Original article

The effects of 1-methylcyclopropene on peach fruit (Prunus persica L. cv. Jiubao) ripening and disease resistance

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Summary

In order to learn how 1-methylcyclopropene (1-MCP) affects ripening and disease-resistance of peach fruit (Prunus persica L. cv. Jiubao) after harvest, they were treated with 1-MCP and some were inoculated with Penicillium expansum. Treating peach fruit with 0.2 μL L⁻¹ of 1-MCP at 22 °C for 24 h effectively slowed the decline in fruit firmness. The minimal concentration of 1-MCP able to inhibit fruit softening was 0.6 μL L⁻¹. Changes in other parameters related to peach ripening, such as content of soluble solids, total soluble sugar, titratable acidity, soluble pectin and ethylene production were also significantly reduced or delayed by 1-MCP. Repeated treatment of peach with 1-MCP resulted in more effective inhibition of ripening. Post-harvest decay of peach fruit was reduced by treatment with 1-MCP and disease progress in fruit inoculated with P. expansum was reduced. The activities of phenylalanine ammonialyase, polyphenoloxidase and peroxidase in the inoculated fruit were also enhanced by 1-MCP.

Keywords: Disease-resistance, ethylene, firmness, post-harvest decay, soluble pectin, titratable acidity.

Introduction

Peaches are perishable fruits that ripen and senesce rapidly at ambient temperatures and require careful and rapid handling after harvest to avoid serious wastage.

Peach is a typical climacteric fruit that exhibits a dramatic increase in ethylene production and respiration rate associated with changes in texture and flavour during ripening. Its ripening can be initiated by either the natural evolution of endogenous ethylene or the commercial application of exogenous ethylene. 1-Methylcyclopropene (1-MCP) is thought to inhibit ripening by irreversibly occupying ethylene-binding sites, thus ethylene is unable to bind and elicit subsequent signal transduction and translation (Serek et al., 1994).

Fruit treated with 1-MCP may remain insensitive to ethylene for some time (Sisler & Serek, 1997). The effects of 1-MCP on inhibiting fruit ripening have been widely observed on various fruits. However, information on how 1-MCP may affect ripening of peach fruit is still lacking.

Decay is an important factor which limits the storage life of peach after harvest and results in appreciable losses at the wholesaler, retailer, and consumer levels (Lill et al., 1989). The control of post-harvest diseases of fruits and vegetables is mostly dependent on controlled atmosphere storage, refrigeration and fungicides (Kader, 1992). Of these, fungicides are the most effective tools to reduce post-harvest decay and extend the shelf-life of produce. However, fungicides are becoming less effective because of the development of pathogen resistance, and there is consumer concerns about possible human health risks associated with the use of fungicides (Charles et al., 1994). The use of
induced resistance in harvested crops holds promise as a new technology for the control of post-harvest diseases. Both physical and biological agents can elicit resistance responses in harvested fruits and vegetables (Wisniewski et al., 1991).

To learn how 1-MCP may affect ripening and decay of peach fruit after harvest, peach fruit were treated with 1-MCP and inoculated with 

**Penicillium expansum**.

**Materials and methods**

**Fruit and treatments**

Peach fruit (*Prunus persica* L. cv. Jiubao) were harvested from a local orchard at the earliest stage of commercial ripening (green mature). 1-MCP was released from a commercial powdered formulation (EthylBloc®, Rohm and Haas China, Inc., Beijing, China) as described in a previous publication (Jiang et al., 2004). The fruit (sixty pieces in each treatment) were treated with 0.5 μL L⁻¹ 1-MCP (or at the concentrations described in the results) in sealed 250-liter plastic-chambers at 22 ± 1 °C, 80–90% RH for 24 h without light. Thereafter, the fruit were stored in air under the same conditions. Control fruit were subjected to the same conditions without exposure to 1-MCP.

**Inoculation and measurement of disease progress**

*Penicillium expansum* was isolated from decayed peach fruit and maintained on potato dextrose agar (PDA). A conidial suspension of the pathogen was prepared by flooding the 14-day-old culture dishes with sterile distilled water containing 0.05% Tween-80. The spore suspension was adjusted to 1 x 10⁷ spore mL⁻¹ with sterile distilled water using a haemocytometer.

The fruit treated with 1-MCP and untreated peach fruit (sixty pieces each) were sterilized with 70% ethanol, then wounded with a syringe at three points (5 mm deep x 4 mm wide) on the equator of each fruit. Twenty microlitres of the conidial suspension was injected into each wound site. The fruit were then incubated at a constant 22 °C under high humidity in enclosed plastic trays containing water. For evaluation of disease progress in the inoculated fruit, lesion diameter and disease-incidence were recorded daily. When the visible rot zone outside the wounded area was more than 1 mm wide, the fruit was counted as affected fruit.

