Concentration Dependence of ‘Redchief Delicious’
Apple Fruit Softening and Chlorophyll Fluorescence
to Repeated Doses of 1-Methylcyclopropene

Sastry S. Jayanty1, Mauricio Cañoles2, and Randolph M. Beaudry3
Postharvest Technology and Physiology Laboratory, Department of Horticulture, Michigan State
University, East Lansing, MI 48824

Additional index words. texture, firmness, ripening, storage, temperature

Abstract. We studied the dose-response of ‘Redchief Delicious’ apple [Malus sylvestris (L) Mill. var. domestica (Borkh.)
Mansf.] fruit to repeated (weekly) dosages of 0.0, 0.02, 0.1, and 1.0 µL·L⁻¹ 1-methylcyclopropene (1-MCP) by measuring
fruit firmness and chlorophyll fluorescence throughout an extended storage period at 0, 5, 10, 15, and 20 °C. The rate of
firmness loss for nontreated fruit increased with increasing temperature. 1-MCP applied at concentrations of 0.1
and 1.0 µL·L⁻¹ slowed firmness loss. The 1-MCP dose-response curve for the rate of firmness loss was essentially the
same for all five temperatures. A concentration of 1.0 µL·L⁻¹ 1-MCP prevented firmness loss at all temperatures for
the duration of the study; however, after holding fruit for an additional 7 days at room temperature, the fruit stored
at 10 °C softened with increasing storage duration, whereas fruit at stored at higher and lower temperatures did not.
The influence of 1-MCP on chlorophyll fluorescence (Fo and Fm) was markedly affected by temperature; Fo increased
during storage at higher storage temperatures and this increase was enhanced by 1-MCP. Conversely, Fm decreased
during storage and the rate of decline was much greater at the higher storage temperatures; the rate of decline was
reduced by 1-MCP, but only at the higher storage temperatures. Photochemical efficiency (Fv/Fm) of nontreated fruit
decayed with time for all storage temperatures. Treatment with 0.1 and 1.0 µL·L⁻¹ 1-MCP only marginally reduced
the rate of decline of photochemical efficiency. Sample loss due to decay increased with temperature, but was reduced
by 1-MCP at all temperatures.

1-Methylcyclopropene (1-MCP) inhibits ethylene action and
can improve the storability of horticultural commodities for which
ethylene plays a role in ripening, abscission, or senescence (Abdi
et al., 1998; Fan et al., 1999a; Golding et al., 1998; Ku and Wills
1999; Porat et al., 1999; Sisler and Blankenship, 1996; Sisler et
al., 1996; Sisler and Serek, 1997). 1-MCP was approved by the
U.S. Environmental Protection Agency (EPA) for application
to edible crops on 26 July 2002 (EPA, 2002) and currently is
labeled by its manufacturer for use on apple fruit. 1-MCP ef-
ectively slows changes in the texture of apple fruit associated
with ripening (Dauny and Joyce, 2002; Fan et al., 1999a; Mir et
al., 2001; Watkins et al., 2000). Apart from negating ethylene
effects during ripening, Fan et al. (1999b) and Rupasinghe et al.
(2000) showed that 1-MCP application also reduced physiologi-
cal disorders such as superficial scald, soft scald, core flush, and
greasiness in different apple cultivars during storage.

