1-Methylcyclopropene Inhibits Degreening But Stimulates Respiration and Ethylene Biosynthesis in Grapefruit

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Abstract. We determined the effects of 1-methylcyclopropene (1-MCP) and ethylene on color change and CO₂ and ethylene production in grapefruit. Treatment with 1-MCP at concentrations equal to or greater than 75 nL·L⁻¹ inhibited ethylene-induced degreening, but increasing 1-MCP concentrations greater than 150 nL·L⁻¹ did not cause additional inhibition of degreening. Although ethylene-induced degreening was inhibited by 1-MCP, the effect was transient. Treating grapefruit with 15 to 75 nL·L⁻¹ 1-MCP resulted in a slight suppression of CO₂ production, whereas treatment with 150 or 300 nL·L⁻¹ 1-MCP resulted in rates of CO₂ production significantly higher than nontreated fruit. 1-MCP treatment also caused a very pronounced increase in the rate of C₂H₄ production that was both dose- and time-dependent. The effects of 1-MCP on respiration and ethylene evolution were reduced if fruit was subsequently exposed to ethylene. Fruit treated with 1-MCP alone had the highest rates of CO₂ production, fruit treated with ethylene after 1-MCP or ethylene alone had intermediate rates of CO₂ production, and control fruit had the lowest rate of CO₂ production. Rates of C₂H₄ evolution were ≈200 nL·kg⁻¹·h⁻¹ from control and C₂H₄-treated fruit compared with ≈10,000 nL·kg⁻¹·h⁻¹ from 1-MCP-treated fruit; fruit treated with ethylene after 1-MCP had ethylene production rates of ≈400 nL·kg⁻¹·h⁻¹. Our results lend further support for a regulatory role for ethylene in degreening of citrus and suggest that endogenous levels of ethylene regulate ethylene production.

Grapefruit (*Citrus paradisi*), like all citrus fruits, produce very low levels of ethylene throughout development and do not exhibit an ethylene climacteric during ripening (Aharoni, 1968; Eaks, 1970). Citrus fruit do exhibit elevated levels of ethylene production in response to a variety of stresses, including wounding (Evenson et al., 1981; Hyodo, 1977; Riov and Yang, 1982), low temperature (Eaks, 1980; McCollum and McDonald, 1991), and infection by pathogens (Achilea et al., 1984; Mullins et al., 2000).

Exposure of citrus fruit to exogenous ethylene induces a number of physiological and biochemical changes, including increases in activity of phenylalanine ammonia lyase (Riov et al., 1969), chlorophyllase (Trebitsh et al., 1993), and cellulase (Kazokas and Burns, 1998), alteration of protein complement (Alonso et al., 1992), and gene expression (Alonso et al., 1995; Alonso and Granell, 1995; Jacob-Wilk et al., 1997, 1999; Kazokas and Burns, 1998). Eaks (1970) reported that exposure of citrus fruits to ethylene resulted in a temporary and repeatable increase in respiration. Ethylene will

also induce abscission of citrus fruits (Goren, 1993). Most notable among the responses of citrus fruits to exogenous ethylene is the loss of chlorophyll (Eilati and Goldschmidt, 1969; Garcia-Luis et al., 1986; Goldschmidt et al., 1977; Purvis and Barmore, 1981; Shimokawa et al., 1978) or “degreening” of commercial practice (Grierson et al., 1986).

Goldschmidt (1997) has pointed out that the lack of a ripening-associated autocatalytic rise in ethylene does not rule out a role for ethylene in the development of nonclimacteric fruits. Several lines of evidence indicate that ethylene does have a regulatory role in citrus fruit physiology. First is the effect of exogenous ethylene on wound-induced ethylene. Exposure of citrus peel discs to ethylene results in the inhibition of wound-induced ethylene, the result of a suppression of ACC, the immediate precursor of ethylene (Riov and Yang, 1982), and suppression of ACC synthase messenger RNA (Mullins et al., 1999). Second is the effect of inhibitors of ethylene action on ethylene-related responses. Goldschmidt et al. (1993) used the ethylene antagonists 2,5-norbornadiene and silver nitrate to block ethylene action and were able to confirm the role of endogenous ethylene in degreening of citrus fruit. More recently, studies with the ethylene action inhibitor 1-methylcyclopropene (1-MCP) (Blankenship and Dole, 2003) have strengthened evidence for a role of endogenous ethylene in citrus fruit physiology. Porat

