



## Effects of 1-methylcyclopropene(1-MCP) on ripening and resistance of jujube (*Zizyphus jujuba* cv. Huping) fruit against postharvest disease

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### ABSTRACT

The effects of 1-methylcyclopropene (1-MCP) on senescence and induction of resistance against postharvest decay in jujube (*Zizyphus jujuba* cv. Huping) fruit were investigated in this study. The results indicated that, compared to control, 1-MCP at 1  $\mu\text{L L}^{-1}$  depressed ethylene production by 28% and respiration rate by 30% at 24 h after treatment, resulting in effective delay in fruit senescence. 1-MCP treatment was beneficial for maintaining quality of jujube fruit stored both at 25 and 0 °C, retarding decline of firmness, vitamin C, titratable acidity (TA) and soluble solids content (SSC) which are important parameters for fruit quality evaluation during the storage periods. Moreover, 1-MCP effectively limited the development of lesion diameter of blue mold rot and significantly reduced the incidence of natural decay. The activities of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), catalase (CAT) and superoxide dismutase (SOD) were significantly induced after 1-MCP treatment. These findings indicate that the induced resistance in jujube fruit by 1-MCP is related with the increase of enzymes involved in scavenging of reactive oxygen species and enzymes associated with phenolics metabolism which produces highly toxic products against pathogen invasion. Our results suggest that 1-MCP has potential effect on maintaining the quality and extending postharvest life of jujube fruit.

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### 1. Introduction

*Hupingzao jujube* (*Zizyphus jujuba* cv. Huping) is one of famous cultivars in China. It is favored for its high nutritional value, good taste. However, the fruit is very perishable and susceptible to browning, decay and water loss after harvest, which greatly affects its edible and commercial value (Tian, Qin, & Xu, 2005; Wu, Tian, & Xu, 2009). Generally, the fruit quality markedly decreases after kept at room temperature for about one week (Wu et al., 2009). Blue mold rot caused by *Penicillium expansum* is the main postharvest decay in jujube fruit during storage periods in China (Tian et al., 2005; Xu, Lai, Jiang, Xu, & Tian, 2009). The traditional approach for decay control in fruit is to use fungicides, however, it is necessary to search new potential approaches to control postharvest diseases, considering the human health risks associated with the use of high concentration of fungicides (Tian & Chan, 2004). In recent years, we have proved that biocontrol agents such as *Rhotorula glutinis* and *Cryptococcus laurentii* could effectively control postharvest decay in jujube fruit (Qin & Tian, 2004; Wan, Tian, &

Qin, 2003). It was reported that fruit ripening and senescence are associated with increased susceptibility to the postharvest pathogens. Some chemicals including oxalic acid (Wang, Qin, Lai, & Tian, 2009) and brassinosteroids (Zhu, Zhang, Qin, & Tian, 2010), which have potential effect on delaying senescence were shown to have the ability to enhance fruit resistance against pathogens.

Ethylene, as one of several plant growth regulators, can affect growth and developmental processes including ripening and senescence (Abeles, Morgan, & Saltveit, 1992). During postharvest storage, ethylene can cause negative effects including senescence, accelerated quality loss, reduced nutrient composition, increased fruit pathogen susceptibility, and physiological disorders in fruit and vegetables (Martínez-romero et al., 2007). Therefore, it is indispensable to inhibit ethylene biosynthesis or its action, in order to slow ripening processes and enhance disease resistance of fruit and vegetables. 1-Methylcyclopropene (1-MCP), the inhibitors of ethylene action, has been shown to compete with ethylene for the binding site on the ethylene receptor in plant tissue, which prevents ethylene from exerting its physiological action (Sisler & Serek, 1997). 1-MCP has been widely used in postharvest storage and proved to be highly effective in maintaining quality of fruit (Blankenship & Dole, 2003; Huber, 2008; Watkins, 2006). By comparison, there are a limited number of reports focusing on the

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effect of 1-MCP on the disease resistance of fruit and vegetables. Existing studies have drawn conflicting conclusions with regard to the benefit of a 1-MCP treatment in controlling postharvest pathogens. For instance, Dong, Lurie, and Zhou (2002) reported that 1-MCP enhance the disease resistance of apricots, while, Jiang, Joyce, and Terry (2001) pointed out 1-MCP can accelerate the disease development in strawberries.

