Superficial Scald, Carbon Dioxide Injury, and Changes of Fermentation Products and Organic Acids in ‘Cortland’ and ‘Law Rome’ Apples after High Carbon Dioxide Stress Treatment

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ABSTRACT. ‘Cortland’ and ‘Law Rome’ apples [*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.] were either nontreated or treated with the inhibitor of superficial scald development, DPA, and exposed to air or CO₂ (40 or 45 kPa) in air at 2 °C for up to 12 days. Fruit exposed to air or 45 kPa CO₂ were sampled during treatment, and peel and flesh samples taken for fermentation product and organic acid analyses. After treatment, fruit were air stored for up to 6 months at 0.5 °C for evaluation of disorder incidence. ‘Cortland’ apples were most susceptible to external CO₂ injury and ‘Law Rome’ to internal CO₂ injury. DPA treatment markedly reduced incidence of both external and internal injury. Fermentation products increased in peel and flesh of both cultivars with increasing exposure to CO₂, but the extent of the increase was cultivar dependant. Acetaldehyde concentrations were about 10 times higher in peel and flesh of ‘Law Rome’ than that of ‘Cortland’ apples. Ethanol concentrations in the flesh were similar in both cultivars, but were about twice as high in ‘Cortland’ than in ‘Law Rome’ peels. Neither acetaldehyde nor ethanol concentrations were affected consistently by DPA treatment. Succinate concentrations, often regarded as the compound responsible for CO₂ injury, increased with CO₂ treatment, but were not affected by DPA application. Citramalate concentrations were reduced by CO₂ treatment in ‘Law Rome’ peel, but other acids were not consistently affected by CO₂. Results indicate that acetaldehyde, ethanol or succinic acid accumulation are not directly responsible for CO₂ injury in apples. Chemical name used: diphenylamine (DPA).

Superficial scald, a physiological disorder that occurs in many apple (*Malus sylvestris* var. *domestica*) and pear (*Pyrus communis* L.) cultivars during cold storage (Ingle and D’Souza, 1989; Meigh, 1970), is usually controlled by a postharvest treatment with the antioxidant DPA. Interest in alternative means of controlling scald development has arisen because of concern that DPA may become unavailable for this purpose due to consumer issues on food safety. Alternative approaches that have been evaluated for scald control include use of stress treatments such as warming, short-term high temperature, and hypoxia exposure (Little and Pegg, 1987; Lurie et al., 1991; Wang and Dilley, 2000; Watkins et al., 1995). Stress treatments using high CO₂ were also tested before commercial acceptance of DPA. Pieniazek (1945) and Pieniazek and Christopher (1945) found that treatment of ‘Rhode Island Greening’ apples at harvest with 30 kPa and 60 kPa CO₂ for 3, 6, or 10 d at 0 °C eliminated scald development. No injury or off-flavors were detected in treated fruit, although ripening was delayed. While further evaluation of responses to 50 kPa CO₂ for 5 d showed scald inhibition without fruit damage for most cultivars tested, CO₂ injury was noted on fruit of ‘McIntosh’ and ‘Baldwin’ (Pieniazek et al., 1946).

Susceptibility of apples to CO₂ injury is greatly affected by cultivar and growing condition (Elgar et al., 1998, 1999; Volz et al., 1998; Watkins et al., 1997; Wilkinson and Fidler, 1973). These injuries have resulted in economic losses for cultivars such as

‘Braeburn’, ‘Empire’, ‘Fuji’, and ‘McIntosh’ apples under commercial conditions. Also, variable sensitivity of single cultivars from different growing regions has restricted utilization of high CO₂ pretreatments to maintain firmness (Bramlage et al., 1977; Lau and Looney, 1978). Recently, it has been found that DPA treatments used for scald control can inhibit development of CO₂ injury on apples (Burmeister and Dilley, 1995; Johnson et al., 1998; Watkins et al., 1997). The mechanism(s) whereby CO₂ injury occurs, or how it is alleviated by DPA, are not clearly understood.