**Assay of ethylene production**

Five peach fruit (about 800 g) were sealed in an 8 L vacuum desiccator with a rubber stopper and incubated for 1 h at 22 °C. A 1 mL gas sample was then withdrawn from the desiccator. Ethylene concentration in the gas sample was measured by injecting into a gas chromatograph (CP-3800, Walnut Greek, CA, USA) equipped with an activated alumina column and a flame ionization detector according to the method of Jiang et al. (2004).

**Measurements of fruit firmness, total soluble sugar concentration, titratable acidity, soluble solids, soluble pectin and vitamin C concentration**

Firmness was measured on the equatorial region of fruit using a firmness tester (Gaoke Inc Jiangshu, China) with a flat round 8 mm diameter head.

Five grams of the flesh tissue was well homogenized with 5 mL distilled water and centrifuged at 4 °C for 10 min at 13 000 x g and the supernatant was collected. The total soluble sugar concentration (SSC) in the supernatant was determined according to the method of Dubois et al. (1956) with glucose as the standard. For titratable acidity (TA) analysis, the supernatant was titrated with 0.1 M NaOH; soluble solids in peach juice were measured using a digital refractometer (Tongfang Inc. Shanghai, China); and the soluble pectin concentration (SPC) was measured by colorimetry with Carbazole-Vitriol (Jiang et al., 2004).

For vitamin C analysis, 2 g of tissue with 20 mL of 6% metaphoric acid in 2 M acetic acid was thoroughly homogenized and centrifuged at 4 °C for 10 min at 13 000 x g. The vitamin C concentration in the supernatant was determined by the dinitrophenylhydrazine method of Terada et al. (1978).

**Assays for the enzymatic activities**

To analyse phenylalanine ammonialyase (PAL), polyphenoloxidase (PPO) and peroxidase (POD) activities, sample tissue was collected from either the pulp around the macerated tissue or the
wound site itself. Five grams of the sample tissue was thoroughly homogenized with 10 mL extraction buffer (pH 7.0, 0.05 M phosphate buffer + 10% (w/w) polyvinypolyvidone for PAL; pH 6.4, 0.2 M phosphate buffer for PPO and POD) and centrifuged at 13,000 × g, 4 °C for 20 min and the supernatant was collected.

For the PAL assay, 1 mL of the extract was mixed with 1 mL 0.02 M L-phenylalanine and 3 mL of phosphate buffer (0.05 M, pH 7.0) and then incubated at 24 °C for 1 h. Afterwards, the absorbance at 290 nm of the reaction solution was measured with an ultraviolet spectrophotometer (Shimadzu UV-2100, Shimadzu, Kyoto, Japan). The PAL activity was expressed as UI290, per UI290 = 0.01 ΔA290 mg protein−1 min−1. For the POD assay, 3 mL of the extract was mixed with 2 mL 0.1% guaiacol and 1 mL 2% H2O2 and was incubated at 22 °C for 2 min. Afterwards, the absorbance at 460 nm of the reaction solution was measured. The POD activity was expressed as UI460, per UI460 = 0.01 ΔA460 mg protein−1 min−1. For the PPO assay, 1.5 mL of the extract was mixed with 3 mL of phosphate buffer (0.05 M, pH 7.0) and 1 mL of 0.5 M catechol and was incubated at 24 °C for 30 min. Afterwards, the absorbance of the reaction solution at 420 nm was measured. The PPO activity was expressed as UI420, per UI420 = 0.01 ΔA420 mg protein−1 min−1.

Protein contents in the extracts were determined according to the method of Bradford (1976) using bovine serum albumin as the standard protein.

Statistical analysis
All statistical analyses were done with SPSS10.0. Data were analysed by Duncan’s multiple test. Mean separations were performed using the least significant difference method.

Results
Effects of 1-MCP on post-harvest quality of peach fruit
Effective concentration of 1-MCP for delaying peach ripening
Firmness of the unripe fruit harvested for this study was about 57 N cm−2. It is at the best edible stage when the firmness of the peach fruit declines to 40 ~ 30 N cm−2. To estimate the concentration of 1-MCP that would be effective in delaying peach fruit ripening, fruit were treated with 0–1 µL L−1 of 1-MCP for 24 h, and then kept in air at 22 °C for 8 days. As shown in Fig. 1, there was a proportional softening inhibition response up to 0.6 µL L−1 1-MCP, no further softening inhibition was observed above this level. On the eighth day, the firmness of fruit treated with 0.6 µL L−1 or higher concentrations of 1-MCP was about 36 N cm−2.

Effect of 1-MCP on ethylene production of peach fruit during storage
Ethylene production in control fruit increased rapidly and reached a peak after 9 days (Fig. 2), then dramatically decreased. During the first 7 days of storage at 22 °C, ethylene production from fruit treated with 1-MCP treated fruit did not change markedly, but thereafter, gradually increased to a peak on the 12th day of storage. Treating peach fruit with 1-MCP significantly delayed the peak of ethylene evolution, however, it did not affect the accumulative rate of production.

