

Effect of Superficial Scald Suppression by Diphenylamine Application on Volatile Evolution by Stored Cortland Apple Fruit

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Cortland apple fruit (*Malus × domestica* Borkh.) stored for 120–140 days in air at 0 °C were warmed to 22 °C and held for 8 days. A portion of the fruit was dipped in a solution of diphenylamine (DPA) at harvest to prevent scald development. Scald occurred only in those fruit not treated with DPA, and its development was accelerated after transfer to 22 °C. Ester production from apple fruit tended to increase from day 0 to day 6 of poststorage holding and declined thereafter in both treatments. However, ester production in scald-developing fruit was reduced by ~50%. The reduction in volatile production remained relatively constant during the rapid development of scald symptoms. Furthermore, the reduction in volatile production appeared to be independent of respiration and ethylene production. Production of esters derived from hexanol was most reduced in fruit developing scald, with hexyl 2-methylbutanoate production being reduced ~15-fold. Interestingly, the production of methyl butanoate was detected only in scalding fruit. α -Farnesene production in fruit developing scald was reduced 43% compared with DPA-treated fruit. In contrast, the primary volatile oxidation product of α -farnesene, 6-methyl-5-hepten-2-one (MHO), was present only in fruit developing scald. The data suggest that inhibition of ester production may occur as a result of the physiological changes associated with susceptibility to, rather than expression of, scald symptoms.

Keywords: *Malus × domestica*; superficial scald; aroma; esters; α -farnesene; DPA

INTRODUCTION

Cortland apple fruit is susceptible to superficial scald during low-temperature storage. The development of superficial scald may be manifested as peel browning, with some pitting if the damage is severe (Emongor et al., 1994). Scald symptom development is accelerated when the stored fruits are warmed to room temperature (~22 °C) 3–7 days following low-temperature storage (Ingle and D'Souza, 1989). However, for commercial storage, fruits are routinely treated with the antioxidant diphenylamine (DPA) to reduce the occurrence of storage scald in apple (Smock, 1955).

Apple fruit volatiles are complex mixtures of organic compounds, the synthesis of which occurs primarily during ripening. The majority of apple aroma compounds are volatile esters; however, apples also produce a relatively large amount of α -farnesene, a sesquiterpene hydrocarbon (Fallik et al., 1997). α -Farnesene has been linked to the formation of superficial scald via its oxidation products. Ester and α -farnesene production peak with the respiratory climacteric following the ethylene burst in apple. However, the mechanistic relationship among volatile production, respiration, and ethylene production is poorly understood.

Apples are often held for several months at low temperature either in air (RA) or in controlled atmosphere (CA) storage. During CA storage, the production of order-active volatiles decreases, and the capacity for their production after storage declines (Willaert et al., 1983). Reduced emission of aroma volatiles has been

reported as the factor most likely responsible for diminished flavor (Smith, 1984). Although the pathways that lead to volatile biosynthesis are still a matter of controversy, it is generally believed that the precursors to or degradation products of fatty acids serve as a major pool of substrates for volatile biosynthesis. It is known that alcohol acyl CoA transferase (AAT) has a capacity to combine primary alcohols with CoA thioesters of short-chain fatty acids to form corresponding esters (Fellman et al., 1993). AAT uses acyl CoA's in an O_2 -dependent manner (Harada et al., 1985) but may have specific isozymes that play an important role in the production of a type of flavor ester being produced (Perez et al., 1993).

Because fatty acid biosynthesis (Ohlrogge and Jaworski, 1997) and formation of mono- and diterpene precursors of sesquiterpene biosynthesis (Steele et al., 1998) take place in chloroplasts, and superficial scald affects the cells of the fruit that contain chloroplasts, we suspected the changes in volatile emissions may accompany development of superficial scald in apple. Our objective was to examine the changes in the emission of volatiles of stored fruit during scald development at 22 °C. The data are compared with fruit rendered resistant to scald development by treating the fruit with DPA at the time of harvest. We also studied the relationship among volatile production, fruit respiration, and ethylene production during poststorage holding at room temperature.

