Variations in Chilling Injury and Epicuticular Wax Composition of White Grapefruit with Canopy Position and Fruit Development during the Season

Harold E. Nordby* and Roy E. McDonald

U.S. Horticultural Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 2120 Camden Road, Orlando, Florida 32803

White grapefruit from interior and exterior tree canopy positions were harvested monthly over an 8-month season, and their epicuticular wax composition and susceptibility to chilling injury during cold storage were determined. Chilling injury was quite pronounced during the entire season in exterior canopy and to a far less degree in interior canopy fruit when stored at 5 °C. Fruit from both canopy positions stored at 15 °C were free of chilling injury. Levels of wax, as measured by gas chromatography, increased throughout the season but at a more rapid rate and to higher levels in exterior canopy fruit. Levels of total wax increased greatly in fruit from both canopies when the fruit were stored at 15 °C, indicating that, in grapefruit, chilling injury is not directly related to the level of total epicuticular wax. The seasonal and 15 °C stored fruit increases were most pronounced in the aldehyde, alkane, and linear alcohol wax fractions and less in the triterpene fraction. Chromatograms of the exterior and interior canopy fruit triterpene fractions were quite different, indicating possible relationships of triterpenes, sun exposure, and chilling injury. The sun-exposed and shaded sides of exterior canopy grapefruit were assayed for chilling injury development and relative percentages of components in the triterpene fraction of the wax. Four major triterpenols were greater on sun-exposed surfaces compared with shaded surfaces, while six triterpenones showed the reverse trend. Ratios of the 10 triterpenes in exterior versus interior canopy grapefruit mirrored the respective ratios of 10 triterpenes in sun-exposed and shaded sides of the fruit, indicating that triterpenes may interact with sunlight and possibly alter the susceptibility of grapefruit to chilling injury.

Keywords: *Grapefruit; chilling injury; triterpenes*

INTRODUCTION

Grapefruit, like many other tropical and subtropical fruits, develop chilling injury (CI) when stored at temperatures below 12 °C. CI symptoms include surface pitting, discoloration, and decay. Several treatments have been reported to reduce CI on fruit (Wang, 1990); however, the mechanism(s) of protection is (are) still being investigated. Early investigations on the epicuticular wax layer of citrus and other fruits showed the wax coating to be related to cuticular resistance and transpiration rates (Bain and McBean, 1967; Possingham et al., 1967), postharvest weight loss (Albrigo, 1972a), and rind disorders (Albrigo, 1972a–c). Temperature conditioning, by holding the fruit at 5 °C for 7 or more days prior to cold storage, has been one of the most successful treatments to reduce CI on grapefruit (Hatton and Cubbedge, 1982, 1983). Nordby and McDonald (1990a,b) found that the increased squalene levels in grapefruit epicuticular wax during conditioning were negatively correlated with CI. Levels of squalene, C_{23}–C_{25} alkanes, and C_{24}–C_{26} aldehydes increased in the epicuticular wax of grapefruit during temperature conditioning, whereas levels of squalene, C_{27}–C_{33} alkanes, and C_{28}–C_{34} aldehydes decreased during low-temperature storage (Nordby and McDonald, 1991). Triterpenes appear to be the major lipid class in grapefruit epicuticular wax (McDonald et al., 1993a; Nordby and McDonald, 1994), in contrast to the compositions reported for other kinds of citrus fruit (Baker et al., 1975; Freeman et al., 1979; Sala et al., 1992; Schulman and Monselise, 1970).

Purvis (1980) found interior canopy grapefruit to be less susceptible to CI compared with exterior canopy fruit and the sun-exposed side of the exterior fruit was more susceptible to CI than the shaded side of the same fruit (Purvis, 1984). Although squalene levels in the fruit from both canopies were the same, the wax of exterior canopy grapefruit had greater relative percentages of alkanes and aldehydes compared with interior canopy fruit (McDonald et al., 1993a). Squalene applied to grapefruit reduced the incidence of CI to a level comparable to the reduction achieved by temperature conditioning. The added coating failed, however, to reduce the postharvest fruit weight loss, indicating that the mechanism by which squalene protects the fruit is different from methods in which waxes, oils, and film wraps reduce CI (McDonald et al., 1993b). Since squalene is a precursor of triterpenes, this study was devised to determine if levels of specific triterpenes are different in exterior versus interior canopy grapefruit and on the sun-exposed versus shaded surfaces of fruit.

