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Superficial Scald of 'Granny Smith' Apples is Expressed as a Typical Chilling Injury

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Abstract. **To examine the hypothesis that superficial scald of apple *(Malus domestics* Borkh.) is a chilling injury, 'Granny Smith' apples were stored at temperatures ranging from 0 to 20C, temperature-conditioned before storage, and warmed during storage. Fruit stored at 0 or 4C developed supeficial scald. At 10C, surface defects occurred but they were not typical symptoms of scald, and at 15 or 20C no symptoms developed. Accumulation of α-farnesene and conjugated trienes in fruit peel correlated with increasing ethylene production, which was greater at higher temperatures. However, concentrations of conjugated trienes were highest at 0 and 4C. When fruit were kept at 10C for 5 or 10 days before storage, scald development after storage was not reduced. An interruption of 0C storage with a single warming period at 10 or 20C reduced scald development after 25 weeks of storage, maximum reduction occurring when fruit were warmed for 3 to 5 days at 20C after 1 to 4 weeks at 0C. Amelioration of scald declined as time at 0C before warming increased. Diphenylamine application after the same intervals at 0C, instead of warming, also was less beneficial as time before treatment increased. α-Farnesene and conjugated trienes increased during warming, but at the end of storage (when scald was developing) the conjugated triene concentrations in peel were reduced in fruit that had been warmed. Warming slightly increased yellowing, softening, and greasiness of fruit after storage, We conclude that chilling induced superficial scald on 'Granny Smith' apples.**

Superficial scald is a physiological disorder of certain apple and pear cultivars that develops during prolonged low-temperature storage. Typically, early-harvested and less-mature fruit are most susceptible, but scald also may develop on fully mature fruit. The disorder is manifest as browning of the skin as a result of damage to the hypodermal cells (Bain and Mercer, 1963). Scald development is widely believed to result from production of α-famesene and its autoxidation to conjugated trienes (Anet, 1972; Du and Bramlage, 1994). Generally, correlations between conjugated triene concentrations and scald occurrence are strong (Anet and Coggiola, 1974; Huelin and Coggiola, 1970b; Meir and Bramlage, 1988; Scott et al., 1980), but those between α-famesene and scald are variable (Anet, 1972; Huelin and Coggiola, 1968; Meigh and Filmer, 1969; Meir and Bramlage, 1988). α-Famesene typically increases rapidly during storage and then declines during the time of scald development, and its accumulation appears to be concomitant with rising ethylene production (Watkins et al., 1993).

Chilling injury generally results from exposure of tropical or subtropical plants to temperatures below 10 to 15C (Saltveit and Morris, 1990). However, Bramlage and Meir (1990) have argued that temperate plants, while resistant to chilling damage, are not immune to it. These fruit have the inherent potential to develop symptoms of chilling injury under rigorous conditions. Apples are a temperate crop and scald occurs after long-term cold storage. Early reports stated that optimum temperature for scald development was 15C, and that in some cultivars it occurred at 25C, but never at 30C (Brooks and Cooley, 1917; Brooks et al., 1919). Smock (1961) also reported scald development on fruit in poly-ethylene bags at 21 C. However, most studies repot-t scald at temperatures no higher than 15C. Martin and Lewis (1961) and Huelin and Coggiola (1970b) showed that, while scald developed earlier at higher storage temperatures (3.5 and 15C), given sufficient time it was more severe when fruit were stored at lower temperatures (–1.5 and 5C). This time–temperature relationship for symptom expression is typical of a chilling disorder (Saltveit and Morris, 1990).

Other features of scald development support the suggestion that it is a chilling injury. Keeping fruit at warn-t temperatures after harvest (conditioning) before imposing cold treatments sometimes reduces scald (Meigh, 1970; Padfield, 1949), and prestorage heat treatment of 38C for 4 days delayed scald development on 'Granny Smith' apples at 0C (Lurie et al., 1991). The disorder can be alleviated by antioxidants such as diphenylamine (DPA) (Huelin and Coggiola, 1968; Lau, 1990), controlled- atmosphere storage (Chen et al., 1985; Lau, 1990), and intermittent warming (Kidd and West, 1935; Smith, 1959), all methods that inhibit development of chilling injury in fruit (Wang, 1993).

