Controlled Atmosphere and Antioxidant Effects on External CO₂ Injury of 'Empire' Apples

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Abstract. The histology of external CO₂ injury of the skin of 'Empire' apples and postharvest factors affecting occurrence of injury were investigated. Injury was greater in a 5% CO₂/2% O₂ atmosphere than in 2% CO₂/2% O₂, but incidence was affected by orchard source. Susceptibility to injury was highest during the first 4 weeks of storage, while a postharvest treatment with diphenylamine prevented the disorder. Ethanol reduced injury, but ascorbic acid increased incidence of the disorder. Keeping fruit in air cold storage for 10 days before application of CO₂ markedly reduced incidence of CO₂ injury. Histological studies showed that external CO₂ injury begins at the hypodermis–cortex boundary and spreads outward into the upper hypodermis and inward into outer cortex cells, although the cuticle and epidermis appear unaffected and unbroken. Radial walls of affected cells collapse and become pleated, so that the skin surface sinks below nearby normal regions. Other cellular events include loss of cytoplasmic integrity, coagulation of the protoplast, loss of organelle structure, and cell wall separation. Nondigested starch can be found in cells of affected fruit at the hypodermis–cortex boundary. We conclude that several factors affect fruit susceptibility to CO₂ injury, including orchard, antioxidant treatment, and delays before application of CO₂.

External CO₂ injury is a disorder of apples that sometimes occurs on fruit of a number of cultivars (Fidler et al., 1973). The injury, described as "bronze epidermal discoloration" (Blanpied et al., 1990), is characterized by irregular-shaped, roughened to pebbly, tan to brown areas of the skin, often partly sunken, with sharply defined edges. It is assumed that injury is associated with collapse of hypodermal cells, although no histological study has been published.

Recently, occurrence of external CO₂ injury has become a concern for storages holding 'Empire', a cultivar produced from a cross between 'McIntosh' and 'Delicious' (Derkacz et al., 1993). 'Empire' is a major commercial cultivar in the northeastern United States for

both domestic and export supplies, and although financial losses to storage operators resulting from CO₂ injury have not been quantified, sporadic occurrence of the injury occasionally results in significant losses of fruit. Injury appears to be worse in storages in which controlled-atmosphere (CA) conditions are rapidly established. Disorder incidence is variable among orchards and fruit from some orchards are extremely susceptible, while those from others are not, under identical storage conditions. Burmeister and Dilley (1995) describe a "scald-like" CA storage disorder of 'Empire' apples that we believe is the same as the external CO₂ injury described in our study.

In 'McIntosh' apples, also susceptible to external CO₂ injury, increased occurrence of the disorder is associated with higher CO₂ concentrations, especially at lower O₂ concentrations and higher storage temperatures, e.g., 3 °C compared with 0 °C (Blanpied and Smock, 1961). Incidence is worse in earlier harvested fruit than in later harvested fruit (Smock and Blanpied, 1963). The potential for external CO₂ injury in 'McIntosh' apples is a major reason for New York recommendations that CO₂ concentrations should be between 2% and 3% for the first month of storage, before being increased to 5% (Smock and Blanpied, 1972). Effects of fruit maturity, surface wetness of the fruit, orchard source, and growing

region on external CO₂ injury have been described in investigations of the use of high (10% to 20%) CO₂ concentrations to maintain flesh firmness of 'McIntosh' and 'Golden Delicious' cultivars (Bramlage et al., 1977; Lau and Looney, 1977, 1978a, 1978b). Burmeister and Dilley (1995) observed that postharvest treatment of 'Empire' fruit with the antioxidant diphenylamine (DPA), used commercially for control of superficial scald, inhibited external CO₂ injury. Industry experience in New York supports this finding. Commercial storage operators who drench postharvest with DPA do not suffer fruit losses from CO₂ injury. DPA may not be available to apple industries in the future, however, because of concerns about postharvest chemical usage. Therefore, we need more information about factors influencing development of the disorder.

The objectives of this study were to investigate postharvest factors influencing external CO₂ injury of 'Empire' apples. These factors include the timing of CO₂ treatments during CA storage, effects of DPA and other antioxidants, and effects of keeping fruit in air storage before application of CO₂. The histology of external CO₂ injury also was examined.

