‘Law Rome’ and ‘Golden Delicious’ Apples Differ in Their Response to Preharvest and Postharvest 1-Methylcyclopropene Treatment Combinations

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Abstract. Experiments were conducted to compare the effects of different preharvest and postharvest 1-methylcyclopropene (1-MCP) treatment combinations on ‘Law Rome’ and ‘Golden Delicious’ apple fruit. Preharvest 1-MCP sprays had minimal effects on postharvest 1-MCP treatment combinations on ‘Law Rome’ and ‘Golden Delicious’ apples to differ in their response to preharvest (spray) and postharvest (gaseous) 1-MCP treatments. The positive effects of preharvest 1-MCP on postharvest quality of ‘Law Rome’ declined in fruit that were harvested 3 days or more after spraying, whereas preharvest 1-MCP continued to have a positive effect on postharvest fruit quality of ‘Golden Delicious’ that were harvested up to 9 days after spraying. The loss in postharvest effects of preharvest 1-MCP treatment on ‘Law Rome’ at delayed harvests was reinstated by exposing fruit to gaseous 1-MCP on the day of harvest. These findings suggest that attached apple fruit of some cultivars may be capable of rapidly generating new ethylene receptors.

Apple fruit must be harvested at an acceptable stage of maturity and carefully managed in the postharvest environment to deliver optimal fruit quality to the consumer. Many of the changes that occur during fruit ripening, including increases in respiration and aroma production and softening of the apple flesh, are triggered by the autocatalytic rise in ethylene production (Schaffer et al., 2007) that defines a climacteric fruit. Currently, the fruit maturation process can be managed in commercial orchards by preharvest application of the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (ReTain; Valent BioSciences Corporation, Libertyville, IL) or by postharvest treatment with the ethylene action inhibitor 1-methylcyclopropene (1-MCP, SmartFresh; AgroFresh Inc., Springhouse, PA). In the absence of ethylene, the ETR family of ethylene receptors directly activate CTR1, resulting in negative regulation of the ethylene response pathway. The activity of CTR1 is inhibited when ethylene binds with the receptor, releasing this negative regulation of the response pathway (Bleecker and Kende, 2000; Huang et al., 2003). 1-MCP is an ethylene antagonist, having a 10-fold higher affinity for the receptor (Blankenship and Dole, 2003), effectively maintaining negative regulation of the ethylene response pathway in the presence of ethylene. Although recent studies have demonstrated the efficacy of preharvest 1-MCP sprays on postharvest fruit quality in apple (Byers et al., 2005; Elving et al., 2007; McArtney et al., 2008; Pozo et al., 2004; Yuan and Carbaugh, 2007), a formulation of sprayable 1-MCP has not been commercially available. Tatsuki et al. (2007) speculate that the efficacy of a postharvest 1-MCP treatment is influenced by various factors, including the number of ethylene receptors that are formed after treatment. Similarly, Blankenship and Dole (2003) assumed that 1-MCP binds permanently to receptors present at the time of treatment and that subsequent generation of new binding sites will result in a return of ethylene sensitivity. Presumably, therefore, the efficacy of a preharvest 1-MCP spray will be influenced by the degree of saturation of the ethylene receptors at the time of treatment and by the rate of formation of new ethylene receptors in attached fruit after field exposure to 1-MCP. Reduced efficacy of a preharvest 1-MCP spray with increasing delay between treatment and harvest might result from the formation of new ethylene receptors in the fruit tissues. Furthermore, if fruit from trees that received a preharvest 1-MCP spray remain responsive to a postharvest gaseous 1-MCP treatment, even at delayed harvests, then this would provide strong evidence in support of the hypothesis that new ethylene receptors were being formed as fruit ripened on the tree. In the present study, different preharvest (spray) and postharvest (gaseous) 1-MCP treatments were applied to ‘Law Rome’ and ‘Golden Delicious’ apples to provide data in support of the hypothesis that new ethylene receptors may be rapidly formed in ripening apple fruit of some apple cultivars.

Materials and Methods

‘Law Rome’. (2007). A block of uniform mature ‘Law Rome’/‘M.7’ apple trees was selected within a commercial orchard in Henderson County, NC. A proprietary formulation of 1-MCP (3.8% a.i.; Harvista; AgroFresh Inc., Spring House, PA) was applied at 160 mg L–1 on 17 Sept. 2007 with a tractor-mounted axial fan sprayer calibrated to deliver 1850 L ha–1. Mixing and sprayer calibration were as described previously (McArtney et al., 2008). Briefly, IAP Hi Supreme spray oil (Independent Agribusiness Professionals, Fresno, CA) was added at 1% of the final volume to the half-filled spray tank and then Silwet L-77 organosilicone surfactant (Helena Chemical Co., Collierville, TN) was added at 0.05% of the final volume before the specified amount of 1-MCP was added and the tank filled to the final volume.

