Using Volatile Emissions and Chlorophyll Fluorescence as Indicators of Heat Injury in Apples

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ADDITIONAL INDEX WORDS. *Malus sylvestris* var. *domestica*, heat stress, ethanol, ethylene, ethyl acetate, flesh browning

ABSTRACT. Volatile emissions and chlorophyll fluorescence were investigated as potential signals of heat injury for apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] fruit. ‘McIntosh’, ‘Cortland’, ‘Jonagold’, and ‘Northern Spy’ apples were exposed to 46 °C for 0, 4, 8, or 12 hours (heat treatments). Following treatments, fruit were kept at 20 °C and evaluated after 1, 2, 4, or 7 days. Heat treatments induced volatile production including ethanol and ethyl acetate. The 8 and 12 hours heat treatments increased ethanol and ethyl acetate production in all four cultivars by as much as 170- and 11-fold, respectively, 1 day after treatments. Heat treatments also reduced ethylene production and chlorophyll fluorescence. Heat for 12 hours caused serious flesh browning. Among the cultivars investigated, ‘Northern Spy’ and ‘McIntosh’ were most susceptible to heat stress based on the degree of flesh browning. Correlation coefficients of heat stress induced ethanol emission and chlorophyll fluorescence with flesh browning were 0.82 and –0.66, respectively. The nondestructive measurements of ethanol emission and chlorophyll fluorescence have potential to identify stressed fruit with reduced quality or compromised storage life.

Environmental stresses that can be detrimental to fruit quality include mineral nutrient deficiencies, freezing, heat, and water deficiency. Heat treatments have been used to induce beneficial modifications of physiological and biochemical characteristics of fruits and vegetables improving their quality retention and storage life. In apple (*Malus sylvestris* var. *domestica*) fruit, postharvest heat treatments delay softening and fruit ripening, increase soluble solids : acid ratios, reduce incidence and severity of superficial scald, (Klein and Lurie, 1990; Lurie and Klein, 1990) and reduce incidence of physiological storage disorders (Porritt and Lidster, 1978). However, high temperatures with long durations may cause damage, such as skin discoloration and flesh browning (Klein and Lurie, 1992). In mild to moderate heat injury, visual symptoms are often not apparent, however, flesh injury may develop during subsequent storage resulting in increased decay and loss of fruit quality. To prevent those losses, the range and duration of temperature which improve or injure fruit quality must be identified.

Volatile compounds, such as ethanol, ethyl acetate, acetaldehyde, and ethylene, have been reported to be indicators of plant injury from freezing, heat stress, and water stress (Forney and Jordan, 1998; Forney et al., 2000; Kimmerer and Kozłowski, 1982). Elevated concentrations of ethanol indicated physiological damage in heat-treated fresh broccoli [*Brassica oleracea* L. (Italica Group)] (Forney and Jordan, 1998) and stressed tree seedlings (Templeton and Colombo, 1995). In addition, ethanol and ethyl acetate concentrations increased in ‘Northern Spy’ and ‘Delicious’ apple fruit as a result of freezing treatment (Forney et al., 2000). Emissions of ethanol and ethyl acetate from apple fruit have also been related to low oxygen stress (Mattheis et al., 1991; Song et al., 1997b) and damage from high concentrations of CO₂ (Volz et al., 1998). Red pine (*Pinus resinosa* Ait.) and paper birch

(*Betula papyrifera* Marsh.) seedlings exposed to SO₂ exhibited increased production of ethylene, which can be a useful indicator of the onset of stress and/or the degree of stress that a plant is experiencing (Kimmerer and Kozłowski, 1982). However, the relationship between volatile emissions and fruit quality of different apple cultivars subjected to heat stress has not been reported.