EXPERIMENTAL PROCEDURES

Sample Collection. Marsh grapefruit (*Citrus paradisi* Macf.) were harvested near the 15th of each month from September 1992 to April 1993, from a grove in central Florida. Each month, 73 exterior and 73 interior canopy fruit from the same tree were separately collected from the interior and the exterior canopies of three trees, the trees being randomly selected from this grove on each of the 8 months. The trees had received normal cultural practices. Fruit were washed and dried; waxes and fungicides were not applied. For determining the wax composition of the sun-exposed and shaded surfaces (sun/shade) of fruit, grapefruit were harvested in February 1994 with the respective surfaces identified, hand

Table 1. Epicuticular Wax Levels (Micrograms per Square Centimeter) of Grapefruit from Exterior (E) and Interior (I) Tree Canopy Positions, Freshly Harvested and Stored at 5 or 15 °C for 4 Weeks[a]

	fresh		5 °C		15 °C			
harvest month	E	I	E	I	E	I	*b*	*c*
Sept	8.0	7.5	7.1	8.5	24.4	14.7	**	***
Oct	14.9	10.9	15.6	12.3	35.5	20.2	***	***
Nov	20.0	12.9	21.6	17.5	44.6	28.7	**	***
Dec	27.8	22.6	16.8	14.0	28.1	25.6	**	***
Jan	38.4	23.2	33.1	23.4	45.9	37.3	***	***
Feb	28.5	21.4	29.6	20.9	40.4	31.3	**	**
March	35.0	24.5	27.9	20.6	45.2	31.1	**	**
April	30.7	22.1	31.1	26.5	42.7	35.4	*	*
mean	25.4	18.2	22.9	17.9	38.3	28.0		

[a] Fruit were sampled over an 8-month period. [b] Significant difference between canopies at $*p = 0.05$, $**p = 0.01$, and $***p = 0.001$. [c] Significant difference between 5 and 15 °C storage at $*p = 0.05$, $**p = 0.01$, and $***p = 0.001$.

washed, and patted dry with paper towels prior to storage and wax sampling.

Storage Treatments. Exterior and interior canopy fruit from each tree were placed (16/box) in four separate nonwaxed, cardboard citrus boxes. Two boxes of each were stored in 5 or 15 °C constant temperature storage rooms without light and at 85% relative humidity for 4 weeks. After 4 weeks of storage, each fruit was classified in one of five CI categories, where 1 is 0%, 3 is ≥1% ≤5%, 5 is ≥5% ≤25%, 7 is ≥25% ≤50%, and 9 is ≥50% ≤100% surface area affected. A CI index was calculated as the mean from the 16 fruit/box CI classifications. In the sun/shade study 15 fruit each were placed on plastic fruit partition boards and stored in 5 and 10 °C storage rooms. Each side of the sun/shade fruit was separately classified as to CI damage after 2, 4, and 6 weeks of storage.

Wax Isolation, Fractionation, and Analyses. Epicuticular waxes were extracted with chloroform from the grapefruit according to a procedure previously reported (Nordby and McDonald, 1994). The surface area of each fruit was measured, and extracts of nine fruit/replicate were prepared prior to and after the storage treatments. Each side of the sun/shade fruit was separately extracted with 100 mL of chloroform according to a procedure previously reported (McDonald et al., 1993a). Five sun/shade fruit were individually extracted prior to storage and after 2, 4, and 6 weeks of storage at 5 or 10 °C.

Each sun/shade extract was placed on silica gel TLC plates, the plates were developed in benzene, and the triterpenol and triterpenone wax components were recovered as previously described (Nordby and McDonald, 1991, 1994). Wax samples in 1 μL of chloroform were injected into a GLC in the no-split, isotherm mode. The injector temperature was 270 °C, the 0.75 mm × 30 m × 1 μm SPB-1 column (Supelco Inc., Bellefonte, PA) temperature was 250 °C, and the FID temperature was 300°C. The carrier gas was helium with a flow rate of 8 mL/min. The chloroform was removed from the extracts for GLC analyses of the triterpenes with nitrogen, and the sample was dissolved in 100 μL of methylene chloride containing 0.0215 μg of cholestane as internal standard. Samples (1 μL) were analyzed using on-column injection with a 0.75 mm × 20 m × 1 μm Supelco SPB-20 column, with injector and FID temperatures at 280 °C and oven temperature at 260 °C. Helium was carrier gas with a flow rate of 8 mL/min. For confirmation of previously reported (Nordby and McDonald, 1994) structures of grapefruit wax components and for determinations on triterpenes not previously resolved, replicate samples were combined and subjected to GC–MS analyses. Mass spectra were determined on triterpenol acetates, free triterpenols and their acetate derivatives, and free triterpenones as well as triterpenones reduced to triterpenols. Mass spectra were determined isothermally at 250 °C and constant GC head pressure of 13 psi of helium (0.63 mL/min) on a HP-5MS 30 m, 0.25 mm i.d., 0.25 μm film column (Hewlett-Packard). The injector and transfer line were at 280 °C, and the detector was at 175 °C. The most prominent *m/e* (%) ions for the triterpenes in Table 4 and Figure 2 with the relative retention times (RRTs) relative to cholestane as run on the SPB-20 column are as follows: β-amyrone, RRT 3.4, 4.24 (14), 409 (5), 218 (100), 205 (49), 203 (61), 189 (25); β-amyrin, RRT 3.6, 426 (4), 411 (2), 218 (100), 203 (55), 189 (14); α-amyrone, RRT 3.8, 424 (18), 409 (12), 245 (14), 218 (100), 205 (38), 161 (25); tentatively assigned structure D:C-friedoolean-?-en-3-one, RRT 3.9, 424 (5), 410 (1), 257 (45), 245 (23), 205 (100), 159 (8), 133 (19), 121 (23); tentatively assigned structure D:C-friedoolean-8-en-3-one, RRT 4.0, 424 (20), 409 (10), 257 (12), 245 (20), 205 (75), 67 (100); α-amyrin, RRT 4.0, 426 (15), 411 (13), 218 (100), 207 (18), 203 (25), 189 (34); lupeol, RRT 4.1 426 (20), 411 (16), 315 (16), 218 (43), 207 (72), 189 (69), 95 (100); 24-methylenecycloartanone, RRT 4.2, 438 (33), 423 (20), 395 (12), 355 (11), 217 (11), 201 (26), 107 (100); tentatively assigned structure D:B-friedo-B′:A′-neogammacer-5-en-3-ol, RRT 4.3, 4.26 (5), 274 (85), 259 (100), 205 (28), 173 (20); friedelin, RRT 5.6, 426 (17), 411 (9), 302 (9), 273 (23), 231 (26), 218 (26), 205 (36), 109 (100).