Eight uniform trees were selected within the orchard with at least one guard tree separating each treatment tree. Four of the trees were treated with 160 mg L–1 1-MCP and the remaining four trees were left untreated. The treatments were arranged as fully guarded single-tree plots in a randomized complete block design experiment with four replications. A random sample of 30 fruit was harvested from each tree 3, 10, 17, and 24 d after treatment and separated into three samples of 10 fruit per plot. The first sample was used to determine treatment effects on fruit maturity as determined by flesh firmness and starch index (SI). At each harvest date, the second sample of 10 fruit was exposed to 1 μL L–1 1-MCP (SmartFresh;
AgroFresh, Inc.) in a closed chamber with a circulation fan for 24 h at 0 °C, whereas the third sample was held in a separate room at 0 °C. Samples two and three were held in the same room at 0 °C in ambient atmosphere after postharvest 1-MCP treatment for 30 d and then held at 20 °C for 7 d before measuring internal ethylene concentration (IEC) and flesh firmness. IEC was measured by injecting a 1-mL gas sample taken from the core cavity of each fruit into a gas chromatograph (Model GC-8A; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and an activated alumina column (Supelco Div., Sigma-Aldrich, Bellefonte, PA). Flesh firmness was measured on opposite pared sides of each fruit with a Guss model GS-20 fruit texture analyzer (QA Supplies, LLC, Norfolk, VA). SI was rated according to the Cornell Starch Chart (Blanpied and Silsby, 1992) where 1 = 100% staining and 8 = 0% staining.

‘Golden Delicious’, (2008). Two groups of eight uniform ‘Golden Delicious’/M.7 apple trees were selected within a mature planting at the Mountain Horticultural Crops Research Station (Mills River, NC). The experimental methodology was identical to that described in the ‘Law Rome’ 2008 experiment except that the spray dates were 30 Aug. and 9 Sept., respectively.

The effects of 1-MCP on fruit maturity at harvest and fruit IEC and firmness after harvest were analyzed by generalized linear models procedures using the SAS program (SAS Institute Inc., Cary, NC). Statistical differences in fruit IEC and firmness values between treatments were described by P values for differences between the least squares means.

Table 1. Flesh firmness and starch index (SI) of ‘Law Rome’ apple fruit harvested at different intervals after a preharvest spray application of 160 mg L⁻¹ 1-methylcyclopropene (1-MCP).z

| Fruit cultivar | Preharvest 1-MCP application date | Time of harvest (d after 1-MCP spray) |
|---------------|----------------------------------|--------------------------------------|
| Golden Delicious | 30 Aug. | | |
| Control | 0.2 | 0.2 | 0.2 | 0.2 |
| 1-MCP | 0.2 | 0.2 | 0.3 | 0.2 |
| Flesh firmness (N) | | | | |
| Control | 71 | 70 | 71 | 68 |
| 1-MCP | 72 | 72 | 68* | 68 |
| SI (1–8) | | | | |
| Control | 2.8 | 2.6 | 2.7 | 3.6 |
| 1-MCP | 2.2 | 2.1 | 2.8 | 4.0 |
| SSC (%) | | | | |
| Control | 13.5 | 13.6 | 13.8 | 13.4 |
| 1-MCP | 13.9 | 13.8 | 13.7 | 13.5 |
| Law Rome | 23 Sept. | | | |
| Control | 70 | 69 | 69* | 71* |
| 1-MCP | 71 | 69 | 69* | 71* |
| Flesh firmness (N) | | | | |
| Control | 4.2 | 3.8 | 4.7 | 4.7 |
| 1-MCP | 3.6 | 3.3 | 4.0 | 4.0 |
| SSC (%) | | | | |
| Control | 13.6 | 14.6 | 13.6 | 14.8 |
| 1-MCP | 14.8 | 14.9 | 14.9 | 14.8 |
| 7 Oct. | | | | |
| Control | ND | ND | ND | ND |
| 1-MCP | ND | ND | ND | ND |
| Flesh firmness (N) | | | | |
| Control | 90 | 89 | 85 | 86 |
| 1-MCP | 90 | 86 | 88 | 86 |
| SI (1–8) | | | | |
| Control | 4.3 | 3.6 | 4.9 | 4.8 |
| 1-MCP | 4.2 | 3.8 | 4.4** | 4.3 |
| SSC (%) | | | | |
| Control | 11.8 | 11.7 | 11.8 | 12.2 |
| 1-MCP | 11.9 | 11.7 | 12.0 | 11.9 |

z1-MCP was applied to each cultivar at two different times before the climacteric.