MATERIALS AND METHODS

Fruit Material. Cortland apple fruits were harvested on September 25, 1996, from the Clarksville Horticulture Experiment Station, Clarksville, MI, at a mature but preclimacteric

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stage of development as judged by internal ethylene levels being $<0.2 \mu\text{L L}^{-1}$. The fruits, ~ 400 in number, were immediately brought to the laboratory and kept overnight at 22°C to equilibrate with ambient temperature.

Half of the fruit (200) was dipped into a DPA solution (5.9 mmol L^{-1}) for 30 s. The DPA-treated fruit was air-dried for $\sim 6 \text{ h}$ at 22°C . DPA-treated fruit and nontreated (control) fruit were held separately in plastic buckets in RA storage at 0°C and at a relative humidity of $>95\%$ for up to 140 days.

After 75 days of storage, seven fruit from nontreated controls were removed from storage at intervals of 7–15 days and held for 7 days at 22°C for assessment of superficial scald. When 100% of the untreated fruit population (with no visible signs of superficial scald at 0°C) developed scald symptoms during room temperature warming (120 days of storage), fruit was used for analyses of volatiles, respiration, and ethylene production.

Two fruit (149–165 g) from each treatment with no visual symptoms of superficial scald were removed from storage at 120 days and held at 22°C for 8 days in 0.5-L glass containers ventilated with humidified air. Each container held one fruit. The flow rate was 10 mL min^{-1} , which ensured aerobic conditions as well as detectable levels of apple volatiles in the exit gas stream. Inlet and exit line tubing was 3 mm i.d. flexible Teflon, which is largely impermeable to all vapors. Volatiles were sampled via glass tees fitted with a Teflon-lined septum at the inlet and outlet of the container. The experiment was repeated at 140 days with the same protocol as above. Hence, the data presented are an average of two independent experiments.

Respiration and Ethylene Production. Using an insulin-type plastic syringe, 0.5 mL (for CO_2) and 1 mL (for C_2H_4) of gas sample were withdrawn daily from the exit gas stream. CO_2 measurements were made with an infrared gas analyzer (model 225-MK3; Analytical Development Co., Hoddesdon, England). Ethylene concentrations were determined using a gas chromatograph (Carle Series 100 AGC; Hach Co., Loveland, CO) equipped with a flame ionization detector. Gas concentrations were calculated relative to a certified gas mixture ($4.85\% \text{ CO}_2$, $1.95\% \text{ O}_2$, and $0.979 \mu\text{L L}^{-1} \text{ C}_2\text{H}_2$ balanced with N_2) and converted to partial pressures by assuming an atmospheric pressure of $1 \times 10^5 \text{ Pa}$.

Volatile Collection, Separation, and Detection. Volatiles were sampled by means of solid-phase microextraction (SPME) using 1 cm long fibers coated with a $100 \mu\text{m}$ thick layer of poly(dimethylsiloxane) (Supelco Co., Bellefonte, PA). Before use, the fibers were conditioned at 250°C for 60 min. The absorption time was 5 min. Separation and detection were performed by gas chromatography (HP-6980, Hewlett-Packard Co.) and time-of-flight mass spectrometry (Pegasus-II, LECO Corp., St. Joseph, MI), respectively, according to the method of Song et al. (1997) except that the GC temperature program was isothermal for 1 min at 40°C , then increased at the rate of $40^\circ\text{C min}^{-1}$ to 250°C , and held for 2 min. Identification of compounds was confirmed by comparison of collected mass spectra with those of authenticated reference standards and spectra in the National Institute for Standards and Technology (NIST) mass spectra library, search version 1.5. Volatiles were measured at 0, 1, 2, 3, 4, 6, and 8 days after transfer of fruit from RA to 22°C . The data were corrected for flow rates and fruit mass for treatment comparisons.

Scald Index. Twenty-nine fruit from untreated control and 50 from DPA-treated fruit were held at 22°C for 7 days. Scald development was recorded daily as scald severity based on percent surface area affected of the total fruit population.

