Carbon Dioxide-induced Flesh Browning in Pink Lady Apples

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Abstract. **To investigate a flesh browning (FB) disorder in Pink Lady apple [*Malus* ×*sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf. cv. Cripps Pink], fruit were harvested from the same orchard each year from 2002 to 2005, at two or three maturity stages each year. Fruit were kept in air or controlled atmosphere (CA) storage (1.5- to 2-kPa O_2 in combination with 1-, 3-, or 5-kPa CO_2) at 0.5 °C. Additional subsets of fruit were exposed to 1 $\mu L \cdot L^{-1}$ 1-methylcyclopropane (1-MCP) for 24 hours and dipped in 2200 $\mu L \cdot L^{-1}$ diphenylamine (DPA) for 5 min or held in air at 0.5 °C for 2 or 4 weeks before CA storage. Flesh browning was not seen in air-stored fruit but appeared in CA-stored fruit as soon as 2 months after harvest. Flesh browning incidence did not increase after longer storage times. Flesh browning increased with increasing CO_2 concentration and decreasing O_2 concentration in storage. 1-MCP did not significantly affect FB incidence, while delaying CA by 2 or 4 weeks reduced it. Diphenylamine eliminated FB incidence. When similar storage atmospheres were compared for the four seasons, FB incidence was high in 2002 and 2004 and low in 2003 and 2005. Concentrations of B, Ca, and Mg in apple flesh and seasonal field temperatures during the growing and harvest periods were related to FB incidence in 2002, 2003, and 2004 but not in 2005. The relationship of these pre- and postharvest factors to FB susceptibility are discussed.**

The Pink Lady apple ('Cripps Pink' apples of an appropriate quality and maturity may be sold using the brand name Pink Lady, a trademark of Apple & Pear Australia Ltd., Victoria, Australia) was developed in Australia in the late 1960s from a cross between 'Golden Delicious' and 'Lady Williams.' Since then, it has been sought for its sweet–tart flavor and crunchy texture. However, in recent years Pink Lady apples have shown a susceptibility to developing internal browning in storage.

Controlled atmosphere (CA) storage extends the life and preserves the quality of Pink Lady apples (Cripps et al., 1993). However, CA can also induce expression of physiological disorders in susceptible apples, such as internal flesh browning in 'Fuji' apples (Volz et al., 1998) and Braeburn browning disorder (BBD) (Elgar et al., 1998; Lau, 1998). Flesh browning in apples occurs intermittently and in unpredictable patterns, perhaps due to the interaction of yet-to-be-identified pre- and postharvest factors that affect the physiological state of the fruit at harvest and resistance to postharvest stresses. There are at least three different manifestations of this physiological disorder. One is diffuse FB, which appears to be related to chilling injury (CI) (Bramlage and Meir, 1990); the second is radial browning, which is related to senescent breakdown (Wilkinson and Fidler, 1973); and the third is CO_2 injury (brown spots that may develop into cavities), which is associated with CA storage (Lau, 1998).

Several factors predispose apple fruit to postharvest storage disorders. Variations in mineral composition are widely recognized to affect fruit quality after harvest (Bramlage et al., 1980; Sharples, 1980). Mineral composition greatly influences postharvest quality retention, and calcium is dominant in this respect. Trees high in nitrogen are usually vigorous trees with low crop loads; as a consequence, fruit is large, high in nitrogen, and low in calcium. Boron has a role in the structure of the plasma membrane (Parr and Loughman, 1983), and calcium is important in all cell membranes to stabilize phospholipids (Marinos, 1962).

Climate after full bloom and before harvest can have a great effect on postharvest development of physiological disorders (de Villiers, 1961; Warrington et al., 1999). Lau (1998) assessed the relationship between the incidence of BBD and cumulative growing degree days higher than 10 °C from 1 May to harvest. Lau (1998) concluded that a cool growing season could increase the susceptibility of the apple to CO_2 injury. Other weather effects are more related with harvest time. Light intensity and other developmental and environmental factors can significantly affect the concentration of sugars and ascorbic acid as well as fruit size (Kondo, 1992).

