Chlorophyll Fluorescence in Relation to Superficial Scald Development in Apple

Nazir Mir,¹ Michael Wendorf, Rufino Perez, and Randolph M. Beaudry

Department of Horticulture, Michigan State University, East Lansing, MI 48824

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ABSTRACT. The relationship between chlorophyll fluorescence of 'Cortland', 'Redchief Delicious', and 'Empire' apple (*Malus × domestica* Borkh.) fruit and the development of superficial scald was studied during 120 days of refrigerated air (RA) storage at 0 °C and during 7 days of poststorage holding at 22 °C. Minimal fluorescence (Fo), maximal fluorescence (Fm), photochemical efficiency (Fv/Fm, where $F_v = F_m - F_o$) and coefficient of photochemical fluorescence quenching (qp) were measured. During storage, while Fv/Fm and Fm declined in 'Cortland' and 'Redchief Delicious' fruit over time, these two measures of chlorophyll fluorescence remained stable in 'Empire' fruit. Of the three cultivars, only 'Empire' is resistant to and did not develop superficial scald. A decline in Fv/Fm preceded scald development in 'Cortland' and 'Redchief Delicious' fruit. After 30 days of storage, qp began to decrease in fruit from all three cultivars. Prestorage diphenylamine (DPA) application had no effect on Fv/Fm, Fo, and Fm and only marginally improved maintenance of qp, but completely prevented the development of superficial scald. Poststorage holding at 22 °C accelerated the rate of change in most fluorescence measurements. The decline in the Fv/Fm ratio and/or qp with storage time may be in response to senescence-related factors that also enhance scald susceptibility, however, Fv/Fm does not appear to be directly related to superficial scald susceptibility *per se*.

Superficial scald is a postharvest physiological disorder manifested as skin browning that develops during storage of many commercially important cultivars of apple and pear (Emongor et al., 1994; Ingle and D'Souza, 1989; Wilkinson and Fidler, 1973). The damage due to the disorder occurs first in the hypodermis, which comprises the outer plastid-bearing cell layers underlying the epidermis, but exterior to the large cortical cells (Bain and Mercer, 1963). While the mechanism of scald development is yet to be elucidated, it has been suggested that it is a low-temperature related disorder, appearing after some period of cold storage (Watkins et al., 1995). The initial symptoms of the disorder often occur primarily on unblushed areas of the fruit, but with advancement of the disorder, the more highly colored portion of the skin may also be affected (Emongor et al., 1994). Severe symptom development is marked by pitting and death of the outer layers of the fruit cortex. Even minor scald damage, however, is sufficient to prevent sale of affected fruit for use in the fresh market. More severe symptom development can result in complete loss of value. Superficial scald can be effectively controlled by dipping the fruit in solution of diphenylamine (DPA), an antioxidant, before cold storage (Ingle and D'Souza, 1989).

Chlorophyll fluorescence measures the primary processes of photosynthesis and has been used as a noninvasive indicator of *in vivo* photosynthetic reactions (Schreiber et al., 1994) and environmental stresses (Gray et al., 1997; Levitt, 1980) of various crop plants. Chlorophyll, found in organized pigment/protein complexes in the chloroplast membrane, absorbs light largely in the blue (≈660 nm) and red (≈680 nm) wavelengths. A portion of the absorbed energy is transferred to electrons in the protein–pigment complex referred to as photosystem II (PSII). The excited electrons are used to fuel the reduction of CO₂ in photosynthesis. A small portion of the energy is not used and is reradiated at

