



Rapid ingress of gaseous 1-MCP and acute suppression of ripening following short-term application to midclimacteric tomato under hypobaria

Xiaoqing Dong^{a,b}, Donald J. Huber^{b,*}, Jingping Rao^{a,**}, James H. Lee^b

^a College of Horticulture, Northwest A&F University, Yangling, Shaanxi Province 712100, PR China

^b Horticultural Sciences Department, IFAS, University of Florida, Gainesville, FL 32611-0690, USA

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ABSTRACT

Our previous studies demonstrated that tomato fruit (breaker or pink) exposed at the midclimacteric stage to hypobaric hypoxia for 6 h exhibited transient increased sensitivity to subsaturating levels of 1-methylcyclopropene (1-MCP). In the present study, we examined the effect of gaseous 1-MCP (500 nL L^{-1} , $20.8 \mu\text{mol m}^{-3}$) applied to mid-climacteric ($>60\%$ peak ethylene production) tomato fruit under hypobaric hypoxia (10 kPa , 2.1 kPa O_2) for 1 h. Application of 500 nL L^{-1} 1-MCP under atmospheric conditions had little effect on softening and timing and magnitude of peak ethylene production, and moderate effects on respiration and lycopene and PG accumulation. By contrast, midclimacteric fruit exposed to 500 nL L^{-1} gaseous 1-MCP under hypobaric hypoxia for 1 h showed acute disturbance of ripening. Firmness and hue angle declines were delayed for ten days and peak ethylene production for eleven days compared with trends for the other treatments. Maximum ethylene production did not exceed 50% of maxima for the other treatments and a definitive respiratory climacteric was not observed. Accumulation of internal gaseous 1-MCP was enhanced under hypobaric hypoxia. Internal 1-MCP in fruit exposed to $20 \mu\text{L L}^{-1}$ 1-MCP ($831 \mu\text{mol m}^{-3}$) under hypobaric hypoxia for 2 or 10 min averaged 7.5 ± 0.5 and $8.7 \pm 1.4 \mu\text{L L}^{-1}$, respectively, compared with 0.8 ± 0.3 and $3.9 \pm 0.7 \mu\text{L L}^{-1}$ in fruit exposed under atmospheric conditions. After 1 h exposure, internal 1-MCP averaged $10.8 \pm 2.2 \mu\text{L L}^{-1}$ under hypobaric hypoxia compared with $5.3 \pm 1.4 \mu\text{L L}^{-1}$ under atmospheric conditions. The results indicate that high efficacy of 1-MCP applied under hypobaric hypoxia is due to rapid ingress and accumulation of internal gaseous 1-MCP.

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1. Introduction

1-Methylcyclopropene (1-MCP), a strong inhibitor of ethylene action (Sisler, 2006), has been extensively researched in a wide variety of fruit and vegetables. The obligatory requirement for ethylene in climacteric fruit (Lelièvre et al., 1997) has drawn much research interest to this ripening class. Although climacteric fruit share a requirement for ethylene for initiation and progression of ripening, response intensity to 1-MCP can vary considerably. Variable responsiveness reflects inherent characteristics of different fruit types as well as cultivar, fruit maturity, delay after harvest, and stage of ripening (Blankenship and Dole, 2003; Watkins, 2006; Huber, 2008). Other factors likely contributing to

response efficacy include 1-MCP binding to non-target sites (Daunay et al., 2003; Nanthachai et al., 2007; Choi and Huber, 2009; Ambaw et al., 2011) and oxidative catabolism (Huber et al., 2010; Lee et al., 2012).