et al. (1999) reported that treatment of ‘Shamouti’ oranges with 1-MCP completely inhibited ethylene-enhanced degreening. In contrast to oranges, neither ethylene nor 1-MCP had significant effect on color change in ‘Oroblanco’ a pummelo × grapefruit hybrid (Porat et al., 2001). Treatment with 1-MCP will also inhibit ethylene-induced abscission in citrus (Sisler et al., 1999; Zhong et al., 2001). Curiously, treatment of citrus rind discs (Mullins et al., 1999; Sisler et al., 1999) and leaves (Zhong et al., 2001) with 1-MCP has been reported to actually stimulate the production of ethylene while at the same time inhibiting the response to ethylene. That treatment with 1-MCP stimulates ethylene production indicates the perception of endogenous ethylene regulates the production of ethylene in citrus. The stimulation of ethylene production by 1-MCP has only been reported for wounded tissues; it is not known if treatment with 1-MCP elicits similar responses in nonwounded tissue. Our objectives in the work presented here were to determine the effects of 1-MCP treatment on ethylene-induced physiological changes in intact grapefruit.

Materials and Methods

Plant material

‘Marsh’ grapefruit harvested from a commercial grove were used in all studies. Fruit was harvested in August and September before color break. Treatment with 1-MCP was conducted within 24 h after harvest.

The number of fruit used for each experimental unit ranged from 5 to 15 depending on the experiment.

Treatments with 1-MCP and ethylene

Treatment with 1-MCP was conducted in 200-L steel chambers. Five milliliters of water containing 0.5% (w/v) sodium dodecyl sulfate was added to the appropriate amount of EthylBloc (0.14% 1-MCP) (Biotechnologies for Horticulture, Inc., Waltersboro, S.C.) to give the desired concentration of 1-MCP gas (15–3000 nL·L⁻¹). The solutions were placed into chambers containing the fruit and the top of the chambers sealed with gasketed lids. The chambers were held at 25 °C for 24 h. Treatment with ethylene (5 μL·L⁻¹) was done at 25 °C in a room with one air exchange per hour.

Five separate experiments were conducted. The objective of the first two experiments was to determine the effects of 1-MCP dose on color loss of grapefruit peel. In the first experiment, grapefruit were exposed to 0, 15, 30, 75, 150, or 300 nL·L⁻¹ 1-MCP for 24 h. After treatment with 1-MCP, the fruit was transferred to air containing 5 μL·L⁻¹ ethylene for up to 72 h. Fruit rind color was measured after 0, 24, 48, and 72 h in ethylene. The second experiment was conducted exactly as the first but with 1-MCP concentrations of 0, 150, 300, 750, 1500, and 3000 nL·L⁻¹. The objective of the third experiment was to determine the effects of 1-MCP dose on grapefruit respiration and ethylene evolution.

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Fruits were exposed to 1-MCP (300 nL·L⁻¹) as described previously but, after exposure to 1-MCP, were transferred to air rather than air containing ethylene. At 0, 24, 48, and 72 h after transfer, rates of CO₂ and ethylene evolution from the fruit were measured. The objective of the fourth experiment was to determine the effects of duration of 1-MCP or ethylene exposure on CO₂ and ethylene evolution from grapefruit. Fruit were held in air, ethylene (5 µL·L⁻¹), or 1-MCP (300 nL·L⁻¹) for up to 72 h. Fruit was removed from the treatments at 24-h intervals and held in air at 25 °C; CO₂ and ethylene production were measured 24, 48, and 72 h after transfer to air. The objective of the fifth experiment was to determine the effects of 1-MCP (300 nL·L⁻¹) alone, 1-MCP followed by ethylene, or ethylene alone on color change, CO₂, and ethylene evolution. Fruit was divided into two groups; one group was treated with 1-MCP for 24 h and the second group held in air for 24 h. After the initial 24-h treatment, the two treatment groups were divided into two subgroups; one subgroup from each of the original groups was transferred to air and the second subgroup from each of the original groups was transferred to air containing 5 µL·L⁻¹ ethylene. The ethylene treatment was done for 72 h, after which the fruit was transferred to air. During the course of treatment, peel color was measured at 24-h intervals. Twenty-four hours after transfer of the ethylene-treated fruit to air, CO₂ and ethylene evolution were determined from fruit in each of the four treatment groups.