The published data for dose-response of apple to 1-MCP are
predominantly derived from studies using single applications of
1-MCP at various temperatures for various durations. Watkins et
al. (2000) used three different 1-MCP concentrations (0.5, 1.0,
and 2.0 µL·L⁻¹) at temperatures between 20 and 25 °C for 7 h
duration. DeEll et al. (2002) tested the influence of three dif-
terent temperature regimes (3, 13, and 23 °C) in combination with
eight different durations of exposure to 0.6 µL·L⁻¹ 1-MCP. Dauny
and Joyce (2002) reported on three separate experiments evalu-
ating the influence of 1-MCP concentration (0.1 to 10.0 µL·L⁻¹),
exposure duration (6 to 48 h), and treatment temperature (0, 5,
10, 15, and 20 °C) on firmness retention by ‘Queen Cox’ and
‘Bramley’ apple fruit stored in air at 3 to 4 °C for 2 or 3 months.
Lurie et al. (2002) compared apple volatile emission with respect
to 1-MCP concentration. The treatments were 0.1 and 1.0 µL·L⁻¹
1-MCP at 20 °C for 20 h. Collectively, the data for single doses
suggest that a maximal response is dependent on the exposure
concentration, duration, temperature, and cultivar. Dose-response
curves indicate that between 0.5 and 1 µL·L⁻¹ 1-MCP is required
for maximal response and an exposure duration of 3 to 6 h is
needed at room temperature, depending on cultivar, whereas a
6- to 12-h exposure is needed at 3 °C. The half-maximal response
can be obtained by a dose of ≈0.05 to 0.1 µL·L⁻¹. Although there
have been no published reports on the influence of temperature
on the dose-response of apple to single applications of 1-MCP,
concentrations of 1-MCP that elicit a full response from apple
are effective at temperatures from 0 to 25 °C.

Multiple and continuous doses of 1-MCP can provide benefits
beyond those obtained by single doses (Mir and Beaudry, 2001;
to 0.7 µL·L⁻¹ 1-MCP resulted in an ≈2-fold increase in the time
required for firmness to fall to a threshold level at 0, 5, 10, 15,
and 20 °C. Multiple (weekly) doses of 1-MCP improved firmness
retention relative to a single exposure at 5, 10, 15, and 20 °C, but
not 0 °C. Furthermore, the time needed to soften to a threshold
firmness level increased as temperature increased for those fruit
receiving weekly applications (Mir and Beaudry, 2001). The
improvement in firmness retention obtained by multiple 1-MCP
applications at elevated temperatures suggests that new ethylene
receptors produced after the initial 1-MCP exposure were occupied
by 1-MCP molecules from subsequent doses as reported in some

Received for publication 14 Oct. 2003. Accepted for publication 11 Feb. 2004.
We thank Erin Curell, Melissa Butkiewicz, and Najma Khan for their expert
technical services. Supported by the Project for Generating Research and
Extension to meet Economic and Environmental Needs (GREEEN) at MSU.
Use of trade names does not imply endorsement of the products named or criti-
cism of similar ones not named.
1Postdoctoral Research Associate.
2Professor, to whom reprint requests should be addressed; e-mail address:
beaudry@msu.edu.
ornamentals (Blankenship and Dole, 2003; Cameron and Reid, 2001; Serek et al., 1994). The lack of a benefit from multiple doses at 0 °C suggested that turnover of the binding site was very limited at this temperature. Thus, it appears that temperature may impact ethylene biology sufficiently in apple that it differentially affects responses to single and multiple doses.

We wanted to determine if the dose-response of apple to 1-MCP changed with temperature for fruit receiving multiple doses. We applied 1-MCP once per week at 0, 5, 10, 15, and 20 °C at concentrations of 0.0, 0.02, 0.1, and 1.0 µL·L⁻¹ while holding the fruit in air.

Materials and Methods

**Plant material.** ‘Redchief Delicious’ apples were harvested from Michigan State University’s Clarksville Horticulture Experimental Station, Clarksville, Mich. at an early climacteric stage as judged by the starch index and internal ethylene concentration (IEC) of 10 representative fruit. The average IEC was 0.11 µL·L⁻¹ (90% having an IEC < 0.2 µL·L⁻¹) and the average starch index was 4.1. At the time of harvest, fruit had a firmness of ≈71 to 73 N. A total of 125 fruit having a diameter >5.7 cm and free from obvious defects were randomly selected and placed in each of 50 plastic mesh bags. One such bag was placed in each of fifty 120-L plastic barrels. After placing fruit in the barrels, 10 barrels were placed in each of five controlled environment chambers held at 0, 5, 10, 15, or 20 °C. Fruit temperature was allowed to equilibrate with the chamber’s temperature by holding the barrels overnight with lids removed. Initial 1-MCP treatment took place on the 2nd day of the study.