Materials and Methods

Fruit used in these experiments were harvested from mature 'Empire' trees growing in the Cornell Univ. orchard at Ithaca, N.Y., or in commercial orchards in western New York. Depending on the experiment, two or four groups of one or two trees each were harvested separately to provide replicates. Fruit were harvested during the optimum period for CA storage as defined by starch pattern index ratings (Blanpied and Silsby, 1992).

Effect of timing of exposure to high CO₂ concentrations. Seven treatments were tested: fruit were kept in a constant 2% CO₂/2% O₂ or 5% CO₂/2% O₂ atmosphere for 20 weeks, or transferred from 2% CO₂/2% O₂ to 5% CO₂/2% O₂ for a single 4-week period (weeks 0–4, weeks 5–8, weeks 9–12, weeks 13–16, or weeks 17–20) before being returned to 2% CO₂/2% O₂ (Table 1). All treatments began 1 day after harvest. Each treatment consisted of four replicates of 40 fruit each, harvested from the Cornell Univ. orchard on 8 Oct. 1994.

Atmospheres were applied via a flow-through gas system. The replicates of 40 fruit each were placed into unstoppered 19-L glass jars and kept overnight at 2 °C. Jars were then stoppered at the times indicated, and connected to the atmosphere mixing system, which delivered humidified premixed gas mixtures containing either 2% CO₂ and 2% O₂ or 5% CO₂ and 2% O₂, the balance being N₂. Flow rates of the gases were maintained at 200 mL·min⁻¹, and atmospheres were monitored at least three times weekly using a paramagnetic oxygen analyzer (David Bishop, England) and a LIRA model 3200 gas monitor (Mine Safety Appliances Co., Pittsburgh) for CO₂. Atmospheres were maintained within 0.2% of the desired concentrations throughout the experiments.

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After 20 weeks of storage, fruit were transferred to a controlled-temperature evaluation room maintained at 20 °C. After 1 d, flesh firmness was measured on opposite sides of each fruit using an EPT-1 pressure tester (Lake City Technical Products, Lake City, Canada) fitted with an 11.1-mm-diameter head. After an additional 6 d, external CO₂ injury was assessed both visually and by rubbing each fruit by hand to detect colorless injury. The percentage of fruit with injury was calculated, regardless of severity of symptoms. Fruits also were cut at least three times at the equatorial plane for assessment of any internal disorders.

Effect of antioxidants. Fruit were untreated or immersed for 1 min in 2.95 mM (500 ppm) or 5.90 mM (1000 ppm) DPA (Shield Brite DPA 15%); water plus 0.1% Tween 20 alone or with 28.4 mM (5000 ppm) or 56.8 mM (10000 ppm) ascorbic acid; 95% ethanol alone or with 6.0 mM (2500 ppm) or 12.1 mM (5000 ppm) ascorbyl palmitate, or with 5.8 mM (2500 ppm) or 11.6 mM (5000 ppm) α -tocopherol (Table 2). The solutions were made up with tap water at ambient temperatures. Fruit were left at 20 °C to dry overnight, and then placed into jars at 2 °C for a further 24 h before being connected to a 5% CO₂/2% O₂ atmosphere. Each treatment consisted of four replicates of 40 fruit each, harvested from the Cornell Univ. orchard on 9 Oct. 1994. Fruit were assessed as described above after 20 weeks of storage.

Effects of DPA treatment and delaying application of CO₂ treatment. Fruit were harvested from two groups of trees in two western New York orchards on 7 Oct. 1995. Fruit from each orchard group were randomized into 12 lots of 40 fruit each. Half of the lots were dipped in 5.9 mM DPA for 1 min and the other half were dipped in water. Fruit were allowed to dry at ambient temperatures for 3 h and placed into jars and held in air at 2 °C for 1, 4, or 10 d. Because free water may increase injury (Lau and Looney, 1977, 1987b), fruit were checked for dryness and wiped if necessary before being placed in jars. After the 1-, 4-, or 10-d air storage period, jars were connected to 2% CO₂/2% O₂, or 5% CO₂/2% O₂ to provide two replicates for water or DPA treatment and for each atmosphere and orchard. Fruit were assessed as described above after 20 weeks of storage.

Statistical analyses. Data were subjected to analysis of variance procedures, using a general linear model.

