How to prevent ripening blockage in 1-MCP-treated ‘Conference’ pears

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Abstract

BACKGROUND: Some European pear varieties treated with 1-methylcyclopropene (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 also to be the case in ‘Conference’ pears. To reverse the blockage of ripening 1-MCP treatments combined with external exogenous ethylene were tested.

RESULTS: 1-MCP treatment of ‘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 behaviour of fruit receiving a lower dose (300 nL L\(^{-1}\)) depended on the year, and this did not depend on maturity at harvest or on 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.

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Keywords: Conference; 1-methylcyclopropene; evergreen; ripening; ethylene

INTRODUCTION

In the majority of European pears, the optimal quality for consumption is characterized by a buttery texture, an appropriate colour and a characteristic taste associated with the content of sugars and acids and with aroma production.1–3 As a climacteric fruit, the ripening process of pears is regulated by ethylene, and inhibition of the biosynthesis of this hormone or its action slows down ripening and increases shelf life.1

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 transduction pathway.4 Postharvest application of 1-MCP delays or decreases softening in ‘Barlett’,5–7 ‘Williams’,8,9 ‘La France’,10,11 ‘d’Anjou’1,5 and ‘Passe-Crassane’.12 Additionally, 1-MCP delays or decreases internal browning, significantly affects the ripening behaviour, storage scald, respiration rate, ethylene production, and 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase activity in pear fruit.1,5–7

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.13 However, the effect of ripening was not totally uniform, with a small percentage of treated fruit reaching their climacteric peak, losing green colour and softening prematurely.14 Repeating the 1-MCP treatment at low doses (25 and 50 nL L\(^{-1}\)) during storage did not resolve this problem.15 With higher doses (300 nL L\(^{-1}\)), ripening can be delayed more effectively,16 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 behaviour). Similar problems have occurred in other European pear varieties, such as ‘d’Anjou’,1 ‘Barlett’,6,7,17 and ‘Blanquilla’.16

To avoid or reverse the evergreen behaviour due to 1-MCP treatment, several strategies have been investigated. One has been the application of a post-storage heat treatment in ‘Blanquilla’ and ‘Conference’.16 In ‘Blanquilla’, the heat treatments restored the ripening process in 1-MCP-treated pears, with efficacy increasing with days at 15°C. Conversely, in ‘Conference’, the evergreen behaviour was not reversed with the thermal treatments tested and the 1-MCP-treated fruit remained green and 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.18 In case...
of winter pears, like ‘d’Anjou’ and ‘Bartlett’, exogenous ethylene has proven successful in inducing ripening, with the dose needed depending on treatment temperature and the duration of storage at chilling temperatures. With this in mind, several authors have applied exogenous ethylene to 1-MCP-treated pears, leading to distinct results. Chen et al. found no reversal of the effects of 1-MCP in ‘Bartlett’ pears after exogenous ethylene application; neither did Calvo, who also studied ‘d’Anjou’ and ‘Packham’s Triumph’ pears, with the same conclusion. Conversely, Manriquez et al. 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.

MATERIALS AND METHODS

**Fruit material**

‘Conference’ pears (Pyrus communis) were harvested at the optimal commercial harvest date for long-term storage from two different orchards. Fruits were selected based on size and absence of defects, and stored at −0.5 °C and 92% relative humidity for 3 months in regular atmosphere (RA) or 6 months in controlled atmosphere (CA; 2% O₂ +1% CO₂) conditions according to standard commercial practice.