Fruit maturity has been demonstrated to be a factor in susceptibility to internal browning in some apple cultivars (Lau, 1998; Volz et al., 1998). In addition, diphenylamine, an antioxidant used commercially to inhibit storage scald in

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apples (Lau, 1990), markedly reduced both external and internal browning from CO_2 injuries in some apple cultivars (Fernández-Trujillo et al., 2001; Watkins et al., 1997). Delaying the establishment of CA has also been shown to reduce internal browning (Argenta et al., 2000).

Our objectives were to determine the most important pre- and postharvest factors that affect the susceptibility of Pink Lady apples to develop CO_2-induced internal FB and investigate possible solutions.

Materials and Methods

Fruit material. On 21 Sept. and 20 Oct. 2002; 6 and 20 Oct. and 6 Nov. 2003; 21 Sept. and 5 and 22 Oct. 2004; and 29 Sept. and 15 Oct. 2005, Pink Lady apples were harvested in the early morning from five 40-tree plots in the same orchard near Stockton, CA. About 50 apples were harvested randomly per tree, and each tree was harvested once. For every harvest, the apples were selected according to average background color and size for that tree and harvest date. Apples were harvested from the middle of the canopy from top to bottom and trunk to exterior.

Quality and internal browning evaluation. On the day of harvest, 30 fruit were selected randomly among the five plots to determine firmness and starch pattern index. Firmness was measured as resistance to penetration with an 11-mm flat-ended cylindrical probe on opposite sides of the fruit using a fruit texture analyzer (Güss Manufacturing, Strand, South Africa) after removing a small area of peel with a regular skin peeler. The apples were then cut in half equatorially, dipped for 2 min in iodine/potassium iodide (3%), and rinsed in fresh water. Starch levels were scored using a Center Technique Interprofessionnel des Fruits et Legumes (CTIFL, Paris) 10-point scale. When FB was evaluated after storage, 80, 105, 125, and 125 fruit per treatment in 2002, 2003, 2004, and 2005, respectively, were cut equatorially in three equally spaced locations resulting in ≈2-cm-thick slices, and the presence of FB was visually determined.

Treatments. Fruit were kept at 10 °C overnight before being sorted to obtain undamaged fruit of uniform size and color among the fruit in each plot before random assignment to the treatments, and the same number of fruit per plot was assigned to each treatment. Immediately after they were sorted, fruit were cooled to 0.5 °C in air for 24 h before the CA treatment was started. Following a determined storage period plus 5 d at 20 °C, fruit were assessed for FB, firmness, and other quality attributes, as described above.

Harvest dates each year were determined by the fruit's CTIFL starch index values: harvest 1, ≈3.5; harvest 2, ≈6.0; and harvest 3, ≈8.5. The CA storage conditions varied between years (Table 1). In 2002, apples were stored in air or in 1.5-, 3.0-, or 21-kPa O_2 in a factorial design with 1.0-, 3.0-, and 5.0-kPa CO_2. In 2003, fruit were stored in air or in 2-kPa O_2 in a factorial design with 1- and 3-kPa CO_2. In 2004, fruit were stored in air or 1.5-kPa O_2 in a factorial design with 1.0-, 3.0-, and 5.0-kPa CO_2 and in 2005, fruit were stored in air or 1.5-kPa O_2 + 5.0-kPa CO_2. The storage temperature was always 0.5 °C, and the storage time varied between 2 and 6 months.

Additional apples from the second harvest in 2003 were precooled at 0.5 °C and placed into 300-L steel tanks for 1-MCP treatment. A small electric fan was placed inside each tank to ensure even distribution of 1-MCP gas around the fruit. The tank lids were constructed to fit into water-filled troughs, forming a hermetic seal. Smart Fresh (AgroFresh, Springhouse, PA) tablets were used to generate 1 $\mu L \cdot L^{-1}$ of 1-MCP gas in the tank. Apples were treated with 1-MCP for 24 h at 0.5 °C and then transferred to air or CA at 0.5 °C. A second set of fruit from the same harvest in 2003, and additional fruit from the second and first harvests in 2004 and 2005, respectively, were treated with 2200 $\mu L \cdot L^{-1}$ DPA in water by immersion for 5 min, air-dried at 20 °C, and precooled overnight at 0.5 °C before storage in air or CA at 0.5 °C. Additional fruit from the same (2005) or another harvest (2003 and 2004) were placed into CA storage after 2 or 4 weeks' delay in air at 0.5 °C. Fruit were then stored for 4 months.