wavelengths (≈685 to 740 nm) that are longer than the absorbed wavelengths in a process known as fluorescence. When the intensity of illuminating light is well below the capacity of the tissue to process the energy, PSII is able to pass on nearly all of the electrons excited by the light to photosynthetic processes, such that its reaction center is fully open for energy influx. Under these conditions, the fluorescence intensity is at a minimum. The measured minimal fluorescence is referred to as Fo or dark, background or initial fluorescence (Fig. 1). Conversely, when the intensity of the illuminating light is well above the capacity of the tissue to process the energy, PSII is able to pass on only a fraction of the electrons excited by the light. The reaction center is essentially 'closed' to energy influx and the excited electrons have a tendency to lose their energy as fluorescence. Under these conditions, the fluorescence intensity is at a maximum and is referred to as Fm or maximal or maximum variable fluorescence (Fig. 1). The relationship between these two responses is more commonly expressed as the ratio between the increase in fluorescence from minimal to maximal ($F_m - F_o = F_v$ or variable fluorescence) and Fm. The Fv/Fm ratio is often found to be at or approaching 0.8 in healthy tissues. Fv/Fm is a measure of the efficiency of the energy transfer process and of chloroplast activity.

When the intensity of the illuminating light is between that needed to determine Fo and that needed to measure Fm, the fluorescence yield increases sharply to an initial maximum (Fms), then declines to a steady-state value (Fs) as the photosynthetic machinery become activated and the energy is conserved in various chemical forms (Fig. 1). The more complete the photochemical energy transfer, the lower the fluorescence yield. The reduction in fluorescence yield due to photochemical reactions is designated as photochemical quenching of chlorophyll fluorescence (Schreiber et al., 1986). Under optimal (low light) conditions, the photochemical energy conversion processes account for >90% of the photosynthetically active light quanta absorbed by the chlorophyll (Björkman and Demmig, 1987).

Using chlorophyll fluorescence techniques, low-temperature injury can be detected in the fruit of banana (*Musa sp.*, AAA group, Cavendish subgroup) (Smillie et al., 1987), mango (*Mangifera indica* L.) (Smillie et al., 1987), cucumber (*Cucumis sativa* L.) (van Kooten et al., 1992), eggplant (*Solanum melongena* L.)

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¹To whom reprint requests should be addressed.

(Abbott, 1994), and green bell pepper (*Capsicum annum* L.) (Lurie et al., 1994). Chlorophyll fluorescence has also been suggested as a tool for detecting low O₂ (DeEll et al., 1995; Mir et al., 1997b; Prange et al., 1997) and high CO₂ stress in stored apple (DeEll et al., 1996; 1995). In addition, Fo, Fv, and Fv/Fm decline with increasing storage duration in 'Starking Delicious', 'Golden Delicious', and 'Rome Beauty' apple fruit in refrigerated air (RA) storage (Song et al., 1997). Reductions in these fluorescence measurements correlate with senescence-related changes in background color and fruit firmness. The decline in Fo, Fv, and Fv/Fm appears to reflect a loss in chloroplast function with advancing senescence (Mir et al., 1997a). Chlorophyll fluorescence has been suggested as having potential use as a nondestructive tool for quality measurement of stored apple (Beaudry et al., 1997; DeEll et al., 1996; Mir et al., 1997c; Song et al., 1997).

Since chloroplasts and chlorophyll molecules reside in the layers of the hypodermis that are prone to scald damage, we suspected that changes in chlorophyll fluorescence may accompany changes in the susceptibility to or development of superficial scald. Our objective was to examine superficial scald development in relation to changes in chlorophyll fluorescence in cultivars which are highly, moderately, and nonsusceptible to scald. Because of the tendency of superficial scald to intensify as fruit age, we were interested in separating the effects of senescence and scald development on fluorescence. To do this, fruit of each cultivar were treated with DPA, which effectively controls scald and has only a minor effect on retarding senescence (Lurie et al., 1989).

Materials and Methods

'Cortland', 'Redchief Delicious' and 'Empire' apple fruit were harvested at a mature, but preclimacteric condition (internal ethylene <0.2 $\mu\text{L}\cdot\text{L}^{-1}$) on 25 Sept. ('Cortland') and 1 Oct. ('Redchief Delicious' and 'Empire') 1996, from the Clarksville Horticultural Experiment Station, Clarksville, Mich. The fruit (240 fruit per cultivar) were immediately brought to the laboratory and were kept overnight at 22 °C to equilibrate with ambient temperature. Half of the fruit were given a drench treatment by dipping them into a solution of 5.9 mmol·L⁻¹ DPA. The DPA-treated fruit were air dried for $\approx$ 6 h. DPA-treated and nontreated (control) fruit were stored separately in plastic buckets at 0 °C in humidified (>95% relative humidity) air for 120 d.

