s Triumph pears by mean of 1-MCP and ethylene applications. Summaries ofXXVIth International Horticultural Congress & Exhibition (IHC2002), 235.

Mitcham, E. J.; Agar, T.; Biasi, W.; Gross, K.; W., D. (2000). Ethylene treatment of 'Bartlett' pears in transit to improve ripening and quality. Washington State University Tree Fruit Research and Extension Center. Postharvest information Network.

Mitcham, E. J.; Crisosto, C. H.; Kader, A. A. (2006). Pear: Bartlett. Washington State University Tree Fruit Research and Extension Center. Postharvest information Network.

Puig L; Varga, D. M.; Chen, P. M.; Mielke, E. A. 1996. Synchronizing ripening in individual 'Bartlett' pears with ethylene. HortTechnology, 6, 24-47.

Reid, M. S. 1992. Ethylene in postharvest technology. In: Postharvest Technology of Horticultural Crops (edited by KADER, A. A.). Pp. 95-108. University of California, Oakland, CA: Division of Agriculture and Natural Resources.

Rizzolo, A.; Cambiaghi, P.; Grassi, M.; Zerbini, P. E. 2005. Influence of 1-methylcyclopropene and storage atmosphere on changes in volatile compounds and fruit quality of Conference pears. Journal of Agricultural and Food Chemistry, 53, 9781-9789.

Sisler, E. C.; Dupille, E.; Serek, M. 1996. Effects of 1-methylcyclopropene and methylcyclopropene on ethylene binding and ethylene action on cut carnation. Plant Growth Regulation, 18, 79-86.

Trinchero, G. D.; Sozzi, G. O.; Covatta, F.; Fraschina, A. A. 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of `Bartlett` pears. Postharvest Biology and Technology, 32, 193-204.

Wang, C. Y.; Mellenth, W.; Hansen, E. 1972. Maturation of ‘d’Anjou’ pears in relation to chemical composition and reaction to ethylene. Journal of the American Society for Horticultural Science, 97, 9-12.

CAPÍTULO 5
EFFECT OF 1-MCP AND EXOGENOUS ETHYLENE ON FRUIT RIPENING AND ACC METABOLISM IN 'CONFERENCE' PEARS

ABSTRACT

BACKGROUND: In some pear varieties like 'Conference', 1-MCP treatment often impairs the ripening process indefinitely and the pears remain 'evergreen'. The application of exogenous ethylene and 1-MCP simultaneously after harvest permitted the recovery of the ripening process after storage. The objective of this study was to describe the effect of such a combined treatment on the ethylene metabolism and to assess whether the effect was maturity dependent.

RESULTS: The efficacy of the treatment was affected by the maturity at harvest and with mature fruit, where the 1-MCP treatment does not cause evergreen the combined treatment did not cause additional softening. The ripening behavior directly depended on the amount of ACC accumulated during cold storage.

CONCLUSION: The combination of a high dose of 1-MCP and ethylene permitted a considerable delay of the softening process without completely blocking it. The exact physiological working principle behind the positive effect is not yet clear but the positive effect on ripening recovery was clearly related to less delay in the start of ethylene production during shelf life.

1. INTRODUCTION

1-Methylcyclopropene (1-MCP) is a synthetic gaseous inhibitor of ethylene action in plants (Sisler et al., 1996). Effective at nanomolar concentrations, it has been reported to significantly extend the shelf life of a range of fruits (Blankenship and Dole, 2003). It is actually registered for use on several fruit crops including apple, banana and tomato (Watkins, 2006). In some pear varieties like 'Conference', 1-MCP treatment often impairs the ripening process indefinitely and the pears remain 'evergreen' (Chiriboga et al., 2011; 2012).

The inhibition of ethylene production by 1-MCP has been well described, and although the exact site in the ethylene biosynthesis pathway where 1-MCP acts is not yet clear it is likely to be one of the two key enzymes in the ethylene production pathway. Down-regulation of 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (ACO) expression and/or activity by 1-MCP treatment is generally seen (Mathooko et al., 2001; Hoeberichts et al., 2002; Owino et al., 2002; Defilippi et al., 2005a; Dal Cin et al., 2006; Zhang et al., 2006) whereas down-regulation of ACC synthase (ACS) expression and activity has been described in apple (Defilippi et al., 2005a; Dal Cin et al., 2006), avocado (Owino et al., 2002) and banana (Zhang et al., 2006). In pears specifically, in 1-MCP treated fruit malonylation had a more limited role and the capacity of the fruit to resume softening was directly related to the inhibition of ACO and to the residual ACS activity which was maintained after 1-MCP treatment during cold storage (Chiriboga et al., 2012).

1-MCP works by binding to the receptors that are normally deactivated by binding of ethylene, which sets the signal transduction cascade in motion. Binding of 1-MCP kept these receptors in a permanently activated state which inhibits ethylene perception and thus prevents ethylene dependent responses (Sisler and Blankenship, 1996; Sisler and Serek, 1997).

The ripening of pears is induced by a threshold concentration of internal ethylene (Chen and Mellenthin, 1981), and the response to 1-MCP treatment depends on fruit maturity at the time of treatment (Calvo and Sozzi, 2004), as well as the presence of ethylene in the fruit at the moment of the 1-MCP treatment (Gamrasni et al., 2010). Based on this information, exogenous ethylene application simultaneously with the 1-MCP treatment was demonstrated to restore the ripening process for 'Conference' pears (Chiriboga et al., 2011).

The objective of this research was to describe the effect of such a combined treatment on the ethylene metabolism in 'Conference' pears as well as the effect of harvest maturity during cold storage and shelf life.

2. MATERIALS AND METHODS

2.1 Fruit Material and Experimental design

Pears (*Pyrus communi*s cv. 'Conference') were harvested from one orchard at three harvest dates in Lleida, Spain. Fruit were collected in the middle of the commercial harvest window (H_0), 7 days (H_7) and 10 days later (H_{10}). Firmness was measured and fruit flesh samples were taken immediately after harvest and after different storage periods up to 105 days as well as during subsequent shelf life at 20 ºC. At each removal time, fruit flesh samples were immediately frozen in liquid N_2 and stored at -80 ºC until analysis.

The experiment was set up using a completely randomised design and the data were analyzed by analysis of variance using SAS statistical software (version 9.1, SAS Institute Inc., USA). Tukey's least significant differences values (LSD; $p \leq 0.05$) were calculated for mean separation.

2.2 Treatments

Immediately after harvest, fruit were placed in cold storage (-0.5 °C) for one day and then treated with 0, 300 nL L^{-1}1-MCP (referred to from here on as single 1-MCP treatment) or a combined treatment of 600 nL L^{-1}1-MCP with 300 nL L^{-1} C_2H_4 (referred to from here on as combined treatment) during 24 hours at -0.5 ºC. 1-MCP was applied using the product Smartfresh™ (Agrofresh Inc., Rohm and Haas, Spring House, PA, USA) and following the company recommendations and as described in Chiriboga et al. (2011). After the treatment, the bag was opened and the chamber thoroughly aired. For the combined treatment, 1-MCP as Smartfresh™ powder (1 g for the 600 nL L^{-1}) was put in the small plastic flask, which was then sealed. Water was added through the seal using a syringe and the treatment device was placed inside the 1 m^3 plastic bag, containing the boxes with fruit, which was then sealed. Gaseous C_2H_4 was withdrawn with a syringe (0.3 ml for the 300 nL L^{-1}) from a gas bottle with pure ethylene (98%) and injected into the plastic bag at the same moment the flask containing 1-MCP was opened. The following day (after 24 h), the plastic bag was opened and the cold room well aired.

2.3 Analytical

Determination of harvest maturity

The harvest maturity of 30 randomly selected fruit from each harvest date was determined using standard protocols. Firmness was determined with a manual penetrometer (Effegi, Milan, Italy) fitted with an 8 mm Magness Taylor probe. Starch index was determined by iodine staining using the radial EUROFRU reference chart (CTIFL, France). Ethylene production was determined on 3 replicates of 2 pears by gas chromatography as described below.

Analysis of ethylene production

Ethylene production (µL kg^{-1} h^{-1}) was measured in an acclimatized chamber at 20 ºC. The pears were placed in 1.5 L respiration flasks continuously ventilated with humidified air at a flow rate of 1.5 L h^{-1}. Samples (1 ml) of effluent air from the flasks were taken using a syringe and injected into a gas chromatograph (Agilent Technologies 6890, Wilmington, Germany) fitted with a FID detector and an alumina column F1 80/100 (2 m x 1/8 x 2.1, Tecknokroma, Barcelona, Spain). The injector and detector were kept at 120 and 180°C, respectively.

Extraction and analysis of 1-aminocyclopropane-1 carboxylic acid (ACC) and malonyl-ACC (MACC) levels:

ACC and MACC levels were analysed using 6 fruit per sample extracting 2 g of frozen flesh tissue from each individual fruit with 30 ml of 80 % ethanol. The ACC concentration was assayed according to the method of Lizada and Yang (1979) with minor modifications as described by Vilaplana et al. (2007) and expressed as nmol ACC g^{-1} fresh weight (FW). MACC was measured by analyzing the ACC content of a hydrolyzed extract as described by Hoffman et al. (1982) . Data were expressed as nmol ACC g^{-1} FW.

Extraction and analysis of ACS and ACO enzyme activity:

ACS and ACO activity were determined using 10 g of frozen flesh tissue from 4 individual fruit per sample. The frozen tissue was homogenized with 10 ml of buffer containing 200 mM Tricine buffer at pH 8.5, 10 mM dithiothreitol (DTT), 20µM pyridoxal phosphate and 2% (w/v) polyvinylpyrrolidone (PVP). The homogenized tissue was then filtered through two layers

of miracloth and centrifuged at 18,000 x g for 20 min at 4 ºC. Subsequently, a 2.5 ml aliquot was loaded into a Sephadex G-25 column (PD 10, Pharmacia, Madrid, Spain), previously equilibrated with 5 mM Tricine buffer (pH 8), 1 mM DTT and 2 µM pyridoxal 5-phosphate. The enzyme was eluted with 3.5 ml of the same buffer and 1.5 ml was incubated for 2 h at 25 ºC with 200 mM Tricine buffer at pH 8 and 100 µM of S-adenosyl-L-methionine (SAM). The reaction was then stopped with 100 mM $HgCl_2$ and 1 ml of the product was mixed and stirred with 100 µl of NaOCl and saturated NaOH (2:1 v/v). After two minutes, a 1 ml headspace gas sample was injected into a gas chromatograph and the results were expressed as nmol C_2H_4 $g^{-1}h^{-1}$.