Table 1. Starch index values at harvest for Pink Lady apples under CA conditions and additional treatments applied in each season from 2002 to 2005.

	Storage conditions and treatments			
	Harvest maturity	Controlled atmosphere (kPa)		
Season	Starch index[z]	O_2	CO_2	Additional treatments
2002	3.5	1.5	1	
			3	
			5	
		3	1	
			3	
			5	
		20	1	
			3	
			5	
	6.0	1.5	1	
			3	
			5	
		3	1	
			3	
			5	
		20	3	
2003	3.5	2	1	
			3	
	6.0	2	1	1-MCP[y], DPA[x]
			3	1-MCP, DPA
	8.5	2	1	Delayed CA[w]
			3	Delayed CA
2004	3.5	1.5	1	
			3	
			5	
	6.0	1.5	1	DPA
			3	DPA
			5	DPA
	8.5	1.5	1	Delayed CA
			3	Delayed CA
			5	Delayed CA
2005	3.5	1.5	5	DPA
	6.0	1.5	5	
	8.5	1.5	5	

[z]Starch index at harvest (CTIFL scale, 1–10).
[y]1 $\mu L \cdot L^{-1}$ 1-methylcyclopropane (1-MCP).
[x]2200 $\mu L \cdot L^{-1}$ diphenylamine (DPA).
[w]2 or 4 weeks in air at 0.5 °C after harvest before CA storage.

Density. Density of apples was calculated every season from 2002 to 2004 using fruit of intermediate maturity (CTIFL starch score, ≈6.0) after 3 months of storage in air at 0.5 °C. Five fruit were selected, one from each plot in the orchard, and weight and buoyancy was recorded. Buoyancy was measured by immersing the apple under water and recording the force exerted by the apple to return to the water surface. The apple was submerged under a metallic funnel with hemispheric shape to hold the apple under the water without forming air bubbles. The funnel was attached with a hard wire to the hook of the bottom of the balance, and the force (grams) was recorded. To calculate the density from the weight and the buoyancy, we used the following formula:

$$\delta(\text{density}) = (\text{weight} - \text{buoyancy})/\text{weight}.$$

Mineral analysis. Apples of average background, color, and size were selected from each plot. Tissue samples consisted of two unpeeled wedges from each of a total of 25 apples each year, five fruit per five orchard plots. The wedges were chopped and a sample of ≈1 g was collected, including some skin, and mixed with the other apple samples so that ≈5 g from each of the five plots was collected for analysis. The samples were dried at 90 °C for 48 h, heated at 500 °C for 4 h, and dissolved in 1 N nitric acid. All samples were sent to a laboratory for analysis [University of California (UC), Division of Agriculture and Natural Resources Analytical Laboratory, Davis, CA]. Samples were analyzed for Ca, B, and Mg by inductively coupled plasma atomic emission spectrometry (ICP-AES) as described by Meyer and Keliher (1992) and by atomic emission spectrometry (AES) for K.

Individual apple mineral composition was also measured in healthy tissue of fruit with and without FB after storage in 1.5-kPa O_2 + 5-kPa CO_2 for 2, 4, and 6 months in 2004 and 2 and 4 months in 2005. A total of 30 apples were analyzed each year. After each storage time, apples were selected, one half with FB and one half without. Samples were freeze-dried and sent to a laboratory for analysis as previously described.

Weather data. The weather data were collected from a weather station very near the orchard in Farmington, CA (UC Statewide Integrated Pest Management Program, Davis, CA). The average daily air temperature was recorded for the 50 d after full bloom (DAFB), beginning on 4, 7, 21, and 17 March for 2002, 2003, 2004, and 2005, respectively.

