

Responses of ‘Bing’ and ‘Rainier’ Sweet Cherries to Ethylene and 1-Methylcyclopropene

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ABSTRACT. ‘Bing’ and ‘Rainier’ sweet cherry (*Prunus avium* L.) fruit treated with 1-methylcyclopropene (1-MCP) were stored at 20 °C in air or 35 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene. Ethylene production by both ‘Bing’ and ‘Rainier’ fruit stored in air was transiently stimulated following 1-MCP treatments, however, there were no significant effects of 1-MCP on respiration rate. Exogenous ethylene stimulated respiration regardless of prior treatment with 1-MCP. Although 1-MCP treatment reduced the increase in ‘Bing’ respiration induced by ethylene, the reduction was less than reported previously for climacteric fruit. These results suggest that stimulation of sweet cherry fruit respiration by ethylene occurs via a process that may be independent of receptors to which 1-MCP binds. Postharvest changes in fruit color and development of stem browning were not altered by 1-MCP treatment, and exogenous ethylene accelerated the development of stem browning regardless of prior treatment with 1-MCP.

The promotion of plant senescence by ethylene can be inhibited by a number of cyclic olefinic compounds including 1-methylcyclopropene, 2,5-norbornadiene, 3,3-dimethylcyclopropene, and diazocyclopentadiene (Sisler et al., 1990). These compounds are effective inhibitors of ethylene action at very low ($\text{h}\cdot\mu\text{L}\cdot\text{L}^{-1}$) concentrations and remain bound to the ethylene receptor much longer than ethylene (Sisler and Serek, 1997; 1999). The progression of ripening and senescence in several fruits, vegetables and cut flowers is delayed following treatment with these compounds (Fan et al., 1999a, 1999b; Fan and Mattheis, 2000; Serek et al., 1994; Sisler and Serek 1997, 1999; Tian et al., 1997a;), and the commercial use of 1-MCP for ornamental crops was approved by the United States EPA in 1999 (Biopesticide Fact Sheet, 2000). 1-MCP treatment of climacteric fruit, such as apple, pear, banana, and plum, results in reduced ethylene production and respiration rate, and fruit ripening is delayed (Abdi et al., 1998; Fan et al., 1999a, 1999b; Golding et al., 1998; Mueller et al., 1999). However, ethylene production by nonclimacteric fruit is low, and changes in fruit quality during ripening are not accompanied by increased ethylene production and respiration rate (Biale and Young, 1981). Tian et al. (1997b) found that treatment of strawberry, a non climacteric fruit, with diazocyclopentadiene (DACP) stimulated ethylene production, but did not affect respiration rate. Strawberry fruit treated with 1-MCP then held in air containing 0.1 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene have an extended postharvest life compared to untreated fruit (Ku et al., 1999). Nonclimacteric and climacteric fruit may have different ethylene receptor(s) (McGlasson, 1985) and these receptor(s) may have different regulatory functions (Tian et al., 2000).

Sweet cherry fruit are nonclimacteric (Biale, 1960; Blanpied, 1972), and exogenous ethylene (ethephon) has no effect on respiration rate or firmness loss during fruit ripening (Li et al., 1994). The objective of this study was to evaluate the responses of sweet cherry fruit ‘Bing’ and ‘Rainier’ to 1-MCP treatment before and after exposure to ethylene. Ethylene production, respiration and fruit quality were measured after the treatments.