For ACO activity, the sample was homogenized in 20 ml of buffer containing 0.1 M Tris-HCl at pH 7.4, 10% glycerol, 30 mM Na-ascorbate, 5 mM DTT and 1% (w/v) PVP. The homogenate was filtered through two layers of miracloth and centrifuged at 16,000 x g for 20 min at 4 ºC. A 2.5 ml aliquot was loaded into a Sephadex G-25 column (PD 10, Pharmacia, Madrid, Spain), previously equilibrated with 20 mM Tris-HCl buffer at pH 7.4, 10% glycerol, 3 mM Na-ascorbate and 1mM DTT. The enzyme was eluted with 3.5 ml of the same buffer and 500 µl of enzyme extract was then mixed with 10 µM $FeSO_4$, 3 mM sodium bicarbonate and 50 µM ACC. The mixture was aired and incubated for 20 min at 26 ºC, after which a 1 ml headspace gas sample was injected into a gas chromatograph and the results were expressed as nmol C_2H_4 $g^{-1}h^{-1}$.

3. RESULTS AND DISCUSSION

3.1 Initial maturity and ripening recovery

As presented earlier (Chiriboga et al., 2012), there was a significant increase in maturity the later the harvest date (Table 1).

Table 1: Maturity indices at harvest in relation to harvest date (H_0, H_7, H_{10}). Values represent the means of 30 fruit. Different letters in the same row indicate significant differences between harvest dates ($p \leq 0.05$).

	Harvest		
Maturity indices	H_0	H_7	H_{10}
Firmness (N)	51.6 a	52.8 a	54.3 a
Starch (1-10)	2.7 c	4.9 b	6.6 a
C_2H_4 (µL kg^{-1} h^{-1})	0.16 b	0.56 a	0.58a

No differences in softening were found between the treatments during cold storage for the fruit from the H_0 and H_7 harvest (Figure 1). The control fruit from H_{10} softened significantly more in cold storage compared with the other two harvests whereas both the single 1-MCP as the combined treatment did not show significant softening. This indicates that during storage the beneficial effect of the 1-MCP treatment remains dominant.

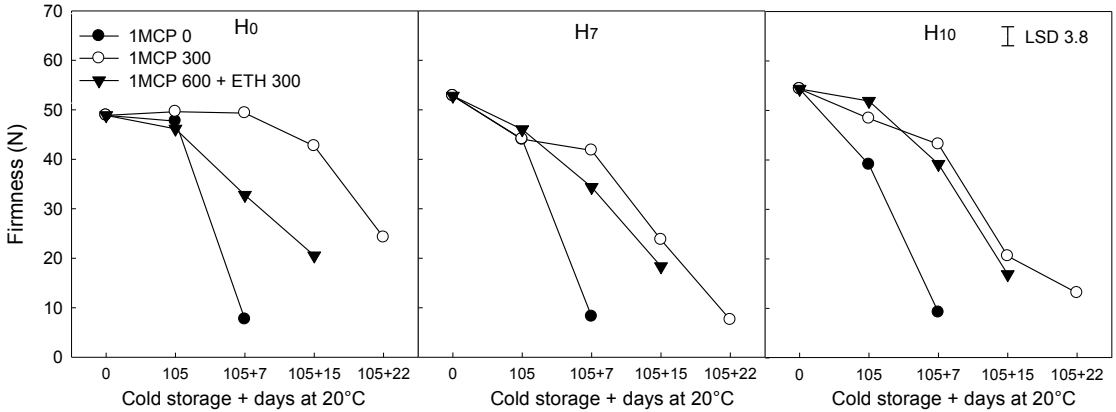

Figure 1. Softening during cold storage and shelf life of 'Conference' pears harvested at three harvest dates: Commercial harvest date (H_0); Commercial harvest date + 7 days (H_7); Commercial harvest date + 10 days (H_{10}). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1} 1-MCP + 300 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 300). Vertical bar represents LSD value ($p \leq 0.05$).

During shelf life, the fruit from the H_0 harvest that received the combined treatment softened faster compared with the fruit from the single 1-MCP treatment but considerably slower compared with the control fruit (Figure 1). For the later harvests that was no significant difference between the single 1-MCP and the combined treatment. The control fruit (1-MCP 0) from all the harvest dates reached the lower limit of eating quality (10 N) within 7 days at 20 ºC. After 15 days of shelf life the fruit that received the combined treatment reached eating quality and exhibited a firmness loss of 39.47 % and 58 % for H_0, H_7 and H_{10} respectively. In contrast, the softening recovery for the fruit from the single 1-MCP treatment was related to harvest date. The fruit from H_0 showed no loss of firmness after 7 days at 20 ºC and after 15 days at 20 ºC the firmness was still above 41 N. The fruit from H_7 exhibited some firmness loss (5 %) after 7 days at 20 ºC and remained just

above the 20 N limit after 15 days of shelf life whereas the fruit from H_{10} exhibited 10% firmness loss after 7 days and was below the limit after 15 days.

This confirms that treating the fruit simultaneously with ethylene and 1-MCP can lead to improved recovery of softening in 'Conference' pears as was reported by Chiriboga et al. (2011). However, what was shown here additionally is that while the effect of a single 1-MCP treatment was influenced by the physiological maturity at harvest in 'Conference' pears, this was not the case for the combined treatment. In the less mature fruit, the single 1-MCP treatment resulted in a complete inhibition of fruit ripening whereas at more advanced stages (hence in the presence of more ethylene) it slowed down the softening process without completely blocking it.

The combined treatment in contrast, allowed the less mature (H_0) fruit to soften adequately but resulted in a similar delay of softening than in the in more mature (H_7 and H_{10}) treated with a single 1-MCP treatment. In other words, the combined treatment resulted in a homogenization between fruit with different maturities. This indicates that even though recovery of softening in 1-MCP treated pears directly depends on harvest maturity and is determined by the ethylene levels at the time of treatment (Chiriboga et al., 2012; Villalobos-Acuña et al., 2011b), exogenous ethylene administered at the moment of treatment can have the same effect that high internal ethylene present in more mature fruit.

3.2 Physiological changes during cold storage

The biochemical basis of the ripening recovery process in 1-MCP treated pear is poorly understood. This process depends on the specific behavior of the cultivar to chilling temperature.

As for control fruit and fruit receiving the single treatment (Chiriboga et al., 2012), cold stress induced a sharp activation of ACC for fruit from the combined treatment and the accumulation clearly depended on harvest date (Figure 2).

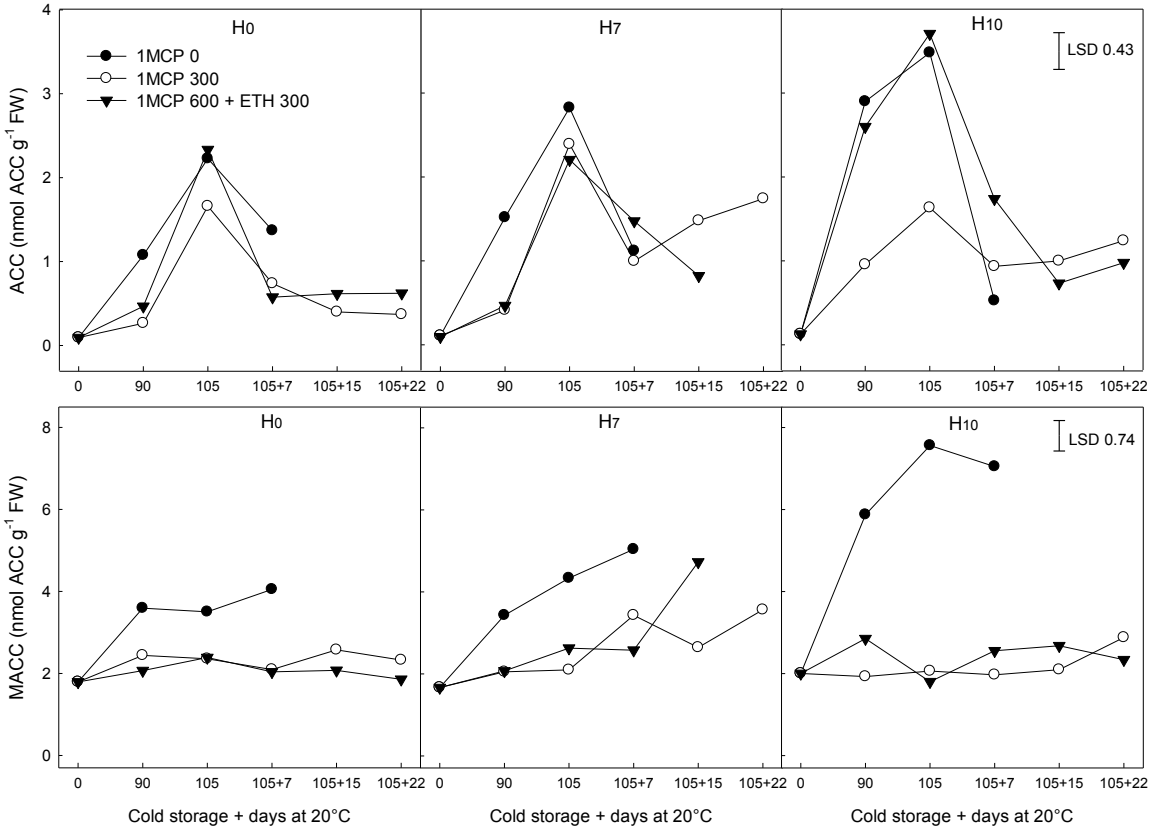

Figure 2. Changes in ACC (top) and MACC (bottom) levels during cold storage and shelf life at 20 ºC in relation to harvest maturity. Commercial harvest date (H_0); Commercial harvest date + 7 days (H_7); Commercial harvest date + 10 days (H_{10}). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1} 1-MCP + 300 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 300). Vertical bar represents LSD value ($p \leq 0.05$).

Irrespective of the harvest date, the increase in ACC was accompanied with a complete inhibition of ACS (Figure 3) and ACO (Figure 4) activity irrespective as well as a reduced capacity of the fruit to convert ACC to MACC (Figure 2) compared with control fruit. This behavior was most similar to that observed in fruit treated only with 1-MCP, except for the ACC level at the end of cold storage, which was similar to the control fruit.