To calculate the growing degree days (GDD) ≥ 10 °C during one period, the base of the calculation (10) was subtracted from the daily average temperature for every day of that period. The positive remainders were added to calculate the cumulative growing degree days (CGDD) ≥ 10 °C. To calculate the low-temperature exposure during the 50 DAFB [cumulative degree days (CDD) < 10 °C], the base of the calculation (10) was subtracted from the daily temperature average for every day of that period, and the negative remainders were added to obtain the total low-temperature exposure.

Statistical analysis. The experimental design consisted of two or three harvest maturities, storage time (2 or 4 or 6 months), and treatment (atmosphere, DPA, 1-MCP, and delayed CA). Each year was analyzed separately by harvest date and time in storage, and a factorial design was employed with five repetitions of 16, 21, 25, and 25 individual fruit each consecutive year. Analysis of variance was computed by SAS (version 8.02; SAS Institute, Cary, NC). Least-square means were employed due to missing values in some treatments. Multiple mean comparisons were performed using Tukey-Kramer adjustment, which was necessary due to the large number of mean comparisons required and the need to maintain a low experimental error rate ($\alpha = 0.05$).

Results

High-CO_2– and low-O_2–related injury. In 2002, the incidence of fruit with FB increased with greater CO_2 and with lower O_2 concentrations during storage (Fig. 1; Table 2). The

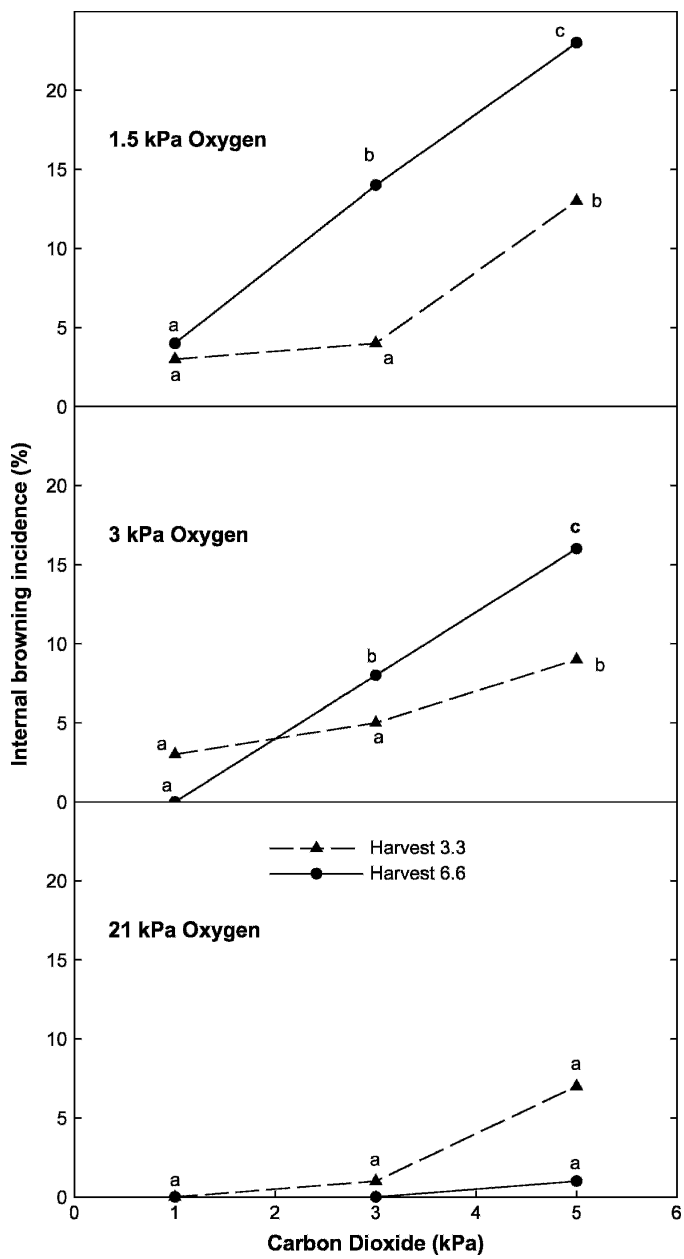

Fig. 1. Relationship between the incidence of flesh browning after 2 months of storage at 0.5 °C in apples harvested at two maturities (starch indices of 3.5 and 6.5) in 2002 and the concentrations of CO_2 and O_2 in storage. Different letters within an O_2 concentration indicate significant differences ($P \leq 0.05$) according to Tukey's test.