Effect of 1-MCP on peach fruit ripening
Titratable acidity of control fruit gradually declined during storage. TA fruit treated with

![Figure 1](image-url)  
**Figure 1** Effects of 1-MCP concentrations on softening inhibition of peach. Peach fruit were treated with 1-MCP at concentrations from 0 to 1.2 µL L−1 for 24 h, then kept in air at 22 °C for 8 days. Data followed by the different letters are significantly different by Duncan’s multiple test, \( P < 0.05; n = 6 \).
1-MCP treated fruit did not change significantly in the first 4 days of storage at 22 °C, but thereafter, gradually deceased (Fig. 3a). The decline of TA in peach fruit during ripening was partially inhibited by 1-MCP.

Soluble sugar concentration increased slowly both in control and fruit treated with 1-MCP during the first 6 days of storage, and then increased rapidly (Fig. 3b). No significant difference in SSC was observed between control and fruit treated with 1-MCP in the later period of storage.

The SPC of control fruit increased gradually after harvest and reached a peak on the sixth day and then dropped dramatically (Fig. 3c). The peak of SPC in fruit treated with 1-MCP was observed on the ninth day of storage.

It was also found that post-harvest decay of peach fruit was reduced by 1-MCP. Disease incidence of peach fruit treated with 1-MCP was about 50% lower than that of control fruit when stored at 24 °C for 14 d (data not shown here).

Effects of repeat treatment with 1-MCP
Repeated treatment of peach with 1-MCP (i.e. peach fruit treated with 0.5 μL L⁻¹ 1-MCP for 24 h on the first day and fifth day after harvest) resulted in more effective softening inhibition. The firmness level in peach treated twice with 1-MCP was significantly higher than that in fruit treated only once (Fig. 4). The total soluble solids level in peach treated twice with 1-MCP was significantly lower, and vitamin C level significantly higher than that in fruit treated with 1-MCP only once (Table 1).
The effects of 1-MCP on changes in level of SPC, SSC and TA in peach were also enhanced by repeated treatments (data not shown here).

Effect of 1-MCP on disease-resistance of peach fruit

To study how 1-MCP may affect disease-resistance of peach fruit, the fruit treated with 1-MCP were inoculated with spores of *P. expansum*. Disease development and relative physiological changes were measured after the inoculation. The evolution of disease development was assessed 4–6 days after the inoculation.

**Effect of 1-MCP on disease-incidence of peach fruit infected by *P. expansum***

Disease progress in inoculated fruit was reduced by treatment with 1-MCP. The lesion area of fruit treated with 1-MCP was about 13% lower than that of control fruit after 9 and 10 days from inoculation (Fig. 5). The disease-incidence of fruit treated with 1-MCP was 81%, which was much lower than that of control (94.4%).

**Effects of 1-MCP on PAL, POD and PPO activities in inoculated peach fruit***

In the early period after inoculation, only a small difference was observed in PAL activity between the fruit treated with 1-MCP and control fruit (Fig. 6a). However, PAL activity of fruit treated with 1-MCP was about 40% higher than that of control fruit on the sixth and ninth days after inoculation.

The POD activity in control fruit quickly increased to a peak on the third day after inoculation and subsequently rapidly decreased. A similar pattern of POD activity was observed in fruit treated with 1-MCP, although, the peak was

Table 1 Effects of 1-MCP treatments on ripening of peach

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total soluble solids (%)</td>
<td>11.6 ± 0.32a</td>
<td>10.5 ± 0.28b</td>
<td>9.2 ± 0.49c</td>
</tr>
<tr>
<td>Total soluble sugar (%)</td>
<td>8.3 ± 0.43a</td>
<td>7.4 ± 0.25b</td>
<td>7.5 ± 0.36b</td>
</tr>
<tr>
<td>Vitamin C (mg kg⁻¹)</td>
<td>230 ± 16a</td>
<td>270 ± 14b</td>
<td>289 ± 19bc</td>
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</table>

M1: peach fruit were treated with 0.5 μL L⁻¹ 1-MCP on the first day for 24 h, then after were constantly stored in air (22 °C, 80–90% RH).

M2: peach fruit were treated with 0.5 μL L⁻¹ 1-MCP on the first day for 24 h and on fifth day treated again and kept in air in the other periods.

The control fruit were kept in the same condition in air. All the analysis was carried by the 10th day of storage.

Data followed by different letters are significantly different within same row by Duncan’s multiple test, *P* < 0.05. For analysis of soluble solids, *n* = 5; for the other analyses, *n* = 3.