Chlorophyll fluorescence is also a useful indicator of several postharvest stresses including exposure of apples to low O₂ or high CO₂ concentrations (DeEll et al., 1998). Chilling stress alters chlorophyll fluorescence in green bell peppers [*Capsicum annuum* L. var. *annuum* (Grossum Group)] (Lurie et al., 1994), cucumbers (*Cucumis sativus* L.) (van Kooten et al., 1992), banana (*Musa acuminata* Colla), and mango (*Mangifera indica* L.) (Smillie et al., 1987). Forney et al. (2000) characterized freezing induced changes of ‘Northern Spy’ and ‘Delicious’ apples using chlorophyll fluorescence. Song et al. (1997a) investigated changes in chlorophyll fluorescence of apple fruit during maturation, ripening, and senescence.

Changes in volatile emissions and/or chlorophyll fluorescence of fruit could serve as indicators of stress. In developing beneficial heat treatments, these indicators could be useful for identifying tolerance limits in order to prevent fruit injury. Therefore, the objectives of this study were to identify and characterize volatiles induced by heat stress, measure heat stress-induced changes in chlorophyll fluorescence, and relate these heat stress indicators to changes in fruit quality.

Materials and Methods

HEAT TREATMENT. Apple fruit, ‘McIntosh’, ‘Cortland’, ‘Jonagold’, and ‘Northern Spy’ were obtained from commercial orchards in September or October 1998, and held at 0 °C. About 4 weeks after harvest, heat treatments were applied by placing fruit in a 46 °C chamber with 85% to 90% relative humidity. Fruit to be heated were placed in trays in unsealed plastic bags to maintain humidity during heating. Apple fruit were placed in the chamber at 4 h intervals so when all fruit were removed, treatment times were 0, 4, 8, or 12 h. The 0 h fruit were used as the control. Temperature of the heating chamber and fruit of each cultivar were monitored during heat treatment by placing three thermo-

Received for publication 6 Jan. 2001. Accepted for publication 21 June 2001. Contribution 2224 of the Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada. We thank Kumudini U.K.G. Nicholas for technical assistance in the evaluation of fruit quality and John DeLong and Charlie Embree for helpful suggestions in preparing the manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

couples in the cortex of three extra apples for each treatment duration, and another thermocouple was placed on the chamber floor. These three extra apples were only used to monitor temperature and were not used for quality evaluation. After heat treatment, fruit were kept at 20 °C until subjected to various evaluations.

FRUIT QUALITY. Samples of 10 apples of each cultivar–heat treatment combination were taken at 1, 2, 4, or 7 d after heat treatment for evaluation. External and internal fruit injury were rated as skin or flesh browning using a scale of 0 to 5 where 0 = none, 1 = up to 20%, 2 = 21% to 40%, 3 = 41% to 60%, 4 = 61% to 80%, and 5 = 81% to 100% of the fruit surface or flesh browning, respectively. To rate flesh browning, apples were cut in half equatorially. Surface color of 10 apple fruit was measured using a chromameter (model 200; Minolta Camera Co., Osaka, Japan) equipped with an 8-mm measuring aperture and calibrated with a white standard tile. L^* (L value), C^* (chroma), and H° (hue angle) were recorded. Color readings were made on both red and green sides of each fruit. Hue angle was used to express color change in this study. Fruit firmness was then determined on both red and green sides of each fruit after peel removal using a Magness-Taylor (MT) fruit tester (type 30 lb, model 30A; Baullaf Manufacturing Co., Laurel, Md.). The juice collected during firmness testing was analyzed for percentage soluble solids content using a hand-held temperature-compensated refractometer (Atago Co., Tokyo), and for titratable acidity by titrating a 2-mL juice sample with 0.1 mol·L⁻¹ NaOH using a semi automatic titrator (Multi-Dosimat E-415; Metrohm AG, Herisau, Switzerland) to a phenolphthalein endpoint of pH 8.1.