RESULTS AND DISCUSSION

Exterior canopy fruit stored at 5 °C developed considerable CI throughout the season, while interior fruit showed slight CI only in April (Figure 1A). No CI developed on fruit from either tree canopy position stored at 15 °C.

Total wax levels of freshly harvested September fruit were 8.0 μg/cm² for exterior and 7.5 μg/cm² for interior canopy fruit (Table 1). During the next 4 months wax levels increased 94% on exterior canopy fruit and only 52% on interior canopy fruit. In January the wax level on exterior fruit was 66% greater than on interior fruit. In previous studies (Nordby and McDonald, 1990b, 1991) it was shown that temperature conditioning of grapefruit prior to cold storage increased wax thickness and reduced CI. Thus, it seems that CI is independent of wax thickness but may be more related to the composition of the wax. Wax levels increased ca. 50% in both exterior and interior fruit when stored at 15 °C for 4 weeks, while wax levels decreased on a monthly average of 10% in exterior and only 1% in interior fruit during storage at 5 °C. The small decreases for interior fruit indicate that their wax components are affected only slightly by cold storage in contrast to the effect on exterior fruit.

With one exception (September triterpenes) during each of the 8 months, levels of the four lipid classes were greater in exterior than interior waxes at harvest (Table 2). Within the aldehyde class, the mean exterior/interior differences were 29, 29, and 34% for fresh and 5 and 15 °C stored fruits, respectively. The similar differences for fresh and stored fruit suggest that the loss of aldehydes during storage is not a cause but a response to CI since the decline would most likely be greater for exterior fruit if aldehydes were causing CI. The mean monthly declines in aldehydes during 5 °C storage, however, were 8.4% for exterior and 8.5% for

Table 2. Levels (Micrograms per Square Centimeter) of Four Major Lipid Classes in the Epicuticular Wax of Exterior (E) and Interior (I) Canopy Grapefruit with Storage over an 8-Month Season