Statistical Analyses. For measurements of volatiles as a function of DPA treatment and poststorage holding duration, a two-factor (treatment and holding duration) completely randomized design was used. For treatment comparisons only, the least significant range test was performed. The data were analyzed by the analysis of variance (ANOVA) procedure using a graphics and statistics software package (PlotIT, Scientific Programming Enterprises, Haslett, MI). Unless otherwise stated, only data significant at $p < 0.05$ are discussed.

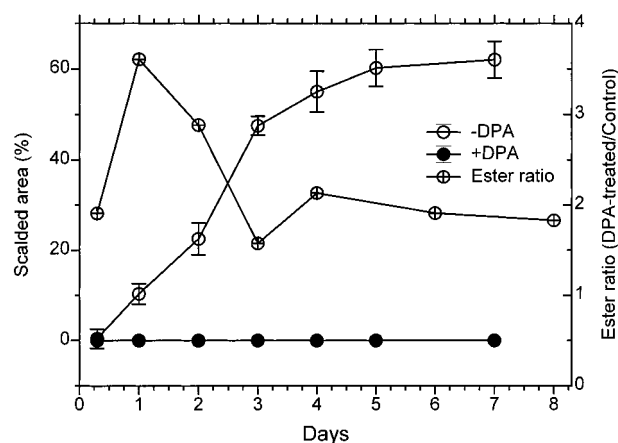


Figure 1. Effect of poststorage warming to 22°C and DPA on the development of superficial scald and ester formation in Cortland apple fruit stored for 120–140 days in air at 0°C . The data shown for scald severity are an average of 29 fruit for controls and 50 fruit for the DPA treatment. The data for the ester ratio (ester production of DPA-treated fruit divided by ester production of non-DPA-treated fruit) are an average of four fruit per treatment at each sampling point.

RESULTS

Scald Development during Storage and Poststorage Holding. Application of DPA at the time of harvest completely prevented the development of superficial scald during both RA storage and subsequent holding at 22°C (Figure 1). In contrast, untreated fruits had 0.4% of the total surface area affected with superficial scald at the time of withdrawal from storage (Figure 1). Poststorage holding at 22°C increased both the intensity and the severity of scald development in untreated fruits (Figure 1). Whereas all fruit showed signs of scald development on day 2 of holding at 22°C (data not shown), the maximal surface area affected by scald (60%) was reached on day 5 of holding (Figure 1).

Effect of DPA on the Production of Selected Volatiles by Stored Cortland Fruit. The production rate of ethyl hexanoate increased during holding, peaking on day 6 in both treatments (Figure 2A). Although the patterns of ethyl hexanoate production were similar in both treatments, control fruits produced higher amounts compared to DPA-treated fruit. However, the differences were statistically nonsignificant.

The production rate of butyl hexanoate increased during holding, peaking on day 1 in DPA-treated and on day 6 in non-DPA-treated fruit (Figure 2B). Although the content of butyl hexanoate was ~ 2.5 -fold higher in DPA-treated fruit compared to that in nontreated fruit during the initial 2 days of holding, the butyl hexanoate contents were similar from day 6 through day 8 in both treatments (Figure 2B).

The production of hexyl hexanoate increased from day 0 to day 6 of holding and declined thereafter in DPA-treated fruit (Figure 2C). Although the patterns of the production rate of hexyl hexanoate in treated and control fruit were similar, the rate of production was 3 times higher for the nonscalding, DPA-treated fruit (Figure 2C).

Whereas the nontreated fruits produced 43% less α -farnesene in comparison to DPA-treated fruits during holding, patterns of α -farnesene production were similar in both treatments (Figure 3A). The primary oxidation product of α -farnesene, MHO, was detected only in scalding fruit (Figure 3B). The MHO production was