Superficial scald is a serious problem on stored apples, and to develop strategies for protection it is necessary to define clearly its relationship with storage temperature. To do this we have reexamined effects of storage temperature, conditioning, and warming of fruit during storage on scald development.

Materials and Methods

Source of fruit. 'Granny Smith' apples were harvested from randomly selected groups of three trees at the Havelock North Research Orchard to provide five replicate lots of fruit for each experiment. The fruit were transported overnight to Auckland, where fruit quality was evaluated and experimental treatments imposed.

Effects of storage temperatures. Fruit were stored at 0,4,10,15, and 20C for 30, 20, 10, 7.5, and 5 weeks, respectively. These

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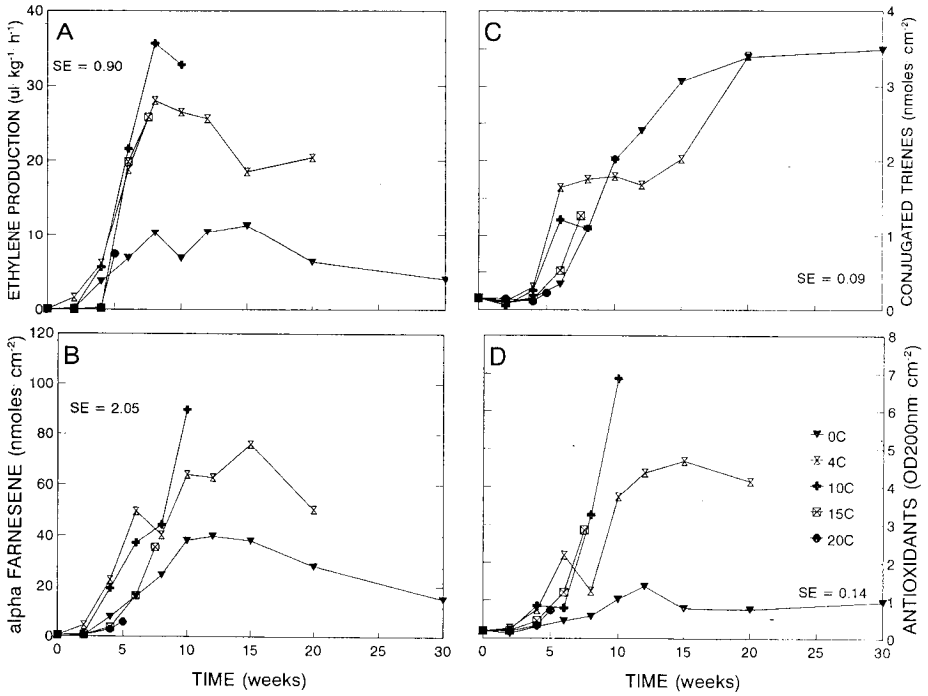

Fig. 1. Ethylene production **(A)** and accumulations of α-farnesene **(B),** conjugated trienes **(C),** and antioxidants **(D)** in peel of 'Granny Smith' apples kept at different temperatures.

intervals were designed to allow all fruit to reach the same stage of ripening and senescence at the different temperatures. At the start of the experiment (12 Apr. 1991) and at regular intervals during storage, ethylene production and background color of 10-fruit samples per replicate were measured before extraction with hexane for measurement of α-famesene, conjugated trienes, and antioxidant concentrations in the peel. At the end of storage at each temperature, *60* fruit per replicate were transferred to 20C for 7 days, after which 10 were extracted in hexane and 50 were evaluated visually for scald.

Effects of warming. Preclimacteric fruit were harvested on 4 Apr. 1991. On the following day and after 2 to 12 weeks at 0C, samples either were kept at 20C for 5 days or treated for 1 min with 1 g·liter^{-1} DPA, and then returned to 0C. Each treatment was warmed only once and the DPA-treated samples were not warmed. All fruit were stored for a total of 25 weeks at 0C. Each replicate consisted of 120 fruit: 10 each for measuring background color,

Table 1. Color measurements of 'Granny Smith' apples at the end of storage at different temperatures and times.