Histological studies. After being kept in a 5% CO₂/2% O₂ atmosphere for 20 weeks plus 5 weeks in air storage, fruit with no visible injury, slight, moderate, and severe injury were selected for further study. Apple skin segments of ≈ 1 mm³ were removed from areas of the fruit exhibiting various degrees of CO₂ injury. These were killed and fixed in 3% glutaraldehyde in Sorenson's phosphate buffer (pH 7.2) at 4 °C for a minimum of 1 d, then rinsed twice in phosphate buffer at 4 °C, and dehydrated-infiltrated in a 4 °C graded series of ethanol-LR White (Polysciences, Warrington, Pa.) plastic resin. The temperature was increased to room temperature when specimens were transferred into pure LR White

resin. Specimens were placed in gelatin capsules filled with pure LR White resin and cured for 24 h in a 60 °C oven at ≈ 50 kPa vacuum. Transverse sections two microns thick were cut from the embedded specimens with a dry diamond knife on a Sorval MT2-B Ultramicrotome. Each section was mounted on a drop of distilled water on a clean microscope slide and then dried overnight on a 50 °C hot plate. Sections were stained with 1% aqueous safranin O for 1 h, counterstained with 0.25% aqueous toluidine blue O for 10 min, rinsed and allowed to dry before coverslipping using Permount (Fisher Scientific, Fair Lawn, N.J.). Selected sections were photographed using a Leitz Laborlux microscope fitted with a Zeiss CS attachment camera and Kodak T-Max 100 film.

Results

Effect of timing of exposure to high CO₂ concentrations. External CO₂ injury was greater in fruit kept in 5% CO₂ than in 2% CO₂ for 20 weeks (Table 1). Injury in fruit exposed to the higher CO₂ concentration for the first 4 weeks was the same as that for 20 weeks. Browncore was observed in all treatments but was not affected by treatment (data not shown). The softest fruit occurred in the 20-week 2% CO₂ treatment and when fruit were exposed to 5% CO₂ after 13 weeks, but there was little difference between the other treatments (Table 1).

Effects of antioxidants. DPA completely inhibited development of external CO₂ injury (Table 2). Ethanol also inhibited injury, the reduction being so marked that it was not possible to determine the independent effects of ascorbyl palmitate or α -tocopherol when combined with ethanol. Ascorbic acid, however, enhanced injury compared to the water plus Tween 20 control. Fruit treated with 5.90 mM DPA were firmer than control fruit, whereas ethanol-treated fruit were softer (Table 2).

Effects of DPA treatment and delaying application of CO₂ treatment. Without DPA treatment, fruit from the 5% CO₂ atmosphere had more external CO₂ injury than did fruit from the 2% CO₂ atmosphere (Table 3). Injury in fruit from one orchard (#1) averaged 8% compared with 36% in fruit from the other (#2). Delays in air before exposure to CO₂

treatment dramatically reduced injury, values averaged over both orchards being 37%, 28%, and 1% for 1-, 4-, and 10-d delays, respectively. However, for the second orchard, the reduction of injury was greater in the 2% CO₂ atmosphere than in the 5% CO₂ atmosphere. Also, injury in fruit from this orchard was not reduced by a 4-d delay before exposure to 5% CO₂.

The only other storage disorder observed was flesh browning (data not shown), which was different between orchards (6.3% vs. 0.3%; $P=0.007$), but no other significant differences were detected.

Flesh firmness was not affected by CO₂ concentration (Table 3). Overall, firmness was 3.1 N greater in fruit from orchard 1 than in fruit from orchard 2, and DPA-treated fruit from orchard 1 were 2.1 N firmer than were nontreated fruit. However, DPA treatment did not affect firmness of fruit from orchard 2. Fruit from both orchards became softer with increasing delays before CO₂ treatment, flesh firmness declining by an average of 0.54 N/day between 1 and 10 d.

Titrate acidity was not influenced by any factor except days before exposure to CO₂, averaging 0.430, 0.387, and 0.381 mg/100 g for a 1-, 4-, or 10-d delay, respectively (data not shown).

Histological studies. 'Empire' fruit not affected ("normal") by CO₂ injury were smooth and free of epidermal discoloration or structural problems (Fig. 1A). In contrast, affected fruit often showed severe symptoms of external CO₂ injury after 20 weeks of storage in 5% CO₂ (Fig. 1B). The skin of such fruit generally had a significant area with light bronzing and a dimpled surface, created as affected patches sunk below unaffected tissues. The border between affected and nonaffected regions was often sharply defined.