VOLATILE ANALYSIS. Three subsamples of four apples each were sampled from each cultivar–heat treatment combination for volatile emissions by sealing the whole fresh fruit in 4-L glass jars with Teflon lids 1, 2, 4, or 7 d after removal from heat treatments. The headspace over the apples was allowed to equilibrate for 1 h, and 1-mL headspace samples were taken and analyzed on a gas chromatograph (model 3400; Varian, Inc., Walnut Creek, Calif.) with a SupelcoWax 10 (30 m × 0.53 mm i.d., 1.0 µm coating thickness) column. The carrier gas was ultra high purity helium (99.999%, Praxair, Mississauga, Ontario, Canada) at a flow rate of 9 mL·min⁻¹. The analysis was performed isothermally at 80 °C. The volatiles were detected by flame ionization using helium as the makeup gas at a flow rate of 30 mL·min⁻¹ and air and hydrogen flow rates were 30 and 300 mL·min⁻¹, respectively. The injection port and detector temperatures were 200 °C. Standards of volatiles were generated by evaporating a known amount of authentic ethanol and ethyl acetate from a piece of filter paper that was dropped into a specially made 4.4-L glass jar fitted with a Mininert gas-tight sampling valve (Alltech Assoc., Deerfield, Ill.). Quantization was done by comparison of the gas chromatograph response of the sample to that of the standard volatile compounds.

ETHYLENE PRODUCTION. One milliliter samples of headspace air from the same three subsamples of apples used for the volatile analysis were analyzed for ethylene. A gas chromatograph with a flame ionization detector (Carle Instruments, Inc., Anaheim, Calif.) equipped with a 1.9 m × 3.2 mm (o.d.) activated alumina column with a helium carrier flow of 50 mL·min⁻¹ was used. Quantization was accomplished by comparison of the gas chromatograph response of the sample to that of a certified standard.

CHLOROPHYLL FLUORESCENCE. Chlorophyll fluorescence was determined using a modulated fluorometer (model OS-500; Opti-Science, Tyngsboro, Mass.). Ten apples of each cultivar–heat

treatment combination were sampled 1, 2, 4, or 7 d after removal from the heat treatment. Fluorescence measurements were made in a darkened laboratory. The fluorometer was operated in the Fv/Fm mode and fluorescence was measured using a photodiode in the 710- to 760-nm range. The fluorometer probe was placed firmly on the apple fruit and a pulse of 10 mmol·m⁻²·s⁻¹ was applied for 0.8 s. Fluorescence parameters of minimal fluorescence (Fo), maximal fluorescence (Fm), and photochemical efficiency (Fv/Fm, where Fv = Fm – Fo) were recorded from the fluorometer digital display. Three readings were taken at equidistant positions around the equator of each fruit and averaged.

STATISTICAL ANALYSIS. The experimental design was completely randomized with a factorial arrangement of treatments: 4 cultivars × 4 heat treatments × 4 evaluation days at 20 °C. For each combination, three subsamples of four apples were used for volatile as well as ethylene analysis, while 10 fruit were used for fluorescence and quality measurement. Data were analyzed using analysis of variance (ANOVA), correlation, and LSD options of Genstat 5 (Genstat 5 Committee, 1993).

Results

FRUIT TEMPERATURE. Fruit were about 5 °C when placed in the heating chamber, and heated rapidly reaching 40 °C after 4 h, 44 °C after 8 h, and 45 °C after 12 h (Fig. 1). Fruit from the three heating durations were all removed from the chamber at the same time.