**Experimental setup**

**Experiment 1: effects of 1-MCP treatment on fruit quality**

This experiment was conducted over three consecutive years (2007–08, 2008–09, 2009–10) using different doses of 1-MCP. In the first two years, the pears were treated with 0, 300 or 600 nL L⁻¹ 1-MCP and in the third year only the treatments with 0 and 300 nL L⁻¹ 1-MCP were repeated. Immediately after harvest, fruits were stored at −0.5 °C. After 1 day of storage, fruits were treated with 1-MCP using Smartfresh™ (AgroFresh Inc.) according to the manufacturer’s recommendations. The fruit was covered with a 1 m³ plastic bag and treatment mixture was placed inside the bag. The reaction mixture contained 1-MCP as a powder (0.5 g for 300 nL L⁻¹ treatment and 1 g for 600 nL L⁻¹) and water at 30 °C (ratio of 1-MCP : water = 1 : 5). After 24 h, 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 combined treatments at the following doses: 300 nL L⁻¹ 1-MCP + 300 nL L⁻¹ C₂H₄ and 600 nL L⁻¹ 1-MCP + 600 nL L⁻¹ C₂H₄. In the second year, 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 C₂H₄ and 600 nL L⁻¹ 1-MCP + 600 nL L⁻¹ C₂H₄.

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 treatment) was placed 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 a 1 m³ plastic bag containing the boxes with fruit, which was then sealed. Gaseous C₂H₄ was withdrawn with a syringe (0.3 mL for the 300 nL L⁻¹ and 0.6 mL for the 600 nL L⁻¹ treatment) 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.

**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* colour space. Hue angle was calculated using the formula arctg b*/a*.

Ethylene production was measured 1 day after harvest in an acclimatized chamber at 20 °C. On the day of harvest, three replicates of two 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 flame ionization detector (model 6890, Agilent Technologies, Wilmington, Germany) and an alumina column 80/100 (2 m × 3 mm) (Teknokroma, Barcelona, Spain).

**Statistical analysis**

Data analysis was performed using the Statistical Analysis System (SAS version 9.1, 1992, SAS Institute, Inc., Cary, NC, USA). 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 least significance difference (LSD) test at P < 0.05.

**RESULTS**

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

**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. 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.

<table>
<thead>
<tr>
<th>Year</th>
<th>Firmness (N)</th>
<th>Background colour (hue angle, °)</th>
<th>Ethylene production (μL kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>64.0 ± 5.9</td>
<td>103.0 ± 7.3</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>2008</td>
<td>72.2 ± 4.3</td>
<td>101.8 ± 2.1</td>
<td>0.11 ± 0.07</td>
</tr>
<tr>
<td>2009</td>
<td>51.7 ± 6.6</td>
<td>115.7 ± 2.3</td>
<td>0.29 ± 0.05</td>
</tr>
</tbody>
</table>

**Table 1. Maturity parameters at harvest of ‘Conference’ pears harvested in three experimental years. Firmness and background colour were assessed immediately after harvest and ethylene production after 1 day at 20 °C. Data represent the mean (±SD 95%)**

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

In 2007 and after 3 months of RA (Fig. 1(A)) or 6 months of CA (Fig. 1(B)) storage at \(-0.5\ ^\circ\ C\), the fruit had softened somewhat (4–7 N) but there were no significant differences in firmness between the control and 1-MCP-treated fruit. Similar behaviour was found in all experimental years, with slightly more softening (13–16 N) in the second experimental year (2008) in both RA (Fig. 1(C)) and CA (Fig. 1(D)) 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 quickly and reached values lower than 9.3 N and 10.2 N within 7 days at 20 °C after RA (Fig. 1(A)) and CA (Fig. 1(B)) storage, respectively. In contrast, no

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**Figure 1.** Softening during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life at 20 °C in three 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). Vertical bars represent the 95% confidence interval surrounding the mean.
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 °C, with values over 50 N under 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 °C after RA storage and within 12 days at 20 °C 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 quickly and had attained eating quality after 7 days at 20 °C following RA (Fig. 1(C)) and CA (Fig. 1(D)) 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 to the previous year when the fruit treated with 300 nL L\(^{-1}\) 1-MCP did soften during shelf life.