Elevated CO₂ may affect respiratory metabolism via effects on glycolysis, fermentative metabolism, the tricarboxylic acid (TCA) cycle or the electron transport chain (Mathooko, 1996). Most research has centered on fermentation and the TCA cycle. A common response to CO₂ treatments is accumulation of acetaldehyde and ethanol (Kader, 1986), however, the relationships between these compounds and CO₂ injury are not clear. Another feature of tissues treated with CO₂ is accumulation of succinic acid (Ke et al., 1993; Fernández-Trujillo et al., 1999; Williams and Patterson, 1964; Yang et al., 1998), and inhibition of succinate dehydrogenase under these conditions has been demonstrated (Frenkel and Patterson, 1973; Ke et al., 1993; Shipway and Bramlage, 1973). Hulme (1956) proposed that, because succinic acid is toxic to plant tissues, its accumulation was responsible for CO₂ injury in apples.

Our initial objective was to investigate use of stress levels of CO₂ for alleviation of scald on ‘Cortland’ and ‘Law Rome’ apples. Our preliminary studies indicated that cultivar variation in response to elevated CO₂ could be high. Therefore, an additional objective was to examine differences in metabolism between the cultivars under these CO₂ treatments either with or without DPA treatments.

Material and Methods

Fruit used in these experiments were harvested in Fall 1997 and 1998 from mature ‘Cortland’ (MM106) and ‘Law Rome’ (M9/MM106 interstems) trees growing at the Cornell Univ. orchards at Ithaca and Lansing, N.Y., respectively. Harvest dates

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each year were during the first few days of commercial harvest for the region (Blanpied and Silsby, 1992). In each year of the experiment, uniformly sized fruit from several trees were randomized to provide experimental units of 45 to 50 fruit.

CARBON DIOXIDE TREATMENTS. In both years, fruit of each cultivar were either nontreated or immersed on the day of harvest for 1 min in DPA (1997: both cultivars with 5.9 mM (1000 mg·L⁻¹) DPA (Shield Brite DPA 15%, Pace Intl. LP, Kirkland, Wash.; 1998: 5.9 and 11.8 mM DPA (No Scald DPA, EC-283, Decco, Elf Atochem North America Inc., Monrovia, Calif.) for 'Cortland' and 'Law Rome', respectively). Fruit were allowed to dry and then placed into unstoppered 19-L glass jars and kept overnight at 2 °C. Jars were then stoppered and connected to an atmosphere mixing system, which delivered a humidified flow (0.20 L·min⁻¹) of air or elevated CO₂ (balance air). Atmospheres were monitored daily by gas chromatography (gas partitioner, model 1200; Fisher Scientific, Springfield, N.J.). The CO₂ concentration was maintained within 5 kPa of the target concentration throughout the experiment.

In 1997, 'Cortland' and 'Law Rome' fruit stored at 2 °C without DPA treatment were exposed to 40 kPa CO₂, and six replicate units removed to air after 0, 3, 6, 9, or 12 d. An additional treatment of DPA without exposure to CO₂ was incorporated. All fruit were transferred to cold storage at 0.5 °C when the last samples were removed from CO₂ treatment. Three replicates were removed from storage after 3 and 6 months, and held at 20 °C for 7 d before analysis. Ten fruit per replicate were used for measurement of firmness, soluble solids, and titratable acidity. Firmness was measured on opposite peeled sides of each fruit using an EPT-1 pressure tester (Lake City Tech. Products, Lake City, Canada) fitted with an 11.1 mm diameter Effegi tip. Fruit were then assessed for incidence of scald and external and internal CO₂ injury. Scald severity was assessed using a subjective scale where 0 = 0%, 1 = 1% to 10%, 2 = 11% to 33%, 3 = 34% to 66%, and 4 = 67% to 100% (Watkins et al., 1995). Soluble solids and titratable acidity were measured on juice extracted from opposite 1/8th segments of composite fruit samples using a refractometer (Atago PR-100, McCormick Fruit Tech., Yakima, Wash.) and an autotitrator (Mettler DL12, Hightstown, N.J.), respectively.

