A Modified Hypothesis on the Role of Conjugated Trienes in Superficial Scald Development on Stored Apples

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Abstract. Three experiments were conducted using 'Cortland' and 'Delicious' apples (*Malus domestica* Borkh.). Fruit varying widely in superficial scald susceptibility, because of either endogenous or experimentally induced conditions, were stored for various intervals at 0C and evaluated for scald development at 20C. Samples were extracted periodically in hexane, and ultraviolet absorption spectra of the extracts were used to evaluate α -farnesene and conjugated triene (CT) relationships to scald development. CT concentrations were calculated using each of the three CT absorption maxima (258 - 290 nm, 269 - 290 nm, and 281 - 290 nm) and expressed accordingly as CT258, CT269, and CT281. The poor association of CT281 concentrations with scald development led us to propose that metabolic products of CT281 species are more likely to be associated with scald development than the species themselves.

Superficial scald of apples is one of the most extensively studied postharvest disorders of fruit (Ingle and D'Souza, 1989; Meigh, 1970). Huelin and Murray (1966) and Murray et al. (1964) first identified α -farnesene as a scald-inducing factor in the natural coating of apples. Huelin and Coggiola (1968, 1970a) found that it was not α -farnesene but its peroxidation to conjugated trienes (CTs) that was related to scald development.

Since the report of Huelin and Coggiola (1968), most studies of the physiology of scald development have included absorbance measurements of hexane extracts of fruit surfaces. Anet (1969) separated two CT species from in vitro peroxidized α -farnesene, both having absorption maxima at $\approx$ 251, 260, 269, and 281 nm. Huelin and Coggiola (1970a) and Meir and Bramlage (1988) observed similar absorption maxima at 258, 269, and 281 nm using hexane extracts of natural apple surfaces. These maxima have been assumed to represent CTs present in apple surfaces. Due to high background absorbance at $\approx$ 260 nm, Anet (1972) proposed measuring spectra only at 281 nm to avoid masking CTs. Although some interference occurred at this wavelength, it could be corrected by subtracting absorbance at 290 nm from that at 281 nm. Since then, these measurements ($OD_{281-290\text{ nm}}$) have been used routinely to calculate CT concentrations. Correlations between $OD_{281-290\text{ nm}}$ and scald occurrences are the basis for the widely accepted hypothesis that α -farnesene is peroxidized to CTs, an action that leads to cellular disruption and scald symptom development. Exogenously applied antioxidants inhibit peroxidation and scald development (Anet and Coggiola, 1974; Huelin and Coggiola, 1970a, 1970b), and endogenous antioxidants are associated with scald resistance (Anet, 1974; Meir and Bramlage, 1988).

CT species never have been isolated and identified directly from hexane extracts of apple surfaces, and their association with scald is based entirely on correlative data. Some inconsistencies among correlation coefficients between $OD_{281-290\text{ nm}}$, $OD_{268-290\text{ nm}}$, and $OD_{258-290\text{ nm}}$ scald occurrences have been noted (Meir and Bramlage, 1988). We therefore undertook to 1) examine the

characteristics of ultraviolet (UV) absorbance of hexane extracts of apple surfaces with different scald potentials and 2) investigate CT changes, as indicated by absorption spectra, during the development of scald symptoms. Results of this investigation led us to reconsider the significance of $OD_{281-290\text{ nm}}$ measurements in relation to the roles of CTs in scald development of apples.

Materials and methods

Apples were grown at the Univ. of Massachusetts Horticultural Research Center, Belchertown, Mass. In 1990, three experiments were conducted using fruit from mature trees grown under standard horticultural conditions.