Table 2. Internal ethylene concentration (IEC), flesh firmness, starch index (SI), and soluble solids concentration (SSC) of ‘Golden Delicious’ and ‘Law Rome’ apple fruit harvested at different intervals after a preharvest spray application of 150 mg L⁻¹ 1-methylcyclopropene (1-MCP).*

| Cultivar | Preharvest 1-MCP application date | Time of harvest (d after 1-MCP spray) |
|----------|----------------------------------|--------------------------------------|
| Golden Delicious | 30 Aug. | | |
| Control | 12.3 | 12.4 | 12.2 | 12.5 |
| 1-MCP | 12.6 | 12.6 | 12.6 | 12.6 |
| IEC (µL L⁻¹) | | | | |
| Control | 0.2 | 0.2 | 0.2 | 0.2 |
| 1-MCP | 0.2 | 0.2 | 0.3 | 0.2 |
| Flesh firmness (N) | | | | |
| Control | 71 | 70 | 71 | 68 |
| 1-MCP | 72 | 72 | 68* | 68 |
| SI (1–8) | | | | |
| Control | 2.8 | 2.6 | 2.7 | 3.6 |
| 1-MCP | 2.2 | 2.1 | 2.8 | 4.0 |
| SSC (%) | | | | |
| Control | 13.5 | 13.6 | 13.8 | 13.4 |
| 1-MCP | 13.9 | 13.8 | 13.7 | 13.5 |
| Law Rome | 23 Sept. | | | |
| Control | 70 | 69 | 69* | 71* |
| 1-MCP | 71 | 69 | 69* | 71* |
| IEC (µL L⁻¹) | | | | |
| Control | 4.2 | 3.8 | 4.7 | 4.7 |
| 1-MCP | 3.6 | 3.3 | 4.0 | 4.0 |
| Flesh firmness (N) | | | | |
| Control | 13.6 | 14.6 | 13.6 | 14.8 |
| 1-MCP | 14.8 | 14.9 | 14.9 | 14.8 |
| SSC (%) | | | | |
| Control | 11.8 | 11.7 | 11.8 | 12.2 |
| 1-MCP | 11.9 | 11.7 | 12.0 | 11.9 |
| Law Rome | 23 Sept. | | | |
| Control | 90 | 89 | 85 | 86 |
| 1-MCP | 90 | 86 | 88 | 86 |
| IEC (µL L⁻¹) | | | | |
| Control | 4.3 | 3.6 | 4.9 | 4.8 |
| 1-MCP | 4.2 | 3.8 | 4.4** | 4.3 |
| Flesh firmness (N) | | | | |
| Control | 11.8 | 11.7 | 11.8 | 12.2 |
| 1-MCP | 11.9 | 11.7 | 12.0 | 11.9 |

*1-MCP was applied to each cultivar at two different times before the climacteric.

**Significantly different from the control at P = 0.01.
**Results and Discussion**

*Fruit maturity at harvest.* Preharvest 1-MCP treatments had only minor effects on fruit maturity at the time of harvest, regardless of the stage of fruit maturity at the time of treatment (Tables 1 and 2). Preharvest 1-MCP treatments included a spray oil in the 2007 experiment, but was applied without oil in the 2008 experiments. The lack of pronounced effects of preharvest 1-MCP on fruit maturity at harvest does not appear to be related to the exclusion of a spray oil in 2008 because there were significant effects on postharvest fruit quality in both years. These data are in conflict with previous findings that preharvest 1-MCP sprays reduced fruit IEC, delayed starch conversion, and increased fruit firmness at harvest (Elfving et al., 2007; McArtney et al., 2008). These authors concluded that 1-MCP applied before harvest held promise as a harvest management tool by enabling fruit to remain on the tree beyond the normal harvest date without sacrificing fruit quality. We reported previously that preharvest 1-MCP sprays delayed fruit maturity of ‘Golden Delicious’ and ‘Law Rome’ in studies in Pennsylvania but was without effect on harvest maturity of ‘Law Rome’ fruit in a study in North Carolina (McArtney et al., 2008). Because harvest maturity of ‘Golden Delicious’ and ‘Law Rome’ were not greatly affected by a preharvest 1-MCP treatment in the current experiments in North Carolina, we suggest that preharvest 1-MCP sprays may not be useful as a harvest management aid in warmer climates.