**1-MCP treatment.** Barrels were sealed by attaching the air-tight lids. 1-MCP was administered by injection of a stock gas through septa into the barrel headspace. Treatment with 1-MCP took place weekly at the indicated storage temperatures; exposure duration was 16 h. Target concentrations were 0.0, 0.01, 0.02, 0.1, and 1.0 µL·L⁻¹ 1-MCP at each temperature. The 0.01 µL·L⁻¹ 1-MCP concentration was below the limits of detectability of the GC so the fruit analyses for this treatment were not included in this report. Barrels were sealed only when 1-MCP was applied. Between 1-MCP exposures, the barrels were continuously flushed with humidified air at the rate of 200 mL·min⁻¹. There were two replicate barrels for each temperature/concentration treatment combination.

A concentrated 1-MCP stock gas was prepared each week in a 1-L glass container by mixing 36.4 g of EthylBloc® (Biotechnologies for Horticulture, Philadelphia) with water using the technique of Mir et al. (2001). After allowing 2 h for the 1-MCP gas to evolve, concentrated 1-MCP gas from the jar headspace was injected into the barrels. Different concentrations were created by altering the quantity of 1-MCP stock gas injected. 1-MCP concentrations in the barrels were measured for three representative barrels for different 1-MCP concentrations. We found that the actual concentration of 1-MCP was less than anticipated based on the amount of EthylBloc®, possibly due to polymerization of the 1-MCP molecules at the high concentrations present in the stock gas. The values given here are actual 1-MCP gas concentration measured in the barrels for each treatment.

Ethylene and 1-MCP concentrations were measured using gas chromatography (Carl Series 100 AGC) as reported previously (Mir et al., 2001). A standard for quantifying 1-MCP was prepared using 1.00 µL·L⁻¹ 1-butene (Matheson Gas Products, Chicago).

**Quality evaluations.** Ten fruit samples comprised of five fruit from each of the two replicates were removed at varying intervals and were analyzed for firmness and chlorophyll fluorescence on 1 and 7 d after removal from storage. Firmness and chlorophyll fluorescence were measured as described previously (Mir et al., 2001). The initial rate of firmness loss in storage was determined by fitting the most linear portion of the firmness data when regressed against storage duration; the first six to nine data points were used. Minimal (Fo) and maximal (Fm) chlorophyll fluorescence data were collected. Photochemical quantum efficiency was calculated from the ratio of (Fm-Fo)/Fm. The study continued until fruit supplies were exhausted.

Fruit losses due to decay were tabulated on storage samples from which fruit were drawn for quality analysis. Decayed fruit were removed from the storage containers when detected. An estimate of the rate of decay was obtained by dividing the number of decayed fruit in each storage chamber by the number of days required to exhaust the supply of 125 fruit.

Results

The firmness of nontreated ‘Redchief Delicious’ apple fruit after 1 d at 20 °C post-storage declined as storage duration increased (Fig. 1). However, the firmness decline appeared to halt after 100 to 150 d of storage and firmness remained relatively constant thereafter at a minimum level that was temperature-dependent. Minimum firmness levels for 0, 5, 10, 15, and 20 °C were ≈58, 40, 35, 31, and 31 N, respectively. The firmness of fruit at minimal softness was not affected by holding them for an additional 6 d at 20 °C (data not shown).

Fruit firmness did not differ between nontreated fruit and those treated with 0.02 µL·L⁻¹ 1-MCP at any temperature after 1 d (Fig. 1) or 7 d post-storage (data not shown). Treatment of fruit with 0.1 or 1.0 µL·L⁻¹ 1-MCP substantially slowed or arrested fruit softening, respectively. Nontreated fruit were softer than those receiving 1.0 µL·L⁻¹ 1-MCP after ≈110, 70, 30, 21, and 15 d for fruit stored at 0, 5, 10, 15, and 20 °C, respectively.