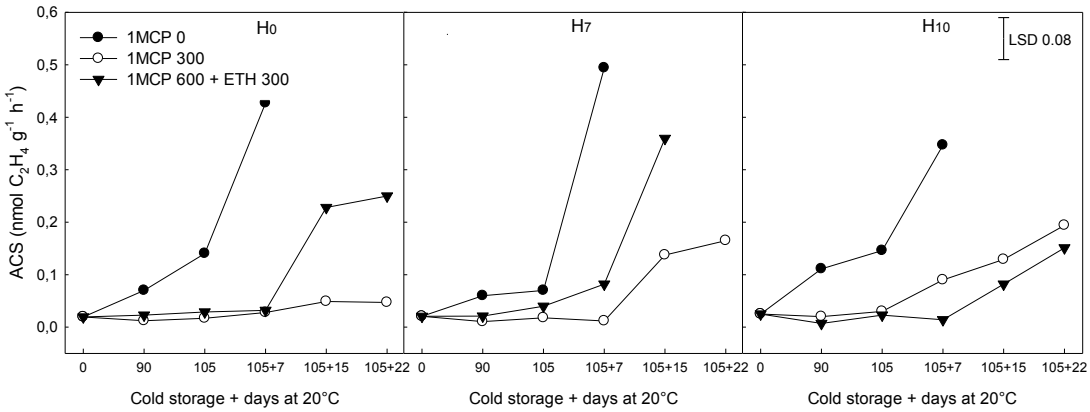

Figure 3. Changes in ACS activity during cold storage and shelf life at 20 ºC in relation to harvest maturity. Commercial harvest date (H_0); Commercial harvest date + 7 days (H_7); Commercial harvest date + 10 days (H_{10}). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1} 1-MCP + 300 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 300). Vertical bar represents LSD value (*p≤0.05*).

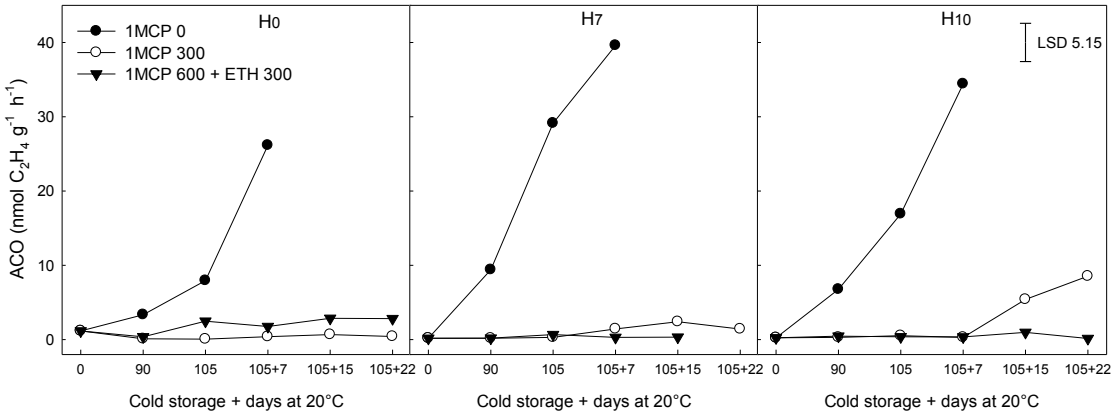

Figure 4. Changes in ACO activity during cold storage and shelf life at 20 ºC in relation to harvest maturity. Commercial harvest date (H_0); Commercial harvest date + 7 days (H_7); Commercial harvest date + 10 days (H_{10}). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1}1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1}1-MCP + 300 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 300). Vertical bar represents LSD value (*p≤0.05*).

3.3 Physiological changes upon removal

The effect of the combined treatment to the ethylene production during shelf life after cold storage was similar to the effect of the single 1-MCP treatment in that ethylene production was significantly lower (Figure 5). Nevertheless, the delay in the peak of ethylene production was not as long as for the fruit receiving the single 1-MCP treatment. After the combined treatment, ethylene production reached the maximum within 10 days of shelf life irrespective of the harvest date, whereas after the single 1-MCP treatment the peak appeared only after 26, 14 or 12 days for H_0, H_7 and H_{10} respectively. This means 16 days earlier in case of H_0 and 3-5 days for the later harvests, which confirms the more pronounced effect of the combined treatment in the fruit from the H_0 harvest. These results also reiterate the homogenization effect of the combined treatment.

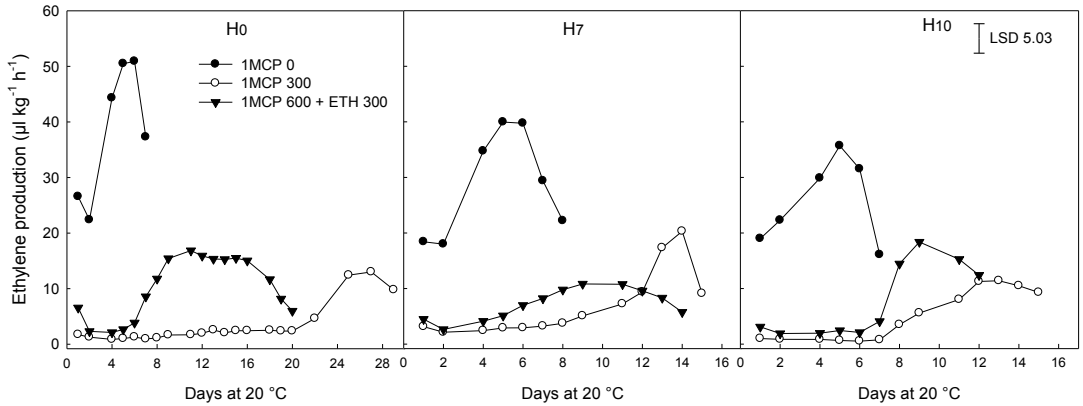

Figure 5. Ethylene production in 'Conference' pears during shelf life at 20 ºC after removal from cold storage (105 days) in relation to harvest maturity. Commercial harvest date (H_0); Commercial harvest date + 7 days (H_7); Commercial harvest date + 10 days (H_{10}). Control fruit (1-MCP 0), fruit treated with 300 nL L^{-1} 1-MCP (1-MCP 300), fruit treated with 600 nL L^{-1} 1-MCP + 300 nL L^{-1} C_2H_4 (1-MCP 600 + ETH 300). Vertical bar represents LSD value ($p \leq 0.05$).

The effect of the combined treatment on the ACC metabolism was very similar to that of the single 1-MCP treatment with two interesting exceptions. The first can be found in the ACS activity during shelf life. In H_0 fruit, the ACS activity sharply increases after 7 days of shelf life for the combined treatment when it stays completely blocked after the single

1-MCP treatment (Figure 3). The second exception, also for H_0 fruit, is a consistent residual ACO activity immediately after removal from cold storage (Figure 4). This small activity might be enough to provoke the earlier ethylene production peak.

4. CONCLUSIONS

The combination of a high dose of 1-MCP and ethylene permitted a considerable delay of the softening process without completely blocking it. The efficacy of the treatment to prevent ripening blockage was most clear in the less mature fruit with no additional effect in more mature fruit thus resulting in a homogenization of ripening between fruit from different harvest dates. The exact physiological working principle behind the positive effect is not yet clear but the positive effect on ripening recovery was clearly related to less delay in the start of ethylene production during shelf life.

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References

Blankenship, S. M.; Dole, J. M. 2003. 1-methylcyclopropene: a review. Postharvest Biology and Technology, 28, 1-25.

Calvo, G.; Sozzi, G. O. 2004. Improvement of postharvest storage quality of 'Red Clapp's' pears by treatment with 1-methylcyclopropene at low temperature. Journal of Horticultural Science and Biotechnology, 79, 930-934.

Chen, P. M.; Mellenthin, W. M. 1981. Effects of harvest date on ripening capacity and postharvest life of ‘d’Anjou’ pears. Journal of the American Society for Horticultural Science, 106, 38-42.

Chiriboga, M. A.; Recasens, I.; Schotsmans, W. C.; Dupille, E.; Larrigaudiere, C. 2012. Cold-induced changes in ACC metabolism determine softening recovery in 1-MCP treated Conference pears. Postharvest Biology and Technology, 68, 78-85.

Chiriboga, M. A.; Schotsmans, W. C.; Larrigaudiere, C.; Dupille, E.; Recasens, I. 2011. How to prevent ripening blockage in 1-MCP-treated 'Conference' pears. Journal of the Science of Food and Agriculture, 91, 1781-1788.

Dal Cin, V.; Rizzini, F. M.; Botton, A.; Tonutti, P. 2006. The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit. Postharvest Biology and Technology, 42, 125-133.

Defilippi, B. G.; Dandekar, A. M.; Kader, A. A. 2005. Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. Journal of Agricultural and Food Chemistry, 53, 3133-3141.

Gamrasni, D.; Ben-Arie, R.; Goldway, M. 2010. 1-methylcyclopropene (1-MCP) application to Spadona pears at different stages of ripening to maximize fruit quality after storage. Postharvest Biology and Technology, 58, 104-112.

Hoeberichts, F. A.; van der Plas, L. H. W.; Woltering, E. J. 2002. Ethylene perception is required for the expression of tomato ripening-related genes and associated physiological changes even at advanced stages of ripening. Postharvest Biology and Technology, 26, 125-133.

Hoffman, N. E.; Yang, S. F.; McKeon, T. 1982. Identification of 1-(malonylamino)cyclopropane-1-carboxylic acid as a major conjugate of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor in higher-plants. Biochemical and Biophysical Research Communications, 104, 765-770.

Lizada, M. C. C.; Yang, S. F. 1979. A simple and sensitive assay for 1-amino cyclopropane-l-carboxylic acid. Analytical Biochemistry, 100, 145.

Mathooko, F. M.; Tsunashima, Y.; Owino, W. Z. O.; Kubo, Y.; Inaba, A. 2001. Regulation of genes encoding ethylene biosynthetic enzymes in peach (Prunus persica L.) fruit by carbon dioxide and 1-methylcyclopropene. Postharvest Biology and Technology, 21, 265-281.

Owino, W. O.; Nakano, R.; Kubo, Y.; Inaba, A. 2002. Differential regulation of genes encoding ethylene biosynthesis enzymes and ethylene response sensor ortholog during ripening and in response to wounding in avocados. Journal of the American Society for Horticultural Science, 127, 520-527.

Sisler, E. C.; Blankenship, S. M. (1996). Methods of counteracting an ethylene response in plants. Pp. 518, 988. U.S. Patent 5.

Sisler, E. C.; Dupille, E.; Serek, M. 1996. Effects of 1-methylcyclopropene and methylcyclopropene on ethylene binding and ethylene action on cut carnation. Plant Growth Regulation, 18, 79-86.

Sisler, E. C.; Serek, M. 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. Physiologia Plantarum, 100, 577-582.

Vilaplana, R.; Soria, Y.; Valentines, M. C.; Larrigaudière, C. 2007. Specific response of apple skin and pulp tissues to cold stress and 1-MCP treatment. Postharvest Biology and Technology, 43, 215-220.

Villalobos-Acuña, M. G.; Biasi, W. V.; Flores, S.; Jiang, C. Z.; Reid, M. S.; Willits, N. H.; Mitcham, E. J. 2011. Effect of maturity and cold storage on ethylene biosynthesis and ripening in 'Bartlett' pears treated after harvest with 1-MCP. Postharvest Biology and Technology, 59, 1-9.

Watkins, C. B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnology Advances, 24, 389-409.

Zhang, M. J.; Jiang, Y. M.; Jiang, W. B.; Liu, X. J. 2006. Regulation of ethylene synthesis of harvested banana fruit by 1-methylcyclopropene. Food Technology and Biotechnology, 44, 111-115.