Table 2. Influence of season, fruit maturity, CA conditions, tree crop load, and average fruit size on the incidence of flesh browning in Pink Lady apples after 2 mo. of storage.

	Harvest maturity	Controlled atmosphere		Season characteristics		Flesh browning	
Season	Starch index[z]	O_2 (kPa)	CO_2 (kPa)	Crop load ($kg \cdot ha^{-1}$)[z]	Avg fruit wt (g)	Flesh browning incidence (%)	"Relative" flesh browning
2002	3.5	1.5	1	36,313	173	3	High
			3			4	
			5			13	
	6	1.5	1			4	
			3			14	
			5			23	
2003	3.5	2	1	29,357	183	2.5	Low
			3			2.5	
	6	2	1			1.3	
			3			1.3	
	8.5	2	1			0,0	
			3			2.6	
2004	3.5	1.5	1	44,372	176	13	High
			3			21	
			5			32	
	6	1.5	1			3	
			3			9.6	
			5			16	
	8.5	1.5	1			1	
			3			6.6	
			5			15	
2005	3.5	1.5	5	35,974	200	6.3	Low
	6	1.5	5			6	
	8.5	1.5	5			4	

[z]Total orchard production in kilograms per hectare.

incidence of FB injury was greater in fruit from the late harvest (starch index 6.0 compared with 3.5) in 2002 for the 1.5- and 3-kPa O_2 atmospheres, and FB incidence was similar among harvests in 2003 and 2005 (Table 2). However, in 2004, FB incidence in fruit from the first harvest (21.3%) was significantly higher than for the following harvests (9.6% and 6.6%) in 1.5-kPa O_2 and 3-kPa CO_2 (Table 2). No change in FB incidence was observed between 2, 4, and 6 months of storage (data not shown), and fruit stored in air with <0.05-kPa CO_2 did not exhibit FB at any time, a pattern that was consistent among years.

Effect of 1-MCP, DPA, and delayed CA. Treatment of fruit with 1-MCP before storage did not reduce the incidence of FB (data not shown), while DPA inhibited FB completely in three consecutive seasons. As an example, in 2004, 16% of fruit were affected with FB in 1.5-kPa O_2 and 5-kPa CO_2 without DPA treatment compared with 0% with DPA treatment. In addition, in 2004, only a delay of 4 weeks in cold storage at 0.5 °C before placing the apples in CA with 1.5-kPa O_2 + 5-kPa CO_2 significantly decreased the incidence of FB after 4 months of storage, with ≈75% decrease in FB incidence compared with no delay before CA storage (Table 3). Fruit from the 4-week delay were significantly softer (4-N lower firmness in 3-kPa CO_2 and 5-N lower firmness in 5-kPa CO_2) than nondelayed fruit, while the 2-week–delayed fruit had similar firmness as the nondelayed controls after 4 months storage (data not shown).

Mineral concentration, fruit size, and crop load. We observed the relationship between FB incidence each year and fruit mineral concentrations (Fig. 2). In 2003, fruit had a lower incidence of FB and had significantly higher Mg, B, and Ca concentrations and significantly lower concentration of K than in 2002 and 2004 when FB incidence was higher. However, the 2005 season also had low FB incidence but had the lowest Ca concentration of all seasons, lower Mg and B, and higher K. There was no relationship between NO_3^-, NH_4^+, Zn, Fe, Cu, P, or S concentration and FB (data not shown). When individual apples were studied, Ca concentration in the flesh was significantly lower in apples stored in CA and damaged by FB than in undamaged apples stored under the same CA conditions [28.1 vs. 36.6 μg Ca per gram fresh weight (FW)]. In 2003 and 2005 when the percentage of FB was low, fruit were large (183–200 g) in comparison with an average fruit size of 173–176 g in 2002 and 2004 (Table 2). At the same time, in 2002 and 2004, when the percentage of FB was higher than in 2003 and 2005, the crop load was also higher (Table 2).