harvest month		triterpenes		aldehydes		alkanes		alcohols	
		E	I	E	I	E	I	E	I
Sept F	*a*	4.4	4.5*	2.8	2.4***	0.42	0.29***	0.21	0.17**
5 °C	*b*	3.8	5.5	2.5	2.4***	0.49	0.27***	0.16	0.16***
15 °C	*c*	5.1	5.6	15.9	7.5***	2.60	1.15	0.52	0.22***
Oct F	*a*	6.9	6.0	6.4	4.0***	0.92	0.41***	0.41	0.28***
5 °C	*b*	6.7	6.6*	7.3	4.8***	0.95	0.49***	0.46	0.3***
15 °C	*c*	8.2	7.7	22.8	10.6***	3.04	1.06***	0.81	0.38**
Nov F	*a*	9.6	7.2	8.1	4.8***	1.34	0.45***	0.54	0.24**
5 °C	*b*	9.5	9.7*	9.3	6.7***	1.53	0.46**	0.76	0.34**
15 °C	*c*	11.4	10.7	28.0	15.3**	3.33	1.31*	1.18	0.60
Dec F	*a*	10.3	9.2	13.9	11.7*	2.14	1.12**	0.86	0.48**
5 °C	*b*	6.1	6.1***	8.2	6.7***	1.50	0.64**	0.66	0.39*
15 °C	*c*	6.3	7.0	17.7	15.7	2.63	1.79	0.88	0.61
Jan F	*a*	13.8	9.5**	20.0	11.9**	2.68	1.00***	1.13	0.51***
15 °C	*b*	12.2	10.7	15.9	10.4***	2.77	0.80**	1.29	0.47
15 °C	*c*	11.7	11.5**	28.7	22.1	3.45	2.05	1.17	0.75
Feb F	*a*	13.6	10.7*	11.8	8.9***	1.92	0.88**	0.71	0.50**
5 °C	*b*	13.4	11.1	13.1	8.2***	1.83	0.74**	0.77	0.58**
15 °C	*c*	11.5	10.6	24.2	17.5	3.03	1.91	1.03	0.75
March F	*a*	15.6	11.2*	14.9	11.2**	2.80	1.07***	1.02	0.62***
5 °C	*b*	11.1	10.2	12.5	8.7**	2.67	0.86**	1.00	0.49**
15 °C	*c*	13.2	11.4	26.3	16.8	3.53	1.62	1.29	0.70
April F	*a*	13.0	10.5*	13.4	10.0*	2.80	0.92	0.86	0.46
5 °C	*b*	12.6	12.1	14.8	11.6**	2.16	1.67	0.89	0.71
15 °C	*c*	12.9	12.0	24.5	19.4	3.37	2.55	1.08	0.82
mean F		10.9	8.6	11.4	8.1	1.88	0.77	0.72	0.41
5 °C		9.4	9.0	10.5	7.4	1.73	0.74	0.75	0.43
15 °C		10.1	9.6	23.5	15.6	3.12	1.68	1.00	0.60

[a] Asterisks following I values indicate statistical difference within a class. Freshly harvested. Significant difference between canopies at $*p = 0.05$, $**p = 0.01$, $***p = 0.001$. [b] Stored at 5 °C for 4 weeks. Significant difference between 5 and 15 °C storage at $*p = 0.05$, $**p = 0.01$, $***p = 0.001$. [c] Stored at 15 °C for 4 weeks. Significant difference between canopies × storage at $*p = 0.05$, $**p = 0.01$, $***p = 0.001$.

interior fruit. Mean monthly aldehyde increases for exterior and interior fruit stored at 15 °C were 106 and 93%, respectively. Squalene levels were not routinely measured during this study since it is found in only trace amounts in fresh fruit and fruit stored at 5 °C (Nordby and McDonald, 1990b) and was not resolved from hexadecosanal under our SPB-1 GLC system. A cursory analysis of the squalene/alkane TLC fractions from fresh and stored fruit, however, showed no difference between exterior and interior fruits (data not presented). Squalene levels in fruit stored at 15 °C were similar to values reported previously (Nordby and McDonald, 1990b).

Alkane and alcohol levels showed the greatest exterior-interior differences of the four lipid classes, the range being 40–60% more in exterior fruit (Table 2). Levels of both lipid classes increased or decreased for the two canopy positions to about the same extent during respective 5 or 15 °C storage, indicating that these two lipid classes, like aldehydes, probably do not play a role in CI development in grapefruit.

Differences in the levels of triterpenes between exterior and interior fruit waxes were much less than for the other three lipid classes. The monthly differences were 21% in fresh and 5% for fruit stored at both 5 and 15 °C. Over the 8-month harvest period, during 5 °C storage triterpenes decreased an average of 13% in exterior fruit and increased an average of 5% in interior fruit. There was an overall decrease of 8% in triterpenes in exterior fruit and an overall increase of 11% in interior fruit. The triterpene decrease over the 8-month season in exterior fruit at both 5 and 15 °C storage temperatures indicates that the slight increase in these lipids within the first 3 months due to maturation was possibly offset by degradation from some external source such as sunlight. Since the levels of the three other lipid classes in both exterior and interior fruit triterpenes all increased and triterpene levels remained about the same in interior fruit with 15 °C storage, the decrease in exterior triterpenes suggests that triterpenes may have a role in causing CI in grapefruit.

In our nonpolar phase GLC system (SPB-1), the majority of triterpenes were eluted between C_{30} and C_{32} aldehydes, the profiles of exterior wax being quite distinguishable from those from interior fruit wax. Seventeen chromatogram peaks were considered, as reported previously (Nordby and McDonald, 1994), to contain triterpenes (Table 3). From the shapes of the peaks, we concluded that several were mixtures of compounds, thus making it difficult to assign specific structures and levels for each of the 8 months. With the exception of peak 12 (mainly friedelin), very little difference was observed in the triterpene levels between fresh and poststorage fruit. This indicates that if CI is influenced by the presence of specific triterpenes, the influence occurs prior to storage.