Color

Fruit peel color (three readings per fruit) was determined using a Minolta chromometer (model CR-300; Minolta Camera Corp., Osaka, Japan). The Commission Internationale de l'Eclairage a* and b* color index scale was used and peel color expressed as a*/b* ratio.

Carbon dioxide and ethylene production

Evolution of carbon dioxide and ethylene from whole fruit was determined using a static system. Fruit were sealed in 1.8-L glass jars with lids fitted with rubber septa. Fruit were incubated at 20 °C for ≈1 h for CO₂ determination and ≈3 h for ethylene. Headspace samples were collected and analyzed by gas chromatography (McCullum and McDonald, 1991). A thermal conductivity detector was used for the detection of CO₂ and a flame ionization detector for the detection of C₂H₄.

Results

Effects of 1-MCP at concentrations from 15 to 3000 nL·L⁻¹ on ethylene-induced degreening of 'Marsh' grapefruit were determined in the first two experiments. Treatment of 'Marsh' grapefruit with 1-MCP at concentrations of 75 nL·L⁻¹ or greater for 24 h resulted in inhibition of ethylene-induced degreening during 48 h in ethylene and an additional 24 h in air (Fig. 1A); however,

increasing the concentration of 1-MCP to greater than 75 nL·L⁻¹ did not result in further inhibition of degreening (Fig. 1B).

There was no significant difference in CO₂ production among grapefruit that had been treated with 1-MCP at concentrations from 0 to 300 nL·L⁻¹ for 24 h at the time of transfer to air (Fig. 2). Twenty-four h after transfer to air, CO₂ production from fruit that had been treated with 15 to 150 nL·L⁻¹ 1-MCP was slightly, but significantly, lower than from fruit that had been treated with 0 or 300 nL·L⁻¹ 1-MCP. At 48 h after 1-MCP treatment, CO₂ production from fruit that had been treated with 150 or 300 nL·L⁻¹ was significantly higher than from all other treatments, whereas CO₂ production from fruit treated with 15 or 30 nL·L⁻¹ 1-MCP was lower than from fruit not exposed to 1-MCP. Seventy-two h after transfer to air, CO₂ production from grapefruit that had been treated with 75 to 300 nL·L⁻¹ 1-MCP was significantly higher than from fruit that had been treated with 0–30 nL·L⁻¹ 1-MCP.

Ethylene production from 'Marsh' grapefruit also exhibited a dose-dependent response to 1-MCP; however, the pattern of the response was considerably different than for CO₂ (Fig. 2B). At the time of transfer from 1-MCP to air, ethylene production was significantly higher in fruit treated with 150 or 300 nL·L⁻¹ 1-MCP than in all other fruit. Twenty-four h after transfer to air, all fruit treated with 1-MCP were producing greater amounts of ethylene than were fruit not exposed to 1-MCP. In addition, the amount of ethylene being produced increased with increasing dose of 1-MCP. Ethylene production increased and reached its maximum between 24 and 48 h after treatment from fruit that had been exposed to 1-MCP at concentrations of 75 nL·L⁻¹ or greater. After transferring to air (48–72 h after exposure to 1-MCP), the rate of ethylene production decreased sharply; however, it was still significantly higher than in fruit that had been exposed to 0 or 15 nL·L⁻¹ 1-MCP.

The effects of duration of ethylene (5 µL·L⁻¹) or 1-MCP (300 nL·L⁻¹) treatment on rates of CO₂ and ethylene evolution from 'Marsh' grapefruit are presented in Fig. 3. In general, compared with controls, treatment with ethylene resulted in only a slight increase in CO₂ evolution and only if the duration of treatment exceeded 24 h. Rates of ethylene evolution from ethylene-treated fruit were for the most part similar to those of control fruit regardless of treatment duration or time after treatment (Fig. 3B, D, F). The response of 'Marsh' grapefruit to treatment with 1-MCP, in contrast to ethylene, was very pronounced. Fruit treated with 1-MCP for 24 h had rates of CO₂ evolution less than control fruit 24 h after transfer to air but, at 48 and 72 h after transfer, had rates of CO₂ evolution greater than control fruit (Fig. 3A). Fruit treated with 1-MCP for 48 or 72 h had rates of CO₂ evolution that were consistently higher than controls (Fig. 3C, E). Additionally, rates of CO₂ evolution from 1-MCP-