Seasonal weather. The incidence of FB in Pink Lady apple varied from season to season. For the intermediate-maturity harvest, the percentage of FB was as high as 14% in 2002 and as low as 1.3% in 2003 in CA with 3-kPa CO_2 (Table 2). The CGDD ≥ 10 °C during the 50 DAFB were high in 2004, corresponding with high FB incidence, and low in 2003, corresponding with low FB incidence (Table 4). However, CGDD ≥ 10 °C during 50 DAFB was not well related to FB incidence in 2002 and 2005. The low-temperature exposure (CDD < 10 °C) during 50 DAFB was highest in 2002, corresponding with high FB incidence; however, in 2004,

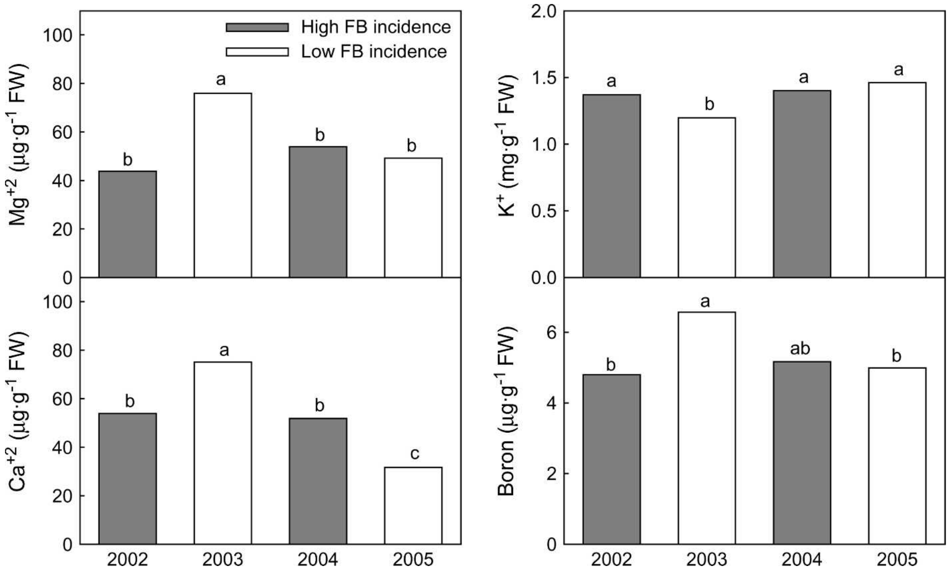

Fig. 2. Calcium, potassium, magnesium, and boron concentration (µg or mg per gram fresh weight) in apples at harvest in 2002–2005. Different letters indicate significant differences within a mineral ($P \leq 0.05$) according to Tukey's test; "High" and "Low" correspond to the severity of flesh browning incidence for that year. Starch index at harvest, ≈6.0.

Table 3. Incidence (%) of flesh browning in Pink Lady apples harvested at starch score 8.5 in 2004 after 4 mo. of storage in air or controlled atmosphere (CO_2 with 1.5-kPa O_2) at 0.5 °C preceded by a 0-, 2-, or 4-week delay in cold storage.

Flesh browning incidence (%)			
	Delay in CA storage (weeks)		
CO_2 (kPa)	0	2	4
1	1 cd	0 d	0 d
3	5 bc	3 cd	4 bcd
5	13 a[z]	8 b	3 cd

[z]Different letters within a row indicate significant differences ($P \leq 0.05$) according to Tukey's test.

Table 4. Relationship between browning severity of Pink Lady apple flesh and cumulative growing degree days (CGDD) ≥ 10 °C and low-temperature exposure [cumulative degree days (CDD) < 10 °C] for the 50 d after full bloom and the density of the fruit at harvest in 2002–2005.