Materials and Methods

‘Bing’ and ‘Rainier’ sweet cherry (*Prunus avium* L.) fruit were obtained from a commercial orchard near Wenatchee, Wash. Experiment 1: Sweet cherry fruit (75 to 80 fruit, 680 to 810 g per treatment) were placed in 20-L glass jars sealed with two layers of plastic wrap. 1-MCP was generated by mixing EthylBloc powder (Floralife, Inc., Walterboro, S.C.) and water in a 50-mL flask, then 1-MCP gas was injected into the jars with a syringe. After the injection, holes in the wrap made by the syringe needle were sealed with tape. The concentration of 1-MCP in the jars was 0 (CK), 0.1 (M0.1), 1.0 (M1) or 10 (M10) $\mu\text{L}\cdot\text{L}^{-1}$ and the jars were held at 20 °C for 15 h. 1-MCP concentration in a 0.5 mL gas sample removed from each jar 15 min after initiation of treatment was analyzed using a gas chromatograph (HP5880A; Hewlett Packard, Avondale, Pa.) fitted with a 30-cm glass column (3.2-mm ID) packed with 80/100 mesh Porapak Q and a flame ionization detector (FID). Gas flows for N_2 , H_2 , and air were 25, 30, and 300 $\text{mL}\cdot\text{min}^{-1}$, respectively. Injector, oven, and FID temperatures were 100, 130, and 200 °C, respectively. A 1-butene standard (Scott, Plumsteadville, Pa.) was used to generate a response factor and 1-MCP quantification was based on this value (Fan et al., 1999c). After the treatment, fruit from the CK and M10 1-MCP treatments were stored at 20 °C in air or air plus ethylene (35 $\mu\text{L}\cdot\text{L}^{-1}$; E and M10 + E) in a flow-through system (15 fruit per replication, five replications per treatment) using 2-L plastic jars with gas flowing at 20 $\text{mL}\cdot\text{min}^{-1}$ for 7 d. Experiment 2: ‘Bing’ cherry fruit held at 20 °C were treated with ethylene (80 $\mu\text{L}\cdot\text{L}^{-1}$) for 6 h or without ethylene (CK) in the flow-through system described above. After 6 h, all fruit were treated with 1-MCP at 0 (E + M0) or 10 $\mu\text{L}\cdot\text{L}^{-1}$ (E + M10) for 15 h as described above. After treatment, all fruit were stored in air at 20 °C for 7 d.

FRUIT QUALITY ANALYSIS. Fruit surface color was measured on the cheek of 30 fruit with a colorimeter (CR-200; Minolta, Japan) using CIE illuminant C and an 8-mm-diameter aperture. L , a^* , b^* color data were converted to hue angle (h°) and chroma (C) (McGuire, 1992). Stem browning of the same 30 fruit was rated visually as 1 = none; 2 = slight; 3 = moderate; 4 = severe. Fruit was analyzed the day of harvest and after 7 d at 20 °C.

ANALYSIS OF RESPIRATION AND ETHYLENE PRODUCTION. The respiration and ethylene production of cherry fruit were measured immediately before and following 1-MCP treatment, and during

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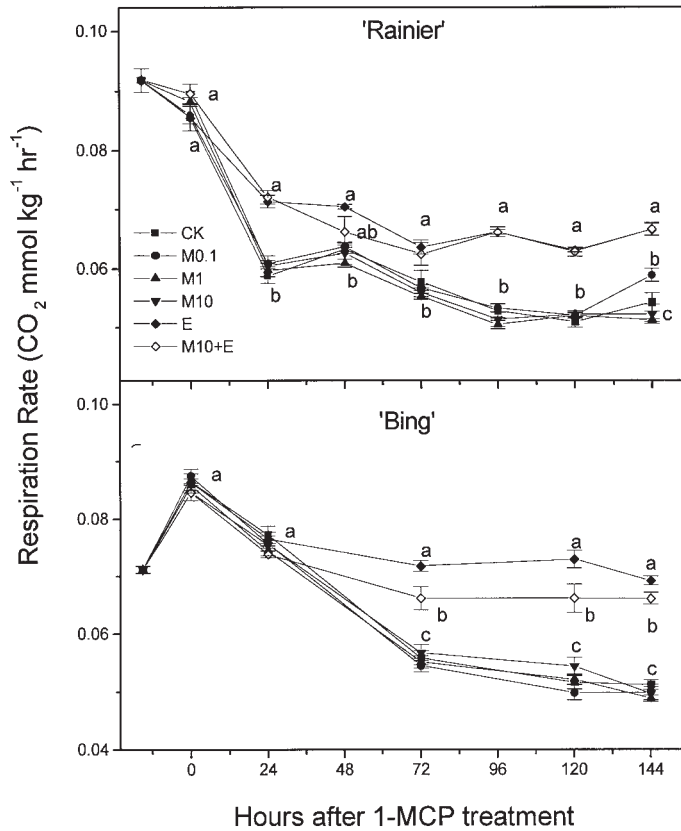


Fig. 1. Respiration rate of sweet cherry fruit ('Rainier' and 'Bing') after 1-MCP treatment exposure to ethylene ($35 \mu\text{L}\cdot\text{L}^{-1}$) or air. Means within same analysis date followed by different letters are significantly different, $P \leq 0.05$, $n = 5$, Duncan's multiple range test.