In 1998, the effects of CO₂ treatment on fruit either nontreated or treated with DPA were investigated. Four replicate samples of nontreated and DPA-treated fruit were exposed to air or 45 kPa CO₂ for 0, 6 or 12 d, after cooling overnight at 2 °C. Additional fruit replicates, nontreated or treated with DPA, exposed to CO₂ were sampled at 3 and 9 d.

At each sampling time, internal ethylene concentrations (IEC) were measured on 1 mL samples of internal gas from the core of 10 fruit from each cultivar, $\pm$ DPA, and atmosphere treatment, as described by Alwan and Watkins (1999), except that the gas chromatograph used was a Hewlett Packard 5890, series II (Hewlett Packard Co., Wilmington, Del.). The fruit were then peeled rapidly and frozen immediately in liquid N₂, and stored at -80 °C for analyses of fermentation products and organic acids. The remaining fruit were transferred to cold storage at 1 °C as in 1997, and stored for 6 months. External and internal disorders were evaluated after 7 d at 20 °C.

FRUIT TISSUE ANALYSES. For analysis of acetaldehyde, ethanol and ethyl acetate, frozen samples were powdered in liquid N₂ and 5 g of powder weighed into a 20 mL vial, which was sealed and put into a water bath maintained at 60 °C for 30 min. Headspace samples of 0.5 mL were removed from the bottles with a gas tight glass syringe and injected immediately into a gas chromatograph

(Hewlett Packard 5890, Wilmington, Del.) fitted with a 15 m $\times$ 0.53 mm wide bore capillary column with a coating thickness of 1.0 mm (Stabilwax, Restek, Bellefonte, Pa.), and attached to an integrator (Hewlett-Packard 3396A). The oven temperature was 45 °C, with injector and detector temperatures of 230 and 240 °C, respectively. Gas flow rates for hydrogen, air, and helium were 35, 175, and 6 mL·min⁻¹, respectively. Acetaldehyde, ethanol, and ethyl acetate were identified from retention times and standard curves obtained using authentic compounds (Fisher Chem. Co., St. Louis, Mo.). Detection limits for acetaldehyde, ethyl acetate, and ethanol were 0.004, 0.005, and 0.002 μ mol·g⁻¹, respectively.

For organic acid analyses, 1 g samples of powdered tissue were extracted and analyzed for acids following the method of Mattick et al. (1970), as described by Fernández-Trujillo et al., (1999).

STATISTICAL ANALYSIS. All data were subjected to analysis of variance (ANOVA) using the general linear model procedure for calculation of least squared means and least significant differences (LSDs), ($P=0.05$) using Minitab software v 11.12 (Minitab, Inc., State College, Pa.). Data were analyzed for effects of cultivar, DPA, atmosphere, and time when appropriate. Where initial analysis confirmed large effects of CO₂ treatment on concentrations of fermentation products and succinate, separate analyses of the effects of cultivar, DPA, and time were carried out on data from CO₂-treated tissues only. Because flesh and peel tissues were obtained from the same fruit, these factors were not independent and therefore data were analyzed separately. Analysis of fermentation products was performed on data transformed to natural logarithms after addition of 0.01 to account for zero values. Scald data from 1997 were analyzed by ANOVA. If the interaction of cultivar $\times$ CO₂ exposure time $\times$ storage time was significant, regressions for scald vs. CO₂ exposure time for each cultivar $\times$ storage time combination were then examined. Carbon dioxide-related disorders were analyzed by ANOVA and LSD tests are reported because of excessive zero values. Significant polynomial trends at $P < 0.05$ for the higher interaction are reported in the text when required. To compare cultivars, the polynomial trends for each cultivar $\times$ storage time combination were compared by calculating LSD for every contrast associated with this interaction.