DISCUSIÓN GENERAL – GENERAL DISCUSSION

DISCUSIÓN GENERAL

1. EFECTO DEL 1-MCP SOBRE LA CALIDAD Y EL BLOQUEO DE LA MADURACION EN PERA 'CONFERENCE'.

Los resultados presentados en esta tesis muestran los efectos que tiene el 1-MCP en distintos parámetros de la maduración en peras 'Conference' durante el período de conservación en frío y en la vida comercial. El tratamiento con 1-MCP es muy eficaz para retrasar la maduración y el ablandamiento, así como la perdida de color verde. Sin embargo, y tal como se ha comprobado durante los diferentes años de estudio, se observa también que el 1-MCP puede llevar a una inhibición permanente de la maduración en esta variedad de pera. Los frutos que mostraron esta incapacidad para madurar se denominaron frutos 'evergreen'. Otros autores han observado también este efecto del tratamiento en otras variedades de pera (Bai y col., 2006; Calvo, 2003; Chen y Spotts, 2005; Gamrasni y col., 2010; Villalobos-Acuña y col., 2011a; Calvo y Sozzi, 2009).

Desde el punto de vista comercial, es muy importante que el tratamiento permita mantener fruta más resistente durante la conservación y el transporte, pero al mismo tiempo es vital que posteriormente esta fruta adquiera una calidad adecuada para ser comestible. A diferencia de las manzanas, las peras tienen que perder firmeza, virar su color y alcanzar la jugosidad suficiente para poder ser consumidas.

Una alta concentración (600 nL L^{-1} 1-MCP) siempre se asocia a un bloqueo completo de la maduración mientras que una menor concentración (300 nL L^{-1} 1-MCP) puede dar resultados muy variables. Estos efectos residuales durante el período de comercialización, cuando el 1-MCP es aplicado en altas concentraciones, se ha demostrado también en otros variedades de peras (Argenta y col., 2003; Bai y col., 2006; Ekman y col., 2004; Trinchero y col., 2004). Por otro lado muchas veces no es conveniente utilizar concentraciones más bajas ya que no serían suficientes para asegurar resultados satisfactorios en cuanto al control de la pérdida de firmeza, producción de etileno y desarrollo del escaldado (comunicación personal Agrofresh).

Por otro lado estos resultados en 'Conference' muestran que las peras pueden ser muy sensibles a la exposición al 1-MCP, por lo que se precisa adaptar el tratamiento a cada variedad y al estado fisiológico del fruto.

El tratamiento con 1-MCP provoca también una retención del color verde de la piel de los frutos durante la conservación y vida comercial. En frutos tratados con 1-MCP se reduce la actividad de la enzima clorofilasa, responsable de la degradación de las clorofila (Hershkovitz y col., 2005). Sin embargo, cuando el 1-MCP causa un bloqueo de la

maduración, no se produce un bloqueo similar en el cambio de color. La pérdida de color verde de los frutos tratados siempre se ve ralentizada pero no se detiene por completo, esto indica un efecto diferencial del 1-MCP sobre los diferentes indicadores de la maduración (firmeza y color). Este efecto diferencial sobre la textura y el color de la piel también fue observado por Ekman y col. (2004).

2. IMPORTANCIA DEL ESTADO DE MADUREZ CAMPO Y CAMPAÑA

Se sabe que la efectividad del tratamiento con 1-MCP depende del estado de madurez de la fruta en el momento de la cosecha (Blankenship y Dole, 2003). En los resultados de las campañas frutícolas del 2008 y 2009 se puede ver este efecto. La fruta temprana (7 días antes de la fecha óptima) ó en la fecha de cosecha comercial (óptima) se mantiene firme tras el tratamiento con 1-MCP y pierde su capacidad para ablandarse incluso después de varios días a 20 ºC. Al contrario, si el tratamiento se da en etapas más avanzadas de madurez (7 y 10 días) la retención de la firmeza es mucho menor, aunque este efecto también depende del campo e incluso de la campaña (año) en que se cosecha la fruta. La menor eficacia del tratamiento en frutos maduros, podría deberse a que éstos recuperan la sensibilidad al etileno en menos tiempo ó bien que ya existen receptores ocupados por etileno en esos frutos en el momento del tratamiento con 1-MCP.

Si observamos únicamente los frutos de la cosecha tardía en el año 2008, el grado de recuperación depende del campo, siendo el campo 3 el que más madura mientras que el campo 1 casi no pierde firmeza. Por otro lado si comparamos el mismo campo 2 en las mismas cosechas (7 y 10 días) en dos años distintos, los frutos no logran perder firmeza el primer año mientras que sí lo hacen en el segundo después de algunos días a 20 °C. Desde el punto de vista comercial, un aspecto interesante del tratamiento con 1-MCP es que permite mantener la calidad de las peras cosechadas en un estado de madurez avanzado, lo cual permite flexibilizar la fecha la cosecha.

Es muy difícil determinar el valor límite de madurez a la cosecha (firmeza o producción de etileno) que pueda evitar el efecto del bloqueo de la maduración. La respuesta sin embargo parece ser ligada principalmente a diferencias en la madurez fisiológica inicial cuando se aplica el 1-MCP (Capitulo 1).

Aunque se observan diferencias en eficacia del tratamiento con 1-MCP entre campos, años de cosecha y fechas de recolección, todo se relaciona con el estado fisiológico de la fruta a la cosecha y al momento del tratamiento. Resulta entonces muy importante determinar la madurez fisiológica de la fruta antes del tratamiento con 1-MCP. En general ninguno de los índices de madurez utilizados generalmente para determinar la fecha de cosecha por si solo, es capaz de describir la madurez fisiológica de manera adecuada a fin de identificar la sensibilidad de la fruta al 1-MCP. El índice Streif

(que incluye firmeza, sólidos solubles y el índice de almidón) combinado con la producción de etileno podría ser un buen marcador de dicha sensibilidad.

Los frutos tratados a la cosecha con un índice de Streif inferior a 0.8 y una producción de etileno inicial superior a 0.23 μL kg^{-1} h^{-1} pierden firmeza durante la vida útil. Al contrario, los frutos cosechados con un índice de Streif de 0.5 y una menor producción de etileno (0.13 μL kg^{-1} h^{-1}) se mantienen firmes durante la vida útil. En este caso, el nivel de etileno producido por las peras (> 0.23 μL kg^{-1} h^{-1}) parece ser suficiente para ocupar los receptores necesarios y evitar que los frutos se bloqueen por el 1-MCP. Lo mismo puede observarse en frutos del 2007 (Capitulo 4) que tienenen la cosecha una producción de etileno de 0.28 μL kg^{-1} h^{-1} y logran perder firmeza durante la vida comercial. Sin embargo, esto no se cumple con el lote de frutos del año 2009 (Capitulo 4), que a pesar de tener esa misma producción de etileno en cosecha (0.29 μL kg^{-1} h^{-1}), se mantienen firmes. Estos resultados muestran pues, que el comportamiento 'evergreen' no esta relacionado exclusivamente con los parámetros de madurez en la cosecha sino también con otros procesos que tienen lugar durante la conservación. En cualquier caso para optimizar la aplicación del 1-MCP y evitar el posterior bloqueo, el índice a la cosecha deberá incluir uno ó más parámetros representativos de la madurez fisiológica del fruto, como la tasa de producción de etileno al momento de la aplicación.

3. EFECTO SOBRE EL METABOLISMO DEL ETILENO

En pera 'Conference', el tratamiento con 1-MCP inhibe la producción de etileno durante la conservación y la vida comercial, en todos los experimentos. De manera similar, otros investigadores han observado en otras variedades de pera el efecto inhibidor del 1-MCP sobre el etileno (Gamrasni y col., 2010; Yazdani y col., 2011; Li y Wang, 2009; Villalobos-Acuña y col., 2011b; MacLean y col., 2007; Yamane y col., 2007; Li y col., 2010). Este efecto inhibidor es el resultado de la acción del 1-MCP sobre los receptores específicos del etileno situados en las membranas celulares, el cual compite con el etileno por los sitios de unión (Sisler y col., 1999). En algunos frutos (Owino y col., 2002; Defilippi y col., 2005; Dandekar y col., 2004; Zhang y col., 2006), y concretamente en peras (Lelièvre y col., 1997; Gamrasni y col., 2010; Yazdani y col., 2011; Li y Wang, 2009; Villalobos-Acuña y col., 2011b; MacLean y col., 2007; Yamane y col., 2007; Li y col., 2010), la inhibición de la producción de etileno por una aplicación de 1-MCP va paralela a una menor actividad y/o expresión de los genes que codifican las dos enzimas claves de la ruta biosintética del etileno: ACS y ACO.

En nuestros resultados el tratamiento con 1-MCP induce una inhibición parcial del aumento de los niveles de ACC y una inhibición completa de la actividad de las enzimas ACS y ACO durante la conservación en frío así como una incapacidad de convertir ACC a MACC en comparación a los frutos sin tratar. Estos resultados son en parte contradictorios

comparado a los obtenidos en manzanas (Vilaplana y col., 2007) en la cual el 1-MCP previene la acumulación de ACC durante la exposición a bajas temperaturas, lo que nos hace pensar que los niveles de ACC durante la conservación en frío juegan un papel muy importante en la maduración de peras 'Conference'.

En el caso de los frutos 'evergreen' no se observa ningún incremento en la producción de etileno durante toda la vida comercial. A contrario, los frutos reanudan el proceso de maduración muestran un pico de etileno después de varios días a 20 °C. Al parecer en estos frutos el 1-MCP retrasa la producción de etileno pero no la bloquea por completo así como el proceso de maduración.

Durante la vida comercial todos los frutos tratados exhiben una disminución de los niveles de ACC, sin embargo, para aquellos frutos considerados como 'evergreen', las enzimas ACS y ACO y la capacidad de convertir ACC en MACC continúan inhibidas durante todo el periodo a 20 °C. Otros autores también han observado esta inhibición de la actividad de las enzimas ACS y ACO así como la acumulación de los mRNAs asociados (Gamrasni y col., 2010; Yazdani y col., 2011; Li y Wang, 2009; Villalobos-Acuña y col., 2011b; MacLean y col., 2007; Yamane y col., 2007; Li y col., 2010).

En el caso de los frutos tratados con 1-MCP que maduran tras el tratamiento y varios días a 20°C, la capacidad para reanudar el proceso de maduración está relacionada con la acumulación de ACC y la intensidad de inhibición de la ACS durante la conservación en frío y con la activación de esta enzima durante la vida comercial a 20°C.