Temp (°c)	Time (weeks)	L*	a*/b*
θ	30	57 c[z]	-0.45 a
4	20	65 b	-0.38 C
10	10	66 b	-0.41 b
15	7.5	73 a	-0.33 d
20	5	66 b	-0.40 bc

[z]Means in a column not followed by a common letter are significantly different at $P \leq 0.05$, using Duncan's new multiple range test.

Table 2. Ethylene production and α-farnesene, conjugated triene, and antioxidant concentrations in 'Granny Smith' apples removed at different intervals from 0C storage (0 days) and kept at 20C for 5 days.

Time of removal from cold storage	Ethylene production (µl·kg^{-1}·h^{-1})		α-Famesene (nmol·cm^{-2})		Conjugated trienes (nmol·cm^{-2})		Antioxidants (OD 200/cm^{-2})	
(weeks)	O days	5 days	O days	5 days	O days	5 days	O days	5 days
o	0.06	0.07	0.1	1.1	0.11	0.07	0.2	0.3
2	0.5	68	1.4	24.1	0.12	0.27	0.2	0.9
4	11	127	15.0	54.8	0.27	1.17	0.5	1.9
6	18	192	30.0	54.5	0.53	1.58	0.6	2.3
8	30	246	50.9	56.8	1.64	2.98	1.3	2.5
10	22	220	41.4	61.3	2.50	5.15	1.1	2.2
12	27	253	48.3	63.7	2.96	6.62	1.2	2.4
Significance								
Warming (W)		**		***		***		***
Weeks (T)		**		***		***		***
Linear		**		***		***		***
Quadratic		**		***		***		***
W × T		**		***		***		***

,*Significant at $P \leq 0.01$ or 0.001, respectively.

Table 3. Effects of warming treatments on hexane-extractable compounds in peel of 'Granny Smith' apples after storage at 0C for 25 weeks.

Weeks at 0C before warming	α-Farnesene (nmol·cm^{-2})	Conjugated trienes (nmol·cm^{-2})	Antioxidants (OD 200/cm^{-2})
o	3.55	4.4	1.14
2	4.82	3.8	1.94
4	5.30	4.3	2.46
6	4.52	3.0	1.72
8	4.65	3.3	2.30
10	5.32	4.7	2.30
12	4.12	4.6	1.86
Significance			
Weeks	***	***	***
Linear	NS	NS	*
Quadratic	***	***	***
Pentic	*	**	NS

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

ethylene production and extraction with hexane a) before and b) after the 5 days at 20C, and c) after 25 weeks at 0C; 30 for DPA treatment; and 60 (30 warmed and 30 not treated) for evaluation of scald after storage plus 7 days at 20C. The DPA-treated fruit were also evaluated for scald at the same time.

In 1992, two additional experiments were conducted to determine the specificity of warming time and temperature. In the first, five replicate preclimacteric samples harvested on 9 Apr. were stored at 0C for 2 weeks before transfer to 20C for between 0 and 14 days, after which they were returned to 0C. At the end of each warming period, internal ethylene concentration and ground color were measured on replicate 10-fruit samples before extraction with hexane. Twenty-five weeks after harvest, all fruit were transferred to 20C, 10 fruit per replicate were tested for firmness after 1 day, and 50 fruit per replicate were evaluated for greasiness and incidence of disorders after 7 days. In the second test, fruit from the same harvest as above were stored at 0C for up to 4 weeks before transfer to 10C or 20C for 5 days, after which they were returned to 0C. At removal and after warming, 10 fruit per replicate were measured for internal ethylene concentration and extracted in hexane. After 25 weeks, all fruit were transferred to 20C. Firmness and background color of 10 fruit were measured after 1 day, and 50 fruit were evaluated for disorders after 7 days.

Effects of conditioning. Fruit were harvested from the same trees and on the same date as in the 1991 warming experiment. Replicates of 80 fruit either were stored immediately at 0C or kept at 10C (conditioned) for 5 or 10 days before storage at 0C. Ten fruit per replicate were taken for measurement of internal ethylene, background color and extraction in hexane at the end of each conditioning period, and for extraction in hexane after 25 weeks at 0C. The remaining 60 fruit were assessed for scald after 7 days at 20C following storage.