Diphenylamine (DPA) treatment (Expt. 1). 'Cortland' apples (internal ethylene, 3 ± 2 ppm) were harvested on 26 Sept. 1990. 'Delicious' apples (internal ethylene, 52 ± 4 ppm) were harvested on 10 Oct. 1990. There were six single-tree replications of samples for each cultivar. Within 3 days of harvest, each replicate sample was separated into two lots of 80 fruit each. One lot was dipped in a solution of DPA at $2\text{ g}\cdot\text{liter}^{-1}$ plus 227 g of 50% methyl 1-(butyl carbamoyl)-2-benzimidazocarbamate (benlate) and 454 grams of 3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thiol]-1 *H*-isindole-1,3(2 *H*)-dione (captan) per 400 liters (to control decay) and the other lot was dipped in a solution of fungicide without DPA. Fruit were dipped for 20 sec, drained, and stored at 0C in air. After 4, 8, 12, and 16 weeks, 10 fruit from each sample were removed from storage and extracted in hexane. After 16 weeks of storage, the remaining fruit were kept at 20C for 7 days and evaluated for scald, which was recorded as percent incidence. However, almost no scald developed on 'Delicious', so the fruit were returned to 0C for an additional 8 weeks plus 1 week at 20C for a second evaluation of scald.

Harvest time (Expt. 2). 'Cortland' apples were harvested on 26 Sept. 1990 (same maturity as in Expt. 1) and on 10 Oct. 1990 (internal ethylene, 57 ± 14 ppm) from three two-tree replications, using the same trees as in Expt. 1. Fruit (70 fruit per replication) were stored at 0C for 30 weeks; an additional 70 fruit per replication from the 10 Oct. harvest were stored for 45 weeks. After storage, scald was evaluated and 10 fruit per replication were extracted in hexane after 0, 2, 4, 7, and 10 days at 20C. Scald development was recorded as percentage incidence and intensity of development, using a system of 1 = 1% to 10%, 2 = 11% to 33%,

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3 = 34% to 66%, and 4 = 67% to 100% of surface area affected.

Storage temperature (Expt. 3). 'Cortland' apples were harvested on 5 Oct. 1990, with 200 fruit collected from each of six replicate trees (internal ethylene, 31 ± 13 ppm). Each replication was divided into four sublots of 50 fruit each. Two sublots were stored at 0C for 12 or 24 weeks and two were kept at 20C for 1 or 2 weeks. At the end of each storage interval, 10 fruit were extracted in hexane and the rest were kept for 7 days at 20C and evaluated for scald.

Hexane extracts were obtained by dipping apples individually and in sequence in 100 ml of high-performance liquid chromatography-grade hexane for 3 min. UV absorbance of properly diluted hexane extracts was measured from 200 to 290 nm. Extracts displayed five absorption maxima at 200, 232, 258, 269, and 281 nm. Concentrations of α -farnesene in hexane extracts were calculated from OD₂₃₂ ($E_{232} = 27,700$) according to Huelin and Coggiola (1968). Concentrations of all CT species in hexane extracts were assumed to have an extinction coefficient of 25,000 (Anet, 1972) and were calculated from OD₂₈₁₋₂₉₀, OD₂₆₉₋₂₉₀, and OD₂₅₈₋₂₉₀. These are referred to as CT281, CT269, and CT258, respectively.

Data were subjected to analysis of variance or regression. Means were separated with least significant difference comparisons when applicable. SAS software (SAS Institute, Cary, N.C.) was used for these analyses.

Results

DPA treatment (Expt. 1). During storage at 0C, concentrations of α -farnesene, CT281, CT269, and CT258 changed significantly over time in 'Cortland' and 'Delicious' apples (Tables 1 and 2). α -

farnesene reached maximum concentrations by 8 weeks and then declined, as commonly observed (e.g., Meigh and Filmer, 1969), and CT increased to a plateau in 12 weeks. However, concentration varied between cultivars and patterns of change over time at 0C varied between cultivars and DPA treatments (Table 2). In 'Cortland' fruit not treated with DPA, CT281, CT269, and CT258 increased over time, but the CT269 : CT281 and CT258 : CT281 ratios decreased over time, varying between only 0.86 and 2.22 (Table 1). In nontreated 'Delicious' apples, CT269 and CT258 increased faster and to higher concentrations than CT281, producing ratios that varied from 2.42 to 8.61 (Tables 1 and 2).

Applying DPA before storage significantly reduced accumulation of α -farnesene, CT281, CT269, and CT258 in 'Cortland' apples (Tables 1 and 2) but concentrations at each wavelength were not reduced proportionally. DPA significantly increased the CT258 : CT281 ratio but did not alter the CT269 : CT281 ratio (Table 2). The effects of DPA on α -farnesene and at least one CT changed over storage time, while the effects on the two ratios did not (Table 2). For 'Delicious' fruit, however, DPA reduced only α -farnesene and CT281 concentrations late in storage (Tables 1 and 2), and this effect was not great enough to alter the CT ratios significantly (Table 2). DPA treatment reduced scald from 23% to 1% for 'Cortland' and from 20% to 5% for 'Delicious' fruit.

