Temperature Conditioning and Surface Treatments of Grapefruit Affect Expression of Chilling Injury and Gas Diffusion

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Abstract. 'Marsh' Grapefruit (*Citrus paradisi* Macf.) were temperature conditioned (7 days at 15C), wiped with hexane, treated with squalene, squalane, or safflower oil (all 10% in hexane), or waxed with a commercial fruit wax (Flavorseal) to determine their effects on weight loss, chilling injury (CI) symptoms on the peel, and gas exchange. Following 3 weeks of storage at SC, wiping fruit with hexane resulted in a significant decrease in weight loss, but not CI. Temperature conditioning and Flavorseal independently inhibited weight loss and CI development. Squalene inhibited CI development, but not weight loss. Chilling injury on fruit treated with squalene or Flavorseal was similar in appearance, but significantly less common than that on nontreated fruit. Grapefruit peel accounted for 92% of the gas diffusion of fruit, and resistance coefficients for peel and whole fruit were similar. Less ethane diffused into fruit that were: temperature-conditioned compared with nonconditioned, hexane wiped compared with nonhexane-wiped, and squalene-treated compared with nonsqualene treated fruit. Ethane influx was significantly restricted into squalane- and squalane-treated fruit compared with Flavorseal- or safflower oil-treated fruit. Oxygen and CO₂ influx was significantly reduced by Flavorseal, safflower oil, squalene, and squalane. Squalane was the most restrictive of ethylene efflux followed by safflower oil, squalene, and Flavorseal. All of these surface treatments are known to reduce CI on grapefruit. These data indicate that water loss is less important to the development of CI than has been previously suggested, and that the beneficial effects of squalene are not the result of an inhibition of water loss. Permeability of grapefruit peel to gases other than H₂O vapor may also influence the expression of CI.

Grapefruit, like many other tropical and subtropical fruits, develop chilling injury (CI) when stored at temperatures <10 to 12C (Chace et al., 1966; Grierson, 1974; Pantastico et al., 1968). High-temperature prestorage conditioning or curing treatments have been successful in preventing or reducing CI in many horticultural crops (Wang, 1990). Although temperature conditioning has been shown to reduce CI in many fruits and vegetables, little is known about its mode of action. Reports describe physical changes to the product as a result of temperature conditioning. The only research relating biochemical changes and the reduction of CI by temperature conditioning were polyamine changes in lemon [*Citrus limon* (L.) Burm.] (McDonald, 1986) and squash (*Cucurbita pepo* L.) (Kramer and Wang, 1990; McCollum et al., 1991) and lipid components of the epicuticular wax of grapefruit (Nordby and McDonald, 1990b, 1991).

It is generally accepted that conditions and treatments that reduce weight loss will reduce the incidence of CI (Wang, 1990). Raising the relative humidity to 100% reduced CI in limes and grapefruit (Pantastico et al., 1968). Moisture loss has been implicated as a causal factor in the CI of cucumber and pepper (*Capsicum annuum* L.) (Morris and Platenius, 1939). Purvis (1984) concluded that moisture loss during low-temperature storage of grapefruit was a contributing factor to CI symptom development.

Waxing has often been reported to decrease the expression of CI in grapefruit (Forney and Lipton, 1990) and to alter gas exchange

between the fruit and its environment (Hagenmaier and Shaw, 1992). The effects of waxing on reducing CI may be related to the waxing effect on permeability to O₂, CO₂, ethylene, or water vapor.

Nordby and McDonald (1990b) reported that squalene reduces CI on grapefruit, but like temperature conditioning, its mode of action is unknown. Squalane also was reported to reduce CI of grapefruit (Nordby and McDonald, 1990a). Safflower oil reduced CI of grapefruit (Aljuburi, 1982), but had no effect on CI symptom development on lemon (McDonald, 1986).

Thus, temperature conditioning and squalene or squalane may act in a physical manner (i.e., by increasing resistance to gas exchange) to reduce CI. Therefore, experiments were conducted to determine the effects of temperature conditioning, waxing, wiping with hexane, and squalene on weight loss, CI expression on the peel, and gas exchange in grapefruit. Because squalane and safflower oil also have been reported to reduce CI, the effects of these compounds and Flavorseal and hexane wiping on gas exchange were also determined. Ethane was used to measure the effectiveness of treatments as diffusion barriers because it is a nonrespiratory gas that provides a quantitative approach to the diffusion of ethylene and other gases in plant tissues.