Analytical methods. Most 10-fruit samples were used both for measuring either internal ethylene concentration or ethylene production, and hexane extraction. Internal ethylene concentrations were measured on 1-ml samples of internal gas drawn into a syringe through a hypodermic needle inserted into the core cavity of each fruit. Rates of ethylene production were measured following sealing of two five-fruit samples in 4.5-liter containers for 1 h followed by analysis of head space gas. Ethylene concentrations were measured by gas chromatography (PU4500 fitted with a flame ionization detector; Philips, England). Background color was assessed using a color reflectance meter (Chroma Meter II; Minolta, Tokyo) under CIE illuminant D65 conditions. Two readings per fruit were taken and the L*, a*, b*, and a*/b* values were used to describe the changes in color; for' Granny Smith', a*/b* ratios have linear relationships with hue angles over the range of –0.60 to –0.35 (Hirst et al., 1990). The 10 fruit of each replicate then were dipped in about 90 ml of high-performance liquid chromatography (HPLC)-grade hexane for 2 min and rinsed with fresh hexane. The solutions were made to 100 ml, and appropriately diluted aliquots were measured for UV absorbance at 200 nm for estimation of antioxidant concentration, and 281 nm and 290 nm for estimation of conjugated triene concentration (Meir

Table 4. Incidence and severity of superficial scald on 'Granny Smith' apples removed from 0C after 2 to 12 weeks and kept at 20C for 5 days before being returned to 0C, or treated with 1 g·liter^{-1} of diphenylamine (DPA) instead of being warmed. The samples placed in storage at 0 weeks either were treated with DPA or had no treatment applied. Scald was evaluated after 25 weeks of storage plus 7 days at 20C.

Time of removal from storage (weeks)	Warmed		DPA-treated	
	Scald (%)	Scald severity'	Scald (%)	Scald severity
o	97	2.7	12	1.2
2	14	0.9	32	1.2
4	33	1.1	26	0.8
6	28	1.1	25	1.1
8	48	1.2	43	1.1
10	61	1.4	51	1.4
12	77	1.6	79	1.9
Significance				
Treatment (T)	**	*		
Weeks (W)	**	***		
T × W	**	***		
Warming vs. DPA:				
0 week	**	**		
2 weeks	**	NS		
4 to 12 wks	NS	NS		

[z]Visual scale: 0 = no scald, 1 = 1% to 10%, 2 = 1l% to 33%, 3 = 34% to 66%, 4 = 67%. to 100'%. of surface affected.
[NS,*,**,***] Nonsignificant or significant at $P \leq 0.05$,0.01, or 0.001, respectively.

Table 5. Effects of warming 'Granny Smith' apples for different times at 20 C after 2 weeks at 0C. Ethylene, α-farnesene, and conjugated trienes (CT) were measured at the end of warming. After 25 weeks at 0C, fruit were transferred to 20C; color, greasiness and firmness were measured after 1 day, and disorders after 7 days.

	Log ethylene production	α-Farnesene	CT	Color		Greasiness		Firmness	Scald	Coreflush		Breakdown
										Total	Severe	
Days at 20C	(μl·kg^{-1}·h^{-1})	(nmol·cm^{-2})	(nmol·cm^{-2})	L*	a*/b*	(%)	(score^z)	(N)	(%)	(%)	(%)	(%)
0	-0.5	1.5	0.06	59.6	-0.48	17	1.0	75	29	71	41	18
1	1.7	9.9	0.09	60.7	-0.48	22	1.3	77	29	67	24	5
3	1.6	31.6	0.41	61.5	-0.48	52	1.4	75	7	77	42	5
5	3.0	40.9	0.66	61.8	-0.46	26	1.0	74	2	66	18	1
7	3.6	55.0	0.88	62.5	-0.46	60	1.5	73	3	53	4	0
11	4.2	62.9	1.34	63.7	-0.40	76	1.8	71	3	44	3	1
14	4.0	78.2	1.47	65.4	-0.37	72	0.9	67	2	40	1	0
Significance												
Days	***	***	***	***	***	**	NS	***	***	***	***	**
Linear	***	***	***	***	***	***		***	***	***	***	**
Quadratic	***	***	*	NS	**	NS		NS	***	NS	NS	*

[z]Score: 1 = slight; 2 = moderate; 3 = severe.

