Epicuticular Wax Morphology and Composition are Related to Grapefruit Chilling Injury

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Abstract. Differences in chilling injury (CI) susceptibility between 'Marsh' grapefruit (*Citrus paradisi* Macf.) from interior and exterior tree canopy positions were analyzed to investigate the hypothesis that epicuticular wax morphology and composition influence CI development during low-temperature storage. The sun-exposed surface of fruit from the exterior canopy had significantly more CI and larger wax platelets than the shaded surface of the same fruit. Interior canopy fruit had significantly less CI and smaller wax platelets than exterior canopy fruit. Hydrocarbons, primarily *n*-alkanes, were significantly more abundant in the epicuticular wax on the surfaces of sun-exposed and exterior fruit compared with surfaces of shaded and interior fruit, respectively. Results of this study suggest that epicuticular wax plays a role in the development of external CI symptoms on grapefruit.

Grapefruit, like many other tropical and subtropical fruit, develop chilling injury (CI) when stored at temperatures below 10 to 12°C. Conditions and treatments that reduce weight loss (e.g., high relative humidity, waxing) will reduce CI incidence (Wang, 1990).

Citrus and other fruit are covered with several layers of hydrophobic material, the outermost being epicuticular wax. Epicuticular waxes have increased cuticular resistance and reduced transpiration rates (Bain and McBean, 1967; Possingham et al., 1967) and reduced postharvest weight loss in 'Valencia' oranges [*Citrus sinensis* (L.) Osbeck] (Albrigo, 1972a). Additionally, Albrigo (1972a, 1972b, 1972c) related morphology and quantitative epicuticular wax changes to rind disorders and fruit quality deterioration in 'Valencia' oranges.

The chemical composition of citrus fruit epicuticular wax has been determined in several studies (Baker and Procopiou, 1975; Baker et al., 1975; El-Otmani and Coggins, 1985; Freeman et al., 1979). The major components of this wax are aldehydes, hydrocarbons, fatty acids, and primary alcohols. Nordby and McDonald (1990, 1991) related increased squalene, C_{23} to C_{25} *n*-alkanes, and C_{28} to C_{34} *n*-

aldehydes in epicuticular wax to reduced grapefruit CI.

Grapefruit from the sun-exposed exterior canopy are more susceptible to CI than those from the shaded interior canopy (Purvis, 1980). Furthermore, the sun-exposed side of exterior canopy fruit is more susceptible than the shaded side (Purvis, 1984). Thus, the morphology and composition of grapefruit epicuticular wax may reduce CI symptom development. The objective of this study was to investigate the role of epicuticular wax in CI symptom development on grapefruit peel. The approach taken used the difference in CI susceptibility in fruit from interior vs. exterior and shaded vs. sun-exposed canopy positions.

'Marsh' grapefruit were harvested from interior and exterior canopy positions of five trees (orientation of exterior fruit noted), hand-washed with tap water, stored 5 weeks at 5°C and 80% to 92% relative humidity, and evaluated for CI development. Thirty-two fruit were harvested from each canopy position of each tree. Depending on the percentage of surface area affected, fruit were classified in one of five CI categories, where 1 is 0%, 3 is $\geq 1\%$ to $\leq 5\%$, 5 is $\geq 5\%$ to $\leq 25\%$, 7 is $\geq 25\%$ to $\leq 50\%$, and 9 is $\geq 50\%$ to $\leq 100\%$. A CI index was calculated from these classifications.

For wax morphology studies, additional fruit were obtained and hand-washed with tap

water. Preliminary studies showed that gentle hand-washing did not disturb the epicuticular wax but removed foreign material that obstructed our view of fruit surfaces. Three samples of the fruit flavedo (≈ 0.25 mm thick) from each of five fruit from different trees and each canopy position and orientation were removed using a razor blade. A 5-mm cork borer was used to excise discs, which were attached to aluminum mounts with double-sided adhesive tape, frozen in liquid N_2 , and freeze-dried. Peel disks were isolated from fruit before and after 5 weeks of storage at 5°C, sputter-coated with 80 gold : 20 palladium, and examined with a Hitachi (Hitachi, Ibaraki, Japan) S-530 scanning electron microscope.