STRESS INDUCED VOLATILES. Heat treatments altered production of ethanol and ethyl acetate. Ethanol production increased significantly 1 d following the 8- and 12-h heat treatments in ‘McIntosh’, ‘Cortland’, and ‘Northern Spy’ (Fig. 2). Compared with the control, the 12 h heat treatment increased ethanol production of ‘McIntosh’, ‘Cortland’, ‘Jonagold’, and ‘Northern Spy’ by 48-, 71-, 9- and 170-fold, respectively. The 8-h heat treatment increased ethanol production of the four cultivar by 41-, 28-, 10- and 114-fold, respectively. The 4-h heat treatment increased ethanol production in ‘McIntosh’ and ‘Northern Spy’ by 5- and 23-fold, respectively, however, no ethanol increase was detected in ‘Cortland’ and ‘Jonagold’ fruit 1 d following heat treatment. Seven days after treatment, ethanol concentrations were 30-, 30-, 1.5-, and 4-fold greater than the controls in the 12-h treated apples in ‘McIntosh’, ‘Cortland’, ‘Jonagold’, and ‘Northern Spy’, respectively; 32-, 22-, 1- and 3-fold greater than the controls in the 8 h heat treatment, respectively, and 1-, 3-, 3- and 1-fold greater than the controls in the 4 h heat treatment, respectively. Results indicated that the 4-h heat treatment had little effect on the induction of ethanol, and only the long duration times of 8 and 12 h exposure to 46 °C induced large quantities of ethanol. Heat stress induced the highest concentrations of ethanol in ‘McIntosh’ and ‘Northern Spy’, which were as high as 1,220 and 1,170 µmol·m⁻³, respectively. The highest headspace concentration for ‘Jonagold’ was only 120 µmol·m⁻³.

Ethyl acetate production also increased but only 1 d following the 12-h heat treatment in these four cultivars. Ethyl acetate was 7-, 11-, 2- and 3-fold greater than that of the control fruit in ‘McIntosh’, ‘Cortland’, ‘Jonagold’, and ‘Northern Spy’, respectively, but there was no significant difference between the 12-h heat treatment and the controls of the four cultivars at day 7 (data not presented).

Unlike changes in ethanol and ethyl acetate, ethylene production decreased following the heat treatments (Fig. 3). One day following the 12-h heat treatment, ethylene production was 26-, 9-, 3-, and 60-fold less than that of the controls in ‘McIntosh’,

Fig. 1. Average fruit temperatures of apples treated for 4, 8, or 12 h in a 46 °C chamber. Solid lines represent the average temperature of three apples for each of four cultivars that were monitored continuously. The dashed line represents the chamber temperature.

'Cortland', 'Jonagold', and 'Northern Spy', respectively. After holding 7 d at 20 °C, ethylene production was 8-, 3-, 4-, and 12-fold less than that of the controls in these four cultivars, respectively. The 8 h heat treatment also reduced ethylene production, but not as much as the 12-h heat treatment. At day 1, ethylene production was 12-, 4-, 2-, and 6-fold less than that of the controls in 'McIntosh', 'Cortland', 'Jonagold', and 'Northern Spy', respectively; at day 7, it was only 1-, 1-, 2-, and 3-fold less than that of the controls, respectively. The 4-h heat treatment had little effect on ethylene production with only a slight decrease of ethylene production after 1 d. After holding the fruit for 7 d at 20 °C, ethylene production remained at nearly the same level as the controls. These results indicated that heat stress immediately reduced ethylene production, which gradually recovered during the 7 d at 20 °C.

FRUIT CHLOROPHYLL FLUORESCENCE. Chlorophyll fluorescence (Fv/Fm) decreased rapidly 1 d following the 12-h heat treatment in 'McIntosh', 'Cortland', 'Jonagold', and 'Northern Spy' (Fig. 4). Fv/Fm declined to 0.28, 0.38, 0.44, and 0.25, which was 36%, 59%, 63%, and 36% of the control fruit values, respectively. Fv/Fm in 'Jonagold' fruit recovered after 7 d at 20 °C, and reached the same level as the control. However, Fv/Fm in 'McIntosh', 'Cortland', and 'Northern Spy' did not recover but remained 0.35, 0.3, and 0.29, which was 49%, 60%, and 49% of the control fruit, respectively. The 8-h heat treatment decreased chlorophyll fluorescence similarly to the 12-h heat treatment but not as severely. The 4 h heat treatment had no significant effect on chlorophyll fluorescence.