At the histological level, the normal skin of mature 'Empire' fruit consisted of a uniseriate epidermis covered by a thick cuticle, subtended by a hypodermis of several cell layers of collenchyma (Fig. 1C and E). Epidermal cells were of tabloid or cuboidal shape, were frequently tangentially separated from each other at fruit maturity, and were more or less embedded in the cuticle. The cells were vacuolate, but much of their volume was occupied by a finely granulated protoplast. The overly-

Table 1. Percent incidence of external CO₂ injury and flesh firmness of 'Empire' apples kept in 2% CO₂/2% O₂ for 20 weeks, or exposed to 5% CO₂/2% O₂ for single 4-week intervals, or 5% CO₂/2% O₂ for 20 weeks.^a

Atmosphere	Time of exposure (weeks)	External CO ₂ injury (%)	Flesh firmness (N)
2% CO ₂ /2% O ₂	0-20	1	60.4
5% CO ₂ /2% O ₂	0-20	10	63.0
	0-4	12	61.9
	5-8	2	63.1
	9-12	2	61.7
	13-16	3	57.9
	17-20	0	58.2
SE		2.9	1.41
P value		0.002	0.019

^aFlesh firmness assessed after 1 d, and external CO₂ injury after 7 d, at 20 °C.

Table 2. External CO₂ injury and flesh firmness of 'Empire' apples treated with antioxidant dips and stored in 5% CO₂/2% O₂ for 20 weeks.

Treatment			External CO ₂ injury	Flesh firmness
Solvent	Chemical	(mm)	(%)	(N)
Water	---	---	20	58.9
Water	DPA	2.95	0	58.0
		5.9	0	61.4
Water	0.1% Tween 20	0	12	57.5
	+ ascorbic acid	28.4	26	56.8
		56.8	37	56.9
95% ethanol	---	---	3	55.9
	ascorbyl palmitate	6.0	7	56.3
		12.1	7	53.1
95% ethanol	α -tocopherol	5.8	7	55.1
		11.6	8	54.2
	SE		7.2	1.77
	P value		<0.001	0.004

ing cuticle (not differentially stained in plate figures) was about as thick as the radial dimension of the epidermal cells. The four to six cell layers of the hypodermis overlaid, and abruptly graded into, the outer cortical (flesh) tissue, which consisted of highly vacuolate, large parenchyma cells surrounded by considerable intercellular space. The hypodermal cell walls were thickened, with the middle lamella and outermost primary wall layer more heavily stained than the inner wall layer. Pits were simple and paired. The small intercellular spaces often were filled with an unknown reticulated or stringy material that was darkly stained with safranin. The outermost hypodermal cells had a large vacuole and a highly vesiculated cytoplasm of a granular nature. These cells also contained lens-shaped chloroplasts and other organelles. The more vacuolate cells of the hypodermis had only a thin peripheral protoplast containing chloroplasts and many droplets, presumably phenolic in nature, at the outer periphery of the large vacuole (see Bain and Mercer, 1963). Droplet size decreased in the inner hypodermal cells. The cells of the outer flesh bordering the hypodermis had large vacuoles, which contained peripheral droplets similar to those of the inner hypodermis, and a peripheral cytoplasm with or without chloroplasts. The intercellular spaces were abruptly much larger than

were those of the hypodermis and these spaces increased in size in more subjacent flesh tissue. Although the hypodermal and cortical cells contained starch at harvest, little to no starch appeared in these cells in normal fruit after storage.

The histological basis for CO₂-induced injury was readily apparent upon close examination of transverse sections (Fig. 1 D, F-H). In fruit showing only faint epidermal discoloration, the hypodermis was the primary affected tissue (Fig. 1F). In sections taken from the border regions between affected and nonaffected tissues, the affected skin region was sunken and the hypodermal and outer cortex cells were radially flattened and distorted (Fig. 1D). The adjacent unsunken area was essentially unaltered from normal skin tissue, except where the tissues were first affected at the hypodermis-cortex interface. The cuticle and epidermal cell layer did not appear to be affected by CO₂-induced injury, such that these structures served as an unbroken covering on affected and nonaffected regions alike.

Closer inspection of the tissues in severely injured regions revealed that injury was confined to the hypodermis and outermost cortex (Fig. 1 G and H). The hypodermal layers in such regions were collapsed radially, primarily as a result of the pleating of the anticlinal (radial) cell walls. Wall separation and degra-

dation were also evident. The outer hypodermal cells appeared in better condition than did the inner hypodermal cells: the outer contained central vacuoles and peripheral protoplasts with intact organelles; the inner cells had a more dispersed granular protoplast, a loss of vacuolar integrity, and often retained undigested starch grains (Fig. 1G). Many collapsing cells contained only darkly stained contents, which probably reflected complete loss of cell function. The inner hypodermis was contiguous with collapsing cells of the outer cortex. Outermost cortex cells also occasionally retained starch. Protoplasts of the outer cortex often were plasmolyzed or otherwise had lost integrity. In both outer cortex and inner hypodermis, distinguishing between cells and intercellular spaces was often difficult.