Chlorophyll fluorescence and firmness were measured at harvest and subsequently at regular intervals of 30 d during a 120 d storage period on five fruit for each cultivar/drench treatment combination. Superficial scald incidence was measured between 60 and 120 d of storage at 15 d intervals for each storage duration/cultivar combination. After 120 d RA storage, chlorophyll fluorescence of fruit not treated with DPA was measured after 0, 2, 4, and 7 d poststorage holding at 22 °C.

CHLOROPHYLL FLUORESCENCE. Fruit removed from 0 °C storage were warmed at 22 °C for 3 h before fluorescence measurements. Chlorophyll fluorescence was measured only on the nonscaled portions of the fruit surface using a fluorometer (OS500, OptiSciences, Tyngsboro, Mass.) as described by Beaudry et al. (1997), with the exception that the fluorometer was run in the kinetics mode of operation to generate a fluorescence induction curve (Fig. 1). Fluorescence was measured using a photodiode in the wavelength range of 710 to 760 nm. The dark-adapted Fo was measured using a modulation intensity setting of 60 ($\approx$ 0.15 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Fm was determined with a saturation light intensity setting of 190 ($\approx$ 7.5 mmol·m⁻²·s⁻¹) for 0.8 s. Turning on the actinic lights with a setting of 50 ($\approx$ 88 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) rapidly increased fluorescence to a maximum

value (Fms), which gradually dropped to a steady state level (Fs). Additional saturating flashes of light provided at 1 min intervals were used to estimate qp (Schreiber et al., 1986). The values for qp reported herein reflect those obtained at steady-state, which occurred after $\approx$ 10 min exposure to actinic light. Photochemical quenching was calculated as (Fms - Fs)/(Fms - Fo). A detector gain of 100 was used for all measurements.

FIRMNESS. The firmness of fruit was measured manually using a drill-stand-mounted Effegi penetrometer (FT-327, McCormick Fruit Tree Inc., Yakima, WA) fitted with an 11-mm-diameter probe. The penetrometer was calibrated at 53.4 N (12 lb) using a top-loading balance. Two skin discs ($\approx$ 2.5 cm in diameter) were removed from opposite sides of the fruit, the first being selected from the most highly colored side of the fruit. The penetrometer probe was pressed into the tissue to a depth of 8 to 9 mm in a single smooth motion for 1 to 2 s. Data were recorded as pounds and converted to Newtons by multiplying by 4.45.

SCALD DEVELOPMENT. Superficial scald development was recorded as percentage of fruits affected after holding fruit for 7 d at 22 °C. Data represent seven fruit per cultivar-drench treatment-storage duration combination except for the last date when fruit numbers were 29, 22, and 25 for 'Cortland', 'Redchief Delicious', and 'Empire', respectively.

STATISTICAL ANALYSES. For measurements of chlorophyll fluorescence and fruit firmness during storage, a three-factor (drench treatment, cultivar and storage duration) completely randomized design was used. For measurements of chlorophyll fluorescence during poststorage holding of untreated fruit held 120 d in RA, a two-factor (cultivar and holding duration) completely randomized design was used. The data were analyzed by the analysis of variance (ANOVA) procedure using PlotIT graphics and statistics package (Scientific Programming Enterprises, Haslett, Mich.). Unless otherwise stated, only data significant at $p = 0.05$ are discussed.