After harvest, epicuticular wax was isolated from each of five fruit from interior and exterior canopy positions. Individual fruit were placed in a porcelain crucible and swirled in 100 ml chloroform for 2 min. Each fruit then was rinsed with 25 ml chloroform, and the combined extracts were reduced to near dryness on a rotary evaporator at 30°C under reduced pressure. Sun-exposed and shaded surfaces of five fruit were bathed separately in chloroform to isolate their wax. Cholestane ($C_{27}H_{48}$) was added as an internal standard, and the extracts were made up to 5 ml with chloroform. A 1.0 μ l aliquot was injected into a gas-liquid chromatograph on a 0.75 mm $\times$ 30 m $\times$ 1 μ m Supelcowax SPB-1 column (Supelco, Bellefonte, Pa.) to separate the wax components. Thirty-four wax components were quantified by the internal standard. These components had been identified previously by Nordby and McDonald (1991) as belonging to four lipid classes: hydrocarbons, *n*-alcohols, *n*-aldehydes, and terpenoids (compounds derived from isoprenes). The oven temperature was kept at 250°C for 8 min, increased 2°C/min to 265°C, and kept there for 30 min. Injection port and flame ionization detector temperatures were 300°C, and the carrier gas (helium) flow rate was 18 ml·min⁻¹. Major wax components are expressed as weight percentage of total. All data were analyzed as a completely randomized design using SAS's analysis of variance procedure (SAS Institute, 1988).

Interior canopy fruit had significantly less CI than exterior canopy fruit (Table 1). Similarly, shaded surfaces of fruit from the exterior canopy had significantly less CI than sun-exposed surfaces; this result confirmed the findings of Purvis (1980). Rindstaining, an important peel disorder of California 'Valencia' oranges, was more severe on exposed surfaces (El-Otmani et al., 1989).

Table 1. Chilling injury (CI) index and epicuticular wax composition of 'Marsh' grapefruit harvested from interior and exterior tree canopy positions.

Fruit location	CI index	Wax components (% of total wax)			
		Hydrocarbons	Aldehydes	Alcohols	Terpenoids
Interior canopy	1.3	3.8	39.2	2.2	54.8
Exterior canopy	4.0	6.2	42.9	2.0	48.8
Significance	***	*	NS	NS	NS
Sun-exposed	6.6	18.2	34.7	3.2	43.9
Shaded	1.4	9.7	38.7	2.9	48.8
Significance	**	*	NS	NS	NS

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

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Average wax platelet size on the peel of interior canopy fruit (Fig. 1A) and on the shaded portion of exterior fruit (Fig. 1B) were similar and smaller than on the sun-exposed portion of exterior fruit (Fig. 1C). Platelet size and shape did not change during 5 weeks of storage at 5C. Thus, larger platelet size was associated with greater CI development. However, with rindstaining, El-Otmani et al. (1989) reported that the sun-exposed side of fruit with no blemish had "crusty" plates, the fruit side with moderate rindstaining had fewer plates and a more amorphous wax structure, and fruit with severe rindstaining had no plates and amorphous wax.

The total mass of wax extracted was not related statistically to canopy position; however, wax composition varied with canopy position and orientation. Components of the epicuticular wax, in decreasing order of abundance, were terpenoids, aldehydes, hydrocarbons, and alcohols. Hydrocarbons, primarily *n*-alkanes, were significantly less abundant in the epicuticular wax on shaded and interior fruit surfaces than on sun-exposed and exterior fruit surfaces, respectively (Table 1). The *n*-alkane fraction also was higher in the epicuticular wax from sun-exposed than from shaded 'Valencia' oranges (El-Otmani et al., 1987). El-Otmani et al. (1987) suggested that fruit maturation and senescence are accompanied by relative and absolute increases in epicuticular wax *n*-alkanes.

In previous work, we observed that squalene was present in trace amounts in the epicuticular wax of freshly harvested fruit or in fruit stored at 5C (Nordby and McDonald, 1990, 1991). Large squalene increases were found only after temperature conditioning at 15C. In this study, we found squalene in trace amounts in interior and exterior canopy fruit; there were no relative differences between canopy positions.

The data from this study indicate that the morphology and composition of crystalline wax are influenced strongly by canopy position. The exposed side of a grapefruit reaches higher temperatures when exposed to sunlight than does the shaded side (Syvertsen and Albrigo, 1980). El-Otmani et al. (1989) suggested that the rind of the more-exposed side of fruit ages more rapidly than rind on the shaded side of the same fruit, and that fruit exhibiting rindstaining display more advanced senescence. Hence, the greater susceptibility of the exposed side to CI may be the consequence of accelerated senescence.

The results of this study also indicate that there is an optimal wax component ratio that confers greater CI tolerance. Present and pre-

Fig. 1. Epicuticular wax deposits on the surface of (A) interior canopy, (B) exterior canopy shaded, and (C) sun-exposed 'Marsh' grapefruit.

vious data (Nordby and McDonald, 1990, 1991) suggest that composition and morphology of grapefruit epicuticular wax influence CI development, possibly by restricting gas exchange. However, differences in epicuticular wax and grapefruit CI susceptibility could be related to senescence, but not necessarily to each other. Although these data are related to external CI symptom development, additional work is needed to determine the underlying cause of CI.

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