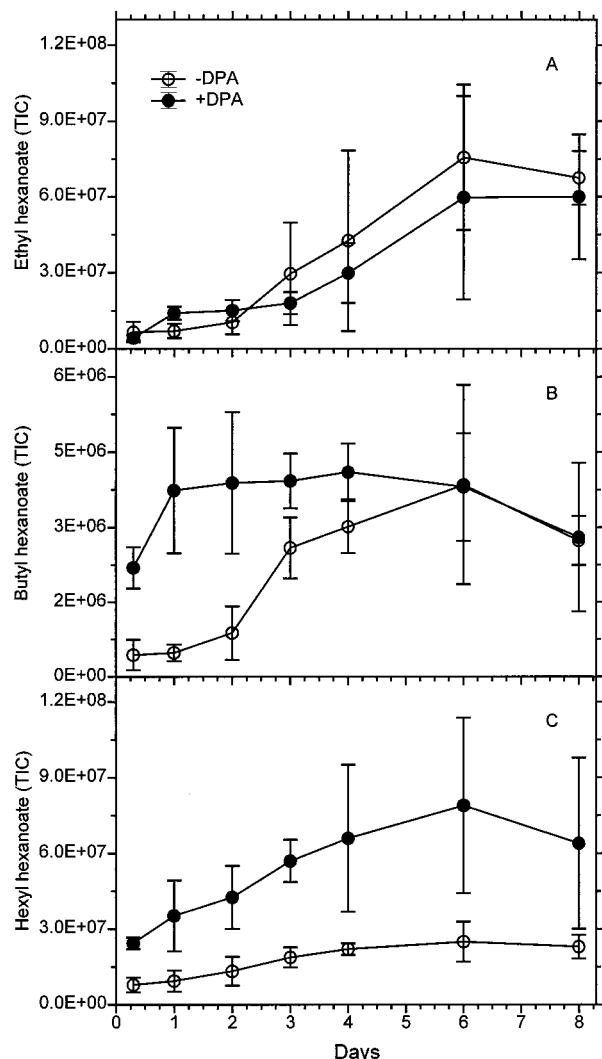


Figure 2. Mass spectral total ion count (TIC) response to vapor phase volatile esters emanating from Cortland fruit held at 22 °C following storage for 120–140 days in air at 0 °C: (A) ethyl hexanoate; (B) butyl butanoate; (C) hexyl hexanoate. The data at each sampling point are an average of four fruit.

maximal on day 2 of the 8-day poststorage holding period and declined thereafter, reaching approximately one-third of maximal production on day 4. The MHO evolution declined gradually from day 4 through day 8.

Effect of Superficial Scald on Volatile Composition. Data for volatile production were pooled over time for comparing DPA-treated and non-DPA-treated fruit (Table 1). α -Farnesene evolution in DPA-treated fruit (i.e., those that did not develop scald) accounted for 72.2% of total chromatographic peak area of the 21 volatile compounds measured (Table 1). The maximal area for volatile esters was 27.4%. The predominant branched-chain ester was hexyl 2-methylbutanoate. The predominant straight-chain esters were, in decreasing order of abundance, butyl hexanoate, hexyl hexanoate, ethyl hexanoate, and ethyl butanoate. Only one precursor alcohol of ester formation, hexanol, was detected.

In non-DPA-treated fruit (i.e., those that did develop scald), there was a reduction in total volatile production by 45%. The maximal reduction occurred in volatile esters (51%) followed by α -farnesene (43%). For those fruit developing scald, the reduction was most severe for esters with hexyl moiety [e.g., hexyl 2-methylbutanoate (94%), hexyl propionate (81%), hexyl 2-methyl-