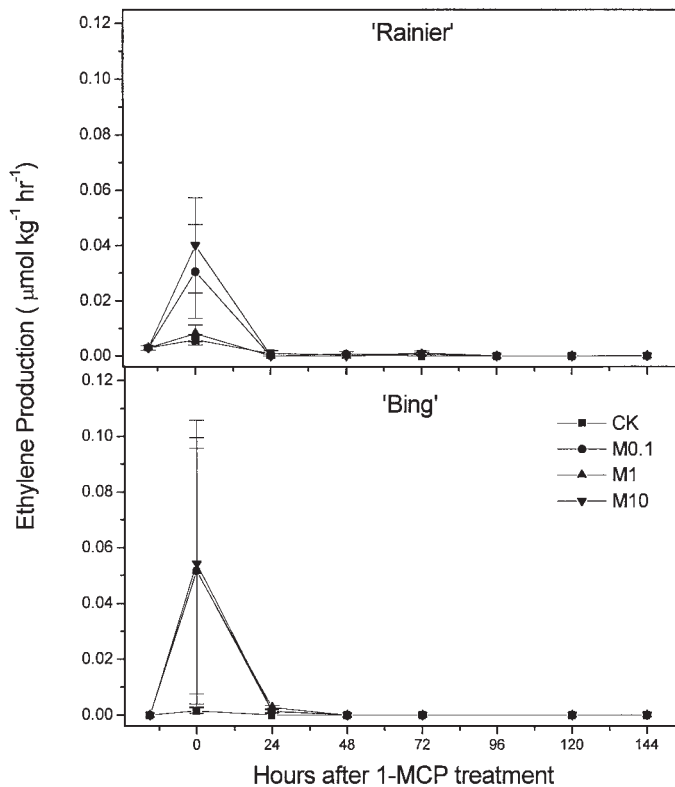


Fig. 2. Ethylene evolution by sweet cherry fruit ('Rainier' and 'Bing') after 1-MCP treatment. Bars indicate standard error, $n = 5$.

storage for treatments CK, M0.1, M1, and M10 in experiment 1. For treatments E and M10 + E, only respiration rate was measured using the same schedule. The measurement immediately following 1-MCP treatment was designated time zero. For experiment 2, respiration and ethylene production were measured immediately before and following ethylene treatment, and during storage of fruit from all treatments. The measurement immediately following ethylene treatment was designated time zero. Fifteen fruit (110 to 120 g) per replicate were sealed in a glass jar (0.96 L) for 1 h (5 replicates per treatment). Two gas samples (1 mL each) were collected from the headspace of each jar and used for CO_2 and ethylene analysis. CO_2 analysis was performed using a gas chromatograph (Hewlett Packard HP 5890, Hewlett Packard, Avondale, Pa.), equipped with a methanizer (John T. Booker, Austin, Texas), a 60-cm stainless steel column (2-mm ID) packed with 80/100 mesh Porapak Q (Alltech Associates Inc., Deerfield, Ill.) and a FID. N_2 , H_2 and air flow rates were 30, 30 and $300 \text{ mL}\cdot\text{min}^{-1}$; injector, methanizer, oven and FID temperatures were 35, 290, 30, and $200 \text{ }^\circ\text{C}$, respectively. Ethylene was measured using a gas chromatograph (HP5880A; Hewlett Packard, Avondale, Pa.) fitted with a 30-cm glass column (3.2-mm ID) packed with 80/100 mesh Porapak Q and a FID detector. Gas flows for N_2 , H_2 , and air were 30, 30, and $300 \text{ mL}\cdot\text{min}^{-1}$, respectively. Injector, oven, and FID temperatures were 100, 60, and $200 \text{ }^\circ\text{C}$, respectively.

DATA ANALYSIS. The data for respiration rate and ethylene production ($n = 5$), stem browning ($n = 30$), and fruit color ($n = 30$) were subjected to ANOVA using Microsoft Excel. Treatment mean separation was performed using Duncan's multiple range test, $P \leq 0.05$.

Results

EFFECT OF 1-MCP AND ETHYLENE ON RESPIRATION RATE AND ETHYLENE PRODUCTION. Fruit respiration by both cultivars decreased during storage at $20 \text{ }^\circ\text{C}$ (Fig. 1) and treatments with 1-MCP did not affect respiration of either cultivar stored in air. Respiration of fruit exposed to ethylene was significantly higher ($P \leq 0.05$) after 24 ('Rainier') or 72 ('Bing') h compared to fruit held in air. After 72 h exposure to ethylene, respiration of untreated 'Bing' fruit (E) was higher compared to fruit previously treated with $10 \text{ mL}\cdot\text{L}^{-1}$ 1-MCP (M10 + E). Ethylene production by both cultivars increased transiently following 1-MCP treatment (Fig. 2) then decreased to the initial rate within 24 h.