Postharvest application of 1-MCP has been shown to retard ripening and maintain the quality in some jujube cultivars. Indian jujube fruits treated with  $0.6 \mu\text{L L}^{-1}$  1-MCP showed better retention of chlorophyll content, soluble solids content, ascorbic acid and fruit firmness. Meanwhile, the climacteric ethylene evolution and respiration rate were delayed (Zhong & Xia, 2007). 1-MCP also showed high efficiency in the quality maintenance of other jujube cultivars, such as Chinese jujube (Jiang, Sheng, Jiang, & Zhou, 2003) and Sihongdazao jujube (Yan et al., 2007). However, little information is available about the effect of 1-MCP on inducing resistance against fruit decay and the mechanisms by which 1-MCP delays ripening. The present paper investigated the effect of 1-MCP on the retention of quality and the induced resistance on Hupingzao jujube. To investigate the mode of actions of 1-MCP to delay senescence and induce resistance, enzymes related with disease resistance such as phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO) and enzymes involved in reactive oxygen species (ROS) scavenging including catalase (CAT) and superoxide dismutase (SOD) were evaluated.

## 2. Materials and methods

### 2.1. Fruit material and pathogen

Jujube (*Z. jujuba* cv. Huping) fruit were harvested from an orchard in Shanxi province of China, and were immediately transported to Institute of Botany, the Chinese Academy of Sciences, in Beijing. Fruit approximate 5 cm long and 3 cm wide without physical injuries or infections were sorted. The firmness of fruit was 75.5 N as determined by a penetrometer (FT-327, UC Fruit Firmness Tester, Milan, Italy), and soluble solids content (SSC) was 23.8°Brix as determined by an Abbe refractometer (10481 S/N, USA).

*P. expansum* strain used in this study was isolated from naturally infected jujube fruit. The pathogen was cultured routinely on PDA plates for 14 days at 25 °C. Fungal spores (2-weeks-old) were harvested by flooding the surface of the culture with sterile distilled water, then filtered through four layers of sterile cheesecloth and adjusted to a concentration of  $5 \times 10^3$  spores per milliliter. Fruit were punctured at the equatorial line (a wound per fruit) by a sterile nail and inoculated with 10  $\mu\text{L}$  of a spore suspension of *P. expansum* at  $5 \times 10^3$  spores  $\text{mL}^{-1}$  in each wound site.

### 2.2. 1-MCP treatment and experimental design

Our preliminary experiments showed that  $1 \mu\text{L L}^{-1}$  of 1-MCP (EthylBloc, Rohm and Haas China, Inc.) was more effective than  $0.5 \mu\text{L L}^{-1}$  and  $1.5 \mu\text{L L}^{-1}$  to inhibit pericarp reddening of Jujube fruit. Jujube fruit were enclosed in a plastic container (18 L), and treated with 1-MCP, which was released from a commercial powder dissolved in sterile distilled water and final concentration was  $1 \mu\text{L L}^{-1}$ , equal volume sterile water as control. The fruit were treated for 24 h at 25 °C, and then divided into two parts randomly. One part was used for analysis of fruit decay caused by postharvest diseases. Another part of fruit was used for quality assays and enzyme activity determination. The fruit were put into plastic trays to maintain a high relative humidity (95%) and stored at 25 or 0 °C.

### 2.3. Ethylene detection and respiration rate assay

Jujube fruit (1 kg) were sealed in a 5 L gas-tight jar at 25 °C for 2 h. Then 1 mL of gas was extracted from the jar by a syringe. Ethylene and  $\text{CO}_2$  was quantified by direct injection on a gas chromatograph (SQ-206, Beijing, China) equipped with a photo ionization detector (PID). The column temperature was set at 80 °C and the oven was at 150 °C. The gas pressure was set at 0.06 MPa for carrier gas (nitrogen), 0.04 MPa for flammable gas (hydrogen), and 0.05 MPa for supporting gas (air). The carrier gas flow rate was  $30 \text{ mL min}^{-1}$ . The  $\text{CO}_2$  level was monitored by the same gas chromatograph fitted with a thermal conductivity detector. The analysis was carried out once a day for up to 9 days after 1-MCP treatment. Each treatment contained three replications. The entire experiment was repeated twice.