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

and Bramlage, 1988). Aliquots also were passed through florisil (Huelin and Coggiola, 1968) and absorbance at 232 nm was used to estimate α-farnesene concentration in the extracts. Fruit surface area was calculated using weight and density of fruit, assuming that each fruit approximated a sphere (Watkins et al., 1988). Data are expressed as nanomoles per square centimeter of peel.

Scald was recorded as percent incidence and severity on affected fruit using a scale where 0 = none, 1 = 1% to 10%, 2 = 11% to 33%, 3 = 34% to 66%, and 4 = 67% to 100% of the surface area affected. Greasiness was recorded as percent incidence and also as severity by touching each fruit and rating it using a scale where 1 = slight, 2 = moderate, and 3 = severe. Severity ratings for scald and greasiness were obtained by dividing the total score only by the number of fruit affected. Firmness was measured on opposite sides of pared fruit using an EPT- 1 pressure tester (Lake City Technical Products, Canada) fitted with an 11.l-mm-diameter Effigi tip.

Results

Effects of storage temperature. During storage at 0 to 20C, ethylene production and α-farnesene and antioxidant concentration patterns showed similar trends at a given temperature (Fig. 1 A, B, and D). For all three variables, levels at 4 and 10C were higher than at other temperatures. Regressions of α-farnesene and antioxidant concentrations against ethylene production provided r^2 values of 0.67 and 0.63 (P <0.001, n = 150), respectively. In contrast, changes in conjugated triene concentrations at the various temperatures were distinctly different from those of the other substances, with the highest concentrations occurring at 0 and 4C (Fig. lC). After storage, injury clearly identifiable as scald only occurred at 0 (100%) and 4C (87%). After storage at 10C, some fruit had browning around the lenticels as well as depressions without discoloration, but no typical scald symptoms.

At the end of storage at each temperature, color measurements were taken as indications of relative senescence of fruit during storage. L* (lightness) and a*/b* (greenness) values indicated that greatest color loss occurred at 15C, and least color loss at 0C, while fruit at 4, 10, and 20C reached almost the same color during storage (Table 1). Thus, slightly less senescence appeared to have occurred at 0C for 30 weeks, and slightly more at 15C for 7.5 weeks, than at the other temperatures. However, these small differences in senescence appear to have had no effect on scald development, since nearly all fruit at 0 and 4C scalded and no fruit at 15 or 20C scalded.

Effects of warming. Ethylene production and concentrations of α-farnesene, conjugated trienes and antioxidants had increased in fruit kept 2 to 4 weeks at 0C without warming (Table 2) in patterns similar to those found in fruit at 0C in the previous experiment (Fig. 1). When fruit were transferred to 20C for 5 days, ethylene production and hexane-extracted compounds rose sharply (Table 2). The increase in ethylene production of fruit that were warmed after 2 weeks at 0C was much greater (135-fold) than that recorded at the other removal times (10-fold), and those for α-farnesene and antioxidants also were greatest at this time. In contrast, conjugated trienes increased the most (333-fold) during warming after 4 weeks at 0C.

During storage, small but significant increases in L*, a*, and b* values of fruit surfaces occurred (data not shown). L* and b* values did not change during warming, but a* values became less negative during warming after 2 to 6 weeks (data not shown). Correlations (n = 35) between increases in ethylene production and changes in a*/b* values during warming were not significant, indicating that color differences were not the direct result of differences in ethylene production.

After storage, fruit that had been warmed after 2 or more weeks at 0C contained more α-farnesene and more antioxidants than those that had been warmed without prior storage at 0C (Table 3). Fruit warmed after 2 to 8 weeks at 0C generally contained less conjugated trienes than those warmed without storage or after 10 or 12 weeks at 0C.