EFFECT OF 1-MCP TREATMENT AFTER ETHYLENE SHOCK. Ethylene production by 'Bing' cherry fruit increased transiently following exposure to ethylene for 6 h at $20 \text{ }^\circ\text{C}$ (Fig. 3A). Treatment with 1-MCP ($10 \mu\text{L}\cdot\text{L}^{-1}$) after exposure to ethylene reduced but did not prevent the increase in ethylene production induced by ethylene shock. Ethylene shock did not affect respiration rate (Fig. 3B); however, fruit treated with ethylene followed by 1-MCP had lower respiration 24 h after the 1-MCP treatment compared to the ethylene shock alone.

EFFECT OF 1-MCP AND ETHYLENE TREATMENTS ON STEM BROWNING. Stem browning was present in all treatments after 7 d at $20 \text{ }^\circ\text{C}$ (Table 1), and treatment with 1-MCP did not prevent stem browning in either cultivar. Exposure to ethylene increased the mean stem browning ratings, and treatment with 1-MCP before exposure to ethylene prevented the increase in stem browning for 'Bing' but not for 'Rainier'. Stem browning of 'Bing' increased after the ethylene shock regardless of subsequent treatment with 1-MCP.

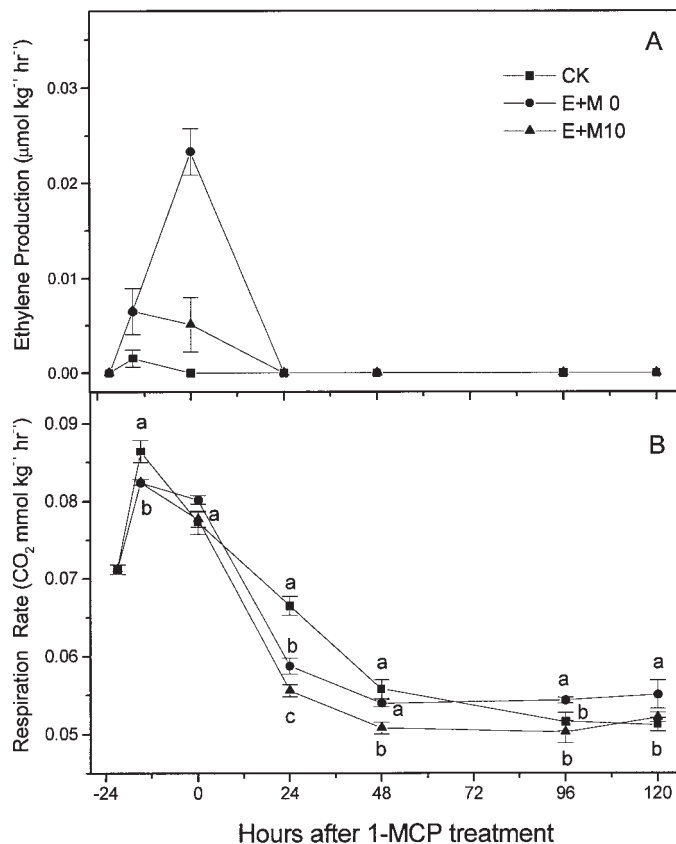


Fig. 3. Ethylene evolution (A) and respiration rate (B) by 'Bing' sweet cherry fruit after exposure to 80 µL·L⁻¹ ethylene, then treatment with 10 µL·L⁻¹ 1-MCP. Bars indicate standard error, n = 5.

EFFECT OF 1-MCP AND ETHYLENE TREATMENTS ON CHERRY FRUIT COLOR. 'Bing' is a solid red cherry, while 'Rainier' is yellow with a red blush; the h° and C values for 'Rainier' are higher compared to those for 'Bing' (Table 2). During 7 d at 20 °C, h° and C values for both cultivars decreased. There were no differences in color between treatments for 'Bing'. Changes in h° between treatments for 'Rainier' were smaller for the low concentration 1-MCP (M0.1) and ethylene treatments than for higher 1-MCP concentration treatments (M1 and M10), and h° values for M10 + E were higher compared to controls. Chroma values for fruit treated with 0.1 µL·L⁻¹ 1-MCP or ethylene were higher than the M1 or M10 treatments. However, h° and chroma values for control fruit were the lowest of all treatments.