Materials and Methods

Weight loss and chilling injury. Two experiments were conducted to determine the effects of temperature conditioning, wiping with hexane, or squalene on weight loss and CI. Freshly harvested 'Marsh' grapefruit were obtained from a commercial grove near Merritt Island, Fla., for three separate tests (replications) in October. On each occasion, 40-count fruit (10 ± 1.3 cm in diameter) were degreened with ethylene at $\approx 5 \mu\text{l-liter}^{-1}$ 30C, 90%

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Abbreviations: CI, chilling injury; R, resistance.

$\pm 2\%$ relative humidity (RH)] for 72 h. Following degreening, the fruit were washed with a nonfungicidal soap (Mold Strip 25; Fresh Mark Chemical, Ocoee, Fla.), surface-dried, and randomized into eight 40-fruit lots to test the effects of temperature conditioning, wiping with hexane, and squalene. In the hexane treatment, fruit were wiped for 15 sec with a paper towel soaked in hexane. Squalene [10% (w/v) in hexane] was applied with a chromatographic sprayer to give ≈ 15 mg/100 cm² per fruit. Four lots were stored 3 weeks at 5C and $86\% \pm 5\%$ RH, and four lots were temperature conditioned for 1 week at 15C and $90\% \pm 2\%$ RH before 3 weeks of storage at 5C.

Weight loss for temperature-conditioned fruit was the weight difference between the start of conditioning and after 3 weeks of storage at 5C, whereas weight loss for nonconditioned fruit was the difference in weight between 0 and 3 weeks of storage at 5C. Ten fruit were weighed individually to determine weight loss in each treatment. Following storage, the fruit were evaluated for visible symptoms of CI, i.e., pitting and rind scald. Fruit were classified into one of five CI categories, depending on the surface area affected, where 1 = 0%, 3 = below 5%, 5 = 5% to 25%, 7 = 26% to 50%, and 9 = more than 50%. A CI index was calculated by summing the products of the number of fruit in each category by the value of each category and then dividing this sum by the total number of fruit evaluated. Data for weight loss and CI were analyzed as a randomized block design with factorial treatments [($\pm$) temperature conditioning X ($\pm$) wiping with hexane X ($\pm$) squalene]] with three harvests (blocks) using the SAS analysis of variance (ANOVA) procedure (SAS Institute, 1988).

A 2×3 factorial experiment was conducted to compare the effects of temperature conditioning with squalene, a commercial wax (Flavorseal, FMC Corp., Lakeland, Fla.) applied with a commercial fruit waxer, and no surface treatment on weight loss and CI. Fruit were obtained, degreened, and washed as described above.

Resistance of fruit to gas diffusion. First-order rate constants and resistance coefficients for the diffusion of ethane through whole fruit, peel, and stem scar of grapefruit were determined according to Cameron and Yang (1982). Gas permeabilities of tomato (*Lycopersicon esculentum* Mill. cv. Sunny), obtained from a local market, were determined to verify if our results gave results similar to those of Cameron and Yang (1982). 'Marsh' grapefruit were harvested from trees in June. Individual fruit were placed in desiccators with a constant flow (500 ml·min⁻¹) of air containing ethane at 900 μ l·liter⁻¹ and kept for 3 h to attain fruit equilibrium.

Ethane influx. Freshly harvested 'Marsh' grapefruit were obtained from a commercial grove near Merritt Island, Fla., in March to determine the effects of temperature conditioning, wiping with hexane, and squalene on diffusion of ethane through their peels. Fruit were prepared for treatments the same as for the weight loss and CI experiments, except that degreening was not necessary. Individual fruit were placed in 1750-ml glass jars and purged with a constant flow (500 ml·min⁻¹) of air containing ethane at 900 μ l·liter⁻¹ (Saltveit and Dilley, 1977) for exactly 90 min. Fruit were then submersed in water and a partial vacuum applied to extract the internal gases (Beyer and Morgan, 1970); ethane analyses were conducted by standard gas chromatography methods. Statistical analysis was the same as for the weight loss and CI experiments.