*Poststorage quality of ‘Law Rome’* in 2007. After 30 d storage at 0 °C plus 7 d at 20 °C, the IEC was ≥100 μL·L⁻¹ and 200 μL·L⁻¹ in fruit harvested 3 d and 24 d after spraying 1-MCP, respectively (Fig. 1). Spraying 160 mg·L⁻¹ 1-MCP on 17 Sept. reduced poststorage IEC of fruit that were harvested 3 d later but was without effect on IEC of fruit harvested 10, 17, or 24 d later. Exposure to 1 μL·L⁻¹ 1-MCP on the day of harvest reduced poststorage IEC of fruit that were harvested on 20 Sept. (SI 4.9), 27 Sept. (SI 5.6), and 4 Oct. (SI 6.0) but not on 11 Oct. (SI 6.4) (Fig. 1A). The poststorage increase in fruit IEC and decline in firmness of ‘Law Rome’ fruit in 2007 indicate a loss in efficacy of preharvest 1-MCP with increasing delay between treatment and harvest. Exposure to gaseous 1-MCP on the day of harvest reduced the loss of firmness during storage in fruit that were harvested before 4 Oct. (Fig. 1B). A preharvest spray of 1-MCP maintained flesh firmness only in fruit that were harvested 3 d after treatment. The loss of poststorage effects in fruit that were harvested 10 or 17 d after spraying 1-MCP was recovered if fruit were exposed to 1 μL·L⁻¹ 1-MCP on the day of harvest (Fig. 1A–B). These data demonstrate that the quality of ‘Law Rome’ fruit could be maintained during storage by postharvest treatment with 1 μL·L⁻¹ 1-MCP on the day of harvest for fruit that were harvested before 4 Oct. (SI 6.0). However, poststorage quality of fruit that were harvested 10 or more days after spraying 1-MCP and that did not receive a gaseous 1-MCP treatment on the day of harvest was not different from the control. These observations indicate that ripening had resumed in ‘Law Rome’ fruit that were harvested 10 d or more after spraying 1-MCP in 2007.

Exposing ‘Law Rome’ apples to gaseous 1-MCP when they were harvested 10 d or 17 d after a preharvest 1-MCP spray reinstated the positive effects of 1-MCP on postharvest fruit quality (Fig. 1). One possible explanation for this result is that new ethylene receptors were formed in the attached fruit by 10 to 17 d after spraying 1-MCP, permitting fruit ripening to proceed in response to increasing ethylene levels unless its action was inhibited by blocking these new receptors with 1-MCP.

**Poststorage quality of ‘Law Rome’** in 2007. Like in 2007, preharvest 1-MCP reduced poststorage IEC (Fig. 2A) and the loss in flesh firmness (Fig. 2B) of ‘Law Rome’ fruit in 2008 only when fruit were harvested relatively soon after treatment. These effects were only observed in fruit that received a preharvest 1-MCP spray at the earlier timing. The effects of preharvest 1-MCP treatment on postharvest fruit quality...
were negligible when fruit were sprayed at a later stage of maturity (Fig. 2C–D). Preharvest 1-MCP spray at the first timing in 2008 was not as effective as a postharvest gaseous treatment even in fruit that were harvested only 1 d after the preharvest spray. One explanation for these observations is that the preharvest spray treatments did not saturate the ethylene-binding sites in ‘Law Rome’ fruit as effectively as a postharvest treatment. The complete loss in efficacy of the preharvest 1-MCP spray at the earlier timing was observed in fruit that were harvested only 3 d after treatment in the case of poststorage IEC (Fig. 2A) and 6 d after spraying the case of poststorage firmness (Fig. 2B). Postharvest 1-MCP treatment at both of these harvest dates effectively reduced poststorage IEC and maintained flesh firmness, indicating that postharvest treatment inhibited ethylene binding to the receptors more effectively than the preharvest treatment. Because the preharvest 1-MCP spray treatment was only partially effective compared with postharvest treatments in the 2008 ‘Law Rome’ study, we cannot conclude that losses in efficacy after delayed harvests are necessarily the result of the formation of new ethylene receptors. An alternative possibility could be that insufficient spray coverage, penetration, or uptake of the preharvest 1-MCP treatment resulted in only partial inhibition of ethylene action.