Fig. 1. Effect of 1-MCP dose (A, 0–300 nL·L⁻¹ treatment; B, 0–3000 nL·L⁻¹) on ethylene-induced degreening of 'Marsh' grapefruit. Fruit was held at 25 °C in the indicated concentrations of 1-MCP for 24 h and then transferred to air containing 5 µL·L⁻¹ ethylene at 25 °C. Values represent the means of 15 fruit. Vertical bars indicate standard error of the mean.

Fig. 2. Effects of 1-MCP treatment on rates of (A) CO₂ and (B) ethylene evolution from 'Marsh' grapefruit. Fruit was treated as described in Figure 1. After 1-MCP treatment, fruit was transferred to 1-MCP free air at 25 °C and CO₂ and ethylene evolution measured every 24 h. Values represent means of three fruit. Vertical bars indicate standard error of the mean.

treated fruit increased with time after transfer to air regardless of treatment duration (Fig. 3A, C, E). Effects of 1-MCP treatment on ethylene evolution from 'Marsh' grapefruit were quite dramatic. Ethylene evolution increased both with increased duration of

Fig. 3. Effects of duration of exposure to ethylene or 1-MCP on rates of CO₂ and ethylene evolution from 'Marsh' grapefruit. Fruit was treated for (A and B) 24 h, (C and D) 48 hours, or (E and F) 72 h and then transferred to air. At 24, 48, and 72 h after transfer to air, rates of CO₂ and ethylene evolution were measured. Bars represent the average of 5 fruit $\pm$ standard deviation.

1-MCP treatment as well as with time after treatment (Fig. 3B, D, F).

In another set of experiments, we determined the effects of 1-MCP (300 nL·L⁻¹, 24 h at 25 °C) alone or followed by ethylene (5 μ L·L⁻¹, 72 h at 25 °C). We also followed color changes in the fruit for a greater duration (3 d vs. 1 d) after ethylene treatment than in the first experiments. Treatment of 'Marsh' grapefruit with 300 nL·L⁻¹ 1-MCP for 24 h inhibited the effect of ethylene-enhanced degreening, but the effect was only temporary because 1-MCP-treated fruit eventually attained nearly the same color as did the nontreated fruit (Fig. 4). Color of grapefruit

treated with 1-MCP alone was similar to control fruit and lower than for fruit that were exposed to ethylene ($\pm$ 1-MCP). However, a/b ratios increased for both control and 1-MCP-treated fruit during the experiment.

Treatment with ethylene for 48 h after 1-MCP resulted in a suppression of 1-MCP-induced increases in CO₂ and C₂H₄ evolution. Rates of CO₂ evolution were highest in 1-MCP-treated fruit, intermediate and similar in fruit treated with C₂H₄ either with or without previous treatment with 1-MCP, and lowest in control fruit (Fig. 5A). Treatment with 1-MCP alone resulted in a 50-fold increase in C₂H₄ evolution compared with

a roughly doubling of C₂H₄ production in fruit treated with ethylene after 1-MCP (Fig. 5B). The rate of C₂H₄ evolution from C₂H₄-treated fruit was slightly higher than from control fruit.

Discussion

The role of ethylene in the development of nonclimacteric fruits has received much less attention than for climacteric fruits. However, it is well known that citrus, as well as other nonclimacteric fruits, respond to treatment with ethylene (Goldschmidt, 1997) and that antagonists of ethylene action can inhibit

Fig. 4. Effects of 1-MCP, 1-MCP followed by ethylene, or ethylene alone on degreening of 'Marsh' grapefruit. Fruit was held at 25 °C in either air or air containing 300 nL·L⁻¹ 1-MCP for 24 h and then transferred to air containing 5 µL·L⁻¹ ppm at 25 °C for 72 h. After ethylene treatment, fruit was transferred to air free of ethylene at 25 °C.