In another experiment, safflower oil, squalene, or squalane (each 10% (w/v) in hexane), were applied with a chromatographic sprayer. Flavorseal was applied as stated above. A hexane spray was also included as a treatment. Ethane influx rates were determined as described above, 1 day after application and following 4 weeks of storage at 10C and $86\% \pm 5\%$ RH to determine if the

treatments were effective over time. The data were analyzed as a completely randomized design with four single-fruit replications.

Oxygen influx. Saffloweroil, squalene, squalane, and Flavorseal were applied as described above to 'Marsh' grapefruit harvested in March. Oxygen influx was determined at ≈ 24 C 1 day after application and following 4 weeks of storage at 10C and $86\% \pm 5\%$ RH. Individual fruit were placed into glass jars and purged with a constant flow (500 ml·min⁻¹) of 99.8% O₂ for exactly 7 min. The fruit were then submersed in water as described above, and an internal gas sample was analyzed for O₂ by standard gas chromatographic methods. Because initial O₂ concentrations depended on individual surface treatments, O₂ influx values obtained were normalized by subtraction of initial O₂ concentrations. Initial concentrations were determined by analyzing similarly treated fruit before O₂ influx. Resulting data were analyzed as a completely randomized design with four single-fruit replications.

Carbon dioxide influx. Surface treatments were applied, fruit were handled, and data analyzed as described above for O₂ influx determinations, with the exception that the fruit were subjected to an atmosphere of 40% CO₂ and 10% O₂ at 24C for exactly 15 min. Carbon dioxide influx values obtained were normalized by subtraction of initial CO₂ concentrations.

Ethylene efflux. 'Marsh' grapefruit, which had been stored at 5C for 9 weeks, beginning in mid-January, to induce CI and ethylene production (McCollum and McDonald, 1992), were warmed in 20C air. Safflower oil, squalene, and squalane [all 10% (w/v) in hexane] were applied with a chromatographic sprayer and the commercial wax, Flavorseal, was applied as above for studying ethylene accumulation in grapefruit. The calyxes on fruit were removed, stopcock grease was applied to the stem scars, and gas chromatograph septums were placed on top of the grease. After 18 h at 20C, ≈ 5 ml internal gas samples were removed through the septums with a 10-ml syringe. A syringe was then used to obtain a 1-ml sample, and ethylene analyses were conducted using standard gas chromatographic procedures. The experiment was a completely randomized design with four single-fruit replications.

Results and Discussion

Weight loss and chilling injury. Following 3 weeks of storage at 5C, weight loss and CI were significantly inhibited in temperature-conditioned compared with nonconditioned fruit (Table 1, Figs. 1 and 2). Wiping with hexane resulted in a significant decrease in weight loss, but not CI. Squalene significantly inhibited CI, but not weight loss when compared with nontreated fruit. Temperature conditioning may decrease CI by inhibiting water loss. Purvis (1984) concluded that moisture loss during low-temperature storage of grapefruit appeared to be a contributing factor to CI symptom development. However, squalene reduced CI without reducing weight loss, and wiping with hexane had the unexpected effect of decreasing weight loss, but had no effect on CI development. Albrigo (1972) and Schulman and Monselise (1970) found that removal of the natural wax from citrus fruits increased water loss. However, both studies used wax-removal procedures that were much harsher than that used in this study. Analysis of the epicuticular wax remaining on the grapefruit after the hexane wiping treatment revealed that about one-fourth of the wax was removed (data not shown). The hexane wiping removed the major portion of the wax alkanes, a substantial amount of the long chain aldehydes C₂₃-C₃₄, and a small amount of the various terpenoids (data not shown). Wiping with hexane may have redistributed the remaining wax on fruit surfaces to effectively block water vapor diffusion. The role that water loss plays in the

Table 1. Analysis of variance of a $2 \times 2 \times 2$ factorial experiment examining temperature conditioning, wiping with hexane, and squalene coating on weight loss, chilling injury, and ethane influx into 'Marsh' grapefruit after 3 weeks at 5C and 86% $\pm$ 5% RH.