Studies with tomato fruit have shown that internal ethylene concentrations (IECs) can strongly modulate 1-MCP efficacy, perhaps providing explanation for the influence of cultivar, fruit maturity and stage of ripening on 1-MCP responsiveness. Experimentally increasing IEC in ripening tomato fruit resulted in unresponsiveness to 500 nL L^{-1} 1-MCP, followed by full recovery after ethylene off-gassing (Zhang et al., 2009). Conversely, reducing IEC through exposure of ripening tomato to hypobaric hypoxia (1.7 kPa O_2 , 8 kPa , 6 h) resulted in transiently increased sensitivity to the ethylene antagonist (Zhang et al., 2010). Levels of 1-MCP causing strong delay of ripening of preclimacteric avocado (*Persea Americana* Mill.) had no effect on midclimacteric fruit (Adkins et al., 2005; Zhang et al., 2011). Delayed ripening of mid-climacteric avocado occurred when 1-MCP was applied after treatment (hypoxia) to reduce IEC or at concentrations 25-fold higher than those effective with preclimacteric fruit (Zhang et al., 2011).

* Corresponding author at: Horticultural Sciences Department, P.O. Box 110690, University of Florida, IFAS, Gainesville, FL 32611-0690, USA. Tel.: +1 352 273 4779; fax: +1 352 392 6479.

** Corresponding author. Tel.: +86 29 87082613; fax: +86 29 87082613.

E-mail addresses: djhuber@ufl.edu (D.J. Huber), raojingping@hotmail.com (J. Rao).

The objective of the present study was to examine the use of short-term, combined hypobaric hypoxia and 1-MCP treatments to influence efficacy of 1-MCP with midclimacteric tomato fruit. Hypobaric hypoxia has been shown to enhance 1-MCP responses in fruit at the preclimacteric stage (Hayama et al., 2005; Chen et al., 2010; Kashimura et al., 2010). Using procedures recently reported for tomato fruit (Dong et al., 2013), we also monitored the rate of gaseous 1-MCP ingress in tomato fruit treated with combined 1-MCP/hypobaric hypoxia.

2. Materials and methods

2.1. Plant material

Unwaxed tomato (*Solanum lycopersicum* L., 'Tasti-Lee') fruit were obtained from a packinghouse in Ruskin, FL, on the day of harvest and transported to the postharvest facilities in Gainesville. After holding overnight at 20 °C, breaker-turning stage fruit of uniform size were selected for the experiments described below.

2.2. Treatment with 1-MCP under hypobaric hypoxia and atmospheric conditions

This experiment used a total of 180 breaker-turning tomato fruit, 45 per treatment. Hypobaric hypoxia treatments employed 9-L desiccators equipped with sleeve valves and side-arm vent. All interfaces (lid, base, sleeve-valve assembly) were coated with a thin layer of vacuum grease. For depressurization, the sleeve-valve side-arm was connected via vacuum tubing to a four-way connector. The line to the vacuum pump contained an in-line shut-off valve. The remaining two ports of the connector were equipped with a pressure gauge and rubber septum for 1-MCP injection.

Preparation and gas chromatographic analysis of stock 1-MCP gas were as described in detail in Choi and Huber (2009). The 1-MCP source was AFXRD-038 powder (3.8% active ingredient, AgroFresh, Inc., Rohm and Haas, Philadelphia, PA) dissolved in diH₂O.

After sealing the fruit in desiccators (15 fruit per desiccator per treatment), the pressure was reduced slowly to 10 kPa (2.1 kPa O₂) over 1 min, and the shut-off valve closed. A volume of stock 1-MCP was immediately injected through the septum using a 1 mL syringe with a 5 cm, 22-gauge needle to generate a concentration of 500 nL L⁻¹. The desiccator sleeve valve was rotated to seal, the four-way connector removed, and the operation repeated in succession for the other two desiccators. No more than 5 min were required to depressurize the three desiccators used for each treatment. After 1 h at 23 °C, the four-way connector was reconnected to the desiccator, the pressure recorded, and the shut-off valve opened slowly to allow pressure equilibration over a 1 min period. The process was repeated for all desiccators. After the hypobaric hypoxia/1-MCP-exposure treatments, an additional 45 fruit (15 per desiccator) were subjected to 1 h exposure to hypobaric hypoxia with the exception that 1-MCP was not added. Desiccator pressures at the end of the 1 h hypobaric hypoxia/1-MCP and hypobaric hypoxia treatments ranged from 12 to 14 kPa. For the treatments under atmospheric conditions, 90 fruit were placed in six desiccators (15 fruit/desiccator) and sealed. Three received 500 nL L⁻¹ 1-MCP and the remaining three received air. Samples were held at 23 °C for 1 h. Desiccator 1-MCP concentrations averaged around 420 nL L⁻¹ after 1 h exposure under both hypobaric hypoxia and atmospheric conditions.