Warming reduced incidence and severity of scald (Table 4). Incidence was reduced the most when fruit were warmed after 2 weeks at 0C, and the effect generally declined with longer time at 0C before warming. Reduction of severity was the same when warming occurred any time between 2 and 8 weeks. Treatment with DPA produced results similar to those from warming except that treatment with DPA before storage reduced scald, and after 2 weeks, warming was more effective than DPA.

Subsequent experiments in 1992 tested effects of different times and temperatures of warming. Scald susceptibility was less than in 1991, with maximum incidence being 29% (Tables 5 and 6). In general, warming at 20C for 3 days or more gave nearly complete scald control (Table 5) and was about equally effective when applied after 1 to 4 weeks at 0C (Table 6). Response to

Table 6. Effects of time at 0C before warming and of warming temperature (10 or 20C) on 'Granny Smith' apples. Ethylene, α-farnesene, and conjugated trienes (CT) were measured before and after warming. After 25 weeks at 0 C fruit were transferred to 20C; firmness was measured after 1 day and disorders were recorded after 7 days.

Time at 0C before warming	Log ethylene production ($\mu l \cdot kg^{-1} \cdot h^{-1}$)			α-Farnesene ($nmol \cdot cm^{-2}$)			CT ($nmol \cdot cm^{-2}$)		
(weeks)	Before	After 10C	After 20C	Before	After 10C	After 20C	Before	After 10C	After 20C
0	–1.0	–1.4	–1.7	2.5	1.0	3.6	0.1	0.2	0.1
1	–1.6	0.1	0.8	0.8	17.1	24.2	0.1	0.4	0.4
2	–0.3	0.6	1.1	1.4	34.3	42.8	0.1	0.6	0.4
3	0.1	0.6	1.4	5.1	50.0	50.6	0.1	0.5	0.7
4	0.5	0.9	1.4	7.7	46.4	60.6	0.1	0.5	0.8
Significance									
Time before Warming (T)		***			***			***	
Linear		***			***			***	
Quadratic		***			***			NS	
Cubic		NS			***			NS	
Temp (°C)		***			***			***	
T × C		***			***			***	

[z]Severe coreflush only. Total coreflush was not significantly affected by treatments in this experiment.
[NS,*,**,***] Nonsignificant or significant at P 0.05,0.01, or 0.001, respectively.

warming at 10C was not significantly different from that at 20C (Table 6). Keeping fruit for increasing periods of time at 20C after 2 weeks of storage at 0C also reduced coreflush and flesh breakdown (Table 5). The extent of reduction of severe coreflush and breakdown was affected by time at 0C before warming (Table 6). Warming temperature did not affect reduction of severe coreflush, but 20C was slightly better than 10C for reducing breakdown (Table 6).

During warming, ethylene concentrations in fruit increased, more so at 20C than 10C, the increase being greater with longer time at 0C before warming (Table 6) and with longer warming time (Table 5). Both α-farnesene and conjugated trienes increased in fruit peel during warming, increases being greater with longer time at 0C before warming (Table 6), higher warming temperature (Table 6) and longer warming time (Table 5). At the end of storage, warming usually resulted in measurable decreases in fruit firmness if fruit were warmed for 5 or more days (Tables 5 and 6). Warming also increased incidence of greasy fruit (but not greasiness score) (Table 5) and caused some loss of green color (Table 7) at the end of storage.

Effects of conditioning. When fruit were conditioned at 10C before storage, no significant changes in α-farnesene, conjugated trienes or antioxidants occurred during 5 or 10 days and no measurable differences existed after 25 weeks at 0C (data not shown). Nearly all of these fruit developed severe scald after storage.