### 2.4. Determination of firmness, soluble solids content, titratable acidity, and vitamin C content

Firmness was measured on the cheeks of the fruit using a hand-held fruit firmness tester (FT-327, UC Fruit Firmness Tester, Milan, Italy), equipped with a cylindrical plunger 8 mm in diameter. Soluble solids content (SSC) was measured by an Abbe refractometer (10481 S/N, USA). Titratable acidity (TA) was determined by titrating the pH value to 8.2 with 0.01 mol/L NaOH. Vitamin C (total ascorbate) contents of the fruit were assayed according the methods as previously reported by Kampfenkel, Montague, and Inzé (1995). The fruits stored at 25 °C were analyzed once every 3 days for up to 15 days. The fruits stored at 0 °C were analyzed once every 10 days for up to 50 days. Each treatment contained three replications, and 15 fruits were used in each replicate. The entire experiment was repeated twice.

### 2.5. Assay for disease and enzyme activity

For pathogen inoculation, fruit were sterilized by dipping in a 20 mL/L sodium hypochlorite solution for 2 min and washed with tap water before use. Incidence of infected wounds and lesion diameter were measured daily after inoculation and observed for 5 days at 25 °C. Each treatment contained three replications, and 15 fruits were used in each replicate. The entire experiment was repeated twice. The incidence of natural decay was measured by observing visible fungal growth or bacterial lesion on the fruits surface. The assay was carried out once every 3 days for up to 18 days in the fruit stored at 25 °C. For fruit stored at 0 °C, decay was detected once every 10 days for up to 60 days. Each treatment contained three replications, and 50 fruits were used in each replicate.

For enzyme activity assay, 10 g of sarcocarp samples around the infection spots from 15 fruits were ground in mortar using liquid nitrogen. All the enzymes were extracted at 4 °C and the absorbance was determined by UV-160 spectrophotometer (Shimadzu, Japan). The activity of phenylalanine ammonia-lyase (PAL) was detected following the methods of Yao and Tian (2005). PAL was extracted with 25 mL of 0.05 mol/L sodium borate buffer (pH 8.8, containing 5 mmol/L  $\beta$ -mercaptoethanol). 1 mL enzyme extract was incubated with 2 mL of borate buffer (50 mmol/L, pH 8.8) and 1 mL of L-phenylalanine (20 mmol/L) for 60 min at 37 °C. The reaction was stopped with 1 mL HCl (1 mol/L). PAL activity was determined by the production of cinnamate, which was measured by absorbance at 290 nm. The activity of polyphenoloxidase (PPO) was assayed following the methods of Tian, Xu, Jiang, and Qin (2002). PPO was extracted with 25 mL 0.2 mol/L sodium phosphate buffer (pH 6.4). For enzyme detection, 3 mL 0.5 mol/L 4-methylcatechol in 0.2 mol/L sodium phosphate buffer (pH 6.4) and 100  $\mu\text{L}$  enzyme

sample were incubated at 25 °C for 1 min, and the change in absorbance at 398 nm with time was recorded. Catalase (CAT) and Superoxide dismutase (SOD) were assayed following the methods of Wang and Tian (2005). CAT was extracted by 50 mmol/L sodium phosphate buffer (pH 7.0). The reaction mixture consisted of 2 mL sodium phosphate buffer (50 mmol/L, pH 7.0), 0.5 mL H<sub>2</sub>O<sub>2</sub> (40 mmol/L) and 0.5 mL enzyme. The decomposition of H<sub>2</sub>O<sub>2</sub> was measured by the decline in absorbance at 240 nm. SOD was extracted by 100 mmol/L sodium phosphate buffer (pH 6.4). The reaction mixture (3 mL) contained 50 mmol/L sodium phosphate buffer (pH 7.8), 13 mmol/L methionine, 75 μmol/L nitroblue tetrazolium (NBT), 10 μmol/L EDTA, 2 μmol/L riboflavin and 0.1 mL enzyme extract. The mixtures were illuminated by light (60 μmol/m<sup>2</sup>/s) for 10 min and the absorbance was then determined at 560 nm. Identical solutions held in the dark served as blanks. Protein content was determined according to Bradford (1976) with bovine serum albumin (BSA) as standard. The enzyme activities were analyzed once a day for up to 5 days. Each treatment contained three replications, and 15 fruits were used in each replicate. The entire experiment was repeated twice.