Results

Fluorescence measures of Fo, Fm, Fv/Fm, and qp were differentially affected by cultivar, DPA treatment, and storage duration (Table 1). Cultivar affected all measures of fluorescence. DPA

Fig. 1. Chlorophyll fluorescence pattern of 'Cortland' fruit at harvest as determined using a pulse-modulated fluorometer. Fm and Fms are the maximum values of fluorescence without and with actinic light, respectively. The numbers represent Fms at each saturation flash. The Fo is the minimum fluorescence measured using a weak modulated light (0.15 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). See text for full descriptions of each term and methodology.

treatment, however, affected only qp, maintaining a slightly elevated level of qp over control. Data for control and DPA-treated fruit were therefore pooled for graphical presentation (Figs. 2–4). Storage duration markedly influenced all measures of fluorescence, however, interactions with cultivar and with DPA treatment were evident. The cultivar $\times$ storage duration interactions reflect markedly differing time-dependent changes in fluorescence for each cultivar. Specific results are therefore presented by cultivar.

'CORTLAND' FRUIT STORAGE. Fo of stored 'Cortland' fruit was not apparently influenced by storage duration, despite the significance of storage duration effects in the study (Table 1). This was likely a reflection of the cultivar $\times$ storage duration interaction for Fo. Fm declined gradually with increasing storage duration, being reduced by $\approx 30\%$ by the conclusion of the study (Table 1, Fig. 2). The Fv/Fm ratio also declined with increasing storage duration up to 90 d of storage, with the ratio declining below 0.70 by 60 d storage (Table 1, Fig. 2). There was also a noticeable drop in qp by

60 d storage, declining from ≈ 0.8 to just above 0.70. Beyond 60 d, qp were not altered appreciably. Superficial scald symptoms appeared by 75 d RA storage (Fig. 3). Fruit firmness declined rapidly from day 30 to day 60 and remained essentially unchanged thereafter (Fig. 4).

DPA application completely prevented scald development during storage (data not shown), but did not influence Fo, Fm, Fv/Fm (Table 1), and fruit firmness (data not shown). Values for qp were slightly higher after 30 d of storage in fruits that were treated with DPA at the time of harvest (Table 1).

During poststorage holding of 'Cortland' fruit at 22 °C after 120 d RA storage, scald development was rapid, increasing from $\approx 20\%$ incidence at removal to 100% incidence after 2 d. During this time, Fm declined from 469 to 342 and the Fv/Fm ratio declined from 0.60 to 0.47 (Table 2). Fo and qp were not influenced by poststorage holding at 22 °C (Table 2).

'REDCHIEF DELICIOUS' FRUIT STORAGE. Fo and Fm of stored

Table 1. Effect of diphenylamine (DPA) and storage duration at 0 °C on various measures of chlorophyll fluorescence in 'Cortland', 'Redchief Delicious', and 'Empire' fruit during refrigerated air (RA) storage.

Cultivar	Treatment	Duration (d)	Fo	Fm	Fv/Fm	qp	
Cortland	DPA	0	170	710	0.76	0.78	
		30	166	673	0.75	0.81	
		60	173	560	0.69	0.73	
		90	203	470	0.57	0.71	
		120	166	439	0.62	0.67	
		0	170	710	0.76	0.78	
	Control	30	172	608	0.72	0.80	
		60	161	553	0.71	0.71	
		90	215	562	0.62	0.68	
		120	170	469	0.64	0.72	
		0	151	567	0.73	0.72	
		Redchief Delicious	DPA	30	145	458	0.74
60	148			571	0.74	0.70	
90	117			324	0.64	0.69	
120	120			337	0.65	0.68	
0	151			567	0.73	0.72	
30	155			554	0.72	0.73	
Control	60		112	424	0.74	0.65	
	90		125	370	0.66	0.65	
	120		95	249	0.62	0.63	
	0		147	637	0.77	0.83	
	30		140	600	0.77	0.83	
	Empire		DPA	60	186	731	0.75
90		200		634	0.68	0.77	
120		158		597	0.74	0.65	
0		147		637	0.76	0.83	
30		162		581	0.72	0.81	
60		171		694	0.75	0.73	
Control		90	179	705	0.75	0.71	
		120	157	618	0.75	0.64	
		ANOVA					
		Cultivar (C)	**	**	**	**	
		DPA (T)	NS	NS	NS	*	
		SE: C × T (n = 25, df = 2)	5.61	14.38	0.009	0.008	
C × T	NS	NS	NS	NS			
Duration (S)	*	**	**	**			
C × S	**	**	**	**			
T × S	NS	*	*	NS			
SE: C × T × S (n = 5, df = 120)	12.55	32.14	0.020	0.019			
C × T × S	NS	NS	NS	NS			

NS,*,** Nonsignificant or significant at $p \leq 0.05$ or 0.01, respectively.