Discussion

Several postharvest factors that can influence incidence of external CO₂ injury of 'Empire' apples have been identified. First, the susceptibility of fruit to injury is greatest in the first four weeks of storage, a distinguishing characteristic of external CO₂ injury (Fidler et al., 1973) and the reason for strict control of CO₂ in 'McIntosh' apple storages during the first month (Smock and Blanpied, 1972). Second, treating fruit with antioxidants influences external CO₂ injury. DPA eliminated injury, thus confirming the results of Burmeister and Dilley (1995) and observations of New York storage operators. Ethanol also reduced injury, but ascorbic acid, a water soluble antioxidant, increased incidence of the disorder. Third, keeping fruit in air in cold storage before exposure to higher levels of CO₂ reduced injury incidence. Although the two orchards we sampled differed significantly in response to a 4-d period in air, injury was completely controlled by a 10-d period in air at 2 °C before being placed in 2% CO₂. Fidler et al. (1973) found that incidence of external CO₂ injury in 'Bramley's Seedling' apples was dramatically reduced by a delay of one week before application of 10% CO₂.

The biochemical basis for external CO₂ injury remains unknown. Dipping or vacuum-infiltrating 'Golden Delicious' apples with

Table 3. External CO₂ injury and flesh firmness of 'Empire' apples treated with water or 5.90 mM DPA on the day of harvest, and kept in air for 1, 4, or 10 d before storage at 2% CO₂/2% O₂ or at 5% CO₂/2% O₂ for 20 weeks.

Observation	Delay before CA storage (days)	Water				DPA			
		2% CO ₂		5% CO ₂		2% CO ₂		5% CO ₂	
		Orchard 1	Orchard 2	Orchard 1	Orchard 2	Orchard 1	Orchard 2	Orchard 1	Orchard 2
External CO ₂ injury (%) ^a	1	3	27	30	88	0	0	0	0
	4	2	8	15	88	0	0	0	0
	10	0	0	0	5	0	0	0	0
	SE				8.3				
Flesh firmness (N) ^b	1	73.5	72.3	74.2	72.4	76.6	72.8	75.5	71.0
	4	72.4	70.2	73.5	70.5	75.6	70.2	73.4	71.7
	10	69.2	67.9	68.8	65.9	72.6	66.5	70.9	67.3
	SE				1.07				

^aAll factors and interactions significant at $P < 0.001$, except delay $\times$ orchard, $P = 0.001$, and delay $\times$ CO₂ $\times$ orchard, $P = 0.010$. Analysis of variance was carried out for the water treatment only as inclusion of DPA data would result in underestimation of the SE.

^bP values: Delay, $P > 0.001$; CO₂, $P = 0.403$; DPA, $P = 0.02$; orchard, $P < 0.001$; DPA $\times$ orchard, $P = 0.024$; all other interactions, NS.

Fig. 1. Comparison of normal 'Empire' apple skin anatomy and skin with CO₂-induced injury during postharvest storage. (A) Normal fruit. (B) Fruit surface with injury, showing dimpled and sunken areas with light discoloration. (C) Normal fruit skin in section, with uniseriate epidermis, hypodermis of thick-walled collenchyma, and outer cortex tissue with large intercellular spaces. (D) Section through border between affected and nonaffected region of skin. Cells of inner hypodermis and outermost cortex are collapsed in affected right half (arrow). Epidermis and cuticle are intact. (E) Normal epidermis and hypodermis. Some vacuoles in hypodermal cells contain polyphenolic droplets (arrows) and organelles can be found in the cytoplasm of many cells. Hypodermal cell walls are thick but unpleated and each cell is well defined. (F) Injured skin of a small fruit that showed mild external symptoms. Many hypodermal cells have senescent coagulated protoplasts and many plasmolyzed cells lack vacuoles. Epidermis and cortex tissues are intact. (G) More severely injured skin showing collapsing radial walls of inner hypodermis and outermost cortex, accompanied by retention of starch (arrows) at the hypodermis-cortex boundary. Cells of outermost cortex show collapse and wall separation. Outer hypodermis and epidermis appear unaffected; note chloroplasts in outer hypodermis (arrowhead). (H) Severely injured skin with general collapse of outer cortex cells and inner hypodermis, with complete loss of cell integrity. Cell separation is evident. Cells of the hypodermis often show coagulated protoplasts and wall degradation. Outer hypodermis and epidermis are intact. Scale bar: A-B is 1 cm; C-H is 100 μm. Abbreviations: C=cortex; E=epidermis; H=hypodermis; IS=intercellular space; P=coagulated protoplast; V=vacuole.