### 2.6. Statistical analysis

All data were analyzed as one-variable general linear model procedure by SPSS. Mean separations were performed by Duncan's multiple range tests. Differences at  $P < 0.05$  were considered as significant.

## 3. Results

### 3.1. Effect of 1-MCP on ethylene production and respiration rate

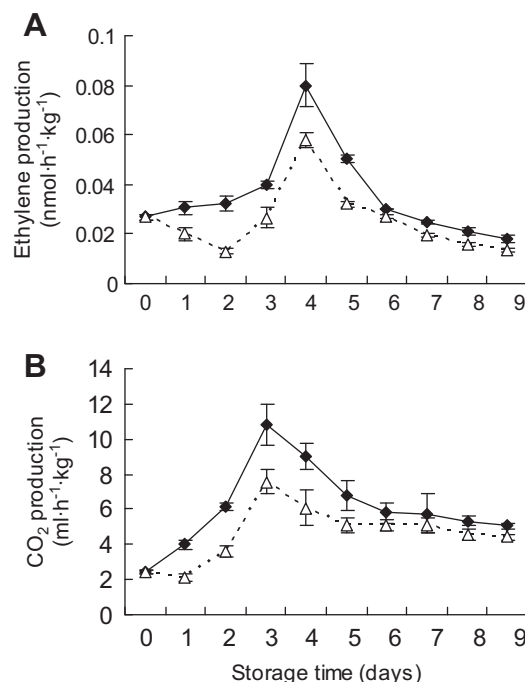
1-MCP treatment significantly impaired the ethylene release after the fruit were treated for 1 day. The reduction of ethylene release was significant until fruit were treated for 5 days. However, 1-MCP didn't delay the peak of ethylene evolution (Fig. 1A). Similarly, the respiration rate of jujube fruit was inhibited after 1-MCP treatment (Fig. 1B), but the rate of CO<sub>2</sub> release reached the maximum value after 3 days at room temperature both in control and in 1-MCP-treated fruit. 1-MCP significantly decreased the respiration rate of jujube fruit after treated for 1 day and lasted for 5 days.

### 3.2. Effect of 1-MCP on fruit quality

Fruit firmness decreased gradually under conditions of room temperature and low temperature. However, the decline was significantly inhibited by 1-MCP treatment (Fig. 2A,B). SSC and TA content showed an increasing trend at the early stage of storage, and then dropped dramatically, thereafter. Fruit treated with 1-MCP maintained higher SSC and TA content at both temperature conditions (Fig. 2C–F). In addition, total ascorbate content declined remarkably at the end of storage. 1-MCP-treated fruit showed an obviously higher level of ascorbate as compared to the control (Fig. 2G–H). The results indicated that 1-MCP treatment had positive effect on maintaining quality of jujube fruit kept both at room and low temperatures.

### 3.3. Effect of 1-MCP on disease resistance in jujube fruit

There was no significant difference between control and 1-MCP-treated fruit in disease incidence after inoculation (Fig. 3A). However, lesion diameter of fruit treated with 1-MCP was lower than that of the control (Fig. 3B). The results showed that 1-MCP treatment can effectively inhibit the development of decay



**Fig. 1.** Effect of 1-MCP on ethylene production (A) and respiration rate (B) at 25 °C. The jujube fruit were treated with 1 μL L<sup>-1</sup> 1-MCP for 24 h at 25 °C. Ethylene and CO<sub>2</sub> levels were quantified with a gas chromatograph. The production of ethylene and CO<sub>2</sub> were expressed as nmol h<sup>-1</sup> kg<sup>-1</sup> fresh weight and ml h<sup>-1</sup> kg<sup>-1</sup> fresh weight, respectively. Each treatment contained three replications, and 1 kg fruits were used in each replicate. The experiment was repeated twice. Vertical bars represent standard error of means. ◆, control; △, 1-MCP.

caused by *P. expansum*. Meanwhile, after treatment with 1-MCP the natural disease incidence was significantly lower than control, especially at 0 °C (Fig. 3C–D).