Discussion

The storage temperature and warming experiments provide clear evidence that superficial scald can be induced by chilling. Injury symptoms that are typical of scald occurred after prolonged storage of 'Granny Smith' at 0 or 4C, but not after storage at 10, 15, or 20C for times that produced generally equivalent changes in ground color. The slight injury observed at 10C, which was distinct from typical superficial scald, may be similar to that observed at 15C by Huelin and Coggiola (1970a). Amelioration of scald by warming (Tables 4, 5, and 6) is characteristic of fruit chilling injuries (Wang, 1993). Chilling injury is viewed as a two-stage event, where rapid damage-inducing events are separated from symptom development events which involve cellular degeneration (Raison and Orr, 1990). Effects of warming meet this criterion since warming altered chemical composition immediately (Tables 2, 5, and 6) but affected scald development many weeks later (Tables 4,5, and 6).

Warming clearly reversed an effect of low temperature, since keeping fruit at 10C for 5 or 10 days before storage did not reduce scald (data not shown), but warming at 20C after 14 (Tables 4 and 5) or 7 (Table 6) days at 0C did reduce it. More than 1 day at 20C was required for reversal (Table 5), and a temperature lower than 20C was effective, although a longer time might be required for consistent results at a lower temperature (Table 6). Smith (1959) used single 5-day warming periods at between 2 and 30 weeks at 0C for 'Bramley's Seedling' apples, and obtained maximum scald reduction from warming at 16 and 20 weeks. The timing of maximum effect of warming therefore was quite different from that on 'Granny Smith' (Table 4), so different cultivars may respond distinctly to warming regimes.

Incidence of coreflush, a recognized chilling disorder in apples (Bramlage and Meir, 1990), also was reduced by warming (Tables 5 and 6). Near elimination took a longer warming time than for scald (Table 5) and this was not as consistent a response as the effect on scald (Table 6). Breakdown of fruit was reduced to about the same extent as scald (Tables 5 and 6), so this disorder also may be chilling-related.

Within the α-farnesene hypothesis of scald development (Anet, 1972), treatments that inhibit scald should lower α-farnesene production, inhibit α-farnesene oxidation to conjugated trienes, or condition fruit tissues against injury by these products. In our study, higher storage temperatures and warming treatments consistently raised α-farnesene concentrations in fruit peel during treatment and at the end of storage, but reduced scald. This reinforces the view (Huelin and Coggiola, 1970b) that α-farnesene itself does not cause scald. α-Farnesene accumulation may have been promoted by the high ethylene concentrations that formed in fruit at higher temperatures (Watkins et al., 1993).

Conjugated trienes also increased during warming (Table 2). Du and Bramlage (1994) presented evidence that α-farnesene oxidation in apples produced scald-inducing and nonscald-inducing species of presumed conjugated trienes. Du (1993) also showed that 20C favored the noninducing species of conjugated trienes while 0C favored the inducing species. The conjugated trienes reported here, measured at 281 nm, are the presumed scald-

Firmness (N)		Scald (%)		Core-flush[z] (%)		Breakdown (%)	
10C	20C	10C	20C	10C	20C	10C	20C
75	76	27	26	29	31	16	14
76	74	13	0	25	32	11	3
75	76	8	2	12	10	4	3
72	74	6	5	32	9	3	0
72	73	4	3	24	14	10	1
$P < 0.07$		**		*		**	
*		**		*		**	
NS		*		NS		**	
NS		NS		NS		NS	
NS		NS		NS		*	
NS		NS		NS		NS	

Table 7. Effects of time at 0C before warming and of warming temperature on surface color of' Granny Smith' apples after storage for 25 weeks at 0C.

Time at 0C before warming	Warming temp	L*	a*	b*	a*/b*
0	10C	58.4	-0.194	0.399	-0.49
1		59.6	-0.195	0.410	-0.48
2		60.3	-0.199	0.418	-0.48
3		61.4	–0.204	0.424	4.47
4		60.5	-0.200	0.423	-0.48
0	20C	59.7	-0.198	0.411	-0.48
1		62.2	-0.197	0.440	4.45
2		61.7	-0.205	0.433	-0.47
3		62.2	-0.201	0.435	-0.46
4		61.4	-0.201	0.424	-0.48
Significance					
Time before warming (T)		***	***	***	**
Linear		***	***	***	NS
Quadratic		**	**	***	*
Cubic		NS	**	NS	*
Quartic		NS	NS	NS	*
Temp (°C)		*	NS	**	NS
T × C		NS	NS	**	***