4. EFECTO SOBRE EL METABOLISMO ANTIOXIDANTE

En esta tesis se observa en todos los frutos un descenso inmediato de la actividad de las enzimas antioxidantes durante los primeros días en frío. Este descenso no es continuo el resto de días en conservación en frío, lo que sugiere que las bajas temperaturas provocan un estrés temporal en los frutos. El tratamiento de 1-MCP provoca un menor descenso de las actividades de las enzimas SOD y CAT en las primeras semanas de conservación en frío a la vez que se observa una mayor actividad de estas enzimas durante toda la conservación en frío. Lo mismo ocurre con el acido ascórbico, lo que sugiere que el tratamiento con 1-MCP provoca un aumento del potencial antioxidante que podría ser ligado a su vez con una disminución de los procesos de senescencia.

El tratamiento con 1-MCP provoca un mantenimiento del metabolismo antioxidante durante la vida comercial a 20 °C, incrementando el potencial del fruto para destruir las especies activas de oxígeno (AOS) especialmente mediante la enzima CAT.

Las peras 'Conference' exhiben un incremento en la conductividad eléctrica después de unos días de vida comercial a 20 °C. Este incremento coincide con la maduración del fruto y la pérdida de firmeza. El aumento en la permeabilidad va generalmente asociado a los procesos normales de maduración y senescencia de los frutos (Marangoni y col., 1996). Esto explica que el incremento en la permeabilidad de las membranas no se observa en los frutos tratados con 1-MCP y probablemente se relacione con que los frutos al mismo tiempo no maduraron.

Los cambios en el ambiente pueden inducir la acumulación de AOS y particularmente de H_2O_2 en las células (Noctor y Foyer, 1998). Durante la vida comercial a 20 °C, las peras en general (tratadas y no tratadas) no acumulan H_2O_2 lo que indica que el tratamiento con 1-MCP no induce un estrés oxidativo.

Sin embargo, la medida en que el 1-MCP puede tener un efecto positivo en el metabolismo antioxidante ya que éste claramente depende de la madurez de la fruta a la cosecha. Los procesos de maduración y senescencia va asociados generalmente a una baja actividad de las enzimas antioxidantes, por lo tanto el tratamiento con 1-MCP puede prolongar la vida útil, no sólo por la inhibición de la biosíntesis de etileno, sino también a través de un mejor mantenimiento de los sistemas antioxidantes de la fruta.

5. APLICACIÓN DE SISTEMAS DE RECUPERACIÓN DE LA MADURACION

El tratamiento con 1-MCP en peras 'Conference' es complicado en parte debido a la alta sensibilidad del fruto al tratamiento y al hecho de que en la mayoría de los casos las peras quedan excesivamente firmes y verdes y pierden su capacidad de madurar. En nuestro caso un tratamiento con 600 nL L^{-1} de 1-MCP provoca un bloqueo del proceso de maduración y de la producción de etileno durante la vida comercial a 20 ºC. Entonces uno de nuestros objetivos fue buscar un sistema que permitiera a los frutos tratados con 1-MCP, recobrar la sensibilidad al etileno y la capacidad de madurar durante la vida comercial.

En otros estudios con peras de la variedad 'Blanquilla' se probaron tratamientos térmicos que resultaron eficaces para lograr una maduración adecuada durante la vida comercial (Chiriboga y col., 2010). En pera 'Conference', ninguno de los tratamientos térmicos funcionó, lo que demuestra que la respuesta a estos tratamientos depende del cultivar. La eficacia del tratamiento en 'Blanquilla' se relacionó con la duración del tratamiento a 15 ºC (Chiriboga y col., 2010) y esto podría ser debido al hecho de que la temperatura podría inducir la síntesis de receptores de etileno, como también se observó en los plátanos (Jiang y col., 2002)

De forma similar Bai y col. (2006) observaron que tratamientos post conservación con temperaturas de 15-20 °C y duración de 10-20 días pueden reiniciar la maduración en peras 'Bartlett' mientras que en peras 'd'Anjou' ninguna combinación de preacondicionamiento permitió alcanzar a los frutos su madurez de consumo.

En la presente tesis se utilizó etileno exógeno como posible estrategia para recuperar la maduración (Capitulo 4). Dado que aplicaciones posteriores de etileno exógeno al tratamiento con 1-MCP ó después de la conservación en frío no permitieron revertir la inhibición causada por el 1-MCP en otras variedades de pera (Argenta y col., 2003; Trinchero y col., 2004), en nuestros experimentos se aplicó un tratamiento simultáneo de 1-MCP con etileno. Se puede suponer que la efectividad de este tratamiento radica en el momento de aplicación de los dos compuestos. Al ser aplicados al mismo momento, los dos tratamientos provocan una competición por los sitios de unión de los receptores, de tal manera que los receptores ocupados por el etileno podrían ayudar a la posterior producción de etileno y a la vez a la generación de nuevos receptores. Así se podría facilitar que las peras tratadas maduraran normalmente después de la conservación, y a su vez tuvieran una vida postcosecha mayor que los frutos sin tratar.

La mejor combinación fue el tratamiento con 600 nL L^{-1}de 1-MCP y 300 nL L^{-1} de C_2H_4 que permitió un retraso considerable del proceso de ablandamiento sin bloquearlo completamente y a su vez permite llegar a la madurez de consumo. Durante el año 2008 esta combinación permitió recobrar la capacidad de madurar de los frutos y extendió la vida comercial hasta 12 días. Resultados similares se obtuvieron en el año 2009 (Capitulo 5) en frutos de cosecha óptima y tardías (7 y 10 días después que la optima) lo que asegura su repetitividad en diferentes años. Este tratamiento no proporciona ningún efecto adicional al aplicarlo en frutos más maduros lo que nos demuestra que la efectividad del tratamiento no depende de la madurez al momento de la aplicación.

La combinación de 600 nL L^{-1} de 1-MCP y 600 nL L^{-1} C_2H_4 se ensayó en 2008 y 2009, sin embargo los resultados fueron distintos en los dos años. En el primero el tratamiento permitió el ablandamiento y mantener una calidad comestible durante 12 días mientras que en el segundo año los frutos maduraron demasiado.

En vista de los resultados obtenidos, es necesario continuar las investigaciones para conocer los efectos concretos que el 1-MCP provoca en las distintas variedades de pera. La respuesta al tratamiento al 1-MCP y las posibles estrategias de recuperación de la maduración dependen del cultivar, por tanto la consideración de cualquiera de estas estrategias por un cultivar determinado no puede ser extrapolada a otros cultivares.

Es importante considerar que todos estos resultados fueron obtenidos a nivel experimental por lo que se requiere ensayar la aplicación de estas estrategias a nivel comercial ó semi comercial. Los protocolos deberán ajustarse a las condiciones reales a fin de optimizar el tratamiento.

Los frutos tratados de manera combinada, con 1-MCP + etileno exógeno y cosechados 7 y 10 días después de la fecha óptima presentan un metabolismo del etileno muy similar a los frutos tratados únicamente con 1-MCP y cosechados en las mismas fechas. Los frutos del tratamiento combinado pero de la cosecha más tardía (10 días después de la óptima) muestran un incremento de ACC muy superior a los tratados únicamente con 1-MCP, aunque este comportamiento no supone diferencias en la firmeza entre los dos tratamientos. Sin embargo en los frutos cosechados en la fecha óptima, las diferencias observadas en el metabolismo del etileno entre los dos tratamientos, marcan después diferencias importantes en la capacidad para madurar. Este resultado muestra otra vez la importancia que tiene la fecha de recolección hasta en los frutos tratados con el sistema de control combinado. Una nueva definición de los índices óptimos de cosecha aparece ser entonces necesaria en caso de una aplicación de esta nueva tecnología a nivel comercial.

Bibliografía

Argenta, L. C.; Fan, X. T.; Mattheis, J. P. 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit. Journal of Agricultural and Food Chemistry, 51, 3858-3864.

Bai, J.; Mattheis, J. P.; Reed, N. 2006. Re-initiating softening ability of 1-methylcyclopropene-treated 'Bartlett' and 'd'Anjou' pears after regular air or controlled atmosphere storage. Journal of Horticultural Science & Biotechnology, 81, 959-964.

Blankenship, S. M.; Dole, J. M. 2003. 1-methylcyclopropene: a review. Postharvest Biology and Technology, 28, 1-25.

Calvo, G. 2003. Effect of 1-methylcyclopropene (1-MCP) on pear maturity and quality. Acta Horticulturae, 628, 203-211.

Calvo, G.; Sozzi, G. O. 2009. Effectiveness of 1-MCP treatments on 'Bartlett' pears as influenced by the cooling method and the bin material. 51, 49-55.

Chen, P. M.; Spotts, R. 2005. Changes in ripening behaviors of 1-MCP treated d'Anjou pears after storage. International Journal of Fruit Science, 5, 3-18.

Chiriboga, M. A.; Schotsmans, W. C.; Larrigaudière, C. 2010. Comparative study of techniques to restore the ripening process in 1-MCP treated 'Blanquilla' and 'Conference' pears. Acta Horticulturae, 858, 149-154.

Dandekar, A. M.; Teo, G.; Defilippi, B. G.; Uratsu, S. L.; Passey, A. J.; Kader, A. A.; Stow, J. R.; Colgan, R. J.; James, D. J. 2004. Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Research, 13, 373-384.

Defilippi, B. G.; Kader, A. A.; Dandekar, A. M. 2005. Apple aroma: alcohol acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene. Plant Science, 168, 1199-1210.

Ekman, J. H.; Clayton, M.; Biasi, W. V.; Mitcham, E. J. 2004. Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears. Postharvest Biology and Technology, 31, 127-136.

Gamrasni, D.; Ben-Arie, R.; Goldway, M. 2010. 1-methylcyclopropene (1-MCP) application to Spadona pears at different stages of ripening to maximize fruit quality after storage. Postharvest Biology and Technology, 58, 104-112.

Hershkovitz, V.; Saguy, S. I.; Pesis, E. 2005. Postharvest application of 1-MCP to improve the quality of various avocado cultivars. Postharvest Biology and Technology, 37, 252-264.

Jiang, Y.; Joyce, D. C.; Macnish, A. J. 2002. Softening response of banana fruit treated with 1-methylcyclopropene to high temperature exposure. Plant Growth Regulation, 36, 7-11.

Lelièvre, J. M.; Tichit, L.; Dao, P.; Fillion, L.; Nam, Y. W.; Pech, J. C.; Latché, A. 1997. Effects of chilling on the expression of ethylene biosynthetic genes in Passe-Crassane pear (Pyrus communis L.) fruits. Plant Molecular Biology, 33, 847-855.

Li, Z. Q.; Qiao, Y. S.; Tong, Z. G.; Zhou, J.; Zhang, Z. 2010. Effect of ethylene and 1-MCP on post-harvest physiology and on expression of the ethylene receptor genes *PpETR3* and *PpERS2* in pear (Pyrus pyrifolia Nakai 'Kikusui') fruit. Journal of Horticultural Science & Biotechnology, 85, 71-77.