water increases injury (Lau and Looney, 1977), but the injury is not associated simply with acidification caused by high CO₂ (Lau and Looney, 1978b). High CO₂ atmospheres cause accumulation of succinic acid in apples (Hulme, 1956). This accumulation probably occurs because of inhibition of succinic dehydrogenase (Frenkel and Patterson, 1973), although a more general effect of CO₂ on activities of mitochondrial enzymes may occur (Shipway and Bramlage, 1973). Burmeister and Dilley (1995) suggest that the mechanism responsible for the CO₂-associated injury in 'Empire' apples is a free radical-catalyzed oxidation of susceptible amino acid residues in proteins essential for cell function and perhaps other macromolecules, such as membrane lipids containing oxidizable reactive groups.

At the morphological level, CO₂ injury of 'Empire' apples is characterized by a skin disorder in which affected areas become sunken, pebbly textured, sometimes with a more or less bronze discoloration, but always with an intact cuticle and epidermis. Cracks and russetting are not a normal symptom. Similarities of CO₂-induced skin injury and superficial scald are also evident. Burmeister and Dilley (1995) have described the injury as "scald-like," and indeed the symptoms of CO₂ injury (Blanpied et al., 1990; Fidler et al., 1973) can appear similar to superficial scald of the skin in that the hypodermal cells and perhaps a few outer cortex layers are affected. The most obvious similarity at the histological level is that sunken skin regions primarily result from radial collapse of the hypodermal and sometimes also the outer cortical cell walls. Such cells show a pronounced pleating of the anticlinal cell walls, with much less dramatic collapse of the tangential walls. Although we did not examine the ultrastructural details of the hypodermis, as did Bain and Mercer (1963), we did see similarities between affected hypodermal cells with scald in their study and affected hypodermal cells in our study of plastic thin sections examined by light microscopy. As injury progressed in the fruit we studied, these cells began to lose integrity as vacuolization ensued. Structures such as chloroplasts lost their distinctive form and the cytoplasm became more granular. Walls also began to lose integrity, often swelling or separating, as amorphous materials accumulated in the intercellular spaces. Severely collapsed cells often contained only dark amorphous materials.

Nevertheless, superficial scald and CO₂-induced skin injury of 'Empire' do differ in site of origin and progression. Whereas superficial scald appears first in the outer hypodermal cell layers and progresses toward the outer flesh tissues (Bain, 1956; Bain and Mercer, 1963), CO₂-induced injury appears first at the boundary of the innermost hypodermis and outermost cortex. The latter injury then spreads into the outer hypodermis and sometimes into several cell layers of the cortex. The epidermal cells may be affected by severe scald, but they and the overlying cuticle remain intact in even severely CO₂-injured fruit.

A pertinent observation, not mentioned in

previous literature discussing superficial scald, is the retention of starch in many hypodermal and outer cortical cells which have senesced in CO₂-injured fruit. Apparently, during storage in high CO₂, many of the starch-containing cells cannot complete starch digestion before senescence begins. Nearby areas of normal skin never show starch after the storage period.

Other interesting parallels exist between CO₂-induced injury and superficial scald. First, development of superficial scald is also inhibited by the antioxidants DPA and ethanol (Chellew and Little, 1995; Lurie et al., 1989; Scott et al., 1995), but the water-soluble antioxidant ascorbic acid is generally less effective (Chellew and Little, 1995; Watkins et al., 1988). The greater effectiveness of compounds such as DPA in reducing CO₂-induced injury and superficial scald may be related to their lipid solubility and ability to migrate across the cuticle. Second, timing of susceptibility to injury appears to be similar. External CO₂ injury appears early in the storage period (Burmeister and Dilley, 1995). However, while visible symptoms of superficial scald develop only during prolonged cold storage, damage occurs within the first several weeks of storage as indicated by decreasing effectiveness of DPA on reducing the disorder as application of the chemical is delayed (Watkins et al., 1995). Thus the timing of the damage may be closer to that for CO₂-induced injury than is apparent from appearance of visual symptoms. A significant difference between the disorders, however, is the effect of holding fruit in cool storage before applying CA conditions. Incidence of external CO₂ injury is decreased by such delays, whereas that of superficial scald usually is unaffected or enhanced (Watkins et al., 1995). Nevertheless, the similarities between the disorders indicate that investigations into either may yield information of potential value to control of both.