Fig. 5. Effects of 1-MCP, ethylene, and 1-MCP + ethylene on (A) CO₂ and (B) ethylene production from 'Marsh' grapefruit. Fruit was treated as described in Figure 4. CO₂ and ethylene production was determined after 48 h in ethylene-free air.

normal developmental changes in citrus (Goldschmidt et al., 1993; Sisler et al., 1999; Zhong et al., 2001) lending further support for the role of ethylene.

Ethylene treatment leads to the loss of green color in grapefruit (Figs. 1 and 4) and other citrus fruits (Eilati and Goldschmidt, 1969; Garcia-Luis et al., 1986; Goldschmidt et al., 1977; Purvis and Barmore, 1981; Shimokawa et al., 1978). The effect of ethylene on color change in citrus fruit has been shown to be related to an increase in chlorophyllase activity (Purvis and Barmore, 1981; Shimokawa et al., 1978; Trebitsh et al., 1993) and also an increase in the abundance of chlorophyllase messenger RNA (Jacob-Wilk et al., 1999). In addition, treatments that inhibit ethylene action have been shown to in-

hibit degreening of citrus fruit (Goldschmidt et al., 1993; Porat et al., 1999). Interestingly, 'Oroblanco' (a grapefruit × pummelo hybrid) fruit show very little color change in response to ethylene treatment and no response to 1-MCP (Porat et al., 2001). Our results with grapefruit fall somewhere in between results with oranges and 'Oroblanco' in that treatment with ethylene does lead to a significant loss of green color and treatment with 1-MCP causes a delay in that loss, but the effect does not persist (Fig. 4).

Although few reports document the effects of 1-MCP on respiration, in those that do, there has been a suppression of respiration (Blankenship and Dole, 2003 and references therein). We found that 1-MCP can inhibit or stimulate CO₂ production in grapefruit depending on dose and time after treatment. Grapefruit treated with 15 or 30 nL·L⁻¹ 1-MCP for 24 h had lower rates of CO₂ production than did control or fruit treated with 1-MCP at rates of 75 nL·L⁻¹ and higher (Figs. 2a and 3A). The 1-MCP-induced increase in respiration was reduced if fruit was subsequently treated with ethylene (Fig. 5A). Ethylene has been reported to cause a transient increase in respiration rates of grapefruit (Eaks, 1970). We found that CO₂ production from ethylene-treated grapefruit was only slightly higher than from control fruit (Figs. 3A, C, E and 5A). How ethylene can inhibit the 1-MCP-induced increase in respiration is difficult to explain.

Treatment with 1-MCP has typically resulted in a suppression of ethylene evolution from a variety of plant tissues (Blankenship and Dole, 2003 and references therein). However, 1-MCP treatment has been reported to increase wound-induced ethylene production (Mathooko et al., 2001; Mullins et al., 2000; Owino et al., 2002). It has also been reported that 1-MCP enhances ethylene biosynthesis in leaves of coriander (Jiang et al., 2002) and parsley (Ella et al., 2003). In the current study, we also found that 1-MCP induces ethylene biosynthesis in grapefruit in a dose-dependent manner (Fig. 2B). In parsley leaves, 1-MCP caused a rapid increase in ethylene evolution followed by a gradual decrease (Ella et al., 2003). The effect of 1-MCP on grapefruit, a nonclimacteric fruit, seems to be similar to that observed for leaf tissue and system 1 ethylene in climacteric fruit (Barry et al., 2000; Nasatsuka et al., 1998). Although ethylene has been found to be autoinhibitory in citrus rind discs (Mullins et al., 1999; Riov and Yang, 1982), in the present study, we found that ethylene production from ethylene-treated intact grapefruit tended to be slightly higher than for nonethylene treated fruit (Figs. 3B, D, F and 5B), although this was not entirely consistent. This suggests that the regulation of wound-induced ethylene differs from the regulation of ethylene in non-wounded tissue in citrus fruit. Blocking the perception of ethylene leads to a massive increase in ethylene biosynthesis (Figs. 2B, 3B, D, F and 5B), whereas exposure to a low dose of ethylene results in a slight stimulation of ethylene biosynthesis.

Blocking the perception of ethylene with 1-MCP has pronounced effects on grapefruit physiology. These results lend further support for a regulatory role for ethylene in citrus fruit development. Although the potential for using 1-MCP as a postharvest treatment to reduce losses of citrus fruit is doubtful, studies with 1-MCP can provide insight into the effects of endogenous ethylene on citrus fruit.

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