Li, Z. Q.; Wang, L. J. 2009. Effect of 1-methylcyclopropene on ripening and superficial scald of Japanese pear (Pyrus pyrifolia Nakai, cv. Akemizu) fruit at two temperatures. Food Science and Technology Research, 15, 483-490.

MacLean, D. D.; Murr, D. P.; DeEll, J. R.; Mackay, A. B.; Kupferman, E. M. 2007. Inhibition of PAL, CHS, and ERS1 in 'Red d'Anjou' pear (Pyrus communis L.) by 1-MCP. Postharvest Biology and Technology, 45, 46-55.

Marangoni, A. G.; Palma, T.; Stanley, D. W. 1996. Membrane effects in postharvest physiology. Postharvest Biology and Technology, 7, 193-217.

Noctor, G.; Foyer, C. H. 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology, 49, 249-279.

Owino, W. O.; Nakano, R.; Kubo, Y.; Inaba, A. 2002. Differential regulation of genes encoding ethylene biosynthesis enzymes and ethylene response sensor ortholog during ripening and in response to wounding in avocados. Journal of the American Society for Horticultural Science, 127, 520-527.

Sisler, E. C.; Serek, M.; Dupille, E.; Goren, R. 1999. Inhibition of ethylene responses by 1-methylcyclopropene and 3-methylcyclopropene. Plant Growth Regulation, 27, 105-111.

Trinchero, G. D.; Sozzi, G. O.; Covatta, F.; Fraschina, A. A. 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of `Bartlett` pears. Postharvest Biology and Technology, 32, 193-204.

Vilaplana, R.; Soria, Y.; Valentines, M. C.; Larrigaudière, C. 2007. Specific response of apple skin and pulp tissues to cold stress and 1-MCP treatment. Postharvest Biology and Technology, 43, 215-220.

Villalobos-Acuña, M.; Biasi, W. V.; Mitcham, E. J.; Holcroft, D. 2011a. Fruit temperature and ethylene modulate 1-MCP response in 'Bartlett' pears. Postharvest Biology and Technology, 60, 17-23.

Villalobos-Acuña, M. G.; Biasi, W. V.; Flores, S.; Jiang, C. Z.; Reid, M. S.; Willits, N. H.; Mitcham, E. J. 2011b. Effect of maturity and cold storage on ethylene biosynthesis and ripening in 'Bartlett' pears treated after harvest with 1-MCP. Postharvest Biology and Technology, 59, 1-9.

Yamane, M.; Abe, D.; Yasui, S.; Yokotani, N.; Kimata, W.; Ushijima, K.; Nakano, R.; Kubo, Y.; Inaba, A. 2007. Differential expression of ethylene biosynthetic genes in climacteric and non-climacteric Chinese pear fruit. Postharvest Biology and Technology, 44, 220-227.

Yazdani, N.; Arzani, K.; Mostofi, Y.; Shekarchi, M. 2011. Alpha-Farnesene and antioxidative enzyme systems in Asian pear (Pyrus serotina Rehd.) fruit. Postharvest Biology and Technology, 59, 227-231.

Zhang, M. J.; Jiang, Y. M.; Jiang, W. B.; Liu, X. J. 2006. Regulation of ethylene synthesis of harvested banana fruit by 1-methylcyclopropene. Food Technology and Biotechnology, 44, 111-115.

GENERAL DISCUSSION

1. EFFECT OF 1-MCP ON RIPENING AND RIPENING BLOCKAGE IN‘CONFERENCE’ PEAR

The results presented in this thesis show the effects of 1-MCP on different ripening parameters in ‘Conference’ pear during cold storage and shelf life. The 1-MCP treatment is very effective in delaying ripening and softening, as well as loss of green color. However, as proven during the different years of study, 1-MCP can also lead to a permanent inhibition of ripening in this variety of pear. The pears that show this failure to ripen were designated ‘evergreen’. Other authors have seen similar effects of the 1-MCP treatment on other varieties of pears (Bai et al., 2006; Calvo, 2003; Chen and Spotts, 2005; Gamrasni et al., 2010; Calvo and Sozzi, 2009; Villalobos-Acuña et al., 2011a)

From a commercial point of view, it is very important that the treatment results in more resilient fruit during storage and transport, but at the same time it is vital that this fruit acquire a adequate quality for consumption. Unlike apples, the consumer prefers pears that have lost firmness, changed color and are juicy for consumption.

A high concentration (600 nL L^{-1} 1-MCP) always resulted in a complete softening blockage while a lower concentration (300 nL L^{-1} 1-MCP) gave variable results. The residual effect during the commercialisation period, when 1-MCP is applied in high concentrations has been shown in other varieties of pears (Argenta et al., 2003; Bai et al., 2006; Ekman et al., 2004; Trinchero et al., 2004). On the other hand it is often not convenient to use lower concentrations because they would not be sufficient to ensure satisfying results in terms of controlling softening, ethylene production and scald development (Personal communication Agrofresh).

‘Conference’ pears were shown to be very sensitive to exposure to 1-MCP, which is why it is necessary to adapt the treatment protocol for each variety and physiological state of the fruit.

The 1-MCP treatment also causes a retention of skin color of the fruit during storage and shelf life. In fruit treated with 1-MCP, the activity of the enzyme chlorophyllase, responsible for the degradation of chlorophyll, is reduced (Hershkovitz et al., 2005). However, when the 1-MCP treatment caused a softening blockage, a similar block in the color change does not occur. The loss of green color in treated fruit is always slower compared with the control but it is not completely stopped, which indicates a differential effect of 1-MCP on the different indicators of ripening (firmness and color). This differential effect on the texture and skin color was also observed by Ekman et al. (2004).

2. IMPORTANCE OF MATURITY, ORCHARD AND YEAR

The effectiveness of treatment with 1-MCP depends on the maturity of the fruit at harvest (Blankenship and Dole, 2003) as can also be seen in the results of 2008 and 2009. The early fruit (7 days before of commercial harvest) or fruit harvested on the commercial harvest date (mid) remains firm after 1-MCP treatment and loses its ability to soften even after several days at 20 °C. On the contrary, if fruit is treated at more advanced stages of maturity (7 and 10 days) the firmness retention is much lower, although this effect also depends on the orchard and even the year when the fruit is harvested. The lower efficacy of the treatment in mature fruit could be related to an inherent faster recovery of their ethylene sensitivity or to the existence of ethylene occupied receptors in these fruit at the time of treatment with 1-MCP.

Only taking into account the data from the late harvest in 2008, the degree of recovery depends on the orchard, with orchard 3 ripening faster whereas orchard 1 almost doesn't. On the other hand, if we compare the same orchard (orchard 2) in the same harvest (7 and 10 days) in two different years, the fruit fail to lose firmness in the first year whereas they do soften in the second year after a few days at 20 °C. From a commercial point of view, an interesting aspect of treatment with 1-MCP is that it allows maintenance of the quality of pears that were harvested in an advanced state of maturity, allowing flexibility in the harvest date.

It is very difficult to determine the optimal maturity at harvest (firmness or ethylene production) to prevent the evergreen behavior. The answer seems to be linked to differences in initial physiological maturity when applying 1-MCP (Chapter 1).

Although there are differences in effectiveness of the treatment between orchards, years of harvest and harvest dates, everything is related to the physiological state of the fruit at harvest and at the time of treatment. Therefore, it is very important to determine the physiological maturity of the fruit before treatment with 1-MCP. In general, none of the individual maturity indices generally used to determine the optimal harvest date are able to describe the physiological maturity adequately to identify the sensitivity of the fruit to 1-MCP. The Streif index (which includes firmness, soluble solids and starch index) combined with the production of ethylene could be a good marker of physiological maturity.

Fruit treated at a Streif index less than 0.8 and a higher initial ethylene production of 0.23 µL kg^{-1} h^{-1}, soften during shelf life whereas fruit harvested with a lower Streif index of 0.5 and a lower ethylene production (0.13 µL kg^{-1} h^{-1}) remain firm during shelf life. In the former case, the level of ethylene produced by the pears (> 0.23 µL kg^{-1} h^{-1}) appears to be sufficient to occupy the necessary receptors and prevent the fruit from becoming blocked. The same can be observed in fruit from 2007 (Chapter 4) that have an ethylene production of 0.28 µL kg^{-1} h^{-1} at harvest and soften during shelf life. However, this is not the case in the fruit harvested in 2009 (Chapter 4). Despite that they had the same ethylene production at harvest (0.29 µL kg^{-1} h^{-1}) this fruit remains firm. These results show that the 'evergreen' behavior is not exclusively related to harvest

maturity, but also to other processes that occur during storage. However, to optimize the application of 1-MCP and avoid resulting blockage, the harvest index used should include one or more parameters representing the physiological maturity of the fruit, like the rate of ethylene production at the time of application.

3. EFFECT ON THE ETHYLENE METABOLISM

In 'Conference' pears, the treatment with 1-MCP inhibited ethylene production during cold storage and shelf life, in all the experiments similar to findings by other researchers in other varieties of pears (Gamrasni et al., 2010; Yazdani et al., 2011; Li and Wang, 2009; Villalobos-Acuña et al., 2011b; MacLean et al., 2007; Yamane et al., 2007; Li et al., 2010). This inhibitory effect is the result of the action of 1-MCP on specific ethylene receptors located in the cell membranes, where 1-MCP competes with ethylene for the ethylene binding sites (Sisler et al., 1999). In some fruits (Owino et al., 2002; Defilippi et al., 2005; Dandekar et al., 2004; Zhang et al., 2006), and specifically in pears (Lelièvre et al., 1997; Gamrasni et al., 2010; Yazdani et al., 2011; Li and Wang, 2009; Villalobos-Acuña et al., 2011b; MacLean et al., 2007; Yamane et al., 2007; Li et al., 2010), the inhibition of ethylene production by an application of 1-MCP is parallel to a lower activity and/or expression of genes encoding the two key enzymes of the biosynthetic pathway of ethylene: ACS and ACO.

Compared to the control, the 1-MCP treatment induces a partial inhibition of the normal increase in the level of ACC and a complete inhibition of ACS and ACO activity during cold storage as well as an inability to convert ACC to MACC. These results are partly contradictory to those obtained in apple (Vilaplana et al., 2007) in which 1-MCP prevents the accumulation of ACC during exposure to low temperatures, which indicates that the levels of ACC during cold storage play an important role in ripening pear 'Conference'.

In the case of 'evergreen' fruit, ethylene production does not increase for the entire shelf life period. In contrast, fruit that resume the ripening process after removal from cold storage show a peak in ethylene production after several days at 20 °C. Apparently in these latter fruit 1-MCP delays ethylene production but does not completely block it nor the maturation process.