As the storage temperature increased, the maximum rate of softening increased similarly for nontreated controls and fruit treated with 0.02 µL·L⁻¹ 1-MCP (Fig. 2). There was a slight increase in softening rate with temperature for fruit treated with 0.1 µL·L⁻¹ 1-MCP, but no relationship between storage temperature and softening rate was apparent for fruit treated with 1 µL·L⁻¹ 1-MCP. Of the fruit treated with 1.0 µL·L⁻¹ 1-MCP, only those held at 10 °C softened substantially over the course of the study (Figs. 1 and 2). At 20 °C, apples repeatedly treated with 1 µL·L⁻¹ maintained firmness at harvest levels of ≈73 N for 180 d.

There was significant loss of fruit at higher temperature due to decay (Table 1). Loss to decay was greater for nontreated fruit and fruit treated with 0.02 µL·L⁻¹ 1-MCP than for fruit treated with 0.1 and 1.0 µL·L⁻¹ 1-MCP. Removal of fruit for quality analysis will have resulted in an underestimate of actual decay rates, but since the number of sampling dates was between 9 and 14 for all treatment combinations, roughly similar numbers of fruit were removed by sampling.

Chlorophyll fluorescence values for Fo, Fm, and Fv/Fm were affected by 1-MCP, temperature, and storage duration for fruit held 1 and 7 d following storage. For nontreated fruit, Fo tended to remain unchanged at the lower temperatures and increased somewhat at elevated temperatures as storage duration increased (Fig. 3). This trend was more pronounced after an additional 7 d
Fig. 1. Effect of 1-MCP on firmness of ‘Redchief Delicious’ apple fruit following storage in air at 20, 15, 10, 5, or 0 °C. Treated fruit were exposed to 1.00, 0.10, and 0.02 µL·L⁻¹ 1-MCP at the storage temperature for 16 h every week. Bars are ± the average SD for all samples of the given temperature; n = 10 fruit, five from each of two replicates. Post-storage holding (data not shown). The 0.1 and 1.0 µL·L⁻¹ treatments with 1-MCP did not affect Fo at 0 and 5 °C, but resulted in a marked increase in this parameter relative to nontreatment at 15 and 20 °C (Fig. 3). Unlike Fo, Fm declined as storage duration increased for all storage temperatures and 1-MCP treatments (Fig. 4). The rate of decline increased as storage temperature increased; however, the decline rate was reduced by 0.1 and 1.0 µL·L⁻¹ 1-MCP at 15 and 20 °C. The measure of photochemical quantum efficiency, Fv/Fm, declined as storage duration increased for all temperatures and 1-MCP treatments (Fig. 5). The higher concentrations of 1-MCP provided some marginal improvement in the maintenance of Fv/Fm at all temperatures, although the effect was least noticeable at 5, 10, and 15 °C.

Discussion

The reduction in softening rate of ‘Redchief Delicious’ apple fruit by 1-MCP was consistent with results from other studies (Dauny and Joyce, 2002; DeEll et al., 2002; Fan et al., 1999a; Mir et al., 2001; Watkins et al., 2000), demonstrating the inhibitory influence of 1-MCP on apple ripening. The 16-h exposure used in the current study should have been sufficient to obtain the full response for the concentrations applied. Dauny and Joyce (2002) and DeEll et al. (2002) obtained no increase in responses to 1-MCP for exposure durations greater than 6 h for 1.0 and 0.6 µL·L⁻¹ 1-MCP, respectively. Our data agree with those of one previous study (Fan et al., 1999a) in which dose-response experiments were...
Fig. 3. Minimal chlorophyll fluorescence (Fo) of ‘Redchief Delicious’ apple fruit after storage in air at 20, 15, 10, 5, or 0 °C with and without weekly exposures to 1.00, 0.10, and 0.02 µL·L\(^{-1}\) 1-MCP. Fluorescence measurements were made after holding fruit for an additional 24 h at 20 °C. Bars are ± the average SD for all samples of the given temperature; n = 10 fruit, five from each of two replicates.