Generally, 'Cortland' fruit contain more α -farnesene and CT281 but less CT258 than 'Delicious' fruit, resulting in much lower CT258 : CT281 or CT269 : CT281 ratios in 'Cortland' than in 'Delicious' fruit (Table 2).

This experiment showed that individual CTs did not change equivalently during storage (Tables 1 and 2). In 'Cortland' apples, CT269 was significantly higher than CT281, while in 'Delicious'

Table 1. Changes in composition of hexane extracts from 'Cortland' and 'Delicious' apples during storage at 0C, with or without DPA treatment before storage. After 16 weeks of storage and 7 days at 20C, 23% and 1%, respectively, of the -DPA and +DPA 'Cortland' apples developed scald but no 'Delicious' apples developed scald. However, 20% and 5%, respectively, of the -DPA and +DPA 'Delicious' apples developed scald after 8 additional weeks at 0C.

Weeks at 0C	α -Farnesene (nmol·cm ⁻²)		Conjugated triene (nmol·cm ⁻²)						Ratio			
			CT281		CT269		CT258		CT269 : CT281		CT258 : CT281	
	-DPA	+DPA	-DPA	+DPA	-DPA	+DPA	-DPA	+DPA	-DPA	+DPA	-DPA	+DPA
<i>'Cortland'</i>												
0	37	37	2.9	2.9	4.9	4.9	5.5	5.5	1.72	1.72	1.99	1.99
4	107	82	2.2	0.4	4.3	0.6	4.4	0.8	2.04	1.71	2.22	2.25
8	121	73	6.7	0.5	9.8	1.2	7.4	1.0	1.47	3.42	1.12	2.25
12	90	63	10.3	2.1	14.1	3.9	9.6	3.6	1.39	1.84	0.96	1.64
16	95	67	11.8	2.7	15.0	4.2	9.9	3.8	1.28	1.54	0.86	1.42
Significance												
Linear	**	NS	***	NS	***	NS	***	NS	***	NS	***	NS
Quadratic	***	***	***	***	***	***	***	***	***	NS	***	NS
Cubic	***	***	***	***	***	***	***	***	***	NS	***	NS
<i>'Delicious'</i>												
0	3	3	0.2	0.2	1.2	1.2	1.5	1.5	6.80	6.80	8.61	8.61
4	74	60	0.8	1.0	4.5	3.6	5.9	3.7	5.39	3.66	3.83	3.68
8	86	68	3.5	3.1	9.4	8.3	9.8	8.0	2.69	2.64	2.83	2.54
12	68	59	4.6	3.7	11.3	11.2	11.3	12.2	2.49	3.01	2.50	3.26
16	43	35	5.0	3.1	12.4	11.2	12.1	12.1	2.49	3.64	2.42	3.92
Significance												
Linear	NS	*	***	***	***	***	***	***	***	**	***	**
Quadratic	***	***	***	***	***	***	***	***	***	***	***	***
Cubic	***	***	***	***	***	***	***	***	***	***	***	***

²Data are means of six replications.

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

apples, CT269 and CT258 were significantly higher than CT281 but similar to each other. When data for the two cultivars were combined, CT269 and CT258 were similar and both were higher than CT281. The differences in CT values, depending on which absorption maximum is used for measurement, are demonstrated in the varying CT258 : CT281 and CT269 : CT281 ratios during storage.

Harvest time (Expt. 2). As time at 0C increased from 16 to 30 weeks, α -farnesene, CT281, CT269, CT258, and the CT258 : CT281 ratio declined greatly in early-harvested 'Cortland' apples, but the CT269 : CT281 ratio remained unchanged (Table 3). However, despite marked reductions of all of the components in fruit peel during this period, the percentage of fruit that developed scald after storage increased from 23% to 60%.