During shelf life treated fruit exhibit decreased levels of ACC; however, in 'evergreen' fruit, ACS and ACO and the ability to convert ACC to MACC remained inhibited throughout the shelf life period. Other authors have also observed this inhibition of the activity of ACS and ACO as well as the accumulation of their respective mRNA (Gamrasni et al., 2010; Yazdani et al., 2011; Li and Wang, 2009; Villalobos-Acuña et al., 2011b; MacLean et al., 2007; Yamane et al., 2007; Li et al., 2010). For treated fruit that do soften after several days at 20 °C, the ability to resume the ripening process seems to be

related to the intensity of ACS inhibition during cold storage and the subsequent activation of this enzyme during shelf life at 20 °C.

All this indicates that in 'Conference' pear, the effect of the 1-MCP treatment probably acts mainly through ACS, which apparently plays an important role in the resumption of softening. Moreover, the resumption of softening is determined during cold storage and is related to the degree of inhibition of ACS.

4. EFFECT ON THE ANTIOXIDANT METABOLISM

During the first days in cold storage, the activity of antioxidant enzymes decrease considerably in 'Conference' pears but this decrease does not continue for the rest of the time in cold storage. This suggests that low temperatures impose a temporary stress on the fruit. The 1-MCP treatment reduces this decline in activity for SOD and CAT in the beginning of cold storage while there is a higher activity of these enzymes throughout the remainder of the cold storage period. The same occurs with ascorbic acid, suggesting that treatment with 1-MCP potentially causes an increase in antioxidant potential that may be linked to a delay in the senescence processes.

Treatment with 1-MCP results inbetter maintenance of the antioxidant metabolism during shelf life at 20 °C, increasing the potential of the fruit to destroy especially active oxygen species (AOS) by CAT.

'Conference' pears exhibit an increase in electrolyte leakage after a few days of shelf life at 20 °C indicating higher membrane permeability. This increase coincides with fruit ripening and loss of firmness as could be expected since an increase in permeability is generally associated with the normal processes of softening and senescence of fruits (Marangoni et al., 1996). This explains why the increase in membrane permeability is not observed in fruit treated with 1-MCP and is probably related to the fact that these fruit were not ripening.

Changes in the environment can induce the accumulation of AOS and particularly of H_2O_2 in cells (Noctor and Foyer, 1998). During shelf life at 20 °C, none of the pears (treated or untreated) experienced an accumulation of H_2O_2, indicating that the 1-MCP treatment does not induce an oxidative stress.

However, the extent to which 1-MCP can have a positive effect on the antioxidant metabolism clearly depends on the maturity of the fruit at harvest. The process of ripening and senescence is generally associated with a low level of antioxidant enzymes; therefore treatment with 1-MCP can prolong shelf life, not only by inhibiting ethylene biosynthesis, but also through a better maintenance of the antioxidant systems of the fruit.

5. RECOVERY SYSTEMS FOR RIPENING BLOCKAGE

Treatment of 'Conference' pears with 1-MCP is complicated because of their high sensitivity to the treatment and because in most cases the pears lose their ability to ripen and remain firm and green. In our case, treatment with 600 nL L^{-1} of 1-MCP causes a blockage of ripening and ethylene production during shelf life at 20 °C. Therefore one of our goals was to find a way to allow treated fruit to recover their ethylene sensitivity and their ability to mature during their commercial life.

In other studies with 'Blanquilla' pear, heat treatments were effective to obtain adequate ripening during shelf life (Chiriboga et al., 2010). The efficacy of treatment in 'Blanquilla' was associated with the duration of a heat treatment at 15 °C (Chiriboga et al., 2010) and this could be due to the fact that the higher temperature could induce the synthesis of ethylene receptors, as was also observed in bananas (Jiang et al., 2002). Bai et al. (2006) also found that post storage heat treatments at temperatures of 15-20 °C during 10-20 days were effective in restarting ripening in 'Bartlett' pears while in 'd'Anjou' none of the treatments allowed the fruit to reach consumption ripeness. We found the same for 'Conference' pear, where none of the heat treatments worked, demonstrating an important cultivar effect.

Besides the heat treatment, exogenous ethylene was also investigated as a possible strategy (Chapter 4). Since applications with exogenous ethylene after 1-MCP treatment or after cold storage did not allow reverse the inhibition caused by 1-MCP (Argenta et al., 2003; Trinchero et al., 2004), in our experiments, exogenous ethylene and 1-MCP were applied simultaneously. This treatment was very effective and the effectiveness of this treatment likely lies in the timing of the application of the two compounds. When applied at the same time, the two treatments lead to a competition for binding sites on the receptors, such that ethylene occupied receptors can assist in the subsequent production of ethylene during shelf life and also in the generation of new receptors. With this treatment the treated pears ripen normally after storage, and in turn have a shelf life greater than untreated fruits.

The best combination was 600 nL L^{-1} 1-MCP + 300 nL L^{-1} C_2H_4 which allowed a considerable delay of the softening process without completely blocking it and allowed the fruit to reach consumption ripeness. In 2008, this combination allowed the recovery of the ability to ripen while still extending shelf life up to 12 days. Similar results were obtained in 2009 (Chapter 5) in optimal and late harvested fruit (7 and 10 days later), which ensures repeatability of the strategy in different years. This treatment provides no additional effect when applied in more mature fruit, which shows that treatment effectiveness does not depend on the maturity at the time of application.

The combination with a higher level of C_2H_4 (600 nL L^{-1}1-MCP + 600 nL L^{-1} C_2H_4) gave different results in the two years it was tested. Whereas in one year the treatment allowed the fruit to soften while maintaining a edible quality for 12 days of shelf life, in the second year the fruit ripened excessively.

In view of the results obtained, further research is necessary to know the specific effects that 1-MCP has on the different varieties of pear. The response to the 1-MCP treatment and possible recovery strategies depend on the cultivar and the harvest maturity, so the consideration of any of these strategies for a particular cultivar cannot be extrapolated to other cultivars.

It is important to consider that all these results were obtained experimentally and the implementation of these strategies needs to be tested on a commercial or semi commercial scale. The protocols need to be adjusted to the actual conditions in order to optimize the treatment.

For fruit that were harvested 7 to 10 days after the optimum harvest date, those that were treated with 1-MCP + exogenous ethylene have a similar ethylene metabolism as do those fruit treated only with 1-MCP. Nevertheless, late harvested fruit (10 days after the optimum) that received the combined treatment show a much higher increase in ACC compared with similar fruit treated only with 1-MCP, although this does not result in differences in softening between the two treatments. However, for the fruit harvested at the optimal harvest date, the differences in the ethylene metabolism between the two treatments were lateron reflected in important differences in their ability to mature. This result again emphasizes the importance of the harvest maturity at harvest also in case of the combined treatment approach. A new set of indices to determine the optimal harvest date for 1-MCP treatment appears to be necessary in case of a commercial application of this new technology in 'Conference' pears.

References

Argenta, L. C.; Fan, X. T.; Mattheis, J. P. 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit. *Journal of Agricultural and Food Chemistry,* 51, 3858-3864.

Bai, J.; Mattheis, J. P.; Reed, N. 2006. Re-initiating softening ability of 1-methylcyclopropene-treated 'Bartlett' and 'd'Anjou' pears after regular air or controlled atmosphere storage. *Journal of Horticultural Science & Biotechnology,* 81, 959-964.

Blankenship, S. M.; Dole, J. M. 2003. 1-methylcyclopropene: a review. *Postharvest Biology and Technology,* 28, 1-25.

Calvo, G. 2003. Effect of 1-methylcyclopropene (1-MCP) on pear maturity and quality. *Acta Horticulturae,* 628, 203-211.

Calvo, G.; Sozzi, G. O. 2009. Effectiveness of 1-MCP treatments on 'Bartlett' pears as influenced by the cooling method and the bin material. 51, 49-55.

Chen, P. M.; Spotts, R. 2005. Changes in ripening behaviors of 1-MCP treated d'Anjou pears after storage. *International Journal of Fruit Science,* 5, 3-18.

Chiriboga, M. A.; Schotsmans, W. C.; Larrigaudière, C. 2010. Comparative study of techniques to restore the ripening process in 1-MCP treated 'Blanquilla' and 'Conference' pears. *Acta Horticulturae,* 858, 149-154.

Dandekar, A. M.; Teo, G.; Defilippi, B. G.; Uratsu, S. L.; Passey, A. J.; Kader, A. A.; Stow, J. R.; Colgan, R. J.; James, D. J. 2004. Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. *Transgenic Research,* 13, 373-384.

Defilippi, B. G.; Dandekar, A. M.; Kader, A. A. 2005. Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. *Journal of Agricultural and Food Chemistry,* 53, 3133-3141.

Ekman, J. H.; Clayton, M.; Biasi, W. V.; Mitcham, E. J. 2004. Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears. *Postharvest Biology and Technology,* 31, 127-136.

Gamrasni, D.; Ben-Arie, R.; Goldway, M. 2010. 1-methylcyclopropene (1-MCP) application to Spadona pears at different stages of ripening to maximize fruit quality after storage. *Postharvest Biology and Technology,* 58, 104-112.

Hershkovitz, V.; Saguy, S. I.; Pesis, E. 2005. Postharvest application of 1-MCP to improve the quality of various avocado cultivars. *Postharvest Biology and Technology,* 37, 252-264.

Jiang, Y.; Joyce, D. C.; Macnish, A. J. 2002. Softening response of banana fruit treated with 1-methylcyclopropene to high temperature exposure. *Plant Growth Regulation,* 36, 7-11.

Lelièvre, J. M.; Tichit, L.; Dao, P.; Fillion, L.; Nam, Y. W.; Pech, J. C.; Latché, A. 1997. Effects of chilling on the expression of ethylene biosynthetic genes in Passe-Crassane pear (*Pyrus communis* L.) fruits. *Plant Molecular Biology,* 33, 847-855.

Li, Z. Q.; Qiao, Y. S.; Tong, Z. G.; Zhou, J.; Zhang, Z. 2010. Effect of ethylene and 1-MCP on post-harvest physiology and on expression of the ethylene receptor genes PpETR3 and PpERS2 in pear (Pyrus pyrifolia Nakai 'Kikusui') fruit. *Journal of Horticultural Science & Biotechnology,* 85, 71-77.

Li, Z. Q.; Wang, L. J. 2009. Effect of 1-methylcyclopropene on Ripening and Superficial Scald of Japanese Pear (Pyrus pyrifolia Nakai, cv. Akemizu) Fruit at Two Temperatures. *Food Science and Technology Research,* 15, 483-490.

MacLean, D. D.; Murr, D. P.; DeEll, J. R.; Mackay, A. B.; Kupferman, E. M. 2007. Inhibition of PAL, CHS, and ERS1 in 'Red d'Anjou' pear (*Pyrus communis* L.) by 1-MCP. *Postharvest Biology and Technology,* 45, 46-55.

Marangoni, A. G.; Palma, T.; Stanley, D. W. 1996. Membrane effects in postharvest physiology. *Postharvest Biology and Technology,* 7, 193-217.