Source	df	Mean squares		
		Wt loss	Chilling injury index	Ethane influx
Replication	2	0.08	0.37	129
Conditioning (C)	1	1.88***	5.95**	1,725**
Wiping (W)	1	1.79***	1.47	871*
Squalene (S)	1	0.01	3.72**	28,262***
C $\times$ W	1	0.01	0.78	675*
C $\times$ S	1	0.02	5.85**	1,023**
W $\times$ S	1	0.15	0.21	371
C $\times$ W $\times$ S	1	0.01	1.14	693*
Error	14	0.04	0.41	2,623

***Significant at P = 0.05, 0.01, or 0.001, respectively.

development of CI symptoms is not clear from these data, but the beneficial effects of squalene appear not to be the result of an inhibition of water loss.

Weight loss was significantly reduced by Flavorseal wax compared with the squalene treatment, but absolute values among the treatments were similar (Table 2). However, Flavorseal wax and squalene were equally effective in reducing CI. The effects of temperature conditioning and Flavorseal wax or squalene application were not synergistic (no interaction detected). Although Flavorseal wax may have been expected to decrease weight loss and CI, this is the first report of temperature conditioning reducing weight loss, and its ability to reduce CI may be related to gas exchange in the fruit. The temperature-conditioned fruit lost less weight even though they were kept longer. Total holding time for temperature-conditioned fruit was 4 weeks (1 week at 15C + 3 weeks at 5C) compared with 3 weeks (5C) for nontemperature-conditioned fruit. Therefore, while temperature conditioning appears to affect CI through permeability of water vapor, squalene acts in a different but unknown manner.

Resistance of fruit to gas diffusion. Cameron and Yang (1982) reported that $\approx$ 97% of gas exchange of a tomato fruit occurs through the stem scar, with the remainder occurring through the

skin. Using their methodology, we found that first-order rate constants for grapefruit peel and whole fruit were similar, as well as constants for tomato stem scars and whole fruit (Table 3). Most (92%) of the diffusion of gases occurred through the peel of grapefruit, whereas most (92%) of the diffusion of gases took place through the stem scar of tomatoes. Resistance of grapefruit peel to gas diffusion was $\approx$ 75-fold less compared with tomato skin, although the resistance coefficients (R) of grapefruit and tomato stem scars were similar. The resistance coefficient of grapefruit stem scars is less important to whole fruit gas exchange because only 0.3% of a grapefruit peel surface is stem scar, whereas 3.8% of a tomato fruit surface is stem scar.

To decrease gas exchange in tomato, it would be necessary to block the stem scar. However, to decrease gas exchange of a grapefruit, it would be more important to reduce the permeability of the peel. This conclusion indicates that treatments that decrease the permeability of grapefruit peel to gas diffusion, as squalene or temperature conditioning do, could play a role in reducing CI.

Ethane influx. It was not possible to apply a surface treatment such as squalene before "loading" the fruit with a noncondensable gas, such as ethane, to determine resistance coefficients because the restrictive nature of the treatment prevented it. It was possible, however, to develop influx values for ethane and respiratory gases to study the effects of treatments as diffusion barriers.

Fig. 1. Effect of temperature conditioning, wiping with hexane, and squalene on weight loss in 'Marsh' grapefruit stored for 3 weeks at 5C. Vertical bars represent SE of the means.

Fig. 2. Effect of temperature conditioning, wiping with hexane, and squalene on chilling injury on 'Marsh' grapefruit stored for 3 weeks at 5C. Vertical bars represent SE of the means.

Table 2. Effects of surface treatments on weight loss and chilling injury of 'Marsh' grapefruit after 3 weeks of storage at 5C and 86% $\pm$ 5% RH.

Surface treatment	Wt loss (%) ²	Chilling injury index
None	1.0 a	2.0 a
Flavorseal wax	0.7 b	1.3 b
Squalene	1.2 a	1.4 b

		Mean squares	
Source	df	Wt loss	Chilling injury index
Replication	2	0.02	0.01
Conditioning (C)	1	0.34***	1.33*
Treatment (T)	2	0.44**	0.85*
C $\times$ T	2	0.04	0.49
Error	10	0.04	0.19

²Values in a column followed by different letters are different at $P = 0.05$.