Results

EFFECTS OF CO₂ ON SCALD AND CO₂ INJURY (1997). Scald incidence increased with storage period in both cultivars (Table 1). 'Cortland' apples from all treatments had low scald incidences after 3 months storage. However, by 6 months, the control fruit (without DPA) had 36% scald and increasing exposure times to 40 kPa CO₂ resulted in a reduction of this incidence. 'Law Rome' fruit showed higher scald incidence at 3 months than 'Cortland', and at this time, incidence was reduced with exposures to CO₂ > 3 d. After 6 months storage, however, differences between control fruit and the CO₂ treatments, while sometimes statistically significant, were small. Carbon dioxide treatment did not affect scald severity of either cultivar (data not presented). Scald incidence was reduced to low levels by DPA treatment at harvest (Table 1).

External CO₂ injury was significant only in 'Cortland' apples, and only after 9 d of CO₂ exposure (Table 1). No effect of storage period was detected. Internal CO₂ injury was found in both cultivars when treated for 9 or 12 d, with injury being greatest for

Table 1. Incidences of superficial scald, and external and internal CO₂ injury in 'Cortland' and 'Law Rome' apples, either nontreated or treated with 5.9 mM DPA and exposed to 40 kPa CO₂ for up to 12 d. Fruit from all treatments were kept at 2 °C for 12 d, transferred to air storage at 0.5 °C for 3 or 6 months, and evaluated after 7 d at 20 °C.

Cultivar	Effect	Superficial scald (%)		External CO ₂ injury (%)	Internal CO ₂ injury (%)	
				Combined ^z	Storage time (months)	
		3	6		3	6
Cortland	DPA	0	2	0	0	0
Law Rome	DPA	0	4	0	0	0
	CO ₂ exposure (d)					
Cortland	0	2	36	0	0	0
	3	4	21	0	0	0
	6	1	10	0	0	0
	9	1	3	27 ^y	5	8
	12	1	11	35	15	21
		NS ^x	L, Q	L, Q	L	L
Law Rome	0	29	77	0	0	0
	3	34	69	0	0	0
	6	11	76	0	0	0
	9	3	69	0	4	7
	12	3	69	1	11	31
		L, C,	NS	NS	L	L, Q

^zCombined data for 3 and 6 months storage because storage period was nonsignificant.

^yBecause of the repeated zero incidence, regressions were performed only on data from days 6, 9, and 12.

^xAll three-way polynomial contrasts were significant at $P < 0.001$ except when indicated (^{NS}Nonsignificant, L = linear, Q = quadratic, and C = cubic).

the longer exposure (Table 1). This injury incidence was slightly greater in 'Law Rome' than in 'Cortland', and injury increased with increasing storage period in 'Law Rome' fruit from the 12 d CO₂ treatment. DPA completely prevented CO₂ injury (Table 1).

At harvest, internal ethylene concentrations (IEC) of 'Cortland' and 'Law Rome' averaged 0.32 and 1.53 mL·L⁻¹, respectively. IEC increased in air over the treatment period, but was suppressed by CO₂ treatment. For example, IEC of 'Law Rome' fruit averaged 22.28 and 0.97 mL·L⁻¹ after 12 d of air and CO₂ treatment, respectively. No effect of DPA was detected (data not presented). After storage, flesh firmness and titratable acidity, but not soluble solids, were affected by cultivar and DPA treatment; DPA-treated fruit of 'Cortland' were softer and had less acidity, and 'Law Rome' apples were firmer with greater acidity, respectively, than control fruit. However effects of CO₂ treatment on these factors

were inconsistent (data not presented).