Fig. 2. The effect of storage duration on qp (A), Fv/Fm ratio (B), and Fm (C) of 'Cortland', 'Redchief Delicious', and 'Empire' fruit. The fruit were warmed to 22 °C before fluorescence measurements. The lack of a diphenylamine (DPA) treatment effect permitted the pooling of treated and nontreated data. The values are averages of 10 fruit, 5 each for DPA and non-DPA treatments.

'Redchief Delicious' fruit began to decline by 60 d of RA storage (Table 1). The time-dependent decline in these two fluorescence measurements was relatively greater than for the other two apple cultivars. The Fv/Fm ratio also declined as storage duration increased, declining below 0.70 between 60 and 90 d RA storage. During the first month of storage, qp accounted for 73% of fluorescence quenching (Table 1). From day 30 to day 60 during storage, qp declined from 0.73 to 0.65; beyond 60 d storage, the quenching components did not change. Superficial scald symptoms appeared after 105 d RA storage, ≈30 d later than for 'Cortland' (Fig. 3). Fruit firmness declined with storage duration, dropping most rapidly from day 90 to day 105 (Fig. 4).

Changes in Fo, Fm, Fv/Fm (Table 1), and fruit firmness (Fig. 4) were not influenced by DPA application. Compared with control fruit, qp was slightly higher after 60 d of storage in fruit that were treated with DPA at harvest (Table 1). DPA application completely prevented scald development during storage (data not shown).

During poststorage holding of 'Redchief Delicious', Fm declined from 337 on day 0 to 200 on day 7 and Fo declined from 120 on day 0 to 68 on day 7 of poststorage holding at 22 °C (Table 2). Despite the rapid drop in Fo and Fm, the Fv/Fm ratio was not influenced by holding at 22 °C (Table 2). Photochemical quenching decreased sharply from 0.65 on day 0 to 0.47 on day 7 (Table 2). As for 'Cortland', poststorage holding at 22 °C increased the incidence of scald symptoms (Fig. 3). A maximum of 40% of the fruit showed

signs of scald development on day 4 of the poststorage holding period.

'EMPIRE' FRUIT STORAGE. Fm of stored 'Empire' fruit tended to increase slightly after 30 d of storage (Table 1), however, the Fv/Fm ratio and Fo were not influenced by the length of storage period (Table 1). During the first month of storage, qp accounted for 83 % of fluorescence quenching and began to decline after 30 d (Table 1). 'Empire' fruit did not develop storage scald (Fig. 3). Fruit firmness declined with increased storage duration, declining most rapidly from day 60 to day 105 and remaining essentially unchanged thereafter (Fig. 4).

DPA application did not affect Fo, Fm, Fv/Fm, and fruit firmness. DPA did, however, help maintain a slightly higher qp relative to controls after 30 d of storage (Table 1).

During poststorage holding of 'Empire', Fm declined from 595 on day 4 to 477 on day 7 of holding at 22 °C (Table 2) but Fo and the Fv/Fm ratio were not influenced by holding at 22 °C (Table 2). The quenching component qp decreased sharply after 4 d of holding at 22 °C (Table 2). Scald symptoms did not develop on 'Empire' fruit during the poststorage holding period (Fig. 3).