Noctor, G.; Foyer, C. H. 1998. Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology,* 49, 249-279.

Owino, W. O.; Nakano, R.; Kubo, Y.; Inaba, A. 2002. Differential regulation of genes encoding ethylene biosynthesis enzymes and ethylene response sensor ortholog during ripening and in response to wounding in avocados. *Journal of the American Society for Horticultural Science,* 127, 520-527.

Sisler, E. C.; Serek, M.; Dupille, E.; Goren, R. 1999. Inhibition of ethylene responses by 1-methylcyclopropene and 3-methylcyclopropene. *Plant Growth Regulation,* 27, 105-111.

Trinchero, G. D.; Sozzi, G. O.; Covatta, F.; Fraschina, A. A. 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of `Bartlett` pears. *Postharvest Biology and Technology,* 32, 193-204.

Vilaplana, R.; Soria, Y.; Valentines, M. C.; Larrigaudière, C. 2007. Specific response of apple skin and pulp tissues to cold stress and 1-MCP treatment. *Postharvest Biology and Technology,* 43, 215-220.

Villalobos-Acuña, M.; Biasi, W. V.; Mitcham, E. J.; Holcroft, D. 2011a. Fruit temperature and ethylene modulate 1-MCP response in 'Bartlett' pears. *Postharvest Biology and Technology,* 60, 17-23.

Villalobos-Acuña, M. G.; Biasi, W. V.; Flores, S.; Jiang, C. Z.; Reid, M. S.; Willits, N. H.; Mitcham, E. J. 2011b. Effect of maturity and cold storage on ethylene biosynthesis and ripening in 'Bartlett' pears treated after harvest with 1-MCP. *Postharvest Biology and Technology,* 59, 1-9.

Yamane, M.; Abe, D.; Yasui, S.; Yokotani, N.; Kimata, W.; Ushijima, K.; Nakano, R.; Kubo, Y.; Inaba, A. 2007. Differential expression of ethylene biosynthetic genes in climacteric and non-climacteric Chinese pear fruit. *Postharvest Biology and Technology,* 44, 220-227.

Yazdani, N.; Arzani, K.; Mostofi, Y.; Shekarchi, M. 2011. Alpha-Farnesene and antioxidative enzyme systems in Asian pear (Pyrus serotina Rehd.) fruit. *Postharvest Biology and Technology,* 59, 227-231.

Zhang, M. J.; Jiang, Y. M.; Jiang, W. B.; Liu, X. J. 2006. Regulation of ethylene synthesis of harvested banana fruit by 1-methylcyclopropene. *Food Technology and Biotechnology,* 44, 111-115.

CONCLUSIONES GENERALES – GENERAL CONCLUSIONS

CONCLUSIONES GENERALES

En base a los resultados obtenidos en la presente Tesis y en relación a los objetivos planteados en la misma, se pueden extraer las siguientes conclusiones:

- En peras 'Conference' el tratamiento 1-MCP reduce la producción de etileno, permite una retención del color verde y reduce la perdida de firmeza respecto a los frutos no tratados.
- El efecto del tratamiento sobre la firmeza depende de la dosis. El tratamiento con 300 nL L^{-1} de 1-MCP en peras 'Conference' da resultados variables sobre la inhibición de la maduración. El tratamiento 1-MCP con 600 nL L^{-1} siempre bloquea el proceso de maduración ('evergreen') obteniendo frutos excesivamente firmes.
- La susceptibilidad de las peras 'Conference' al bloqueo de la maduración está ligada principalmente al estado de madurez del fruto en el momento de la recolección. Frutos recolectados con madurez más avanzada son menos susceptibles al 'evergreen'. Una cosecha más tardía (más de 10 días) posibilita la aplicación del tratamiento 1-MCP y evita el bloqueo de la maduración.
- Ninguno de los parámetros de calidad por sí solos podrían indicar la sensibilidad de los frutos al 'evergreen', aunque la producción de etileno a la cosecha parece ser el mejor indicador. Sería necesario integrar la producción de etileno con otros parámetros de calidad (por ejemplo el Indice de Streif) para definir el mejor estado fisiológico del fruto para la aplicación con 1-MCP.
- Las pautas de maduración durante la vida útil y la eficacia del tratamiento con 1-MCP vienen determinados por los eventos fisiológicos que tienen lugar durante la conservación al frío, las cuales a su vez están influenciadas por la madurez de la fruta y en menor medida por el campo.
- En principio el 'evergreen' parece estar más relacionado con un cambio en la capacidad de producción de etileno y en el metabolismo del ACC que con cambios en los procesos oxidativos durante la conservación.
- Durante la vida útil, el tratamiento con 1-MCP en peras 'Conference' tiene un papel importante en la mejora de la capacidad de la fruta para eliminar especies activas de oxígeno mediante el aumento de la actividad antioxidante enzimático y no enzimático. Sin embargo, la intensidad de esta mejora depende claramente de la madurez de la fruta a la cosecha y de factores de campo que quedan aún por determinar.

- El tratamiento combinado con 1-MCP (600 nL L^{-1}) + etileno exógeno (300 nL L^{-1}) parece ser una buena opción para prevenir el 'evergreen' en peras 'Conference'. El tratamiento extiende la vida comercial a 20 °C y permite alcanzar la calidad de consumo. Se precisan sin embargo, otras optimizaciones para ajustar los protocolos de aplicación a las condiciones comerciales reales.

GENERAL CONCLUSIONS

Based on the results obtained in this thesis and in relation to the objectives stated herein, we can come to the following conclusions:

- In 'Conference' pear, the 1-MCP treatment reduces ethylene production, allows the retention of the green color and reduces the softening rate compared to untreated fruit.
- The effect of the treatment on firmness depends on the dose. Treatment with 300 nL L^{-1} of 1-MCP gives variable results for the inhibition of ripening in 'Conference' pears. The treatment with 600 nL L^{-1} of 1-MCP always blocks ripening resulting in too firm fruit.
- The susceptibility of 'Conference' pear to 'evergreen' is linked mainly to the harvest maturity of the fruit. More mature fruit are less susceptible to become 'evergreen'. A very late harvest (more than 10 days after the current commercial optimal date) is preferred for the application of 1-MCP and to avoid blockage.
- None of the individual quality parameters are able to indicate the sensitivity of fruit to become 'evergreen', but ethylene production at harvest appears to be the closest. Combining the ethylene production with other quality parameters (for instance the Streif index) could help determine the best physiological state for 1−MCP application.
- The ripening pattern during shelf life and the effectiveness of the 1-MCP treatment are determined by the physiological events that occur during cold storage, which in turn are influenced by the harvest maturity at harvest and to a lesser extent by the orchard.
- The 'evergreen' behavior seems to be more related to a change in the capacity of the fruit to produce ethylene and in the ACC metabolism than with changes in the oxidative processes during storage.
- During shelf life, the treatment with 1−MCP improves the capacity of the fruit to remove reactive oxygen species by increasing the enzymatic and non-enzymatic antioxidant activity. However, the intensity of this improvement clearly depends on the harvest maturity as well as on orchard factors that remain to be determined.
- The combined treatment of 1-MCP (600 nL L^{-1}) + exogenous ethylene (300 nL L^{-1}) allows for a considerable delay in the softening process while at the same time permitting the restoration of the ripening process during shelf life following cold storage in normal as well as controlled atmosphere. This combination appears to be a good option to prevent 'evergreen' in 'Conference' pears. The combined treatment extends the shelf life of the pears at 20°C and allows them to reach eating quality. Nevertheless, more optimization is needed before application in commercial conditions is possible.

Publicaciones

A lo largo de los años de realización de la Tesis, se han elaborado presentaciones de los resultados preliminares que se han expuesto en los siguientes congresos:

(1) IX Simposio Nacional y VI Ibérico sobre Maduración y Postcosecha
Restauración de la maduración en peras 'Blanquilla' y 'Conference' tratadas con 1-MCP y sometidas a un tratamiento térmico (Poster)
23-26 Septiembre 2008, Zaragoza, España.
Avances en maduración y posrecolección de frutas y hortalizas, 387-395.

(2) PostHarvest Unlimited
Comparative study of techniques to restore the ripening process in 1-MCP treated 'Blanquilla' and 'Conference' pears (Presentación oral)
4-7 November 2008, Potsdam, Germany
Acta Horticulturae 858, 149-154

(3) 10th Controlled and Modified Atmosphere Research Conference
Physiological aspects of the ripening blockage in 'Conference' pears treated with 1-MCP (Poster)
4-7 April 2009, Antalya, Turkey

(4) 8th International Symposium on the Plant Hormone Ethylene
Ethylene metabolism does not entirely explain softening during storage of 1-MCP treated 'Conference' pears (Presentación oral)
21-24 June 2009, Ithaca, NY, USA.

(5) 28th International Horticultural Congress,
Cold treatments does not prevent blockage of ripening in Conference pears treated with 1-MCP (Poster)
22-27 August 2010, Lisboa, Portugal

(6) X Simposio Nacional y VII Ibérico sobre Maduración y Postcosecha de Frutas y Hortalizas
Effect of simultaneous 1-MCP and exogenous ethylene treatment on fruit ripening and ACC metabolism in 'Conference' pears (Poster)
1-4 Octubre 2012, Lleida, España

Se han realizado también presentaciones relacionados con otros temas:

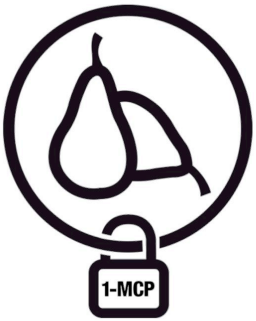
(1) COST action 924
Specific effects of 1-MCP treatment on physiological disorders in 'Blanquilla' pears (Poster)
3-5 Mayo 2007, Bolonia, Italia.
Novel approaches for the control of postharvest diseases and disorders, 284-289.

(2) International Conference on Ripening Regulation and Postharvest Fruit Quality
Biochemical changes in 1-MCP treated skin tissue during cold storage, relationship with physiological disorders (Poster)
12-13 Noviembre 2007, Weingarten, Alemania
Acta Horticulturae 796, 119-123.

(3) 6th International Postharvest Symposium,
Temperature dependent quality changes in peach fruit during storage determined using destructive and non-destructive (acoustic, VIS) techniques (Poster)
08-12 April 2009, Antalya, Turkey

(4) 8th International Symposium on the Plant Hormone Ethylene
Temperature dependent ethylene metabolism during storage of 'Rich Lady' peach (Presentación oral)
21-24 June 2009, Ithaca, NY, USA
Acta Horticulturae (En Prensa)

(5) 7th International Postharvest Symposium
Advanced maturity at harvest enhances oxidative stress of 'Blanquilla' pears during cold storage (Poster)
25-29 June 2012, Kuala Lumpur, Malaysia



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