EFFECTS OF CO₂ ON SCALD AND CO₂ INJURY OF DPA-TREATED FRUIT (1998). Pretreatment of fruit with 45 kPa CO₂ for increasing time reduced, but did not prevent, scald development in either cultivar (Table 2). DPA treatments controlled the disorder in both cultivars.

External CO₂ injury was observed in 'Cortland' apples after 6 d and reached very high levels in the 12 d treatment, while injury was found only in the 12 d treatment for 'Law Rome'. DPA reduced this injury to negligible levels. In contrast, internal CO₂ injury was essentially absent in 'Cortland', but progressively more severe with increasing CO₂ exposure in 'Law Rome'. DPA reduced, but did not prevent injury development.

EFFECTS OF CO₂ AND DPA ON FERMENTATION PRODUCTS. When measured at the different removal times, concentrations of acetal-

Table 2 Incidences of superficial scald, and external and internal CO₂ injury, in 'Cortland' and 'Law Rome' apples either nontreated or treated with 5.9 or 11.8 mM DPA, and exposed to 45 kPa CO₂ in air for 6 or 12 d at 2 °C. Fruit were evaluated after 6 months storage at 0.5 °C and 7 d at 20 °C^z.

Cultivar	CO ₂ exposure time (d)	DPA treatment	Superficial scald (%)	External CO ₂ injury (%)	Internal CO ₂ injury (%)
Cortland	0	—	100	0	0
	0	+	0	0	0
	6	—	96	7	0
	6	+	0	0	0
	12	—	69	71	1
	12	+	0	0	0
Law Rome	0	—	87	0	0
	0	+	0	0	0
	6	—	51	0	3
	6	+	0	0	4
	12	—	47	11	60
	12	+	0	1	31

^zCultivar × exposure time × DPA interactions were significant at $P < 0.001$, 0.002, and < 0.001 for superficial scald, and external and internal CO₂ injury, respectively.

dehyde, ethanol, and ethyl acetate increased in peel (Fig. 1) and flesh (Fig. 2) of both cultivars treated with 45 kPa CO₂. The effects of CO₂ on fermentation product accumulations were large, whereas in air-treated fruit accumulations were negligible. Therefore, analysis was performed only on data obtained from the peel and flesh samples of CO₂-treated fruit of both cultivars (Table 3).

Accumulations of acetaldehyde were higher in 'Law Rome' than 'Cortland' in peel and flesh tissues (Figs. 1 and 2). DPA did not affect acetaldehyde in flesh tissue of either cultivar, or peel tissue of 'Cortland', but had variable effects in peel tissue of 'Law Rome'. In 'Law Rome', acetaldehyde increased earlier in DPA-treated fruit than in control fruit, but patterns of change were inconsistent. Ethanol accumulations increased over time, but were greater in the peel and flesh of 'Cortland' than in 'Law Rome'. The only significant interaction detected for ethanol was between cultivar, DPA, and time. Its accumulation in DPA-treated peel tissue of 'Cortland' was higher than that in the peel of control fruit on day 12 of treatment. Ethyl acetate in peel or flesh tissues was not affected by cultivar. DPA treatment did not affect ethyl acetate accumulation in flesh tissues of either cultivar or peel tissue of 'Cortland', but in 'Law Rome' peel, ethyl acetate

in DPA-treated fruit exposed to high CO₂ was initially greater than in the fruit not treated with DPA.

EFFECTS OF CO₂ AND DPA ON ORGANIC ACIDS. Succinate accumulated rapidly in peel and flesh of both cultivars treated with CO₂, while levels in air-stored fruit remained low (Fig. 3, Table 3). Succinate concentrations were 25% higher in 'Cortland' than 'Law Rome', and 57% higher in the peel than in flesh tissues. Succinate accumulation in 'Cortland' tended to plateau or decline after 9 d, but in 'Law Rome' continued over the 12 d period. DPA did not affect accumulation of succinate; except for relatively small differences in 'Law Rome' peel tissue at 9 and 12 d, and flesh tissue at 3 and 9 d.