When stored for 30 weeks at 0C, the late-harvested fruit contained higher concentrations of α -farnesene, CT281, CT269, and CT258 than early-harvested fruit (Table 3). Differences in CTs measured at the three absorption maxima were not proportional, however, because the CT258 : CT281 ratio was higher in late-harvested than in early-harvested fruit. Despite having higher concentrations of α -farnesene and CTs, late-harvested fruit developed much less scald than the early-harvested fruit (21% vs. 60%) (Table 3).

When the late-harvested fruit were kept for an additional 15 weeks at 0C, α -farnesene concentration declined and CT269 and CT258 rose, while CT281 remained unchanged (Table 3). These CT changes produced higher CT258 : CT281 and CT269 : CT281 ratios after 45 weeks of storage. However, scald susceptibility did

Table 2. Statistical analyses of changes in α -farnesene and conjugated trienes in apple peel during storage at 0C, as affected by storage time, DPA treatment, and cultivar.

Variable	α -Farnesene (nmol·cm ⁻²)	Conjugated triene (nmol·cm ⁻²)			Ratio	
		CT281	CT269	CT258	CT269 : CT281	CT258 : CT281
<i>Cortland</i>						
Storage (S)	***	***	***	***	NS	*
DPA	***	***	***	***	NS	*
S × DPA	***	***	***	***	NS	NS
<i>Delicious</i>						
S	***	***	***	***	***	***
DPA	***	***	NS	NS	NS	NS
S × DPA	*	***	NS	NS	NS	NS
<i>Comparison of cultivars</i>						
Cortland (C)	77	4.26	6.30	5.17	1.84	1.67
Delicious (D)	50	2.51	7.54	7.97	3.96	4.52
Significance	***	***	NS	***	***	***
<i>Comparison of conjugated trienes²</i>						
C		4.26 b	6.30 a	5.17 ab		
D		2.51 b	7.51 a	7.78 a		
C + D		3.39 b	6.91 a	6.47 a		

²Means within a row not followed by a common letter are significantly different at $P \leq 0.05$, by Duncan's multiple range test.

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

Table 3. Comparisons of hexane-extractable components from early-harvested (26 Sept., with 16 or 30 weeks of storage at 0C) and late-harvested (10 Oct., with 30 or 45 weeks of storage at 0C) 'Cortland' apples on removal from cold storage. Data are means of three replications. Scald was evaluated after 7 days at 20C.

Harvest date	Weeks at 0C	α -Farnesene (nmol·cm ⁻²)	Conjugated triene (nmol·cm ⁻²)			Ratio		Scald (%)
			CT281	CT269	CT258	CT269 : CT281	CT258 : CT281	
<i>Early-harvested fruit, increasing storage time</i>								
26 Sept.	16	95	11.8	15.0	9.9	1.28	0.86	23
	30	9	2.9	3.4	1.4	1.20	0.53	60
Significance	***	***	***	***	NS	*	**	**
<i>Early- vs. late-harvested fruit</i>								
26 Sept.--	30	9	2.9	3.4	1.4	1.20	0.53	60
10 Oct.	30	36	5.8	9.1	7.2	1.58	1.27	21
Significance	***	***	***	***	***	NS	***	**
<i>Late-harvested fruit, increasing storage time</i>								
10 Oct.	30	36	5.8	9.1	7.2	1.58	1.27	21
	45	22	5.7	15.5	15.2	2.73	2.67	24
Significance		**	NS	***	***	**	***	NS

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

Fig. 1. Changes of (A) α -farnesene, (B) CT281, (C) CT258, and (D) percentage scald in 'Cortland' apples at two harvest dates (26 Sept. or 10 Oct.) and storage durations (30 or 45 weeks). *., **., ***Significant at $P \leq 0.05$, 0.01, or 0.001.

not change with extended storage time for these late-harvested, scald-resistant fruit.