***, **, *Significant at $P = 0.05$, 0.01, or 0.001, respectively.

Ethane influx was significantly greater in fruit that were: nonconditioned compared with temperature-conditioned, nonhexane-wiped compared with hexane-wiped, and nonsqualene-treated compared with squalene-treated (Fig. 3, Table 1). Although temperature conditioning and wiping with hexane had an effect on reducing the permeability of grapefruit peel to ethane, the effect of squalene was much greater. Temperature conditioning increased R by >3-fold and squalene increased it by $\approx$ 19-fold (data not shown). Wiping with hexane had no effect on R. Apparently, applying squalene to the surface of fruit creates a barrier that effectively reduces the permeability of the peel to ethane.

The amount of ethane that diffused into grapefruit treated with various surface coatings is expressed as a percentage of ethane in the "loading" atmosphere (900 μ l·liter⁻¹), in Fig. 4. One day following application, control, hexane spray, and Flavorseal-treated fruit had the least resistance (highest percentage) to ethane influx (Fig. 4A). However, ethane influx was significantly restricted by safflower oil, squalene, and squalane.

Ethane influx was slower in all treatments following 4 weeks of

storage at 10C (Fig. 4B) compared with determinations made 1 day following application (Fig. 4A). Squalene, safflower oil, and squalane had significantly higher resistance to ethane influx than Flavorseal, hexane spray, and control fruit (Fig. 4B).

Oxygen influx. Safflower oil, squalene, squalane, and Flavorseal significantly increased resistance to O₂ influx compared with nonconditioned and hexane spray-treated fruit 1 day following

Fig. 3. Effect of temperature conditioning, wiping with hexane, and squalene on ethane influx of 'Marsh' grapefruit stored for 3 weeks at 5C. Vertical bars represent SE of the means.

Fig. 4. Effect of surface treatments on ethane influx of 'Marsh' grapefruit 1 day following treatment (A) and after 4 weeks at 10C (B). Letters indicate mean separation by Duncan's multiple range test, $P = 0.05$.

Table 3. Diffusion of ethane through grapefruit and tomato fruits.

Fruit	K^2 ($s^{-1} \times 10^{-5}$)	Diffusion contribution ² (%)	R ($s \times cm^{-1} \times 10^3$)
Grapefruit			
Peel	118.6	91.7	2.2
Stem scar	10.8	8.3	0.2
Whole fruit	129.4	100	2.2
Tomato			
Skin	5.8	7.7	164.6
Stem scar	69.6	92.3	0.5
Whole fruit	75.4	100	12.5

²Fruit-order rate constants (k) for stem scars were calculated by subtraction.

²Percent diffusion contribution and resistance coefficients (R) were calculated from k.

application (Fig. 5). Although safflower oil was as effective as Flavorseal, squalene, and squalane initially, the latter three compounds were significantly better than the oil at increasing resistance to O_2 influx following 4 weeks of storage at 10C. As was the case with ethane influx, O_2 influx was also reduced over time regardless of surface treatment. The increase in resistance to gaseous diffusion in all treatments and controls may have been due to peel dehydration during storage (Ben-Yehoshua, 1967, 1969).

Carbon dioxide influx. It was not possible to "load" grapefruit previously treated with various surface coatings with similar

amounts of CO_2 to study CO_2 efflux. Therefore, CO_2 influx was considered as a measure of resistance to CO_2 diffusion.

Resistance to CO_2 influx was significantly increased by Flavorseal, safflower oil, squalene, and squalane initially, and after 4 weeks of storage at 10C (Fig. 6). Squalene and squalane significantly increased resistance to CO_2 influx, both initially and following storage, compared with Flavorseal and safflower oil.

In general, the surface treatments increased the CO_2 content and decreased the O_2 content of the internal atmosphere of grapefruit. The degree of change depended upon the coating used and the

Fig. 5. Effect of surface treatments on O_2 influx of 'Marsh' grapefruit 1 day following treatment (A) and after 4 weeks at 10C (B). Letters indicate mean separation by Duncan's multiple range test, $P = 0.05$.

Fig. 6. Effect of surface treatments on CO_2 influx of 'Marsh' grapefruit 1 day following treatment (A) and after 4 weeks at 10C (B). Letters indicate mean separation by Duncan's multiple range test, $P = 0.05$.