Citramalate also was affected by CO₂, with lower concentrations in peel of CO₂-treated than air-treated 'Law Rome' fruit (Fig. 4). No differences were detected in 'Law Rome' flesh, or either tissue type in 'Cortland'.

Overall, malate concentrations averaged 35.2 and 32.2 $\mu\text{mol}\cdot\text{g}^{-1}$ in 'Cortland' peel and flesh respectively, compared with 28.7 and 30.5 $\mu\text{mol}\cdot\text{g}^{-1}$ in 'Law Rome' peel and flesh, but cultivar differences were detected only for peel tissue ($P < 0.001$). Malate

Fig. 1. Acetaldehyde, ethanol, and ethyl acetate concentrations in peel tissues of 'Cortland' and 'Law Rome' apples, either nontreated (open symbols) or pretreated with DPA (closed symbols), and exposed to air (circles) or 45 kPa CO₂ in air (triangles) for up to 12 d at 2 °C. Vertical bars represent pooled LSD ($P = 0.05$) for the highest significant interaction using a four-way analysis of variance. The ANOVA table for data is shown in Table 3.

Fig. 2. Acetaldehyde, ethanol, and ethyl acetate concentrations in flesh tissues of 'Cortland' and 'Law Rome' apples, either nontreated (open symbols) or pretreated with DPA (closed symbols), and exposed to air (circles) or 45 kPa CO₂ in air (triangles) for up to 12 d at 2 °C. Vertical bars represent pooled LSD ($P = 0.05$) for the highest significant interaction using a four-way analysis of variance. The ANOVA table for data is shown in Table 3.

Table 3. Analysis of variance for acetaldehyde, ethanol, and ethyl acetate in CO₂-treated 'Cortland' and 'Law Rome' peel and flesh tissue either nontreated or treated with DPA for 3, 6, 9, or 12 d of storage at 2 °C. Fermentation product data were transformed to log (0.01 + value) before analysis.

	df	Peel				Flesh			
		Acetaldehyde	Ethanol	Ethyl acetate	Succinate	Acetaldehyde	Ethanol	Ethyl acetate	Succinate
Cultivar (C)	1	***	***	NS	***	***	***	NS	***
DPA	1	***	NS	**	NS	NS	NS	NS	NS
Time (T)	3	***	***	***	***	***	***	***	***
C × DPA	1	***	NS	*	*	NS	NS	NS	NS
C × T	3	***	NS	NS	*	***	*	***	***
DPA × T	3	***	NS	***	NS	NS	NS	NS	NS
C × DPA × T	3	***	*	*	NS	NS	NS	NS	**

NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01 or 0.001, respectively.

concentrations in the flesh were not affected by any factor (data not presented). In peel tissues, malate increased in air-treated fruit, but decreased in CO₂-treated fruit ($P < 0.05$). DPA treatment did not affect malate concentrations.

In general, concentrations of other organic acids (citrate/isocitrate, aconitate, and quinate) were not affected by atmosphere or DPA treatment (data not presented). Some quantitative differences were detected: the highest quinate concentration (2.5 $\mu\text{mol}\cdot\text{g}^{-1}$) was detected in 'Law Rome' peel followed by 'Law Rome' flesh (1.6 $\mu\text{mol}\cdot\text{g}^{-1}$), and both 'Cortland' tissue types (1.1 $\mu\text{mol}\cdot\text{g}^{-1}$).

Discussion

Efficacy of CO₂ exposure in alleviating scald was cultivar and storage-time dependent (Tables 1 and 2). Scald development was delayed, but not prevented by CO₂ treatment, which is similar to the effects of other nonchemical methods. For example, heat treatments controlled scald for 3, but not 6, months of storage (Lurie et al., 1991). In contrast to DPA, which appears to control scald by preventing oxidation of α -farnesene (Huelin and

Fig. 3. Succinate concentrations in the peel and flesh of 'Cortland' and 'Law Rome' apples, either nontreated (open symbols) or pretreated with DPA (closed symbols), and stored in air (circles) or 45 kPa CO₂ in air (triangles) at 2 °C for up to 12 d. Vertical bars represent pooled LSD ($P = 0.05$) for the highest significant interaction using a four-way analysis of variance. The ANOVA table for data is shown in Table 3.