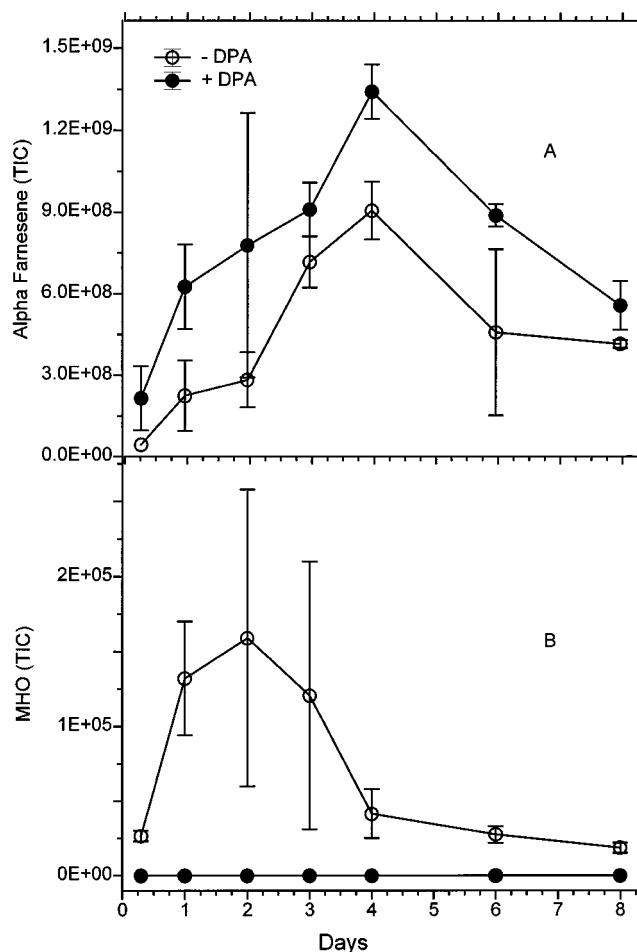


Figure 3. Mass spectral TIC response to vapor phase volatile sesquiterpene, α -farnesene, and its oxidation product, MHO, emanating from Cortland fruit held at 22 °C following storage for 120–140 days in air at 0 °C: (A) α -farnesene; (B) MHO. The data at each sampling point are an average of four fruit.

2-propenoate (79%), hexyl acetate (77%), hexyl hexanoate (68%), and hexyl butanoate (50%]. In contrast, the evolution of two abundant esters with an ethyl moiety, ethyl butanoate and ethyl hexanoate, were higher by 20 and 33%, respectively, in scalding fruit as compared with nonscalding fruit. However, these differences in esters with an ethyl moiety were statistically nonsignificant between the two treatments. Developing scald was also associated with a reduction in hexanol. MHO, an oxidation product of α -farnesene, and methyl butanoate, were produced only by fruit developing scald.

Ethylene Production. Ethylene production rates were similar in scalding and nonscalding fruit (Figure 4A). The ethylene evolution increased sharply from 0.6 $\text{nmol kg}^{-1} \text{s}^{-1}$ on day 0 to 1.3 $\text{nmol kg}^{-1} \text{s}^{-1}$ on day 1 and increased gradually thereafter, reaching 1.8 $\text{nmol kg}^{-1} \text{s}^{-1}$ on day 9 of holding.

Respiration Rate. The rates of CO_2 production were similar in scalding and nonscalding fruit (Figure 4B). The CO_2 evolution increased from 75 $\text{nmol kg}^{-1} \text{s}^{-1}$ on day 0 to 190 $\text{nmol kg}^{-1} \text{s}^{-1}$ on day 1. The rate of CO_2 evolution was stable from day 1 through day 5 and declined thereafter in both treatments (Figure 4B).

DISCUSSION

There are >300 volatile compounds that emanate from apple fruit during ripening or are produced as a

Table 1. Effect of Developing Scald on Production of Aroma Compounds (Percent Peak Area of Total Compounds in Nonscalded Fruit) in Stored Cortland Apple during Holding at 22 °C^a