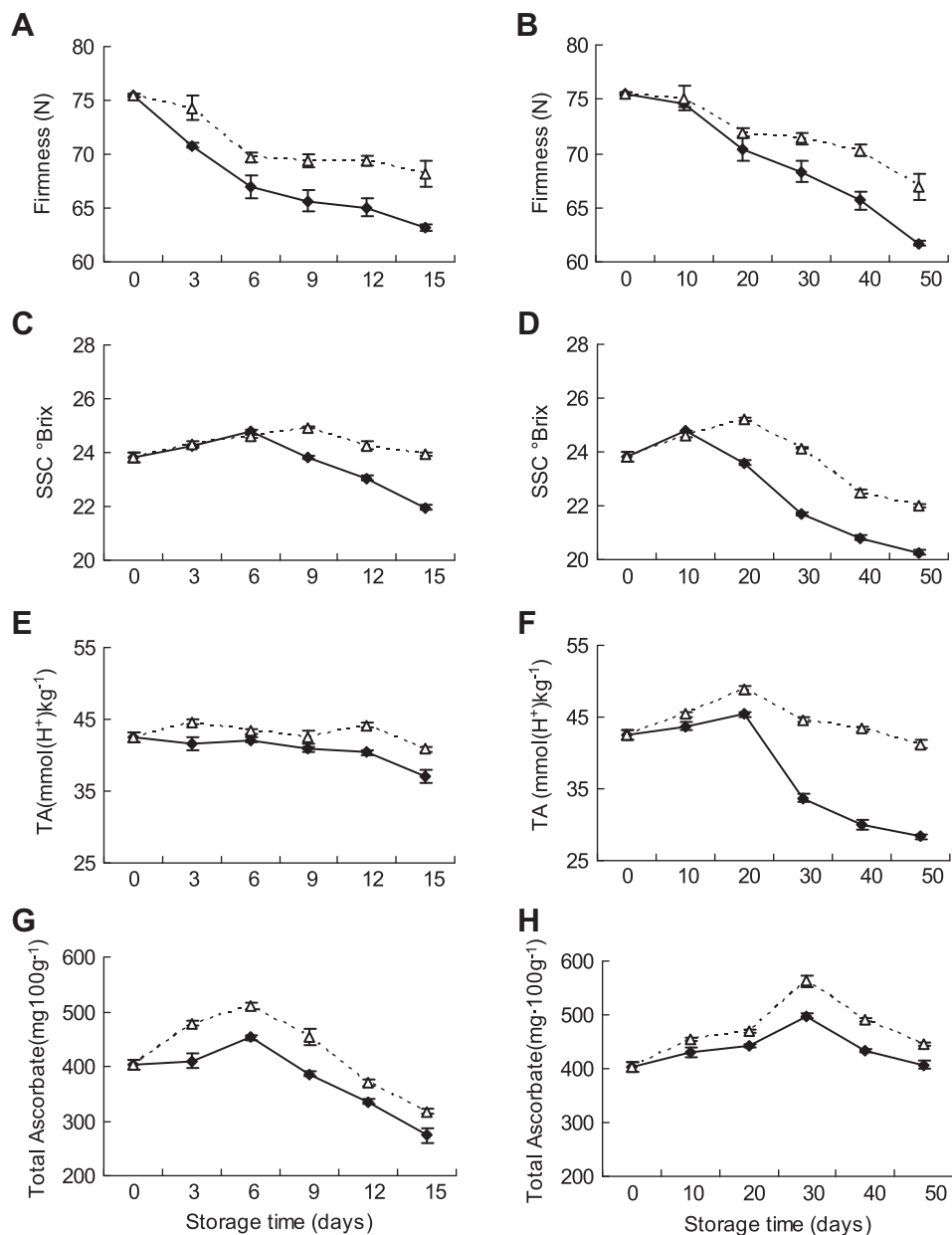
### 3.4. Effect of 1-MCP on the activity of defense-related enzymes

It was clear that PAL activity was significantly higher in 1-MCP-treated fruit than that of the control after 2 days of treatment, indicating 1-MCP possibly induced resistance (Fig. 4A). In contrast, PPO activity in 1-MCP-treated fruit showed significantly higher level compared to the control at the first day after treatment and there was no significant difference after 2 days of treatment (Fig. 4B).

CAT activity showed a peak on the first day, and then declined (Fig. 4C). SOD activity had a constant decrease during storage (Fig. 4D). However, 1-MCP showed an induction effect on CAT and SOD activity throughout the storage periods.