Table 4. Effect of surface treatments on the resistance of 'Marsh' grapefruit to ethane, O₂, and CO₂.

Gas	Resistance ^c (s × cm ⁻¹ × 10 ³)					
	Control	Hexane spray	Flavorseal	Squalene	Safflower oil	Squalane
Ethane	5.9	5.8	10.1	62.5	19.1	115.0
O ₂	1.4	1.7	45.0	29.0	21.9	42.8
CO ₂	2.6	2.3	14.1	43.6	14.1	72.1

^aComputations based on data in panel A of Figs. 4, 5, and 6 assuming that the fruit were uniform in internal volumes and surface areas.

length of storage. These results support earlier work with oranges [*Citrus sinensis* (L.) Osb.] where the degree of change depended upon the wax used (Davis et al., 1967; Eaks and Ludi, 1960).

Ethylene efflux. Treatment of grapefruit with squalane, safflower oil, or squalene resulted in considerably greater ethylene retention than treatment with Flavorseal, sprayed with hexane, or none of them (control) (Fig. 7). The flavedo and the outer cuticle of orange fruit were found to be important structures in directing the diffusion of endogenously produced ethylene, thereby increasing ethylene accumulation in the fruit (Barmore and Biggs, 1972). Thus, the surface treatments used in this study, particularly squalane, safflower oil, and squalene, effectively enhanced the outer peel in further reducing diffusion of endogenously produced ethylene.

Generally, the order of effectiveness of the surface coatings as barriers to gas diffusion in this study was squalane > squalene > safflower oil > Flavorseal. Squalane is a completely saturated hydrocarbon; safflower oil is intermediate in saturation; squalene is a highly unsaturated hydrocarbon; and Flavorseal wax is composed of a mixture of aliphatic and aromatic hydrocarbons. Because squalane and squalene differ only in their degree of saturation, it appears that more highly saturated molecules make more effective diffusion barriers.

The effect of the surface treatments on the resistance of grapefruit to ethane, O₂, and CO₂ is presented in Table 4. These computations are based on data in Figs. 4-6. Waxing with Flavorseal and the coatings of safflower oil, squalene, and squalane all increased the resistance to the noncondensable gases. The latter two compounds were more effective as gas diffusion barriers than the former two. Waxing and sealing citrus fruit in plastic films has been reported by Ben-Yehoshua et al. (1985) to increase their

resistance to ethylene, CO₂, and O₂ diffusion.

Since squalene was shown to decrease CI without reducing moisture loss, it may be that the beneficial effects of squalene and squalane, and, to some extent safflower oil, are due to the creation of a barrier that hinders O₂ and CO₂ diffusion. The effectiveness of modified atmospheres in reducing CI varies with commodity, O₂ and CO₂ concentrations, and storage temperature (Wang, 1982). Beneficial effects of low O₂ and high CO₂ to reduce the development of CI in zucchini squash have been reported (Mencarelli et al., 1983; Mencarelli, 1987; Wang and Ji, 1989). Spalding and Reeder (1975) found atmospheres of low O₂ and high CO₂ to be effective in reducing CI in avocados (*Persea americana* Mill.).

We do not know why Flavorseal did not reduce the permeability of the fruit surfaces to noncondensable gases as well as the other surface treatments. Coatings are known to differ substantially in their permeability to noncondensable gases and water vapor (Hagenmaier and Shaw, 1992). The amount of solids of squalane, squalene, and safflower oil applied to grapefruit averaged 15 mg/100 cm² and that of Flavorseal averaged 19 mg/100 cm². This small difference suggests that it is the form that the material takes on the fruit surface and not the quantity of material per unit of fruit area that affects the permeability of fruit peels.

In summary, temperature conditioning appears to act in reducing CI on grapefruit by restricting moisture loss, while squalene acts to reduce CI in another manner. Safflower oil and the terpenes squalane and squalene were found to be effective barriers to gas exchange when applied to grapefruit. These coatings may work in this manner to reduce CI, and all may be used on grapefruit.

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Fig. 7. Effect of surface treatments on ethylene efflux of 'Marsh' grapefruit. Letters indicate mean separation by Duncan's multiple range test, $P = 0.05$.

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