Discussion

Ethylene responses occur via a metal-containing receptor (Burg and Burg, 1967). Many cyclopropene compounds can interact with the ethylene receptor and prevent ethylene action by blocking ethylene binding to the receptor in plant tissue (Sisler and Serek, 1997, 1999). Many studies indicate ripening of climacteric fruit is delayed following treatment with cyclic olefinic compounds including 1-MCP (Blankenship and Sisler, 1989, 1993; Fan et al., 1999a, 1999b, 1999c; Golding et al., 1998); however, responses to 1-MCP by nonclimacteric fruit vary. Treatment of 'Shamouti' orange (*Citrus sinensis* L. Osbeck) with 1-MCP does not prevent development of volatile off-flavors and chilling injury promoted by ethylene, but treatment with 1-MCP inhibits peel degreening promoted by ethylene (Porat et al.,

1999). Tian et al. (1997b) reported no significant differences in the progression of color change or firmness loss in strawberry fruit treated with DACP. Similarly, no significant differences in the rate of color change or firmness loss (data not shown) were detected in sweet cherry fruit treated with 1-MCP in our study. An ethylene releasing compound, ethephon, applied to 'Bing' cherry fruit (Li et al., 1994), also did not impact changes in fruit firmness during ripening. These results together indicate the regulation of color changes and softening during sweet cherry ripening occur independent of ethylene action or the lack thereof. Treatment with 1-MCP does not reduce development of stem browning. Stem browning was enhanced by exposure to ethylene after 1-MCP treatment in 'Rainier' and before 1-MCP treatment in 'Bing'. This indicates the processes resulting in browning of sweet cherry stems may not be related to endogenous ethylene, and enhancing stem browning by exogenous ethylene is not regulated via the ethylene receptor to which 1-MCP binds. Differences in responses between 'Bing' and 'Rainier' may be the result of cultivar variation.

Studies using cyclic olefin inhibitors of ethylene action have demonstrated increases in ethylene production and respiration during climacteric fruit ripening require active ethylene receptors (Fan et al., 1999a, 1999b, 1999c; Golding et al., 1998; Gong and Tian, 1998; Tian et al., 1997a). For sweet cherry, a nonclimacteric fruit, 1-MCP treatment transiently stimulates ethylene production. Tian et al. (1997b) reported a similar result from strawberry fruit treated with DACP, and suggested that the stimulation was due to increased ACC content in the fruit without changes in ACC oxidase activity. In addition, ethylene diffuses from receptors to which 1-MCP binds. The $t_{1/2}$ (the time for 1/2 of the receptors to become free after being exposed to the compound) of 2 to 10 min (Sisler and Serek, 1999). This departure is necessary for the formation of the active complex (Sisler and Serek, 1997). The $t_{1/2}$ for 1-MCP is much longer (i.e., 30 d in banana, Sisler and Serek, 1999). Therefore, diffused ethylene may be released from fruit tissue after 1-MCP treatment and may appear to enhance ethylene production. In climacteric fruit, because ethylene production is much higher, this enhancement may not be observed.

Table 1. Effect of ethylene and 1-MCP on sweet cherry fruit stem browning. Browning was rated visually as 1 = none; 2 = slight; 3 = moderate, 4 = severe. Stems were rated the day of harvest (Initial) and after 7 d at 20 °C following treatments. Fruit were treated with 1-MCP for 15 h at 20 °C following treatments. Fruit were treated with 1-MCP for 15 h at 0 (control), 0.1 (M0.1), 1 (M1), or 10 (M10) µL·L⁻¹. Fruit were exposed to 35 µL·L⁻¹ ethylene during 7 d at 20 °C: non-1-MCP treated fruit exposed to ethylene (E), fruit previously treated with 10 µL·L⁻¹ 1-MCP exposed to ethylene (M10 + E). Fruit exposed to 80 µL·L⁻¹ ethylene in air for 6 h before treatment with 1-MCP for 15 h: ethylene exposure then no 1-MCP treatment (E + M0), ethylene exposure then treatment with 10 µL·L⁻¹ 1-MCP (E + M10).

Treatment	Rainier	Bing
Initial	1.1 d ²	1.8 c
Control	3.0 c	2.9 b
M0.1	3.1 c	2.9 b
M1	3.0 c	2.8 b
M10	3.0 c	2.8 b
E	3.3 b	3.2 a
M10 + E	3.5 a	2.9 b
E + M0	---	3.3 a
E + M10	---	3.5 a

²Means with same letter are not significantly different within the column, $P \leq 0.05$, Duncan's multiple range test.