Fig. 4. Citramalic acid concentrations in the peel and flesh of 'Cortland' and 'Law Rome' apples, either nontreated (open symbols) or pretreated with DPA (closed symbols), and stored in air (circles) or 45 kPa CO₂ in air (triangles) at 2 °C for up to 12 d. Vertical bars represent pooled LSD ($P = 0.05$) for the highest significant interaction using a four-way analysis of variance.

Coggiola, 1970), the mechanism of stress treatments such as low O₂, high CO₂, and heat is more likely associated with delaying of ripening processes. Inhibition of scald development also may be related to endogenous ethanol produced under these conditions as suggested by Wang and Dilley (2000) for O₂ stress treatments. In separate experiments, however, we were not able to satisfactorily control scald by storing high CO₂-treated fruit under normal CA conditions (unpublished data).

The limited effects of CO₂ in controlling scald in the current study contrast with total control of scald observed by Pieniazek (1945), and Pieniazek and Christopher (1945), but are comparable with their subsequent work (Pieniazek et al., 1946). Under New York growing conditions, both 'Cortland' and 'Law Rome' are scald-susceptible cultivars. Nevertheless, scald severity observed in susceptible cultivars can vary greatly in different years and growing regions (Bramlage and Watkins, 1994; Emonger et al., 1994; Meigh, 1970). Cultivars also can vary in response to different treatments applied for control of the disorder (e.g., Wang and Dilley, 2000; Watkins et al., 2000; Wilkinson and Fidler, 1973). According to the α -farnesene hypothesis for the mechanism of scald development, these differences could be attributable to physical and metabolic factors such as skin permeability, substrate (α -farnesene) production, and antioxidant defense systems. These factors may account for variations in inherent susceptibility of fruit to the disorder relating to season, harvest date, and treatment conditions and lead to differences in efficacy of CO₂ treatment for scald control. Carbon dioxide treatment cannot be recommended as a reliable means of controlling scald, especially when compared with DPA. Moreover, only a narrow CO₂ exposure time resulted in reduced scald without causing external or internal CO₂ injuries.

'Cortland' and 'Law Rome' apples differed greatly in susceptibility to external and internal CO₂ injury (Tables 1 and 2); 'Cortland' was more susceptible to external CO₂ injury than 'Law Rome', whereas 'Law Rome' was more susceptible to internal CO₂ injury than 'Cortland'. Cultivar variations to CO₂ injuries are well known (Wilkinson and Fidler, 1973), though the reasons for these differences are not fully understood.

To study the metabolism of fruit with different incidences of external and internal CO₂ injury we also applied DPA, which has been shown to inhibit development of these injuries (Burmeister and Dilley, 1995; Johnson et al., 1998; Watkins et al., 1997). DPA treatment markedly reduced incidence of both external and internal CO₂ injury of 'Cortland' and 'Law Rome' apples (Tables 1 and 2). We focused on treatment effects on fermentation product and organic acid concentrations in the skin and flesh of each cultivar.

No relationships between accumulation of acetaldehyde, ethanol or ethyl acetate (Figs. 1 and 2) and the incidences of either external or internal CO₂ injury (Table 2) were detected. 'Cortland' and 'Law Rome' had the highest incidences of external and internal injury, respectively, but 'Cortland' consistently had lower acetaldehyde, and higher ethanol and ethyl acetate, accumulations in the peel and flesh tissues than 'Law Rome'. These concentrations were generally unaffected by DPA, and treatment differences when detected, were inconsistent.