Scald usually develops after fruit are transferred from cold storage to higher temperatures. At 20C, linear increases in percentage scald occurred over time, but they usually were accompanied by linear decreases in α -farnesene and CT281 in apple peel. However, CT258 did not change consistently (Fig. 1). Typical UV absorption spectra of hexane extracts from scald-susceptible and scald-resistant 'Cortland' apples during this time showed that, in the early-picked, scald-susceptible fruit (Fig. 2A), all CTs (at 258, 269, and 281 nm) decreased about equivalently during 7 days at 20C. However, in late-picked, scald-resistant fruit (Fig. 2B), CTs were much higher when fruit were removed from storage, and CT281 did not decline significantly during 10 days at 20C, while CT258 and CT269 increased significantly ($P \leq 0.01$). These differences were illustrated by the CT258 : CT281 ratios, which remained <1.0 in the early-harvested fruit but rose from ≈ 1.4 to ≈ 2.4 during 10 days at 20C in the late-harvested fruit (data not shown). Differences were less pronounced when CT269 : CT281 ratios were compared for the two lots of fruit (data not shown).

When random samples of fruit were taken for the measurements shown in Fig. 2, fruit with different amounts of scald were included. To obtain an indication of differences among fruit with different amounts of scald after 7 days at 20C, single (nonreplicated) 10-fruit samples with specific scald intensities were selected from among the replications and extracted. Those with the most scald seemed to contain the most α -farnesene, but there was little difference among them in CT281, CT269, or CT258, except when harvest dates were compared (Table 4). In the early-harvested fruit, the CT258 : CT281 ratios were close to unity; but, in the late-harvested, less scald-susceptible fruit, they were close to 2.0.

Fig. 2. Typical ultraviolet absorbance spectra of hexane extracts from (A) early-harvested and (B) late-harvested 'Cortland' apples at 20C after 30 weeks of storage at 0C.

Storage temperature (Expt. 3). To test the effects of temperature on scald induction, 'Cortland' apples were kept at 20C for 1 or 2 weeks or at 0C for 12 or 24 weeks. More α -farnesene

accumulated at 20C than at 0C (Table 5). In contrast, more CT281 accumulated at 0C than at 20C. CT269 values had become slightly higher at 0C than at 20C, but CT258 values were slightly lower in fruit at 0C than in those at 20C. CT258 : CT281 and CT269 : CT281 ratios declined over time at 20C, but these ratios still seemed to remain higher at 20C than at 0C. Fruit stored at 0C developed scald after transfer to 20C. About 2% of fruit kept continuously at 20C exhibited slight scald-like symptoms, but these symptoms were present at harvest and their origin is unknown.

Discussion

Since evidence was first presented that CTs (later defined as CT281) are oxidation products of α -farnesene (Anet, 1969; Huelin and Coggiola, 1970a) and correlated with scald development (Anet and Coggiola, 1974; Huelin and Coggiola, 1970b), this concept has dominated scald research, and no alternative hypothesis of scald development has been advanced, even though data sometimes have been hard to rationalize with this scheme.

Of the data reported here, most are not consistent with this concept.

1) After long periods of storage, later-harvested 'Cortland' apples contained twice the CT281 concentrations as early-harvested fruit, yet the later-harvested fruit developed only one-third as much scald and its intensity was less (Tables 3 and 4).

2) The 'Cortland' apples harvested 26 Sept. had one-fourth as

much CT281 (2.9 vs. 11.8 nmol·cm⁻²) but developed three times more scald (60% vs. 23%) after 30 than after 16 weeks of storage at 0C (Table 3).

3) Apples with greatly varying intensities of scald development in the same harvest contained almost equal concentrations of CT281 (Table 4).

4) Apples stored continuously at 20C developed substantial concentrations of CT281 but did not develop scald (Table 5).

These inconsistencies may result, at least in part, from using CT281 values to represent the total CT concentrations in fruit-surface extracts. Anet (1969) showed that at least two hexane-soluble CT compounds formed in vitro. α -Farnesene oxidation in vivo may produce a complex mixture of CT compounds, the composition of which may vary greatly with oxidative conditions. Huelin and Coggiola (1970a) mentioned that hexane extracts from scald-resistant apples often displayed a high peak at 258 nm, but they did not expand on this observation. We found that changes over time in the absorption spectra of fruit extracts varied among absorption maxima (Fig. 2). When overall values for CT281, CT269, and CT258 were compared, CT269 was different from CT281 in 'Cortland' and 'Delicious', CT258 was different from CT281 in 'Delicious', and, in the combination of 'Cortland' and 'Delicious', CT269 and CT258 were not different from each other (Table 2). The absorption maximum of the hexane extract may represent the maxima of certain CT species that dominate in this mixture, or it may represent common absorption characteristics of

Table 4. Changes in components of hexane extracts of 'Cortland' apples with a range of scald intensities after 7 days at 20C following storage for 30 weeks at 0C. Single unreplicated samples of 10 apples with the same scald intensity were selected for hexane extraction from among the harvest-date replicates.