Differences in levels between fresh exterior and interior waxes ranged from 0 to 50% for triterpenes 3, 4, 6, 8, 9, 10, 12, 13, 16, and 17 and over 50% for triterpenes 1, 2, 5, 7, 11, 14, and 15. Although the difference for friedelin was only 21%, on a microgram

Table 3. Mean Monthly Levels (Micrograms per Square Centimeter) of Triterpenes in Exterior (E) and Interior (I) Canopy Grapefruit at Harvest and after 4 Weeks of Storage at 5 or 15 °C

triterpene peak[a]	fresh		5 °C		15 °C					
	E	I	E	I	E	I	*b*	*c*	*d*	*e*
1	0.09	0.16	0.10	0.18	0.10	0.18	***	**	***	***
2	0.57	0.33	0.47	0.36	0.47	0.36	***	**	*	***
3	0.24	0.23	0.22	0.24	0.23	0.25	***			
4	0.26	0.26	0.23	0.28	0.22	0.27	***	***		
5	0.68	0.16	0.60	0.25	0.68	0.31	***	***		***
6	0.30	0.30	0.24	0.28	0.16	0.20	***		***	*
7	0.19	0.09	0.17	0.07	0.22	0.09	***	***	***	***
8	0.20	0.30	0.16	0.35	0.17	0.38		***		***
9	0.20	0.14	0.18	0.11	0.23	0.19		***	**	
10	0.38	0.33	0.33	0.37	0.34	0.32	***			
11	0.68	0.34	0.59	0.39	0.64	0.53	*	***	**	***
12	6.69	5.53	5.75	5.70	6.06	5.84	***	***	*	*
13	0.20	0.20	0.22	0.18	0.24	0.18	***	***		***
14	0.03	0.14	0.03	0.15	0.04	0.17	***	***		***
15	0.06	0.03	0.07	0.03	0.06	0.03	***	***		
16	0.03	0.02	0.03	0.02	0.03	0.04	*			
17	0.09	0.06	0.07	0.05	0.14	0.20			***	
total	10.89	8.60	9.44	8.99	10.05	9.55				

[a] Peaks from total wax analysis on SPB-1 GC column, see text. [b] Significant difference between months for triterpene peak at $*p = 0.05$, $***p = 0.001$. [c] Significant difference between canopies at $**p = 0.01$, $***p = 0.001$. [d] Significant difference between storage temperatures at $*p = 0.05$, $**p = 0.01$, $***p = 0.001$. [e] Significant difference between month × canopy at $*p = 0.05$, $***p = 0.001$.

Table 4. Major Triterpenes (Relative Percent of Total Triterpenes) in Sun-Exposed (EX) and Shaded (SH) Sides of Grapefruit Stored at 5 or 10 °C for 0, 2, 4, and 6 Weeks

storage			triterpenones[a,b]						triterpenols[a,b]			
temp (°C)	time (weeks)	side	3.4	3.8	3.9	4.0	4.2	5.6	3.6	4.0	4.1	4.3
	0	EX	1.0	0.5	0.5	0.3	1.4	45.8	12.1	12.0	12.8	4.5
		SH	2.4**	1.1*	1.0	0.9	3.0**	62.0*	6.5**	4.4**	7.2**	2.5*
5	2	EX	1.4	0.8	0.9	0.6	2.7	59.5	7.2	6.2	5.9	3.1
		SH	1.8	0.9	0.9	0.9	2.9	57.7	6.7*	5.4	6.7	3.1
	4	EX	1.5	0.8	0.8	0.4	2.4	53.3	9.0	9.0	9.1	3.8
		SH	1.8	1.2	1.0	0.5	2.4	52.6	9.0	8.6	9.4	4.2
	6	EX	1.1	0.6	0.6	0.4	2.2	47.7	9.5	9.6	9.6	4.9
		SH	1.8	0.9	1.0	0.9	2.9	53.3	7.9	5.0	7.2	5.9
10	2	EX	1.2	0.7	0.8	0.4	1.9	37.9	13.9	12.3	13.4	5.0
		SH	1.8	0.9	1.0	0.7	2.7	61.7*	6.7*	5.7*	6.8	2.9*
	4	EX	1.2	0.7	0.8	0.3	1.9	43.2	11.9	11.6	12.3	4.6
		SH	2.4*	1.2	1.2	1.1*	3.3	59.7*	6.2*	6.4*	6.4*	2.6
	6	EX	1.0	0.6	0.7	1.0	1.6	32.9	13.1	12.0	11.5	7.9
		SH	1.9*	1.0*	1.0*	0.8	2.3	58.8*	7.0	5.6	6.1	3.6*

[a] Significant difference between sides at $*p = 0.05$, $**p = 0.01$. [b] See text for structures, RRTs relative to cholestane on SPB-2.

basis the 1.16 $\mu g/cm^2$ difference accounted for 51% of the level differences between total triterpenes in exterior and interior grapefruit waxes. Other triterpene peaks that showed major $\mu g/cm^2$ exterior–interior differences were 2, 5, and 11. Twelve of the 17 triterpene peaks showed highly significant ($p = 0.001$) differences between months. Twelve, but not always the same 12, showed differences between canopy positions. In contrast to this, only four triterpene peaks showed highly significant differences due to storage, again evidence to suggest that exterior fruit triterpenes have their influence, if any, on CI prior to storage.