[z]NS,*,**,***Nonsignificant or significant at P 0.05,0.01, or 0.001, respectively.

inducing species. In our experiments, warming did not reduce scald unless fruit were first stored at 0C (Tables 4 and 6). While the conjugated trienes increased during warming (Tables 2,5, and 6), they were lower at the end of storage (when scald was developing) in the warming treatments that produced the most scald reduction (Table 3). Thus, warming may have induced events that gradually suppressed accumulation of scald-inducing conjugated trienes during long-term storage. However, as time at 0C before warming increased the effect of warming on the induction on scald was diminished (Table 4). Furthermore, conjugated trienes at the end of storage were not suppressed (Table 3). This indicates that conjugated triene accumulation could not be suppressed after extensive exposure to 0C. DPA, which suppresses accumulation of the scald-inducing conjugated trienes (Du and Bramlage, 1994), also had diminishing effects on scald development as chilling time increased (Table 4).

High storage temperatures (Fig. 1 D) and warming during storage (Table 2) increased 0D 200 nm values of peel extracts. A number of antioxidants have absorption maxima near 200 nm (Anet, 1974), and these values correlated strongly with assays of lipid-soluble antioxidant activity of apple peel extracts (Meir and Bramlage, 1988). While the results suggest that higher temperatures and warming may have reduced the formation of conjugated trienes by increasing endogenous antioxidants, Barden and Bramlage (1994b) found that 0D 200 nm values can increase with fruit ripening without corresponding increases in the principal lipid-soluble antioxidants in fruit peel. Therefore, the 0D 200 nm values recorded in peel extracts may not be reliable indices of antioxidant concentrations.

Rather than simply modifying α-farnesene oxidation, warming also may have conditioned fruit tissue against damage from injurious products generated at low temperature. Antioxidants

such as DPA can maintain a high degree of fatty acid unsaturation in fruit during chilling (Wang and Baker, 1979), and synthesis of unsaturated fatty acids may occur during warming (Wang, 1993). Since benefits from both warming and DPA diminished as time at 0C before treatment increased (Table 4), increasing amounts of cell damage from longer periods of chilling may either have inhibited this conditioning or rendered it less beneficial. Higher metabolic activity during warming also may remove toxic or inhibitory substances that accumulate at low temperature, or replenish deficiencies that have occurred (Wang, 1993). Perhaps longer warming times are needed as preceding chilling time increases.

An interesting issue is raised by the different stimulations of ethylene production by warming after different periods at 0C (Table 2). After 2 weeks at 0C, ethylene production was still low but it was stimulated 135-fold during warming. At subsequent removals initial rates of ethylene production were much higher, but they increased only 10-fold during warming.α- Farnesene and conjugated triene accumulations, in proportion to ethylene production rates, were far greater at 0C than they were at 20C (Table 2). Ethylene-stimulated ripening and metabolism in the cold might result in metabolic imbalances that can lead to cell damage, and be different from that at higher temperatures which might condition fruit tissues against cell damage.

Conditioning fruit at 10C for up to 10 days before storage did not influence scald susceptibility, but during this time, no significant increase in internal ethylene concentration was observed. Initiation of the climacteric in 'Granny Smith' apples is delayed at higher temperatures (Jobling et al., 1991). We propose that if the climacteric had been initiated during the time at 10C, then scald may have been reduced by conditioning. Further experiments are in progress to test this hypothesis. However, the failure of conditioning to reduce scald (data not shown) shows clearly that postharvest conditioning at 10C is not equivalent to preharvest exposure to temperatures of 10C or below, in which as few as 100 h can reduce scald susceptibility of preclimacteric fruit (Barden and Bramlage, 1994a).

Warming maybe an effective nonchemical scald control method for 'Granny Smith' apples, and may have commercial implications for fruit that are not stored under controlled atmospheres. While some loss of fruit quality by ripening maybe a risk associated with warming (Tables 5, 6, and 7), some of our treatments greatly reduced scald with only minor effects on ground color or flesh firmness. Whether similar results occur for cultivars with higher rates of ethylene production remains to be determined.

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