Scald score ^z	α -Farnesene (nmol·cm ⁻²)	Conjugated triene (nmol·cm ⁻²)			Ratio	
		CT281	CT269	CT258	CT269 : CT281	CT258 : CT281
<i>Harvested 26 Sept.</i>						
0	5.7	1.2	2.0	1.1	1.7	0.9
1	7.0	1.5	2.5	1.7	1.7	1.1
2	3.4	1.3	2.3	1.4	1.8	1.1
3	5.9	1.2	1.3	0.4	1.1	0.3
4	9.5	1.5	1.5	0.7	1.0	0.5
5	9.8	1.5	1.5	0.9	1.0	0.6
<i>Harvested 10 Oct.</i>						
0	17.3	4.7	11.0	10.3	2.3	2.2
1	22.0	5.1	13.5	13.6	2.7	2.6
2	40.4	6.4	12.7	12.9	2.0	2.0

²Scald score: 0 = no scald, 1 = 1% to 10%, 2 = 11% to 33%, 3 = 34% to 66%, 4 = 67% to 100%, 5 = apples with >67% of surface affected at removal from storage.

Table 5. Changes in 'Cortland' apples during storage at 0 or 20C after harvest on 5 Oct. 1990. Scald on stored fruit was determined after an additional 7 days at 20C. Data are means of six replications.

Storage time (weeks)	Storage temp (°C)	α -Farnesene (nmol·cm ⁻²)	Conjugated triene (nmol·cm ⁻²)			Ratio		Scald (%)
			CT281	CT269	CT258	CT269 : CT281	CT258 : CT281	
Early-harvested fruit, increasing storage time								
0	---	17	1.3	4.5	5.2	4.3	5.1	---
1	20	134	3.4	9.9	11.5	3.0	3.5	---
2	20	112	4.4	10.5	10.5	2.4	2.4	2
12	0	93	8.1	12.1	9.8	1.5	1.2	---
24	0	47	6.3	13.1	7.9	1.7	1.3	34
LSD _{0.05}		16	1.6	2.2	1.7	1.4	1.9	18

most or all of these species. However, even in the latter case, the specific absorption characteristics of different species may vary. In the experiments reported here, the CT258 : CT281 and CT269 : CT281 ratios varied greatly with experimental conditions, and we interpret these differences to indicate the relative abundances of different CT species or groups of species under different conditions. Viewed in this way, the data offer a new perspective on scald development.

In these experiments, a negative relationship repeatedly occurred between the CT258 : CT281 ratio and scald development under varying conditions, as follows.

1) In 'Cortland' and 'Delicious' apples, the CT258 : CT281 ratios of fruit not treated with DPA dropped with time during storage at 0C (Tables 1 and 3), while scald incidence increased with storage time (Table 3).

2) After 16 weeks at 0C, among 'Cortland' apples with a CT258 : CT281 ratio of 0.9, 23% developed scald, while 'Delicious' apples with a CT258 : CT281 ratio of 2.4 developed no scald unless they were stored for an additional 8 weeks, after which 20% developed scald (Table 1).

3) Later-harvested, scald-resistant 'Cortland' apples had higher CT258 : CT281 ratios than early-harvested, more scald-susceptible fruit (Tables 3 and 4).

4) 'Cortland' apples kept continuously at 20C had high CT258 : CT281 ratios and did not scald, while those at 0C had low CT258 : CT281 ratios and scalded after transfer to 20C (Table 5).

Thus, the CT258 values and CT258 : CT281 ratios seemed to be better indicators of scald development than the CT281 values. In these experiments, a CT258 : CT281 ratio of about 22.0 was associated with little or no scald development in each of the postharvest conditions tested, while ratios of about 11.0 were associated with significant scald development. The CT269 : CT281 ratios generally reflected the CT258 : CT281 ratios, but differences usually were less distinct when comparing lots of fruit with different scald potentials.