FRUIT QUALITY. Flesh browning of apple fruit occurred in response to heat stress. Generally, the longer the heating period, the more severe the flesh browning. However, the response of apples to heat stress can vary according to cultivar. Among the cultivars investigated, 'Northern Spy' fruit was the most sensitive to heat stress, and slight flesh browning was found even with the 4-h heat treatment (Fig. 5). The 12-h heat treatment resulted in more severe flesh browning in 'McIntosh' and 'Northern Spy' than in 'Cortland' and 'Jonagold'. After holding fruit at 20 °C for 7 d, flesh browning reached up to 60% to 80% in 'McIntosh' and 'Northern Spy' while it was 25% to 40% in 'Cortland' and 'Jonagold'.

In addition to flesh browning; fruit firmness, titratable acidity, soluble solids, and fruit skin color were also measured to investigate additional effects of the heat treatments (Table 1). After heat treatment, fruit firmness decreased greatly in 'McIntosh'

and 'Northern Spy' which coincided with higher flesh browning; 'Jonagold' firmness decreased slightly, while it increased in 'Cortland'. Surface color (hue angle) and soluble solids were not affected by heat treatment. Fruit titratable acidity declined significantly with increasing heat treatment duration.

CORRELATIONS WITH FLESH BROWNING. To further understand the effect of heat stress on fruit quality, flesh browning was correlated with the induced volatiles, chlorophyll fluorescence (Fv/Fm), firmness, titratable acidity, soluble solids, and fruit skin color from the

Fig. 2. 'McIntosh', 'Cortland', 'Jonagold', and 'Northern Spy' apple headspace ethanol concentration during 1, 2, 4, or 7 d at 20 °C following a 0, 4, 8, or 12 h exposure to 46 °C.

Fig. 3. 'McIntosh', 'Cortland', 'Jonagold', and 'Northern Spy' apple ethylene production during 1, 2, 4, or 7 d at 20 °C following a 0, 4, 8, or 12 h exposure to 46 °C.

four individual cultivars, the four treatment times, and the four evaluation times (Table 2). Overall, flesh browning was highly correlated with ethanol concentration at $r = 0.94, 0.73$, and 0.93 in 'McIntosh', 'Cortland', and 'Northern Spy', respectively. Flesh browning was negatively correlated with ethylene production and Fv/Fm as indicated by the high r value of $-0.77, -0.57$, and -0.84 and $-0.80, -0.84$, and -0.88 , respectively. Ethyl acetate correlated poorly with flesh browning. For 'Jonagold', only ethyl acetate could be used as an indicator of heat stress with $r = 0.57$, and flesh browning was not correlated with ethanol, ethylene, or Fv/Fm. Flesh browning had a highly negative correlation with firmness in 'McIntosh', 'Jonagold', and 'Northern Spy' but not 'Cortland'. Heat treatment decreased titratable acidity, and flesh browning was highly negatively correlated with titratable acidity at $r = -0.74, -0.86, -0.50$, and -0.67 in 'McIntosh', 'Cortland', 'Jonagold', and 'Northern Spy', respectively. Surface color and soluble solids were not correlated with flesh browning.

Discussion

Effects of stress on apple fruit may not be immediately apparent making it impossible to identify injured fruit until they break down in storage, resulting in significant financial loss. Injury caused by stress can manifest itself as a variety of postharvest disorders including surface discoloration, lesions, internal flesh browning, and breakdown (Meheriuk et al., 1994). Nondestructive indi-

cators of stress could provide methods to identify fruit with poor storage potential and thus minimize wastage. Results in this study indicated that changes in ethanol, ethyl acetate, and ethylene emission, as well as chlorophyll fluorescence were useful indicators of heat stress. Ethanol and ethyl acetate were major volatiles induced after heat treatment in our study. Large rapid increases in ethanol were observed 1 d following injurious heat treatments and these elevated concentrations were maintained for 7 d at 20 °C. In noninjurious heat treatments (exposure to 46 °C for 4 h), a slight rise in ethanol was observed, which in some cultivars declined 2 d following treatment. Ethanol concentrations correlated well with development of flesh browning. However, elevated ethanol was not always present in association with flesh browning as seen in 'Jonagold' fruit 7 d after heat treatments.