Season	Flesh browning severity	CGDD ≥ 10 °C	Low-temp. exposure (CDD < 10 °C)	Density at harvest ($kg \cdot L^{-1}$)
2002	High	178	17.3	0.822 a[z]
2003	Low	156	2.4	0.797 b
2004	High	322	0.0	0.802 b
2005	Low	178	1.6	—[y]

[z]Different letters indicate significant differences ($P \leq 0.05$) according to Tukey's test.
[y]No data collected.

another season with high FB incidence, low-temperature exposure was very low.

We also determined the CGDD ≥ 10 °C from 1 May to harvest. Season 2003 presented the highest CGDD ≥ 10 °C accumulation for every harvest, corresponding with a low incidence of FB (Table 5). The CGDD ≥ 10 °C measured from 1 May to 31 Oct. (average commercial harvest date) were also higher in 2003 than the 7-year average from 1998 to 2005. However, the 2005 season, which also had a low incidence of FB, had intermediate CGDD ≥ 10 °C.

Discussion

Our results show that CO_2-induced FB was strongly associated with the CO_2 concentration in storage. A low concentration of O_2 increased the incidence of the disorder, but 21-kPa O_2 did not prevent it when CO_2 was elevated. These results agree with Watkins et al. (1997), Lau (1998), and Volz et al. (1998), who proposed that the incidence of FB and external browning were greater with higher CO_2 concentration. However, other factors appear to modulate fruit susceptibility, as we can conclude from the differences in incidence of FB among seasons.

In 2002, there was a trend toward more FB in the late-harvested than in the early-harvested fruit. On the contrary, in 2004, fruit from the earliest harvest had higher FB incidence. The pattern of 2004 in our study seems contradictory to previously reported trends by Volz et al. (1998) and Lau (1998) in which more mature fruit would show higher FB incidence. Both groups found that delaying harvest generated a higher incidence of FB and internal cavities (IC) in 'Fuji' and 'Braeburn' apples, respectively. Lau (1998) attributed this to an increase in respiration rate, skin resistance, and sensitivity to low O_2 and elevated CO_2 atmospheres in the late-harvested fruit compared with the early-harvested fruit. However, Elgar et al. (1999) found that early-harvested 'Braeburn' apples showed a higher incidence of internal cavities than the delayed-harvest fruit. Additionally, Smock and Blanpied (1963) and Meheriuk (1977) associated higher levels of external CO_2 injury with earlier harvests.

DPA completely inhibited CO_2 injury. Others have also shown that this antioxidant can inhibit CO_2-induced external

Table 5. Relationship between incidence of flesh browning in Pink Lady apples and cumulative growing degree days (CGDD) ≥ 10 °C for the period 1 May to harvest for each of the harvests and for the period 1 May–31 Oct. (average time for last harvest) in 2002–2005 and the average value for 1998–2005.

		1 May–harvest (CGDD ≥ 10 °C)			1 May–31 Oct. (CGDD ≥ 10 °C)
Yr	FB incidence	H1	H2	H3	H3
2002	High	1571	1609	—[y]	1838
2003	Low	1820	1940	2004	2004
2004	High	1605	1732	1831	1846
2005	Low	1634	1738	1828	1842
7-Year avg[z]					1923

[z]Average value for 1998–2005.
[y]No third harvest in 2002.

and internal browning as well as storage scald in stored apples (Fernández-Trujillo et al., 2001; Lau, 1990; Watkins et al., 1997). Delaying the introduction of CA in cold storage has also been shown to reduce CO_2 injury, both externally (Watkins et al., 1997) and internally (Argenta et al., 2000; Colgan et al., 1999). The reduction in damage was not seen until the delay was longer than 5 or 6 weeks for external or internal injury, respectively, for Watkins et al. (1997) and Argenta et al. (2000), although in 'Fuji' a 4-week delay has been shown to eliminate CO_2-induced FB (R.K. Volz and E.J. Mitcham, unpublished data). For Pink Lady, we observed a significant reduction in CO_2 injury after a 4-week delay in CA with 5-kPa CO_2. Perhaps this cold period before CA storage allows for a reduction in respiration rates, stabilization of membranes, and production of ascorbic acid after the stress of harvest in which the fruit is separated from the tree, its main source of nutrients. Firmness of Pink Lady apples that were held in cold storage for 4 weeks before CA storage was lower than that of apples placed directly in CA with 1.5-kPa O_2 plus 3- or 5-kPa CO_2. After 5 months in CA, Watkins et al. (1997) and Argenta et al. (2000) found that 'Empire' and 'Fuji' apples were softer following 8 months of storage when they were delayed 2–12 weeks in air before CA than when they were placed directly in CA. Treating apples after harvest with 1-MCP did not have any effect on FB incidence. The FB disorder does not appear to be associated with ethylene action.