Fig. 4. Maximal chlorophyll fluorescence (Fm) of ‘Redchief Delicious’ apple fruit after storage in air at 20, 15, 10, 5, or 0 °C with and without weekly exposures to 1.00, 0.10, and 0.02 µL·L\(^{-1}\) 1-MCP. Fluorescence measurements were made after holding fruit for an additional 24 h at 20 °C. Bars are ± the average SD for all samples of the given temperature; n = 10 fruit, five from each of two replicates.
The response to 0.1 µL·L\(^{-1}\) 1-MCP was nearly as effective as 1.0 µL·L\(^{-1}\). In the present study, there were no obvious differences in the effectiveness of the two highest concentrations for the first 100 d of the study for all temperatures. However, after this time, it was evident the efficacy of 0.1 µL·L\(^{-1}\) in preventing firmness loss and, to some extent, chlorophyll fluorescence changes, waned in a temperature-dependent manner. By the conclusion of the studies at the various temperatures, it was evident that, as storage temperature increased, the effectiveness of the 0.1 µL·L\(^{-1}\) 1-MCP treatment declined relative to the 1.0 µL·L\(^{-1}\) treatment. The loss in efficacy of the 0.1 µL·L\(^{-1}\) treatment may be due to an inability of the low level of 1-MCP to saturate binding sites such that the fruit continued to ‘sense’ available ethylene, enabling ripening to proceed slowly.

Blankenship and Dole (2003) noted that 1-MCP is thought by some to bind the ethylene binding site(s) in an essentially irreversible manner, although they further assert that there are little direct supporting data. If binding is irreversible in apple, then one would expect that some sites not occupied following the first exposure to nonsaturating levels of 1-MCP, (e.g., 0.1 µL·L\(^{-1}\) 1-MCP and less) very likely would be bound by 1-MCP during subsequent exposures. Thus, in an irreversible binding scenario, the efficacy of 0.1 µL·L\(^{-1}\) should have increased with time as long as the turnover rate of the ethylene receptors resulted in no net gain in receptors over time. Given that a single exposure of 0.7 µL·L\(^{-1}\) 1-MCP prevented firmness loss in ‘Redchief Delicious’ for over 30 d at 20 °C (Mir et al., 2001), binding site turnover in apple would be expected to be quite slow such that some of the binding sites are retained for as long as 4 weeks at high temperatures, and likely longer at low temperatures. Since the efficacy of weekly 0.1 µL·L\(^{-1}\) 1-MCP treatments declined, it is more likely, therefore, that 1-MCP does not bind irreversibly, but rather slowly dissociates from the ethylene binding sites (Serek and Sisler, 2001; Sisler et al., 1996; Sisler and Serek, 1999). This view is consistent with previous measures of 1-MCP and ethylene dissociation constants (Sisler and Serek, 1997) and further supports the suggestion by Watkins et al. (2000) that high levels of ethylene found in ‘McIntosh’ apple fruit may compete actively with 1-MCP.

Temperature had a marked influence on the rate of fruit softening, which increased 9-fold as temperature increased from 0 to 20 °C. The relatively linear increase in the rate of softening with increasing temperature suggests that softening processes in apple are not affected by temperature in the same manner as metabolism as judged by respiratory rate, which increases with temperature in a curvilinear manner (Hardenburg et al., 1986 and citations therein).

The interaction between temperature and 1-MCP efficacy in reducing firmness loss appeared to be somewhat complex. The greatest rate of softening of ‘Redchief Delicious’ apple fruit treated weekly with 1 µL·L\(^{-1}\) 1-MCP occurred at 10 °C. This was consistent with the findings of Mir et al. (2001) using multiple exposures to 0.7 µL·L\(^{-1}\) 1-MCP. They showed that the storage life of 1-MCP-treated ‘Redchief Delicious’ apple fruit, when measured in terms of firmness retention, was minimized at 10 °C and increased as temperature increased and declined. However, the slight increase in firmness at 0 °C for fruit treated with 0.1 and 1.0 µL·L\(^{-1}\) 1-MCP was not found previously by Mir et al. (2001). The slight increase in firmness at 0 °C may be due to moisture loss, although no shriveling was evident; alternatively, 1-MCP may alter cell wall metabolism such that synthetic reactions can, in some instances, marginally outpace catabolic reactions at low temperature.