In other studies, apples and other plants produced stress induced volatiles. Apple fruit injured by freezing (Forney et al., 2000) or low O₂ or high CO₂ (Song et al., 1997b)

produced large quantities of ethanol. In addition to ethanol, freezing induced production of large quantities of ethyl acetate in 'Delicious' and 'Northern Spy' fruit (Forney et al., 2000). However, heat stress only induced a transient increase in ethyl acetate in the 12-h heat treatment indicating potential differences in the response of apples to freezing and heat stress. Thomas (1931) found an association of internal breakdown and soft scald in stored apples with increases in ethanol and acetaldehyde in the

Fig. 4. 'McIntosh', 'Cortland', 'Jonagold', and 'Northern Spy' apple chlorophyll fluorescence (Fv/Fm) during 1, 2, 4, or 7 d at 20 °C following a 0, 4, 8, or 12 h exposure to 46 °C.

Fig. 5. 'McIntosh', 'Cortland', 'Jonagold', and 'Northern Spy' apple flesh browning index during 1, 2, 4, or 7 d at 20 °C following a 0, 4, 8, or 12 h exposure to 46 °C.

fruit. In other plants, production of ethanol and ethyl acetate have also been induced by stress caused by heat treatments (Forney and Jordan, 1998), SO₂ fumigation, and water deficit (Kimmerer and Kozłowski, 1982), and low O₂ and high CO₂ treatments (Zuckermann et al., 1997). In addition, Templeton and Colombo (1995) used ethanol emissions from stressed tree seedlings to predict seedling vigor and demonstrated strong correlations between ethanol and losses of root growth, and foliage and bud viability. Ethylene production was reduced as heat injury developed in this study. A decrease in ethylene production may indicate severe heat injury in apple such as that occurring after 8 or 12 h of treatment. Freezing stress also reduced ethylene production in apple fruit (Forney et al., 2000). However, the change in ethylene production is dependent on fruit ripeness. In this study,

Table 1. Quality measurements of 'McIntosh', 'Cortland', 'Jonagold', and 'Northern Spy' apples after exposure to 46 °C for 0, 4, 8, or 12 h. Values are means of 10 fruit samples held at 20 °C for 1, 2, 4, or 7 d. F probabilities for the effects of cultivar (C), heat treatment (T), evaluation day at 20 °C (E), C × T, C × E, T × E, and C × T × E were derived from the ANOVA.

Cultivar	Time at 46 °C (h)	Firmness (red) (N)	Firmness (green) (N)	Hue angle (°)	Fv/Fm	Peel browning rating ^z	Flesh browning rating ^z	Soluble solids (%)	Titrateable acidity (mg malic acid/100 mL juice)
McIntosh	0	47.7	46.8	8.5	0.75	0	0	12.0	576
	4	47.2	47.2	8.5	0.73	0.03	0	12.3	604
	8	40.5	41.0	9.0	0.58	0.11	2.01	12.0	461
	12	39.7	41.9	5.1	0.37	0.4	2.8	11.5	398
Cortland	0	51.7	49.9	17.3	0.60	0	0	13.8	563
	4	48.1	49.5	20.1	0.62	0.06	0.11	13.6	530
	8	52.1	52.6	19.5	0.46	0.09	0.55	13.1	498
	12	55.2	53.9	20.4	0.32	0.24	1.08	13.5	469
Jonagold	0	58.4	58.4	19.3	0.67	0	0	15.8	540
	4	57.0	56.6	19.9	0.67	0	0.01	15.2	541
	8	58.4	57.9	16.8	0.63	0	0.19	16.1	501
	12	53.5	53.5	16.0	0.56	0.04	0.84	15.6	497
Northern Spy	0	75.7	75.3	25.4	0.67	0	0.04	15.6	944
	4	70.8	68.2	26.4	0.62	0.53	0.51	14.9	702
	8	57.9	53.5	31.7	0.57	0.96	2.54	15.4	583
	12	46.3	47.2	27.7	0.30	2.16	4.28	14.8	634
LSD _{0.05}		1.58	1.60	9.85	0.08	0.46	0.74		
Significance	df								
Cultivar (C)	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Heat treatment (T)	3	<0.001	<0.001	0.371	<0.001	<0.001	<0.001		
Evaluation day at 20 °C (E)	3	<0.001	<0.001	0.085	<0.001	<0.001	<0.001		
C × T	9	<0.001	<0.001	0.181	<0.001	<0.001	<0.001		
C × E	9	0.058	0.005	0.181	<0.001	0.232	0.021		
T × E	9	0.015	0.003	0.727	<0.001	0.022	<0.001		
C × T × E	27	0.001	<0.001	0.237	<0.001	0.627	0.067		