The ameliorating effect of keeping fruit in air before exposure to CO₂ exposure indicates that the etiology of the disorder is complex, as many changes associated with ripening and adjustment to cold storage conditions take place during this time. Whatever the mechanism, the reduction in incidence of injury by this treatment may explain why the disorder appears to be more severe for those storage operators who are using rapid CA storage procedures. Delaying application of CA is an alternative means of avoiding losses due to external CO₂ injury. However, such a delay is counter to the direction of modern apple industries, which are seeking to establish CA conditions as rapidly as possible to maintain fruit quality. Delaying CA is thus unlikely to be an acceptable alternative. Moreover, in one of our test orchards the incidence of disorder was 8% in the 2% CO₂ treatment, even after a 4-d delay in air storage, and by 10 d fruit were 4.4 N softer than after 1 d.

Incidence of CO₂-induced injury is variable, with fruit from some orchards being extremely susceptible and others only slightly susceptible under identical storage conditions. Significant injury can occur in susceptible

fruit even in a 2% O₂/2% CO₂ atmosphere. Commercial storage records also indicate that injury occurs on fruit at CO₂ concentrations lower than 2% to 3%; nevertheless, injury is greatest in storages using rapid CA storage techniques. Most problems appear to occur most when CO₂ concentrations are not kept lower than those of O₂ during the first few weeks of storage. The ratio between CO₂ and O₂ may be critical to the development of injury, rather than absolute concentrations of each gas within the normal range of atmospheres used in apple storages. The data of Burmeister and Dilley (1995) partially support this view, as incidence of the disorder tended to be higher when CO₂ concentrations were lower than O₂ concentrations, although relationships were not always consistent.

Because the risk of superficial scald development in 'Empire' appears to be low under New York growing conditions, many storage operators have ceased using DPA treatments for this cultivar. Advantages include cost savings, avoidance of a postharvest chemical, and reduced incidence of decay. However, prevention of external CO₂ injury by DPA, when it is applied for scald control, may be critical when using rapid CA procedures, as fruit from certain orchards appear highly susceptible to injury even when CO₂ levels in the storage atmosphere are relatively low. Also, observations that DPA can maintain firmness of fruit from some orchards have been confirmed in experiments on a larger number of orchards (unpublished data), and has commercial implications. The mechanism of this effect is not certain. Little is known about the effects of DPA on fruit physiology, although Lurie et al. (1989) found that 17.7 mM (3000 ppm) DPA resulted in firmer 'Granny Smith' fruit after 6 months in air storage and that DPA influenced several oxidative processes in the fruit, rather than just preventing oxidation of α -farnesene.

Inhibition of external CO₂ injury in the 'Empire' apple by DPA raises the interesting possibility that similar effects may occur in other apple cultivars. It is recommended that CO₂ concentrations be strictly controlled during early stages of CA storage of 'McIntosh' to avoid injury (Smock and Blanpied, 1972). However, CO₂ control may be less critical for DPA-treated fruit, as the research upon which the recommendation was based (Blanpied and Smock, 1961; Smock and Blanpied, 1963) was developed before the advent of routine DPA treatment to control scald. Johnson et al. (1997) recently reported that DPA treatment inhibits both external and internal CO₂ injury in 'Bramley's Seedling' apples. In addition, the possible benefits of DPA on maintenance of flesh firmness in other cultivars are not known, and are currently being investigated.