Discussion

The possibility that superficial scald development may affect, or be affected by chloroplast physiology is supported by a number of observations. Perhaps the most suggestive link is that chloroplasts reside in the hypodermal layers of the skin where scald damage occurs (Bain, 1956; Bain and Mercer, 1963). The fact that the most highly colored portion of the fruit, which is also the portion most directly exposed to the sun, is most resistant to superficial scald also argues for the involvement of some photochemical component in scald development. Further, fruit harvested in a less mature state will maintain a greater green life as measured by chlorophyll retention relative to fruit harvested at more advanced stages of ripeness and are more susceptible to superficial scald (Emongor et al., 1994). In addition, chloroplasts are the seat of the biosynthesis of isoprenoids, which are the building blocks for sesquiterpenes such as 3,7,11-trimethadodeca-1,3(E),6(E),10-tetraene or *trans,trans*- α -farnesene, the oxidation products of which are hypothesized to cause storage scald in apple (Anet, 1972; Huelin and Coggiola, 1970; Ingle and D'Souza, 1989). In that chlorophyll fluorescence provides a measure of electron transport in PSII, a link between superficial scald and

Fig. 3. Effect of storage duration on percentage of non-diphenylamine (DPA)-treated 'Cortland', 'Redchief Delicious', and 'Empire' fruit developing superficial scald. The fruit ($n = 7$ in each treatment at each sampling date except at 120 d, when $n = 29$ for 'Cortland', $n = 22$ for 'Redchief Delicious', and $n = 25$ for 'Empire') were held at 22 °C for 7 d after removal from refrigerated air (RA) storage.

Fig. 4. Effect of storage duration on firmness loss of 'Cortland', 'Redchief Delicious', and 'Empire' fruit during refrigerated air (RA) storage at 0 °C. The lack of a diphenylamine (DPA) treatment effect permitted the pooling of treated and nontreated data. The values are averages of 10 fruit, 5 each for DPA and non-DPA treatments.

fluorescence would provide additional evidence for a chloroplast involvement in scald development.

There was some indication that the presence of superficial scald was associated with changes in chlorophyll fluorescence of the fruit. In our study, the scald-resistant cultivar 'Empire' underwent essentially no drop in the F_o , F_m , and the F_v/F_m ratio over 120 d in RA. In contrast, scald-susceptible 'Redchief Delicious' and 'Cortland' experienced a significant decline in F_m and the F_v/F_m ratio during cold storage before superficial scald development. Song et al. (1997) found a similar relationship for 'Starking Delicious' fruit in which superficial scald development occurred after the F_v/F_m ratio had declined below 0.7. The decline in the F_v/F_m ratio, as measured by the point in time when the ratio declined below 0.7, appeared to coincide with or slightly precede scald development in 'Cortland' and 'Redchief Delicious' fruit in this study. The F_v/F_m ratio of 'Empire' generally remained well above 0.7.

It is interesting to note that the fruit of the scald-resistant cultivar 'Empire' underwent the greatest relative change in q_p , yet did not develop superficial scald symptoms during storage. 'Empire' fruit may accumulate relatively higher concentrations of antioxidants in comparison with 'Cortland' and 'Red Delicious' that could compete with natural reactions of energy conservation in these fruit. In intact chloroplasts of spinach, a decline in q_p was found to be in response to membrane energization due to an ascorbate-requiring, Mehler-peroxidase reaction (Schreiber and Neubauer, 1990). While antioxidant levels in 'Empire' have not been reported, endogenous antioxidant activities contributed to inhibition of scald development in 'Delicious' and 'Cortland' fruit (Barden and Bramlage, 1994). Interestingly, the levels of ascorbate were 2-fold higher in 'Red Delicious' fruit with moderate susceptibility to superficial scald compared with 'Cortland' fruit, which are highly susceptible to scald. These data can be interpreted to suggest that the more rapid decline in q_p for 'Empire' during storage may, in part, be associated with high endogenous antioxidant levels that imparts resistance to superficial scald development in 'Empire' (Fig. 3).

The lack of a marked effect of DPA on the pattern of fluorescence changes in RA-stored fruit indicates that DPA, while preventing scald development, does little to influence PSII activity. However, some effect of DPA on chloroplast metabolism is evident in its effect on fluorescence photochemical quenching. The preservation of a slightly elevated q_p during RA storage relative to untreated fruit suggests DPA confers a slightly improved maintenance of photosynthetic pigment systems in apple. DPA has been shown to have some effect on maintaining the integrity of a number of oxidation systems in 'Granny Smith' apple fruit (Lurie et al., 1989).