A major objective of this study was to determine triterpene structure with degree of sun exposure. To define a narrower temperature range between pronounced and near absence of CI in grapefruit, the storage temperature on "control" fruit was lowered from 15 to 10 °C for this sun-exposed–shaded study. Table 4 presents the relative percentages of individual triterpenes in the wax from sun-exposed and shaded sides of grapefruit. Levels of four of the six triterpenones and the four triterpenols were significantly different in the waxes from the two fruit surfaces. To a lesser degree this exposed–shaded difference was observed in fruit stored at 10 °C for 2, 4, and 6 weeks. Fruit stored at 5 °C over the 6 weeks failed to show a significant difference between exposed and shaded sides of the fruit even though CI was more prevalent on the sun-exposed side after 4 weeks (Figure 1B). For the exposed–shaded study improved resolution of individual triterpenes within the same lipid class was obtained by utilizing on-column injection with a slightly more polar SPB-20 column for GLC analyses. Figure 2A shows the relative percentages of the four most prevalent triterpenols in sun-exposed and shaded sides of exterior grapefruit along with the percentages of these triterpenols on whole exterior and interior fruit. The profiles of the two sets are similar, with percentages of triterpenols in exterior and sun-exposed waxes being more abundant

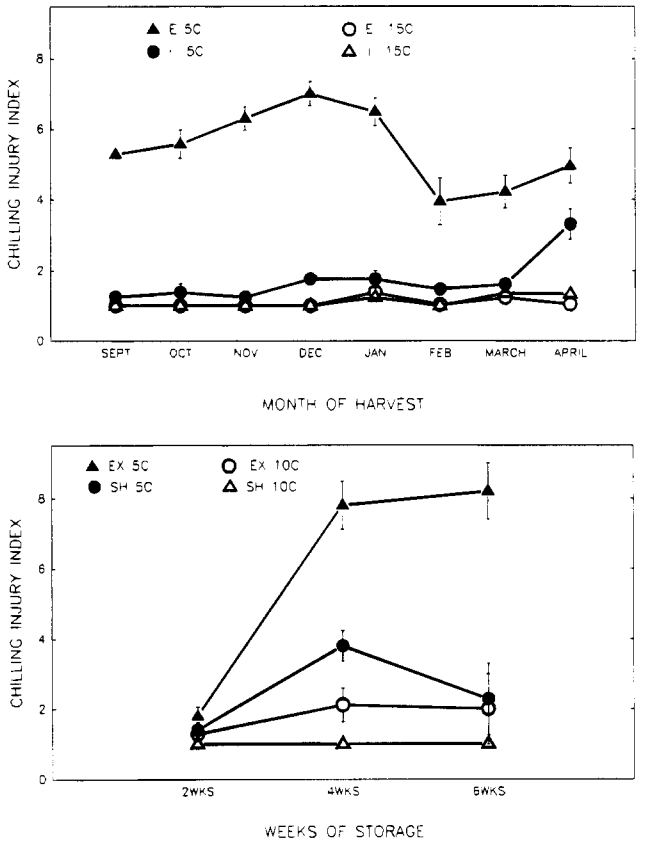

Figure 1. (A, top) Chilling injury in exterior (E) and interior (I) canopy fruit stored at 5 or 15 °C for 4 weeks. (B, bottom) Chilling injury in sun-exposed (EX) and shaded (SH) sides of exterior canopy February 1994 grapefruit stored at 5 or 10 °C.