Literature Cited

- Bain, J.M. 1956. A histological study of the development of superficial scald in Granny Smith apples. *J. Hort. Sci.* 31:234-238.
Bain, J.M. and F.V. Mercer. 1963. The submicroscopic cytology of superficial scald, a physiological disease of apples. *Aust. J. Biol. Sci.* 16:442-449.
Blanpied, G.D., D.S. Johnson, O.L. Lau, P.D. Lidster,

- E.C. Loughheed, and S.W. Porritt. 1990. Apples, p. 7-22. In: P.D. Lidster, G.D. Blanpied, and R.K. Prange (eds.). *Controlled-atmosphere disorders of commercial fruits and vegetables*. Agr. Can. Publ. 1847/E.
Blanpied, G.D. and K.J. Silsby. 1992. Predicting harvest date windows for apples. *Info. Bul.* 221, Cornell Coop. Ext.
Blanpied, G.D. and R.M. Smock. 1961. Two-factorial experiments on controlled atmosphere storage of McIntosh apples. *Proc. Amer. Soc. Hort. Sci.* 78:35-42.
Bramlage, W.J., P.H. Bareford, G.D. Blanpied, D.H. Dewey, S. Taylor, S.W. Porritt, E.C. Loughheed, W.H. Smith, and F.S. McNicholas. 1977. Carbon dioxide treatments for 'McIntosh' apples before CA storage. *J. Amer. Soc. Hort. Sci.* 102:658-662.
Burmeister, D.M. and D.R. Dilley. 1995. A 'scald-like' controlled storage disorder of Empire apples—A chilling injury induced by CO₂. *Postharvest Biol. Technol.* 6:1-7.
Chellew, J.P. and C.R. Little. 1995. Alternative methods of scald control in 'Granny Smith' apples. *J. Hort. Sci.* 70:109-115.
Derkacz, M., D.C. Elfving, and C.G. Forshey. 1993. The history of the 'Empire' apple. *Fruit Var. J.* 47:70-71.
Fidler, J.C., B.G. Wilkinson, K.L. Edney, and R.O. Sharples. 1973. Chapter 2: Injuries caused by incorrect concentrations of carbon dioxide and/or oxygen, p. 81-87. In: *The biology of apple and pear storage*. Res. Rev. 3, Commonwealth Bureau of Horticulture and Plantation Crops, East Malling, England.
Frenkel, C. and M.E. Patterson. 1973. Effect of carbon dioxide on activity of succinic dehydrogenase in 'Bartlett' pears during cold storage. *HortScience* 8:395-396.
Hulme, A.C. 1956. Carbon dioxide injury and the presence of succinic acid in apples. *Nature* 178:218-219.
Johnson, D.S., C.J. Dover, and R.J. Colgan. 1997. Effect of rate of establishment of CA conditions on the development of CO₂-injury in Bramley's Seedling apples. *Acta Hort.* (In press.)
Lau, O.L. and N.E. Looney. 1977. Water dips increase CO₂-associated peel injury in 'Golden Delicious' apple. *HortScience* 12:503-504.
Lau, O.L. and N.E. Looney. 1978a. Effects of a pre-storage high CO₂ treatment on British Columbia and Washington 'Golden Delicious' apples. *J. Amer. Soc. Hort. Sci.* 103:341-344.
Lau, O.L. and N.E. Looney. 1978b. Factors influencing CO₂-induced peel injury of 'Golden Delicious' apples. *J. Amer. Soc. Hort. Sci.* 103:836-838.
Lurie, S., J. Klein, and R. Ben-Arie. 1989. Physiological changes in diphenylamine-treated 'Granny Smith' apples. *Israeli J. Bot.* 38:199-207.
Scott, K.J., C.M.C. Yuen, and F. Ghahramani. 1995. Ethanol vapor—A new anti-scald treatment for apples. *Postharvest Biol. Technol.* 6:201-208.
Shipway, M.R. and W.J. Bramlage. 1973. Effects of carbon dioxide on activity of apple mitochondria. *Plant Physiol.* 51:1095-1098.
Smock, R.M. and G.D. Blanpied. 1963. Some effects of temperature and rate of oxygen reduction on the quality of controlled atmosphere stored McIntosh apples. *Proc. Amer. Soc. Hort. Sci.* 83:135-138.
Smock, R.M. and G.D. Blanpied. 1972. Controlled atmosphere storage of apples. *Info. Bul.* 41, Cornell Univ., Ithaca, N.Y.
Watkins, C.B., J.E. Harman, and G. Hopkirk. 1988. Effects of lecithin, calcium, and antioxidant formulations on superficial scald and internal breakdown of 'Granny Smith' apples. *N.Z. J. Expt. Agr.* 16:55-61.
Watkins, C.B., W.J. Bramlage, and B.A. Cregoe. 1995. Superficial scald of 'Granny Smith' apples is expressed as a typical chilling injury. *J. Amer. Soc. Hort. Sci.* 120:88-94.