The patterns of decline in F_o , F_m , and F_v/F_m during storage and the poststorage holding for the superficial scald susceptible cultivars examined here are consistent with those reported previously (DeEll, 1996; Song et al., 1997). The marked decline in F_m for 'Cortland' and 'Redchief Delicious' fruit was probably related to a decline in chlorophyll pigment content during storage as found by Mir et al. (1997a) for 'Golden Delicious' fruit. The decline in the F_v/F_m ratio likely indicates a loss of chloroplast activity occurred and may serve as an indirect measure of chloroplast senescence (DeEll, 1996; Mir et al., 1997a, 1997c). The decline in photochemical quenching and hence a decline in the associated photochemical electron transport in

Table 2. Changes in measures of chlorophyll fluorescence in untreated stored fruit of 'Cortland', 'Redchief Delicious', and 'Empire' during poststorage holding at 22 °C.

Cultivar	Time (d)	F_o	F_m	F_v/F_m	q_p
Cortland	0	187	469	0.60	0.66
	2	161	342	0.47	0.67
	4	182	367	0.49	0.65
	7	131	296	0.55	0.67
Redchief Delicious	0	120	337	0.65	0.65
	2	93	291	0.68	0.58
	4	82	245	0.66	0.54
	7	68	200	0.67	0.47
Empire	0	158	597	0.74	0.64
	2	161	599	0.73	0.66
	4	171	595	0.71	0.70
	7	134	477	0.71	0.60
ANOVA					
Cultivar (C)		**	**	**	**
Holding (H)		**	**	NS	**
SE: C $\times$ H (n = 8, df = 84)		9.75	31.34	0.020	0.016
C $\times$ H		NS	NS	**	**

NS, **, *** Nonsignificant or significant at $p \leq 0.05$ or 0.01, respectively.

apple chloroplasts with time also suggests advancing stages of senescence of the chloroplasts for all three cultivars.

There is some indication that an advanced stage of senescence is needed for scald symptom development (Ingle and D'Souza, 1989; Lurie et al., 1989). In that fruit softening is one of the primary criteria of senescence in apple (Dull and Hulme, 1971), the data for fruit firmness in this study indicate advancing senescence with time for all three cultivars. In addition to fruit softening, there is general agreement that membrane deterioration due to lipid peroxidation is a fundamental aspect of fruit senescence (Meir et al., 1992). The reported changes in chloroplast activity are consistent with advancing senescence. However, the similar patterns of senescence in DPA-treated and nontreated fruit for all three cultivars are an indication that senescence, like changes in chloroplast metabolism, may be a necessary, but not sufficient condition for scald development during storage.

The pattern of changes in Fo, Fm, the Fv/Fm ratio, and quenching components reported here are consistent in form with fluorescence responses to chilling in other fruit tissues (Abbott, 1994; van Kooten et al., 1992; Lurie et al., 1994; Smillie et al., 1987) and support the supposition that scald is a chilling-type disorder (Watkins et al., 1995). However, where chilling temperature perception can be detected via rapid and often striking changes in these measures of chlorophyll fluorescence, the changes reported here were relatively slow.

Fluorescence at harvest has been suggested as a potential tool for predicting superficial scald in 'Delicious' apples (DeEll, 1996; DeEll et al., 1996). It has been previously noted that superficial scald development was only detected on 'Starking Delicious' fruit that had undergone a significant decline in fluorescence yield (Song et al., 1997), which is consistent with the results obtained for 'Cortland' and 'Redchief Delicious' in the present study (Table 1, Fig. 2). These results indicate that fluorometry may have some promise in assessing the predisposition of apple fruit to the development of superficial scald. Nonetheless our data did not suggest a causal relationship between the physiological status of the chloroplast and superficial scald development in apple fruit.

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