During the first three seasons of this study, a relationship between the concentrations of Ca, Mg, K, and B and FB incidence was observed. While there appeared to be a trend with high Mg^{2+}, Ca^{2+}, and B and low K^{+} related to low FB incidence in 2002, 2003, and 2004, this relationship did not hold in 2005. Lau and Looney (1978) investigated differences in mineral concentration of 'Golden Delicious' apples and found a greater incidence of CO_2 injury associated with higher fruit N, Mn, and Zn and lower K and Mg, but no association with Ca concentrations. Low Ca concentrations have long been recognized to increase the susceptibility of apple fruit to physiological disorders (Ferguson and Watkins, 1992). However, in 2005, apples had the lowest Ca and B concentrations of all four seasons, but the incidence of FB was also low. This result may indicate that high Ca can reduce the susceptibility to FB, such as in 2003, but low Ca concentration does not necessarily lead to high susceptibility to the disorder. We saw a similar pattern with Mg and the opposite pattern with K. When we investigated the mineral concentration of individual apples, those apples with FB after storage were the apples with lower Ca concentration. The relationship of Ca with CO_2-induced FB remains uncertain. High concentration of Ca appears to reduce the susceptibility of the apple to the disorder; however, Ca alone does not explain all the variability we have observed among seasons.

Fruit from light cropping seasons are more susceptible to postharvest disorders, like BBD (Elgar et al., 1999), but in our study lower crop-load years resulted in low FB incidence, and seasons with higher crop load had high FB incidence. There appears to be a biennial pattern to FB susceptibility in our study, with 2002 and 2004 being the years of more severe FB incidence and highest crop loads. Kondo (1992) reported that overcropping treatments reduced significantly the content of ascorbic acid. Ascorbic acid content has been related to FB incidence in fruit (de Castro et al., unpublished data), and this may explain why high crop-load years will present a high FB incidence.

Light crop loads have been associated with higher density fruit than fruit from trees with more commercial-sized crop loads (Bussakorn et al., 2001). However, in our study we found that the average fruit density in 2003, a light-cropped year, was similar to the average in 2004, the highest crop-load year, In addition, fruit density was not related with FB incidence in our study.

Low- or high-temperature exposure during the first 50 DAFB does not seem to be related with the seasonal pattern in FB. The high amount of chilling 50 DAFB in 2002 might explain the high severity of FB incidence; however, in 2004, the other season with high FB severity, low-temperature exposure was low.

The climate during the growing season seems to influence FB susceptibility. Our results agree with previous studies that found a relationship between cool growing seasons (May to harvest) and increased incidence of BBD (Lau, 1998). Lau concluded that a total number of CGDD $\geq$ 10 °C less than 1300 resulted in an increased incidence of internal browning. For our trial with Pink Lady, it is difficult to propose a specific threshold number; however, it is clear that 2002, with the lowest number of CGGD $\geq$ 10 °C had high FB incidence and 2003, the year with largest number of CGDD $\geq$ 10 °C, had the lowest incidence of FB. However, 2005 was nearly as low as 2002 in CGDD $\geq$ 10 °C but had low FB incidence.

It is clear that fruit susceptibility to FB cannot be related to a single condition before harvest or during storage. High CO_2 concentration is the main factor causing FB; however, its effect is modulated by a number of known—and not yet known—interacting factors. Among these factors, harvest date, mineral nutrition, and seasonal weather conditions have shown some relationship with apple fruit susceptibility to high-CO_2–induced FB and warrant further study.

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