^zWhere 0 = none, 1 = up to 20%, 2 = 21% to 40%, 3 = 41% to 60%, 4 = 61% to 80%, and 5 = 81% to 100% of the fruit peel or flesh browning.

Table 2. Correlation coefficients (*r*) of flesh browning with stress volatiles, chlorophyll fluorescence, and fruit quality parameters after heat exposure to 46 °C for 4, 8, or 12 h and holding at 20 °C for 1, 2, 4, or 7 d.

Parameter	McIntosh	Cortland	Jonagold	Northern Spy
Ethanol	0.94**	0.73**	0.32 ^{NS}	0.93**
Ethyl acetate	-0.11 ^{NS}	0.16 ^{NS}	0.57*	0.31 ^{NS}
Ethylene	-0.77**	-0.57*	-0.31 ^{NS}	-0.84**
Fv/Fm	-0.80**	-0.84**	-0.16 ^{NS}	-0.88**
Firmness (green)	-0.73**	0.12 ^{NS}	-0.58*	-0.91**
Firmness (red)	-0.88**	0.19 ^{NS}	-0.61*	-0.93**
External rating	0.83**	0.66*	0.81**	0.92**
Titrateable acidity	-0.74**	-0.86**	-0.50*	-0.67*
Soluble solids	-0.33 ^{NS}	-0.31 ^{NS}	-0.22 ^{NS}	-0.31 ^{NS}
Hue angel (green)	0.05 ^{NS}	-0.38 ^{NS}	-0.42 ^{NS}	-0.01 ^{NS}
Hue angel (red)	0.64*	0.11 ^{NS}	-0.21 ^{NS}	0.29 ^{NS}

^{NS},*,** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

ethylene production by control fruit ranged from 0.6 to 14 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. Without control fruit, it would be difficult to determine whether low ethylene production was a result of heat stress or fruit maturity. Thus, using changes in ethylene production as an indicator of heat stress is limited.

There is considerable evidence that the photosynthetic apparatus is an initial site of damage induced by heat stress (Ho, 1987). Heat stress influences the structure and composition of the thylakoid membrane. Use of chlorophyll fluorescence as a non-destructive tool to monitor physiological disorders has been widely reported (Schreiber and Bilger, 1993) and Fv/Fm is one of the most powerful and fastest measurements to monitor physiological injury (van Kooten et al., 1992). In this study, a sharp decrease in Fv/Fm of 0.3 or more was a good indicator of irreparable damage expressed as flesh browning. When apple fruit are injured, chloroplast integrity declines. As a result, the ability of the chloroplast to use light energy also declines and is reflected in Fv/Fm. In addition to stress, fruit age can affect chlorophyll fluorescence. After 6 months storage at 0 °C, Fv/Fm of 'Starking Delicious' apples declined from 0.8 to 0.5 to 0.6 (Song et al., 1997a). Thus, changes in Fv/Fm is an indicator that can be used effectively in assessing heat injury, but fruit age also should be considered when using it for commercial purpose. Damaging heat treatment also affected chlorophyll fluorescence in other plants. In heat treated broccoli, an immediate decline of Fv/Fm by 0.3 or more was used to discriminate between beneficial or detrimental heat treatments (Tian et al., 1996). A sudden drop of Fv/Fm from 0.75 to 0.67 was used to indicate heat stress in mango fruit (Joyce and Shorter, 1994).