The effects of 1-MCP on changes of level of SPC, SSC and TA in peach were also enhanced by repeated treatments (data not shown here).

![Figure 4](image1.png)

*Figure 4* Effects of multiple treatments with 1-MCP on peach fruit. MCP1: peach fruit were treated with 0.5 μL L⁻¹ 1-MCP on the first day for 24 h, then stored in air at 22 °C for 8 days. MCP2: peach fruit were treated with 0.5 μL L⁻¹ 1-MCP on the first and fifth days, and were kept in air at 22 °C for the remainder of the period. Control fruit were constantly kept in air. Each point represents the mean value of six replicates ± SE (as vertical bars).

![Figure 5](image2.png)

*Figure 5* Effects of disease progress in peach inoculated with *P. expansum*. Peach fruit were treated with 0.5 μL L⁻¹ 1-MCP or kept in air (control) for 24 h, and then inoculated with *P. expansum* and incubated at 22 °C. Data are the mean values of three replicates (thirty fruits of each replicates) ± SE (as vertical bars).
3 days later and higher than that in control fruit (Fig. 6b). PPO activity gradually increased in both fruit treated with 1-MCP and control fruit during the period after inoculation (Fig. 6c).

**Discussion**

The effects of 1-MCP on fruit ripening inhibition have been widely reported. In the present study, it was shown that treatment with 1-MCP could effectively prevent a decline in the firmness of peach. In addition, changes in the other parameters related to peach ripening, such as content of soluble solids, total soluble sugar, TA, soluble pectin and ethylene production were also significantly reduced or delayed by 1-MCP. These results are consistent with those reported on apple (Fan et al., 1999) and banana (Golding et al., 1999; Jiang et al., 2004).

Responsiveness to ethylene is related to the quantity of ethylene receptors that exist in the plants. 1-MCP is thought to inhibit ripening by irreversibly occupying ethylene-binding receptors (Sisler & Serek, 1997). It was observed in this study that treating peach fruit with 0.2 l LL \(^{-1}\) 1-MCP at 22 °C for 24 h could slow the decline in fruit firmness, however, the concentration of 1-MCP for effectively inhibiting fruit softening was 0.4 l LL \(^{-1}\). Treating peach fruit with higher concentrations of 1-MCP did not result in a greater effect (Fig. 1). This suggests that treatment with 0.4 l LL \(^{-1}\) 1-MCP at 22 °C for 24 h is enough to block all the ethylene receptors in peach fruit. The question remained as to how long the blocking effect lasted? It is generally believed that 1-MCP is irreversibly bound to the ethylene receptors (Sisler & Serek, 1997). It was reasonable to expect that peach fruit ripening would be stopped after treating the peach fruit with sufficient concentrations of 1-MCP. However, this hypothesis was not supported by the experimental results (Figs 2 and 3). Furthermore, repeat treatments with 1-MCP resulted in more effective softening inhibition (Fig. 4). This effect could be explained on the basis that new ethylene receptors are constantly formed in the fruit during storage.

Fruit softening is usually considered to be a result of protopectin degradation. This view was further supported by the present study. It was observed that the increase in soluble pectin in peach fruit was inhibited by 1-MCP along with the inhibition of fruit softening.

Decay is an important factor that limits the storage life of peach fruit after harvest and results in appreciable losses at wholesaler, retailer and consumer levels (Lill et al., 1989). In the present study we observed that post-harvest decay of peach fruit was reduced by treatment with 1-MCP. Disease progress in the fruit inoculated with *P. expansum* was also reduced by 1-MCP.
It is a general view that disease resistance of fruit is closely related to the degree of ripeness. As ripening of peach fruit was significantly inhibited by 1-MCP, the disease resistance would be expected to be enhanced by 1-MCP. However, the mechanisms that are involved in 1-MCP inhibiting fruit ripening and enhancing fruit disease-resistance may not be same. It has been suggested that PAL, PPO and POD may play important roles in disease-defensive systems of plants. Increase of PAL activity is associated with biosynthesis of toxic metabolites in the plant defence pathway, such as phytoalexins, phenols, lignins and salicylic acid (Chappell et al., 1984). POD is involved in cross-linking extension molecules to form lignin. PPO oxidize phenols to form more toxic quinones, which directly influence invading pathogens in plant-pathogen interactions (Mohammadi & Kazemi, 2002). It was observed that the activities of PAL, PPO and POD in the fruit inoculated with P. expansum were effectively enhanced by 1-MCP. The size of the fruit lesion area correlated with the increase of the activities of the three defensive enzymes. Similar results were observed in several other studies, for instance 1-MCP treatments can reduce decay development in avocado (Pesis et al., 2002).

In conclusion, post-harvest ripening of peach fruit can be effectively inhibited by treating the fruit with 1-MCP; 1-MCP treatment could also enhance the disease resistance of peach.

Acknowledgment

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References


