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Chlorophyll Fluorescence as a Potential Indicator of Controlled-atmosphere Disorders in 'Marshall' McIntosh Apples

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Abstract. **Chlorophyll fluorescence was evaluated as a rapid and nondestructive technique to detect low-O_2 or high-CO_2 stress in apples (*Malus domestica* Borkh.) during storage. 'Marshall' McIntosh apples were held for 5, 10, 15, 20, or 25 days at 3C in 1) standard O_2 (2.5% to 3%) and low CO_2 (<1%), 2) low O_2 (1% to 1.5%) and low CO_2 (<1%), 3) standard O_2 (2.5% to 3%) and standard CO_2 (4% to 4.5%), or 4) standard O_2 (2.5% to 3%) and high CO_2 (11% to 12%). Only 10% of the apples had skin discoloration after 5 days in 1% to 1.5% O_2; 80% developed skin discoloration after 20 days in low O_2. Small desiccated cavities in the cortex, associated with CO_2 injury, developed in 10% of the apples after 20 days in 11% to 12% CO_2. Five days in 1% to 1.5% O_2 or 11% to 12% CO_2 caused variable fluorescence (Fv) of apple fruit to decrease compared to those held in standard atmospheres. Additional exposure did not significantly affect Fv in either the low-O_2 (1% to 1.5%) or high-CO_2 (11% to 12%) treatment. Our results suggest that chlorophyll fluorescence techniques can detect low-O_2 and high-CO_2 stress in apples before the development of associated disorders.**

During the past decade, interest has developed in the practical application of chlorophyll fluorescence as a rapid and nondestructive method to detect stress in plants. Stress or injury to plant tissue that disrupts photosynthesis changes the characteristic fluorescence pattern of that tissue. There have been numerous studies using chlorophyll fluorescence to detect environmental, chemical, and biological stresses in plants (Lichtenhaler, 1988). However, most studies have used leaves, and there has been little application of this technique to postharvest studies of fruit. Chlorophyll fluorescence has been used to follow the development of chilling injury in banana (*Musa*, AAA Group, Cavendish subgroup) and mango (*Mangifera indica* L.) (Smillie et al., 1987) and to detect chilling injury in cucumbers (*Cucumis sativus* L.) (van Kooten et al., 1992) and green peppers (*Capsicum annuum* L.) (Lurie et al., 1994).

Apples are often stored in a controlled atmosphere (CA) in which the O_2 is reduced to 1% to 3% and CO_2 is elevated to 1.5% to 5% (Lidster et al., 1988). However, if the O_2 concentration is too low or the CO_2 concentration is too high, apples may become injured (Meheriuk et al., 1994). Our objective was to evaluate chlorophyll fluorescence as a potentially rapid and nondestructive method to detect low-O_2 or CO_2 stress in stored apples before the development of associated disorders. This report gives only preliminary results on the potential applicability of this technique to apples held under low-O_2 or high-CO_2 stress.

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Materials and Methods

Plant material. 'Marshall' McIntosh apples were harvested from orchards in the Annapolis Valley, Nova Scotia, Canada, in late Sept. 1993 at optimum maturity for long-term storage (based on internal ethylene concentrations and starch index). Apples were cooled to 0C within 12 h of harvest.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence was determined using a plant productivity fluorometer (model SF-20; Richard Brancker Research, Ottawa, Ontario, Canada). Apples were dark-adapted for at least 4 h and warmed to 20C before fluorescence measurements were taken. With a light intensity of 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the fluorometer probe was placed firmly on the blush side of the apple. Each apple was numbered and marked where fluorescence was measured to ensure that subsequent readings would be from the same apple at the same location. The peak (P) and terminal (T) values of the fluorescence induction curve were recorded from the fluorometer digital display. The T value was obtained after 50 secs. Variable fluorescence (Fv) was calculated as (P – T)/P, which was used in the statistical analyses.

Low-O_2 and high-CO_2 treatments. Apples were held in ambient air at 0C for ≈1 month or in standard CA (2.5% O_2 and 4.5% CO_2) at 3C for 4 months. On removal from storage, apples were warmed to 20C and divided into twenty 10-apple samples; initial chlorophyll fluorescence measurements were taken. Each 10-apple sample then was placed in a 4-liter jar and attached to a gas mixing board at 3C. Appropriate gas mixtures from bottled N_2, CO_2, and medical air were flushed through the jars every 3 h for 3 min at a rate of 0.7 liter·min^{-1} to ensure gas concentrations were maintained. Gas concentrations flowing through the jars were monitored using a gas analyzer (Nova Analytical Systems, Hamilton, Ontario, Canada); concentrations within the jars were monitored daily by withdrawing 1-ml gas samples through septa attached to the jars and injecting them into a gas chromatograph (model 3400; Varian Canada, Georgetown, Ontario).