Table 2. Color of sweet cherry fruit. Fruit were treated with ethylene or 1-MCP then stored at 20 °C for 7 d. Initial color measurement conducted on the day of harvest. 1-MCP treatments were applied for 15 h at 0 (control), 0.1 (M0.1), 1 (M1), or 10 (M10) $\mu\text{L}\cdot\text{L}^{-1}$. Fruit were exposed to 35 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene during 7 d at 20 °C: non-1-MCP treated fruit exposed to ethylene (E), fruit previously treated with 10 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (M10 + E). Fruit exposed to 80 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene in air for 6 h before treatment with 1-MCP for 15 h: ethylene exposure then no 1-MCP treatment (E + M0), ethylene exposure then treatment with 10 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (E + M10).

Treatment	Rainier	Bing
Hue angle		
Initial	87.0 a ^z	10.5 a
CK	70.8 c	8.9 b
M 0.1	78.5 bc	8.3 b
M 1	73.5 c	8.5 b
M 10	71.3 c	8.1 b
E	79.0 bc	8.1 b
M10 + E	82.2 ab	8.2 b
E + M 0	---	8.5 b
E + M 10	---	7.8 b
Chroma		
Initial	76.0 a	13.4 a
CK	50.4 cde	9.3 b
M 0.1	60.6 b	8.9 b
M 1	43.4 e	8.8 b
M 10	49.3 de	8.8 b
E	59.4 bc	8.3 b
M10 + E	56.3 bcd	9.0 b
E + M 0	---	8.9 b
E + M 10	---	7.9 b

^zMeans with same letter are not significantly different within the column, $P \leq 0.05$, Duncan's multiple range test.

Treatment of sweet cherry fruit with 1-MCP does not induce changes in respiration rate. This is similar to results reported by Tian et al. (1997b, 2000) where DACP or 1-MCP treatment did not impact respiration of ripened (red) or pink strawberry fruit. There are two ethylene production systems in fruit, system one and two (McMurchie et al., 1972). Only system one, characterized by a low level of ethylene production and autoinhibition of ethylene production in response to exogenous ethylene treatment, is present in nonclimacteric or immature climacteric fruit (McGlasson, 1985). Wound-induced ethylene production in citrus flavedo tissue is also inhibited by exogenous ethylene treatment (Riov and Yang, 1982; Mullins et al., 1999). Propylene treatment of intact green bananas suppresses ethylene synthesis and delays the peak of ethylene production (McMurchie et al., 1972). Our results indicate that while ethylene production by sweet cherry fruit can be stimulated by an ethylene treatment of 80 $\mu\text{L}\cdot\text{L}^{-1}$ for 6 h, the stimulation can be inhibited in part by 1-MCP treatment after exposure to ethylene. Increased respiration after exposure to ethylene is partially inhibited in 'Bing' fruit previously treated with 1-MCP treatment. This suggests exogenous ethylene may induce system two ethylene production, but when exogenous ethylene is removed, system two production stops.

Treatment of banana (climacteric fruit) (Golding et al., 1998) or floral vegetable broccoli (Fan and Mattheis, 2000) with 1-MCP totally inhibits the effect of exogenous ethylene on respiration. However, in nonclimacteric fruit, the increase in respiration induced by exogenous ethylene is dependent on ethylene concen-

tration (Biale and Young, 1981), and may not be regulated by an ethylene receptor (Tian et al., 1997b). Our results with sweet cherry fruit are consistent with this hypothesis, as treatment with 1-MCP did not prevent the ethylene-induced increase in respiration for 'Bing' or 'Rainier' sweet cherries. Partial inhibition by 1-MCP of the ethylene-induced increase in respiration by 'Bing' fruit may result from less system two ethylene receptor induced by exogenous ethylene.

In summary, 1-MCP treatment of the sweet cherry cultivars 'Bing' and 'Rainier' transiently stimulated ethylene evolution but had no impact on respiration, color change, stem browning or firmness during fruit ripening. These responses illustrate differences between sweet cherry, a nonclimacteric fruit and climacteric fruit such as apple, pear, and tomato to the ethylene action inhibitor 1-MCP. Regulation of respiration in sweet cherry fruit may not be regulated by the ethylene receptor.

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