In the third year, as in both previous years, control fruit exhibited a significant loss in firmness to 7.6 N for RA (Fig. 1(E)) and 10.3 N for CA (Fig. 1(F)) stored fruit after 7 days at 20 °C. Treatment with 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 did appear to start softening.

Hue angle also slightly decreased during storage in RA and CA (Fig. 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 colour was similar in both years, whereas CA-stored fruit behaved slightly differently in both years. In 2007, 1-MCP-treated fruit did not lose green colour whereas in 2008 the hue angle decreased for all three treatments.

Comparing the evolution of colour in the first and second year (Fig. 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, 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.

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

In the first year (Fig. 3), no differences were found in softening between the treatments during storage. Although directly after removal from storage (0 days at 20 °C) all the treatments started from similar fruit firmness of around 57 N, significant differences in firmness were observed after 7 days at 20 °C. As mentioned before, the control fruit (1-MCP 0) softened quickly and reached their eating quality within 7 days at 20 °C. The fruit treated with 600 nL L\(^{-1}\) 1-MCP showed no significant loss of firmness, whereas the fruit treated with 300 nL L\(^{-1}\) 1-MCP and the combined treatments

**Figure 2.** Skin colour (hue) during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life at 20 °C in two 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). Vertical bars represent the 95% confidence interval surrounding the mean.
Ripening recovery in 1-MCP-treated pear

Figure 3. Softening during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life at 20 °C 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\(_2\)H\(_4\) (1-MCP 300 + ETH 300), fruit treated with 600 nL L\(^{-1}\) 1-MCP and 600 nL L\(^{-1}\) C\(_2\)H\(_4\) (1-MCP 600 + ETH 600). Vertical bars represent the 95% confidence interval surrounding the mean.

Figure 4. Softening during cold storage in regular atmosphere (RA) or controlled atmosphere (CA) followed by shelf life at 20 °C 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 300 nL L\(^{-1}\) 1-MCP and 300 nL L\(^{-1}\) C\(_2\)H\(_4\) (1-MCP 300 + ETH 300), fruit treated with 600 nL L\(^{-1}\) 1-MCP and 600 nL L\(^{-1}\) C\(_2\)H\(_4\) (1-MCP 600 + ETH 600). Vertical bars represent the 95% confidence interval surrounding the mean.

exhibited significant firmness loss even though firmness remained higher compared with the control fruit. Fruit treated with 300 nL L\(^{-1}\) 1-MCP with or without 300 nL L\(^{-1}\) C\(_2\)H\(_4\) 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, 5 days later, fruit had softened beyond eating quality. Conversely, the fruit treated with 600 nL L\(^{-1}\) 1-MCP + 600 nL L\(^{-1}\) C\(_2\)H\(_4\) had not reached eating quality after 7 days of shelf life, and took five more days to reach this eating quality.

In the case of CA, softening of treated fruit was slower compared with RA and only fruit treated with 600 nL L\(^{-1}\) 1-MCP with and without 600 nL L\(^{-1}\) C\(_2\)H\(_4\) behaved exactly the same under both storage conditions. The fruit treated with 300 nL L\(^{-1}\) 1-MCP and 300 nL L\(^{-1}\) C\(_2\)H\(_4\) still reached eating quality after 7 days of shelf life, comparable to RA stored fruit, but fruit treated with 300 nL L\(^{-1}\) 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\(^{-1}\) 1-MCP + 600 nL L\(^{-1}\) C\(_2\)H\(_4\).