Accumulation of acetaldehyde and ethanol in fruit and vegetables treated with high CO₂ is a well-described phenomenon (Kader, 1986), usually associated with nonmarketability or death of the product. In apples, such accumulations have been associated with development of a number of physiological disorders including CO₂ injury (Smagula and Bramlage, 1977; Thomas,

1925; Volz et al., 1998). Smagula and Bramlage (1977), however, suggested that acetaldehyde accumulation was a result, rather than a cause, of tissue disorganization. More recently, Volz et al. (1998) found that while flesh browning in 'Fuji' apples was associated with acetaldehyde and ethanol accumulations, relationships between damage and these products were not simply causal. Our results also cast doubt on a role for acetaldehyde or ethanol in directly causing internal or external CO₂ injury when different cultivars, tissue types, and DPA treatment effects are compared.

Succinate accumulated in CO₂-treated tissues, to a greater extent in peel than in skin, irrespective of cultivar and DPA treatment (Fig. 3). Accumulation of succinate is also a commonly observed response to CO₂ treatment in many plant tissues (Fernández-Trujillo et al., 1999; Williams and Patterson, 1973; Yang et al., 1998), and is usually thought to result from CO₂-induced inhibition of succinate dehydrogenase (SDH) (Frenkel and Patterson, 1964; Ke et al., 1993; Shipway and Bramlage, 1973). Hulme (1956) suggested that accumulation of succinate (1.8 mmol·g⁻¹ in his single sample from injured fruit) was responsible for cell death in CO₂-damaged apples, and this concept has not been seriously questioned.

Our results do not support the aforementioned view as cultivar and treatment differences in external or internal CO₂ injury were not reflected in differences in succinate accumulation. Recent studies with strawberries (*Fragaria × ananassa* Ducesne) also have shown that fruit with low levels of fermentation product accumulation had higher levels of succinic acid accumulation than fruit with high fermentation product accumulation (Fernández-Trujillo et al., 1999). Ke et al. (1993) did not find a relationship between CO₂ injury and succinate concentrations in lettuce (*Lactuca sativa* L.) rib and leaf tissues. Succinate accumulation could be a symptom of resistance to stress rather than the cause of injuries, and may involve the action of several other pathways in addition or response to inhibition of SDH activity (Fernández-Trujillo et al., 1999).

Of the other acids, no treatment differences were detected, with the interesting exception of citramalate, which declined in peel of 'Law Rome' apples in response to CO₂ treatment. Citramalic acid is a methyl-malic acid associated with development of anthocyanin in apple skin at concentrations of 0.26 to 1.04 $\mu\text{mol}\cdot\text{g}^{-1}$ (Noro et al., 1988). A similar range of concentrations was found in our experiment (Fig. 4). High levels of citramalate have been reported in mature apples of pigmented red cultivars (Noro et al., 1988). A decrease in this compound in 'Law Rome' peel treated with CO₂ could be associated with decreased anthocyanin synthesis as reported in other CO₂-treated fruit (Holcroft and Kader, 1999), but there was no evidence of a direct relation with CO₂ injury.

In conclusion, we examined effects of high CO₂ partial pressures on inhibition of scald in 'Cortland' and 'Law Rome' apples and have shown that CO₂ effects are cultivar and time dependent. In addition, the two cultivars differed greatly in susceptibility to external and internal CO₂ injury, and the incidences of injury can be reduced by DPA treatment before exposure to CO₂ treatment. Accumulations of fermentation products, and of succinate, thought to be responsible for CO₂ injury were not affected in a causal manner. Accumulations of these compounds were independent of injury incidence in different tissue types or in response to DPA treatment. Development of CO₂ injury is likely to be a complex process involving more than poisoning of tissues by any single compound. Death of cells may be the result of progressive failure

to maintain energy balance and metabolic cell function in response to cytoplasmic acidification and induction of several toxic products under elevated CO₂ conditions.

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