## 4. Discussion

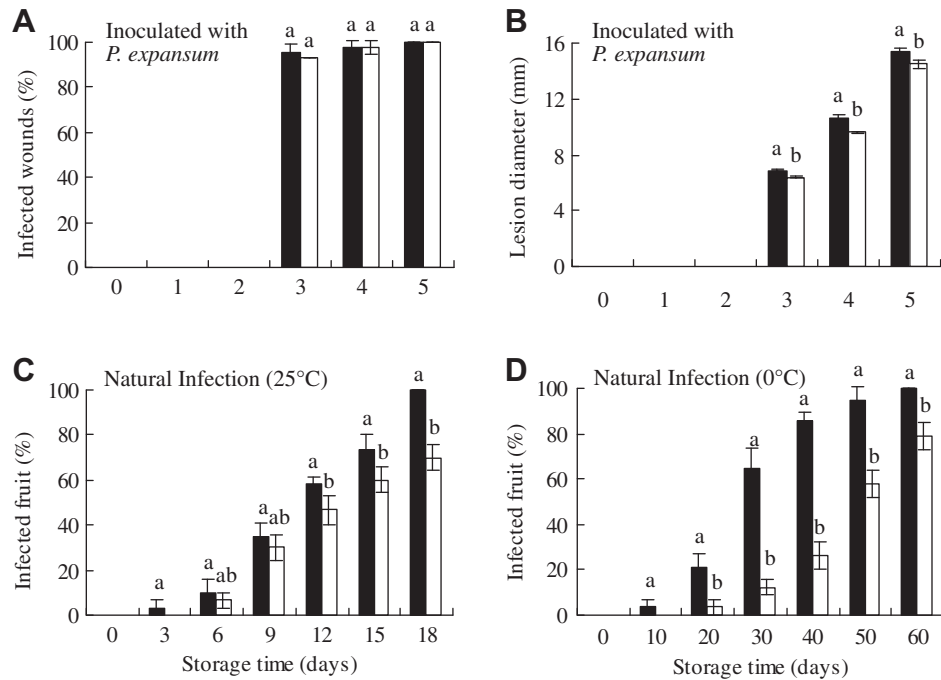
Fruit are usually classified in two types, according to their changes in respiration pattern, namely climacteric and non-climacteric fruit. Different jujube cultivars may differ in respiration pattern and ethylene production. Some cultivars, such as Dazao and Yuanzao, are considered to be non-climacteric (Kader, Li, & Chordas, 1982; Lu, Li, & Lu, 1993), while others, like Mallacy and Bambawi, were reported to be typical climacteric fruit (Abbas, Al-Niami, & Al-Sareh, 1994; Abbas & Sagggar, 1989). Our data suggest that jujube fruit cv. Huping belongs to climacteric fruit, because the fruits showed remarkable peaks of ethylene production and respiration (Fig. 1). 1-MCP, as an ethylene action inhibitor, has been proven to be effective in retarding senescence process by binding



**Fig. 2.** Effect of 1-MCP on firmness, soluble solids content (SSC), titratable acidity (TA), vitamin C (total ascorbate) content of jujube fruit at 25 °C (room temperature, left) and 0 °C (low temperature, right). Jujube fruit were treated with 1-MCP at 1  $\mu\text{L L}^{-1}$  for 24 h at 25 °C and stored at 25 or 0 °C. The contents of TA and vitamin C were expressed as  $\text{mmol (H}^+) \text{kg}^{-1}$  fresh weight and  $\text{mg} \cdot 100 \text{g}^{-1}$  fresh weight, respectively. Each treatment contained three replications, and 15 fruits were used in each replicate. The entire experiment was repeated twice. Vertical bars represent standard error of means. ◆, control; △, 1-MCP.

irreversibly to ethylene-binding receptor, thereby inhibiting the ethylene signal transduction pathway, such as mango (Wang, Qin, et al., 2009; Wang, Wang, et al., 2009), pear (Lu, Curetaz, & Toivonen, 2009), peach (Ortiz, Graell, López, Echeverría, & Lara, 2010) and plum (Khan & Singh, 2009), and widely used to control ripening and senescence, and extending postharvest storage life in climacteric fruits. Previous studies showed that 1-MCP treatment can effectively inhibit the release of ethylene and  $\text{CO}_2$  and delay the release peaks. In our study, we found the absolute decrease of ethylene and  $\text{CO}_2$  production, but did not observe the delay of the peaks of ethylene and  $\text{CO}_2$  production. This may be associated with the type of jujube fruit. It was reported that jujube fruits are different from the typical type of climacteric and non-climacteric fruit. Some of the jujube fruit showed two peaks of ethylene and