![Fig. 5. Photochemical efficiency as measured by chlorophyll fluorescence (Fv/Fm) of ‘Redchief Delicious’ apple fruit after storage in air at 20, 15, 10, 5, or 0 °C with and without weekly exposures to 1.00, 0.10, and 0.02 µL·L\(^{-1}\) 1-MCP. Fluorescence measurements were made after holding fruit for an additional 24 h at 20 °C. Bars are ± the average SE for all samples of the given temperature; n = 10 fruit, five from each of two replicates.](image-url)
The chlorophyll fluorescence data (Fo, Fm, and Fv/Fm) for 0.1 and 1.0 µL·L⁻¹ 1-MCP treatments are consistent with those published previously for repeat applications of 0.7 µL·L⁻¹ 1-MCP (Mir et al., 2001) as well as nontreated (Mir et al., 2001) and air-stored fruit (Song et al., 1997). The similarity in the dose-dependency of changes in firmness with that of Fo, Fm, and Fv/Fm in the current study suggests that textural and chloroplastic responses have a similar dependence on the concentration of 1-MCP. However, the magnitude of the influence of 1-MCP on firmness retention was not reflected in the magnitude of the effect on Fv/Fm. The marked time-dependent decline in Fv/Fm demonstrated that chloroplast function was not protected appreciably by 1-MCP treatment, suggesting ethylene has a real, but minor, role to play in the loss in chloroplast function during storage. The temperature dependence of the influence of 1.0 µL·L⁻¹ 1-MCP on Fo and Fm in stored apple was similar to that reported by Mir et al. (2001). The data suggest that at high temperatures, ethylene limits the time-dependent decline in Fm and enhances the increase in Fo, but at low temperature, changes in these parameters are governed by a factor other than ethylene.

Our studies confirmed the earlier published results (Mir et al., 2001) that 1 µL·L⁻¹ application to ‘Redchief Delicious’ apples once per week was highly effective at preserving fruit firmness at higher temperatures. Under special circumstances (e.g., lack of access to refrigeration and/or modified-atmosphere storage), 1-MCP may be helpful in maintaining apple fruit firmness at elevated temperatures. However, while firmness is one of the most important quality attributes for apples (Arthey, 1975; Liu and King, 1978), storage at elevated temperatures likely would lead to losses in other quality attributes. For instance, the high rates of fungal decay found in the current study at higher storage temperatures, while lower for 1-MCP-treated fruit, would be expected to severely limit storage of apple at nonoptimal storage temperatures.

The dose-response for multiple doses of 1-MCP appears to be very similar to that for single doses. The lack of a difference between the dose-response of single and repeated doses suggests that binding is not reversible, or else subsequent doses of sub-saturating concentrations of 1-MCP in the multiple dose system would have improved the response of fruit by binding to a portion of the remaining unbound ethylene binding sites. One would anticipate, therefore, that a continuous dosage at concentrations below those that saturate the response would behave similarly to sub-saturating repeated and single doses and result in incomplete inhibition of ethylene action.

There appeared to be no shift in dose-response with temperature. A previous study documenting the slight increase in the exposure duration required at lower temperatures (DeEll et al., 2002) may describe the influence of temperature on the diffusion of 1-MCP into and through the fruit, rather than a shift in tissue responsiveness. The relative insensitivity of the 1-MCP dose-response to temperature suggests that binding is influenced little by temperature and may be characterized by a low apparent enthalpy. The influence of temperature on the dose-response of plant material for ethylene has, to our knowledge, not yet been reported, nor has the enthalpy of ethylene binding been published.

Literature Cited