Each of five jars received one of the following treatments: 1) standard O_2 (2.5% to 3%) and low CO_2 (<1%), 2) low O_2 (1% to 1.5%) and low CO_2 (<1%), 3) standard O_2 (2.5% to 3%) and standard CO_2 (4% to 4.5%), and 4) standard O_2 (2.5% to 3%) and high CO_2 (11% to 12%). The low-O_2 (1% to 1.5%) treatment examined the effects of low-O_2 stress on chlorophyll fluorescence, using standard O_2 (2.5% to 3%) as a control, whereas the high-CO_2 (11% to 12%) treatment examined the effects of high-CO_2 stress on fluorescence, using standard CO_2 (4% to 4.5%) as a control. Although apples stored in <3% O_2 may develop symptoms of low-O_2 injury (Autio et al., 1990), no low-O_2 injury occurred in 'Marshall' McIntosh after long-term CA storage in 2.5% O_2 (DeEll and Prange, unpublished data).

One jar from each treatment was removed after 5, 10, 15, 20, or 25 days, and chlorophyll fluorescence measurements were repeated. Individual apples also were examined for external and internal symptoms of low-O_2 or CO_2 injury on removal.

Statistical analyses. The data were tested by the analysis of variance (ANOVA) procedure on the statistical program Genstat 5 (Payne, 1993). The factors and levels used in the analyses were atmosphere (four treatments), removal time (5, 10, 15, 20, and 25 days), and two replications, which were confounded within time (after 1 month in air at 0C and after 4 months in standard CA at 3C) because of space constraints of the gas board. In the ANOVA, there was no significant difference between the two replications in initial fluorescence values (data not presented).

Results and Discussion

Only 10% of the apples had skin discoloration after 5 days in 1% to 1.5% O_2 (Table 1), but 80% developed skin discoloration after 20 days in low O_2. Strong off-flavors also were

detected (subjective tasting) in apples after 20 days in 1% to 1.5% O_2. No visual symptoms of CO_2 injury were observed in apples held for 15 days in 11% to 12% CO_2. However, six of the 40 apples evaluated after 20 and 25 days in high CO_2 developed small desiccated cavities in the cortex—a symptom of CO_2 injury. Strong off-flavors also were detected (subjective tasting) in apples after 20 days in 11% to 12% CO_2.

Table 1. Incidence of low-O_2 or CO_2 injury and chlorophyll fluorescence (Fv[z]) values of 'Marshall' McIntosh apples after 5 days in standard O_2 (2.5% to 3%), low-O_2 (1% to 1.5%), standard CO_2 (4% to 4.5%), and high-CO_2 (11% to 12%) atmospheres at 3C.

		Fv	
	Injury incidence	Interval (days)	
Atmosphere	(%)	0	5
Low O_2			
Standard	0	9.9	12.5***
Low	10	11.5	7.1
High CO_2			
Standard	0	10.4	8.7***
High	0	11.0	3.3
SEM (n = 20, df = 399)[y]			1.52

[z]Fv = [(P – T)/P] × 100.

[y]To compare the effects of treatment × time. The statistical analysis was on Fv data from evaluations after 5, 10, 15, 20, and 25 days. However, because Fv did not change after 5 days, only these data are presented.

***Difference significant at $P < 0.001$.

The Fv of apple fruit decreased from 11.5 to 7.1 after 5 days in low O_2; the Fv of apples in standard O_2 increased from 9.9 to 12.5 (Table 1). The Fv of apple fruit decreased from 11.0 to 3.3 after 5 days in high CO_2, and the Fv of apples in standard CO_2 decreased from 10.4 to 8.7. Exposure for >5 days did not significantly affect Fv in either the low-O_2 or high-CO_2 treatment. Low-O_2 or high-CO_2 stress was detected by changes in chlorophyll fluorescence before any major physical symptoms of low-O_2 injury or CO_2 injury developed.

Based on our studies, chlorophyll fluorescence may have potential as a rapid and nondestructive method to screen for stress tolerance in apples. For example, the relative tolerance of different cultivars and strains to low-O_2 or high-CO_2 concentrations in storage could be determined before storage using chlorophyll fluorescence techniques. However, more research is needed to determine if this technique works on other apple cultivars and strains and if low O_2 or high CO_2 affects fluorescence within hours or days of exposure.

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