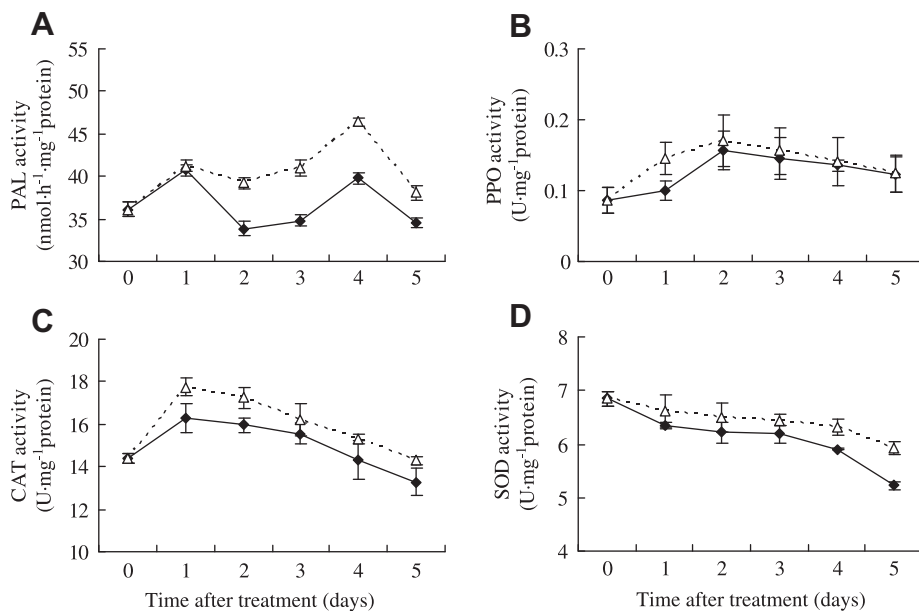
$\text{CO}_2$  production during the processes of ripening (Xue, Zhang, Zhang, & Wang, 2003). In our experiment, the maturity of the fruit that we choose may lead to the missing of the first peak, which may be delayed by 1-MCP treatment. Fruit ripening and senescence are related to resistance against fungal pathogens (Chan et al., 2008). Although there are many reports focused on effects of 1-MCP on ripening and quality, little information is available about the function of 1-MCP against fungal decay, particularly jujube fruit. Blue mold rot, caused by *P. expansum*, is the major postharvest disease in jujube fruit during storage periods in China (Qin & Tian, 2004; Tian et al., 2005; Xu, Lai, Jing, Xu, & Tian, 2009). Here, we firstly provide the evidence that 1-MCP could effectively limit lesion development of blue mold rot in jujube fruit (Fig. 3B), and 1-MCP-treated fruit showed significantly lower incidence of natural



**Fig. 3.** Effect of 1-MCP on the decay of jujube fruit caused by *P. expansum*. The jujube fruit were treated with  $1 \mu\text{L L}^{-1}$  1-MCP for 24 h at 25 °C, punctured at the equatorial line, and inoculated with  $10 \mu\text{L}$  of a spore suspension of *P. expansum* at  $5 \times 10^3$  spores  $\text{mL}^{-1}$ . Fruit were stored at 25 °C, and the disease incidence and lesion diameter were measured daily. The incidence of natural decay was measured by observing visible fungal growth or bacterial lesion on the fruits surface. Fruit were stored at 25 and 0 °C. Decay was observed once every 3 days at 25 °C and once every 10 days at 0 °C. (A) Disease incidence of blue mold at 25 °C. (B) Lesion diameter of blue mold at 25 °C. (C) Natural disease incidence at 25 °C. (D) Natural disease incidence at 0 °C. Each treatment contained three replications, and 15 fruits were used in each replicate. The entire experiment was repeated twice. Vertical bars represent standard error of means. Values followed by different letters are significantly different according to Duncan's multiple range test at  $P < 0.05$ . ■, control; □, 1-MCP.

decay as compared to control fruit (Fig. 3C–D). This result coincides with previous reports that treatment with  $0.6 \mu\text{L L}^{-1}$  1-MCP result in lower stem-end rots severity than control in Indian jujube fruit and the storage life can be extended by 7 days (Zhong & Xia, 2007).