In the second year (Fig. 4), the treatment with 300 nL L\(^{-1}\) 1-MCP and 300 nL L\(^{-1}\) C\(_2\)H\(_4\) was replaced by a treatment with 600 nL L\(^{-1}\) 1-MCP and 300 nL L\(^{-1}\) C\(_2\)H\(_4\). 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 fruit treated with 1-MCP alone and fruit treated with 1-MCP and ethylene became more apparent. In this year the fruit stored in RA and in CA showed the same behaviour. Softening of the pears was markedly inhibited in the fruit treated with only 1-MCP.
MCP, with commercially negligible loss of firmness for both doses and under both storage conditions. The fruit treated with 600 nL L\(^{-1}\) of 1-MCP + 600 nL L\(^{-1}\) C\(_2\)H\(_4\) 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\(^{-1}\) 1-MCP and 300 nL L\(^{-1}\) C\(_2\)H\(_4\) also softened, but more slowly compared with the control fruit, and was still within the eating quality window after 7 days at 20 °C and for CA fruit even after 12 days, whereas the fruit treated with 600 nL L\(^{-1}\) 1-MCP and 600 nL L\(^{-1}\) C\(_2\)H\(_4\) was already below the 15 N limit at that point.

Differences in colour could be noted between the 2 years. In the first year (Fig. 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\(_2\)H\(_4\) doses used. After CA storage, the control fruit had also lost significantly more of their green colour. Additionally, significant differences were found between the treatment with only 600 nL L\(^{-1}\) 1-MCP and the other treatments, where the former remained greener. In the second year (Fig. 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.

**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\(^{-1}\) 1-MCP) gave the same result, with a complete blockage of softening in both years, whereas the lower concentration (300 nL L\(^{-1}\) 1-MCP) resulted in slower softening (as desired) in the first year and no softening in the second and third. This blockage is not paralleled by a similar blockage in the background colour changes, which were slowed down but not completely stopped by 1-MCP, indicating a differential effect of 1-MCP on different ripening indicators (firmness, colour). It is well known that the maturity at treatment strongly influences the effect of 1-MCP treatment, but in this case a difference in maturity could not explain this discrepancy. At harvest, the maturity parameters (firmness, colour and ethylene production) were different between
the years, the larger difference being 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 is not an appropriate indicator for reliable prediction of the response of pears to 1-MCP.

Therefore, evergreen behaviour does not seem to be linked exclusively with maturity at harvest and is likely due to another trigger process in this cultivar. Evergreen behaviour 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,26 the new synthesis of ethylene cannot be the key to reversing evergreen behaviour – not until new receptors are generated. Plant tissues have been shown to vary widely in their ability to regenerate new receptors.4 The difference in ripening behaviour in 1-MCP-treated ‘Conference’ pears might also be related to the abundance of ethylene receptors at the moment of 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 ethylene receptor proteins), as well as related to changes in ethylene receptor turnover during storage.29

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 ethylene had no effect on softening.7 In ‘d’Anjou’ pears treated with 1-MCP, exogenous ethylene had some effect but only caused a minor increase in softening (maximum difference 8.9N).1 In the results presented here, the differences between single 1-MCP and combined 1-MCP + C2H4 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, we have to consider that these results relate to three different cultivars that react differently to 1-MCP for this discrepancy. First, we have to consider that these results after 7 days was 27 N. There are several possible explanations.

These results show the potential interest of the combined treatment of 1-MCP and ethylene in ‘Conference’ pears to prevent ‘evergreen’ behaviour and allow the fruit treated with 1-MCP to recover their ability to ripen. On one hand, although it seems that 600 nL L−1 of 1-MCP and 300 nL L−1 of C2H4 combination is the most promising treatment, this needs to be repeated and verified. On the other hand, the combination of 600 nL L−1 1-MCP and 600 nL L−1 C2H4 which was repeated over the 2 years did not give the same results. This calls for more research.

CONCLUSIONS

The big challenge for the successful application of 1-MCP in ‘Conference’ pears is to delay ripening, maintaining the firmness and the green colour without totally blocking this process. Considering our results, the combined treatment appears to be an interesting tool to counteract evergreen behaviour 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.

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REFERENCES


18 Reid MS, Ethylene in postharvest technology, in Postharvest Technology of Horticultural Crops, ed. by Kader AA. Division of Agriculture and Natural Resources, University of California, Oakland, CA, pp. 95–108 (1992).