Scald development consists of induction and symptom-expression periods (Bramlage and Meir, 1990). Induction apparently occurs only at low temperatures, while symptoms develop at either low or high temperatures, most commonly the latter (Fig. 1D). The role of CTs in this process has not been defined, but they could be involved in the peroxidation of membrane lipids, an action that ultimately disrupts cellular organization, and, thus, be agents of symptom expression after slow cell disruption.

If CT281 is involved directly in scald development, its concentrations likely would be highest when symptoms develop. However, like α -farnesene, CT281 reached a maximum and then declined during prolonged storage of 'Cortland' apples (Table 3), a result that is similar to the CT281 changes in early-picked apples reported by Anet (1972). Yet, 23% of the fruit scalded after 16 weeks and 60% scalded after 30 weeks. Similarly, during symptom development after transfer to 20C, percentage scald increased linearly during 10 days, while CT281 concentrations declined linearly (Fig. 1).

Since CT formation is autocatalyzed, declining concentrations should indicate increasing rates of metabolism-catabolism of these free-radical species, and, since declines accompanied scald development (Fig. 1), CT metabolites-catabolites may be involved more in symptom development than CT281. Since conditions of low and high scald development produced high and low CT258 : CT281 ratios, respectively, regardless of CT281 concentrations, this ratio may reflect CT281 metabolism-catabolism to CT258 in apple peel.

We propose that, if CTs are associated with scald development,

the metabolites-catabolites of CT281 are involved more directly in cellular disruption than CT281. We interpret our results to suggest that CT281 is metabolized to CT258 and some unknown compound in one of two ways, as follows:

- A) α -farnesene $\rightarrow$ CT281 $\rightarrow$ CT258 $\rightarrow$? $\rightarrow$ scald or
 B) α -farnesene $\rightarrow$ CT281 $\rightarrow$ CT258 $\rightarrow$?
 $\rightarrow$ CT281 $\rightarrow$? $\rightarrow$ scald

In either sequence, we envision two critical control points: α -farnesene $\rightarrow$ CT281 and either CT258 $\rightarrow$? or CT281 $\rightarrow$? DPA apparently suppressed the first of these and may or may not have suppressed the second.

In sequence A, if the second control point is impeded, a high CT258 : CT281 ratio should be produced and little scald should develop, regardless of CT281 concentration. In sequence B, impeding the second control point diverts CT281 to CT258, which is inactive in scald development. Therefore, the toxic metabolite of CT281 is avoided, and again a high CT258 : CT281 ratio should occur and little scald develop, regardless of CT281 concentration. In either sequence, if the second control point is "open," then scald development is only a function of the peroxidation of α -farnesene to CT281.

Other interpretations of these results are possible, but we believe that they are less likely to represent the conditions of scald development. For example, the sequence could be α -farnesene $\rightarrow$ CT258 $\rightarrow$ CT281, or it could be that α -farnesene may be oxidized directly to either CT258 or CT281. However, neither of these sequences addresses three important points. First, CT281 is not the direct determinant in scald development. Second, CT258 and CT281 had opposite associations with scald. Third, CT258 : CT281 ratios had greater associations with scald than CT281 or CT258 alone. (We have extensive unpublished data supporting the second and the third points.) If CT258 is metabolized to CT281, then scald development should be a function of CT281 formation, which it was not (Tables 3 and 4), and CT258, as a precursor of CT281, should not be inversely related to scald development, which it was (Tables 3-5). In the second of these sequences, scald should be a function of CT281 concentrations, assuming that it can be metabolized freely to its toxic product. This second alternative sequence explains why α -farnesene is not a direct determinant of scald, but does not explain why CT281 is not the direct determinant. Nevertheless, considerably more research is needed to clarify the pathway for α -farnesene oxidation in apple peel.

CT258 and CT281 probably represent groups of compounds rather than individual species, with CT269 being influenced by both groups. Thus, the proposed sequence may represent metabolic fluxes being directed by various redox conditions existing in the cells. While this proposal is tentative, we believe that it represents possible CT relationships to scald development better than a simple peroxidation of α -farnesene to CT281, since a highly complex family of compounds probably exists in this system.

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