However, there are some opposite results showing that pre-treatment with 1-MCP increase susceptibility toward *Botrytis cinerea* in tomato fruit (Diaz, ten Have, & van Kan, 2002) and high 1-MCP concentration accelerate the disease development in



**Fig. 4.** Effect of 1-MCP on the activities of PAL (A), PPO (B), CAT (C) and SOD (D). The jujube fruit were treated with  $1 \mu\text{L L}^{-1}$  1-MCP for 24 h at 25 °C and inoculated with a spore suspension of *P. expansum* at  $5 \times 10^3$  spores  $\text{mL}^{-1}$ . Ten gram of FW sarcocarp samples around the infection spots from 15 fruits were used for assays of enzyme activity. Each treatment contained three replications, and 15 fruits were used in each replicate. The entire experiment was repeated twice. Vertical bars represent standard error of means. ◆, control; △, 1-MCP.



strawberries (Jiang et al., 2001). The difference may be related to the different fruit species and concentrations of 1-MCP used.

The mechanism by which 1-MCP limits decay development in jujube fruit was investigated by analyzing enzyme activities related to senescence and defense response. The results indicated that 1-MCP treatment induced activities of PAL, PPO (Fig. 4), CAT and SOD (Fig. 4). PAL, as the first enzyme of phenylpropanoid pathway, is involved in the biosyntheses of phenolics, phytoalexins and lignins (Mauch-Mani & Slusarenko, 1996). The resulting phenolics could be oxidized by PPO to produce slightly colored compounds, such as quinines (Campos-Vargas & Saltveit, 2002). Quinines can polymerize to produce colored compounds which are highly toxic to invading microorganisms (Campos-Vargas & Saltveit, 2002). Lignin plays an important role in barricading the pathogen from invading the plant through physical exclusion (Bruce & West, 1989). The induction of PAL and PPO suggests that 1-MCP can induce resistance of jujube fruit against fungal pathogen. On the other hand, the activities of  $\beta$ -1,3-glucanases and chitinases in jujube fruit were not significantly enhanced by 1-MCP (data not shown), indicating that  $1 \mu\text{L L}^{-1}$  1-MCP is not able to induce these two enzymes which can inhibit the growth of fungi directly. CAT and SOD are important enzymes that can protect cells from oxidative damage by scavenging reactive oxygen species (ROS) (Lee & Lee, 2000; Scandalios, 1993). ROS accumulation causes oxidative injury, accelerating the progression of senescence and various senescence-associated disorders (Stadtman, 1992). Our recent studies demonstrate that ROS can cause oxidative damage of mitochondria and accelerate senescence processes in peach fruit (Qin, Meng, Wang, & Tian, 2009). According to present result, we consider that 1-MCP limiting decay in jujube fruit is mainly related to the induction of defense-related enzymes and retarding fruit senescence by alleviating the damage of ROS.

In addition, some reports indicated 1-MCP treatment can maintain quality and extends storage life in plum (Khan & Singh, 2009), pear (Lu et al., 2009) and peach fruit (Ortiz et al., 2010). In the present research, we found that 1-MCP-treated fruit kept both at room temperature and low temperature had higher levels of SSC, TA and vitamin C (Fig. 2). Fruit softening was delayed (Fig. 2), indicating 1-MCP treatment is beneficial for maintaining quality of jujube fruit in postharvest storage.

## 5. Conclusions

In the present study, 1-MCP at a concentration of  $1 \mu\text{L L}^{-1}$  effectively suppressed ethylene production and respiration rate. The quality parameters including firmness, SSC, TA and vitamin C in 1-MCP-treated fruit kept at a much higher level than those in untreated fruit stored at both 25 and 0 °C. Additionally, 1-MCP increased the resistance of jujube fruit against *P. expansum*. The delayed senescence and induced resistance by 1-MCP may be related to the enzyme activity of CAT and SOD, which are associated with ROS scavenging, and PAL and PPO, which are involved in phenolics metabolism. Our results suggest that 1-MCP can provide a feasible technique for maintaining the quality and extending storage life of jujube fruit.

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