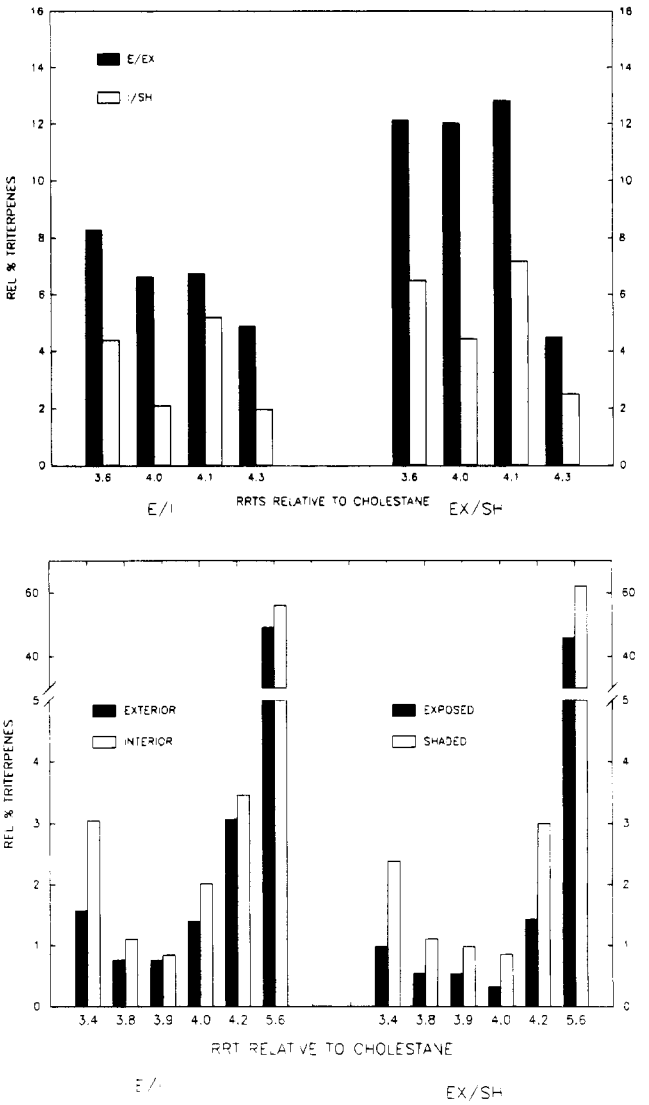

Figure 2. (A, top) Major triterpenols in epicuticular wax of exterior (E) and interior (I) canopy and on sun-exposed (EX) and shaded (SH) sides of freshly harvested grapefruit. (B, bottom) Major triterpenones in epicuticular wax of exterior (E) and interior (I) canopy and on sun-exposed (EX) and shaded (SH) sides of freshly harvested grapefruit.

than respective interior and sun-shaded waxes in approximately the same ratios.

Structures of the four triterpenols were determined by their mass spectra and the mass spectra of their acetate derivatives. Triterpenol 3.6 was β-amyrin. Triterpenol 4.0 was α-amyrin. Lupeol (RRT 4.1), which was not resolved from α-amyrin on the nonpolar, SPB-1 column used in the exterior–interior study, was fairly resolved on the SPB-20 column using on-column injection but was baseline separated from α-amyrin by GC–MS with electronic pressure control (EPC). The triterpenol with RRT of 4.3 has been tentatively characterized as D:B-friedo-B':A'- neogammacer-5-en-3-ol (3.β), with a base peak of 259, a major peak at 274, and no other peaks between these peaks and the parent peak at 426 (Ogunkoya, 1981). The spectrum was the same as that of no. 55701 in the NBS75K library.

Figure 2B presents the relative percentages of the major triterpenones in exterior–interior (E/I) and sun-exposed–shaded (Ex/SH) fresh grapefruit wax. For all six triterpenes the relative percentages in E and EX waxes are less than in I and SH waxes, respectively. This is especially evident for β-amyrone (RRT 3.4) in both sets of waxes and for 24-methylenecycloartanone (RRT 4.2) in the EX/SH set of waxes. The higher percentages of triterpenones in interior or shaded side of the fruit in contrast with the triterpenols having higher percentages in exterior or exposed side of the fruit suggest a solar influence on the selective degradation of triterpenones to *sec*-triterpenes. The influence of ultraviolet light on triterpenes such as the amyrins, lupeol, and friedelin has been reported for leaves (Baas, 1985, and references cited therein) and *sec*-triterpenes have been synthesized from triterpenones with the aid of an ultraviolet lamp (Baas, 1983). In the syntheses, the triterpenol is first oxidized chemically to the triterpenone prior to the photolysis treatment. Since in *in vitro* synthesis the oxidation of the triterpenol is the limiting chemical reaction, it can be reasoned that in the citrus fruit this same limiting factor exists, thus explaining the higher relative percent of triterpenols in exposed fruit.

The mechanisms by which these changes in triterpene percentages may affect chilling injury still need to be studied. It is probable that during the oxidation stages of the triterpenes free radicals are generated similar to those generated by autoxidation of cholesterol (Smith, 1981). It seems likely that the mechanism resembles that observed in superficial scald of cold stored apples when farnesene, a sesquiterpene, is oxidized. Resulting oxidized compounds then initiate the membrane lipid degradation, causing the skin blemish (Huelin and Coggiola, 1970). The triterpenes reported here for grapefruit wax have the structural moieties present in cholesterol and farnesene which are responsible for photooxidation or autoxidation.

ACKNOWLEDGMENT

We thank Jaritza Figueroa-Detres for competent technical assistance during the course of this work.

LITERATURE CITED

Albrigo, L. G. Distribution of stomata and epicuticular wax on oranges as related to stem end rind breakdown and water loss. *J. Am. Soc. Hortic. Sci.* **1972a**, *97*, 220–223.