volatile compound	+DPA	-DPA	relative change (%)	
methyl butanoate	0.00	0.06	+	* ^b
ethyl butanoate	1.93	2.31	+20	ns ^b
ethyl 2-methylbutanoate	0.47	0.31	-34	ns
1-hexanol	0.33	0.19	-42	*
propyl butanoate	0.11	0.04	-63	*
methyl hexanoate	0.10	0.08	-20	ns
2-methylpropyl butanoate	0.06	0.02	-66	*
6-methyl-5-hepten-2-one	0.00	0.02	+	*
ethyl hexanoate	2.73	3.63	+33	ns
butyl butanoate	0.71	0.32	-45	*
hexyl acetate	0.31	0.07	-77	*
butyl 2-methylbutanoate	0.19	0.14	-26	ns
pentyl butanoate	0.16	0.19	+19	ns
pentyl 2-methylbutanoate	0.15	0.07	-53	*
hexyl propionate	0.42	0.08	-81	*
hexyl 2-methyl-2-propenoate	0.24	0.05	-79	*
hexyl butanoate	0.36	0.18	-50	*
butyl hexanoate	8.80	3.86	-56	*
hexyl 2-methylbutanoate	5.70	0.36	-94	*
hexyl hexanoate	5.00	1.62	-68	*
α -farnesene	72.22	41.39	-43	*
methyl esters	0.16	0.15	-6	
ethyl esters	5.13	6.25	+22	
propyl esters	0.11	0.04	-64	
butyl esters	9.70	4.32	-55	
pentyl esters	0.31	0.26	-16	
hexyl esters	12.04	2.35	-80	
total esters	27.44	13.37	-51	
total volatiles	100	54.97	-45	

^a The data of all the collection times were pooled. The values shown are the average of four fruit. ^b ns, * = nonsignificant or significant at $p < 0.05$, respectively.

result of processing (Dimick and Hoskin, 1983). However, only a few of these compounds have a decisive impact on the sensory quality and are, therefore, designated "impact compounds" (Cunningham et al., 1986). In general, impact compounds are characteristic of each cultivar, and variation in the type and amount of these impact compounds exists within a cultivar (Berger and Drawert, 1984). The apple-like or fruity smell in various cultivars of apple is largely due to a class of volatile compounds known as esters (Flath et al., 1967). Apple cultivars may be either "acetate-ester producing" such as Golden Delicious, Red Delicious, and Fuji (Dixon and Hewett, 1997; Fellman et al., 1993) or "non-acetate-ester" producing such as Cortland and McIntosh (Yahia et al., 1990). Although esters in apple are important for flavor perception, it was recently shown that α -farnesene accounted for 70% of the total volatile compounds in Golden Delicious apple fruit (Fallik et al., 1997). The qualitative composition of aroma volatiles for Cortland in this study is similar to that found by other investigators (Yahia et al., 1990).

There is some indication that an advanced stage of senescence is needed for scald symptom development (Ingle and D'Sousa, 1989; Lurie et al., 1989). Although the mechanism of scald development has not been elucidated, the impairment of active oxygen species (AOS) metabolism during senescence may be related to the disorder (Anet, 1969; Rao et al., 1998). The metabolism of AOS differed markedly between fruit of scald-resistant and scald-susceptible lines (Rao et al., 1998), and DPA application at harvest reduced the activities of AOS-degrading enzymes in stored fruit of Granny Smith apple (Lurie et al., 1989). Furthermore, it was