Poststorage quality of ‘Golden Delicious’ in 2008. Poststorage IEC in untreated control fruit from the ‘Golden Delicious’ study was greater than 200 μL·L⁻¹ regardless of time of harvest (Figs. 3A and C). A preharvest spray application of 1-MCP on 30 Aug. reduced poststorage IEC (Fig. 3A) and maintained flesh firmness after storage (Fig. 3B) similar to a postharvest gaseous 1-MCP treatment on the day of harvest, even in fruit that were harvested as late as 9 d after field application of 1-MCP. The similar poststorage responses to preharvest and postharvest 1-MCP treatments, regardless of time of harvest, indicate that preharvest application of 1-MCP to ‘Golden Delicious’ at the first date effectively saturated the ethylene-binding sites. It is also hypothesized that in the case of ‘Golden Delicious’, no new binding sites were formed in fruit remaining on the tree for at least 9 d after treatment.

Preharvest application of 1-MCP on 9 Sept., when the fruit were at a later stage of maturity, but still preclimacteric because fruit IEC was less than 1 μL·L⁻¹, inhibited poststorage IEC and maintained firmness during storage (Fig. 3C–D). Application of preharvest 1-MCP to more mature fruit did not suppress poststorage IEC to the same extent as a postharvest gaseous 1-MCP treatment (Fig. 3C). Both flesh firmness and IEC data suggest a loss of efficacy of preharvest 1-MCP treatment when fruit were harvested 9 d after treatment (Fig. 3C–D; P < 0.05 and P < 0.0001 for differences in IEC and firmness, respectively, between preharvest and postharvest 1-MCP treatments). These responses suggest that although fruit had not reached the climacteric at the time of the second 1-MCP application or indeed up to 9 d after treatment, as determined by IEC concentration, ethylene levels in the fruit were sufficient at that time to initiate ripening to some degree. The slight loss in efficacy of a preharvest 1-MCP spray when it was applied to more mature fruit was recovered if the fruit received a postharvest 1-MCP treatment on the day of harvest. New ethylene receptors may have been formed in attached fruit at this time, although to a lesser extent than the ‘Law Rome’ fruit in the previous experiment.

Preharvest application of 1-MCP as a foliar spray failed to have commercially relevant effects on ripening of ‘Law Rome’ and ‘Golden Delicious’ apple fruit in the present study, suggesting that growers may not be able to rely on 1-MCP as a harvest management aid in warmer climates. Preharvest 1-MCP sprays reduced poststorage IEC and reduced softening during short-term storage in ambient air. The effects of a preharvest 1-MCP spray treatment on postharvest fruit quality of ‘Law Rome’ was as effective as a postharvest 1-MCP gaseous treatment if fruit were harvested 3 d after the preharvest spray in 2007. However, a preharvest 1-MCP spray was less effective than postharvest treatment in 2008. In contrast, the positive effects of preharvest and postharvest 1-MCP treatments on postharvest fruit quality of ‘Golden Delicious’ were similar in 2008. Data from the ‘Law Rome’ study in 2007 demonstrate that delaying harvest resulted in a loss in the efficacy of preharvest 1-MCP sprays but that fruit were still responsive to 1-MCP when they were subsequently exposed to a gaseous 1-MCP treatment on the day of harvest. These findings suggest that attached fruit of some apple cultivars may be capable of rapidly generating new ethylene receptors and that the rate of formation of new ethylene receptors may be greater in warmer climates.

Information describing the formation of ethylene receptors in attached apple fruit is limited and apparently contradictory. Blankenship and Sisler (1989) reported that the number of ethylene-binding sites in apple cortical tissue did not change as the fruit ripened. The same authors subsequently found that the concentration of ethylene required to saturate the binding sites in tissues from ripening ‘Delicious’ apples increased consistently during a 5-week period that included the respiratory climacteric (Blankenship and Sisler, 1993). They reconciled these results by concluding that because the total number of binding sites in apple did not change significantly during fruit ripening, then perhaps the affinity of the binding sites for ethylene declined or another compound was competing with ethylene for the binding sites. Li and Yuan (2008) found that expression of the ethylene receptor genes MdETR1 and MdERS2 decreased in cortical tissue of ‘Delicious’ apple fruit during ripening, whereas MdETR2 and MdERS1 transcript levels did not change until fruit were at a late stage of ripening. Expression of MdETR1, MdETR2, MdERS1, and MdERS2 in ‘Golden Delicious’
increased during fruit ripening but remained constant during ripening of ‘Fuji’ (R. Yuan, personal communication). The apparently divergent results from these studies might be explained by an effect of cultivar on ethylene receptor formation in ripening fruit. As a consequence, preharvest 1-MCP sprays may have less usefulness as a harvest management tool in cultivars that rapidly form new ethylene receptors as fruit ripen.

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