Albrigo, L. G. Ultrastructure of cuticular surfaces and stomata of developing leaves and fruit of the Valencia orange. *J. Soc. Hortic. Sci.* **1972b**, *97*, 761–765.

Albrigo, L. G. Variation in surface wax on oranges from selected groves in relation to fruit moisture loss. *Proc. Fla. State Hortic. Sci.* **1972c**, *85*, 262–263.

Baas, W. J. Dihydronyctanthic acid methyl ester and other 3,4-seco-pentacyclic triterpenoids from Hoya lacunosa. *Phytochemistry* **1983**, *22*, 2809–2812.

Baas, W. J. Naturally occurring Seco-ring-A-triterpenoids and their possible biological significance. *Phytochemistry* **1985**, *24*, 1875–1889.

Bain, J. M.; McBean, D. M. The structure of the cuticular wax of prune plums and its influence as a water barrier. *Aust. J. Biol. Sci.* **1967**, *20*, 895–900.

Baker, E. A.; Procopiou, J.; Hunt, G. M. The cuticles of Citrus species. Composition of leaf and fruits waxes. *J. Sci. Food Agric.* **1975**, *26*, 1093–1101.

Freeman, B.; Albrigo, L. G.; Biggs, R. H. Cuticular waxes of developing leaves and fruit of blueberry, Vaccinium ashei Reade cv. Blugem. *J. Am. Soc. Hortic. Sci.* **1979**, *104* (3), 398–403.

Hatton, T. T.; Cubbedge, R. H. Conditioning Florida grapefruit to reduce chilling injury during low-temperature storage. *J. Am. Soc. Hortic. Sci.* **1982**, *107*, 57–60.

Hatton, T. T.; Cubbedge, R. H. Preferred temperature for prestorage conditioning of Marsh grapefruit to prevent chilling injury at low temperatures. *HortScience* **1983**, *18*, 721–722.

Huelin, F. E.; Coggiola, I. M. Superficial scald, a functional disorder of stored apples. V. Oxidation of alpha-farnesene and its inhibition by diphenylamine. *J. Sci. Food Agric.* **1970**, *21*, 44–48.

McDonald, R. E.; Nordby, H. E.; McCollum, T. G. Epicuticular wax morphology and composition are related to chilling injury in grapefruit. *HortScience* **1993a**, *28*, 311–312.

McDonald, R. E.; McCollum, T. G.; Nordby, H. E. Temperature conditioning and surface treatments of grapefruit affect expression of chilling injury and gas diffusion. *J. Am. Soc. Hortic. Sci.* **1993b**, *118*, 490–496.

Nordby, H. E.; McDonald, R. E. Method for protecting citrus fruit from chilling injury, and fruit protected thereby. U.S. Pat. 4921715, May 1, 1990a.

Nordby, H. E.; McDonald, R. E. Squalene in grapefruit wax as a possible natural protectant against chilling injury. *Lipids* **1990b**, *25*, 807–810.

Nordby, H. E.; McDonald, R. E. Relationship of epicuticular wax composition of grapefruit to chilling injury. *J. Agric. Food Chem.* **1991**, *39*, 957–962.

Nordby, H. E.; McDonald, R. E. Friedelin: the major component of grapefruit epicuticular wax. *J. Agric. Food Chem.* **1994**, *42*, 708–713.

Ogunkoya, L. Application of mass spectrometry in structural problems in triterpenes. *Phytochemistry* **1981**, *20*, 121–126.

Possingham, J. V.; Chambers, T. C.; Radler, F.; Grncarevic, M. Cuticular transpiration and wax structure and composition of leaves and fruit of Vitis vinifera. *Aust. J. Biol. Sci.* **1967**, *20*, 1149–1153.

Purvis, A. C. Influence of canopy depth on susceptibility of March grapefruit to chilling injury. *HortScience* **1980**, *15*, 731–733.

Purvis, A. C. Importance of water loss in the chilling injury of grapefruit stored at low temperature. *Sci. Hortic.* **1984**, *23*, 261–267.

Sala, J. M.; Lafuente, T.; Cunat, P. Content and chemical composition of epicuticular wax of Navelina oranges and Satsuma mandarins as related to rindstaining of fruit. *J. Sci. Food Agric.* **1992**, *59*, 489–495.

Schulman, Y.; Monselise, S. P. Some studies on the cuticular wax of citrus fruits. *J. Hortic. Sci.* **1970**, *45*, 471–478.

Smith, L. L. *Cholesterol Autoxidation*; Plenum Press: New York, 1981; pp 49–62.

Wang, C. Y. Alleviation of chilling injury of horticultural crops. In *Chilling Injury of Horticultural Crops;* Wang, C. Y., Ed.; CRC Press: Boca Raton, FL, 1990; pp 281–302.

Received for review January 24, 1995. Accepted April 24, 1995.[⊗]

JF9500602

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1995.