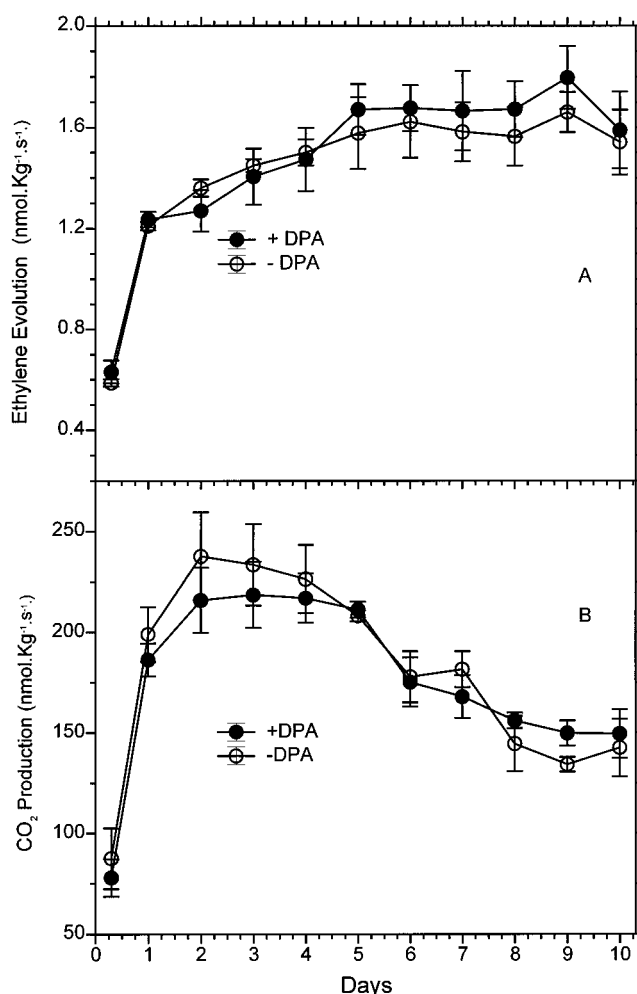


Figure 4. Ethylene evolution (A) and respiration (B) at 22 °C of Cortland fruit stored for 120–140 days in air at 0 °C. The data at each sampling point are an average of four fruit.

suggested that DPA appears to have some beneficial physiological effects on delaying senescence in apple fruit (Lurie et al., 1989). Thus, the reported higher volatile production in DPA-treated fruit compared with nontreated control in this study may have been due to reduced rates of senescence-related processes in these fruit.

The reduction of ester production in scalding fruit as compared with nonscalding fruit may indicate that some key enzymes in the synthesis pathways are affected by DPA or developing scald in apple fruit. These changes in volatile production at a time when superficial scald development occurs seem to be independent of respiration or ethylene-associated changes in ripening. Interestingly, the reduction in ester production was evident prior to full symptom development and was maintained at a constant level relative to DPA-treated fruit during holding (Figure 1). This is consistent with the earlier studies of Huelin (1964), who reported ester formation in scald-susceptible Granny Smith fruit during scald symptom development at 22 °C was half that of fruit treated with DPA. The possibility that esters or their metabolic products may be causally related with the development of superficial scald in apple has been ruled out (Huelin, 1964), but it is conceivable that scald development could alter fatty acid metabolism and subsequently ester biosynthesis. In that esters contribute to a great extent to the perception of fruit flavor in apple (Fellman et al., 1993), scald-susceptible cultivars

may have a lower consumer preference much before the actual symptoms of scald on the surface of the fruit develop.

The reduction in free hexanol production by control fruit may indicate that fruit developing scald have a reduced capacity to synthesize alcohol precursors for ester formation. If reduced alcohol synthesis results in reduced ester synthesis, the control point of ester production in Cortland may be at the level of alcohol availability. If the control of ester formation was due to thioester availability, one would expect that the reduction in hexyl esters in the control fruit would be associated with an increase in free hexanol. However, the reverse was found. In addition, the data on ester production in this study support the preference for esterification of long carbon chain alcohols in Cortland (Berger and Drawert, 1984).

Volatile compounds produced by apple fruit during low-temperature storage were first suggested to be associated with the development of superficial scald by Brooks et al. (1919). During low-temperature storage, lipophilic volatile compounds such as α -farnesene (Huelin and Coggiola, 1970) and its oxidation products (conjugated trienes and MHO) are continuously produced. These compounds partition into the fruit cuticle and potentially accumulate to high levels (Huelin and Coggiola, 1970; Mir et al., 1998). The linear increase in α -farnesene evolution during the initial few days of holding may have been due to enhanced release of this compound from the fruit cuticle due to warming or an increase in its production or both. However, the 43% reduction of α -farnesene evolution in scalding fruit in comparison to that in nonscalding fruit may be a result of α -farnesene loss due to oxidation (Mir et al., 1998). The fact that MHO was only detected in scalding fruit suggests α -farnesene oxidation did occur and that DPA prevented its oxidation. There is some possibility that MHO may be causally related to scald development in that induction of scald-like skin browning by exogenous application of MHO vapor has been found in nine cultivars of apple, and the sensitivity of the cultivar to MHO was shown to be highest in scald-susceptible as compared with scald-resistant cultivars (Song and Beaudry, unpublished results).

This study demonstrates the improved maintenance of volatile flavor compounds in stored Cortland apple fruit in addition to scald suppression by application of DPA at the time of harvest.

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