After treatments, fruit were placed in 30-cell Styrofoam trays and placed in storage at 20 °C. Fifteen fruit from each treatment were used for firmness and hue angle, and six fruit of each treatment were used for respiration rate and ethylene production. The remaining fruit were used for measuring lycopene content and PG

activity. For PG and lycopene measurements, three fruit were sampled at each measurement interval. Fruit were sliced, the stem and blossom regions along with seeds and placental tissue discarded, and outer and radial pericarp from the equatorial region (± 4 cm from equator) collected and stored at -80 °C until analyzed. The experiment was conducted twice with similar results.

2.3. Fruit firmness and surface color measurement

Fruit firmness was determined using the TA.HD plus Texture Analyser (Stable Micro Systems Ltd., Godalming, England) with Exponent Texture Analyser Software. Firmness was measured by compression using 5 N load cell and a 76-mm flat probe. Fruit (15 per treatment) were nested in a concave device and compressed 2.5 mm at the equatorial region of each fruit with a probe trigger force of 0.03330 N and probe speed of 1 mm s⁻¹. Measurements were taken at a single point at the equatorial axis of each fruit. The maximum force (N) generated during probe travel was used for data analysis.

Fruit surface color (hue angle) was determined using a Minolta CR-400 colorimeter (Minolta camera Co., Ltd., Japan) with a standard C illuminant. The beam diameter was 11 mm with a viewing angle of 0°. A white calibration plate was used for calibration ($L^* = 96.88$, $C^* = 2.05$, $h^\circ = 89.4$, $a^* = 0.02$, $b^* = 2.05$). The data were expressed by the CIE $L^*a^*b^*$ system and converted to hue angle using the formula $hab = \tan^{-1}(b^*/a^*)$. Two measurements were done at two equidistant points on the equatorial axis of each fruit. Firmness and color were measured every other day.

2.4. Ethylene production and respiration rate

Ethylene production and respiration rate were conducted by placing individual fruit in 1.76 L glass containers equipped with sampling septa and sealed for 2 h at 20 °C. Three-microliter samples of the headspace atmosphere were withdrawn with a 3-mL gas syringe. Ethylene production and respiration rate were analyzed using a Varian CP-3800 gas chromatograph as described in detail in Zhang et al. (2011). Ethylene production and respiration rate are expressed as ng kg⁻¹ s⁻¹ and μ g kg⁻¹ s⁻¹, respectively.

2.5. Lycopene content and polygalacturonase activity

Lycopene content was measured as described in Sadler et al. (1990) with the modifications of Fish et al. (2002). Briefly, partially thawed pericarp tissue (5 g) and 50 mL of hexane–acetone–ethanol (2:1:1, v/v) were homogenized for 1 min in a 100 mL test tube wrapped in aluminum foil. Afterward, 15 mL of water was added and samples shaken for 10 s. After allowing phase separation on ice, lycopene concentration was determined by measuring the absorbance of the hexane at 503 nm. Lycopene content was calculated using the molar extinction coefficient of 17.2 L mol⁻¹ cm⁻¹ (Zechmeister et al., 1943) and was expressed as mg kg⁻¹ fresh weight. Three independent samples derived from three fruit were used for analyzing lycopene content.

Polygalacturonase activity was extracted using 5 g partially thawed pericarp using the procedure described in Huber and O'Donoghue (1993). Activity was measured reductometrically using the Milner–Avigad assay (Milner and Avigad, 1967). Three independent samples derived from three fruit were used for measurements. Activity is expressed as mmol galacturonic acid equivalents generated per kg of protein per second (mmol kg⁻¹ s⁻¹).

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