A strong negative correlation has been observed between stress induced ethanol production and chlorophyll fluorescence. 'McIntosh', 'Cortland', 'Jonagold', and 'Northern Spy' apples held at 46 °C for 8 or 12 h had lower Fv/Fm and greater ethanol accumulation compared to the control fruit and correlations of ethanol and Fv/Fm were $r = -0.84$, -0.88 , -0.71 , and -0.90 , respectively. Prange et al. (1997) also found that Fv/Fm decreased as ethanol production rate increased, suggesting that ethanol accumulation in apple fruit reduced the exciton energy transfer to photosystem II in the thylakoid membranes.

Heat stress stimulated ethanol production in apple fruit, which was also associated with the loss of firmness, titrateable acidity, chlorophyll fluorescence, and the development of skin and flesh browning. Physiological response of fruit to heat exposure depends on many factors such as fruit maturity, fruit size, cultivar, and treatment conditions (Lurie, 1998). Differences in cultivar

response to heat injury have been identified in this study. Among the tested cultivars, 'Jonagold' was the most resistant cultivar, having minimal flesh browning, less production of ethanol and ethyl acetate, and only a slight decline in Fv/Fm, which recovered to control levels after 7 d. The ethanol level in 8- or 12-h heat-treated 'Jonagold' fruit was the same or nearly the same as the control after holding fruit at 20 °C for 7 d. 'Cortland' fruit also showed relative strong tolerance to heat stress, and stimulation of ethanol production was much less than in 'McIntosh' and 'Northern Spy'. 'McIntosh' and 'Northern Spy' apples were more sensitive to heat stress and ethanol was induced even with the 4-h heat treatment in these two cultivars. As a result of the high concentrations of ethanol and the greater development of severe flesh browning in the 8- or 12-h heat-treated apples, it was easy to differentiate these stressed fruit from the controls. However, following mild heat stress, visual symptoms were often not obvious, although ethanol was induced under these circumstances. Kim et al. (1993) also found cultivar differences in heat resistance, reporting that 'Golden Delicious' and 'Delicious' showed relative strong tolerance to heat among 11 apple cultivars tested.

Maturity of apple fruit may have influenced their response to heat treatments. The low levels of ethylene in 'Jonagold' control fruit compared to the other cultivars, indicate it may have been less mature, although 'Jonagold' fruit were harvested at commercial maturity and stored under the same conditions as the other cultivars prior to treatment. Less mature apples may have been more resistant to heat treatments. Therefore, in addition to apparent cultivar differences, maturity may affect the fruit response to heat and other stresses. The effects of fruit maturity on stress responses needs to be characterized to aid in the interpretation of stress-induced physiological responses.

Physiological indicators of apple fruit injury could be useful to correlate fruit injury from heat, freezing, or other stresses. Non-destructive measurements such as the emission of ethanol or ethyl acetate, or changes in chlorophyll fluorescence could be applied to identify stressed fruit with reduced quality or compromised storage life. Ethanol was found in low concentration in healthy apples, however, it increased rapidly following a heat stress, which could prove to be a useful indicator of heat stress. In addition to the volatiles measured in this study, other volatiles induced by stress, such as acetaldehyde, butanol, propanol, hexanol, and the hexenals may provide additional indicators of physiological damage. There is potential to develop models to predict apple storage life based on these and other stress indicators in order to reduce postharvest losses.

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