

# Resistance of Citrus Fruit to Mass Transport of Water Vapor and Other Gases<sup>1</sup>

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## ABSTRACT

The resistance of oranges (*Citrus sinensis* L. Osbeck) and grapefruit (*Citrus paradisi* Macf.) to ethylene, O<sub>2</sub>, CO<sub>2</sub>, and H<sub>2</sub>O mass transport was investigated anatomically with scanning electron microscope and physiologically by gas exchange measurements at steady state. The resistance of untreated fruit to water vapor is far less than to ethylene, CO<sub>2</sub> and O<sub>2</sub>. Waxing partially or completely plugs stomatal pores and forms an intermittent cracked layer over the surface of fruit, restricting transport of ethylene, O<sub>2</sub>, and CO<sub>2</sub>, but not of water; whereas individual sealing of fruit with high density polyethylene films reduces water transport by 90% without substantially inhibiting gas exchange.

Stomata of harvested citrus fruits are essentially closed. However, ethylene, O<sub>2</sub> and CO<sub>2</sub> still diffuse mainly through the residual stomatal opening where the relative transport resistance (approximately 6,000 seconds per centimeter) depends on the relative diffusivity of each gas in air. Water moves preferentially by a different pathway, probably through a liquid aqueous phase in the cuticle where water conductance is 60-fold greater. Other gases are constrained from using this pathway because their diffusivity in liquid water is 10<sup>4</sup>-fold less than in air.

The commercial practice of waxing fruits inadequately reduces transpiration, and yet it is so effective in restricting O<sub>2</sub> and CO<sub>2</sub> transport that off-flavors sometimes result (4–7). Conversely, sealing fruits individually in HDPE<sup>4</sup> film reduces water loss 10-fold without changing the fruit's endogenous O<sub>2</sub>, CO<sub>2</sub>, or ethylene content (6). Consequently, seal-packaging is more effective than waxing in preventing shrinkage and in extending the storage life of citrus and certain other fruits (6, 7, 10, 20).

The different effects which seal-packaging with a plastic film 10 μm in thickness, and waxing, which forms a noncontinuous membrane 1 μm in thickness, have on gas exchange are not easily explained by previous morphological studies concerning the distribution of applied waxes (4, 7, 8, 31), nor by most current theories of gas mass transport in citrus and other fruits. Opinions differ widely concerning the relative contributions of the various mechanisms proposed to account for gas exchange in harvested fruits, although usually it is tacitly assumed that water and gases move by the same pathway. Air-filled stomata



FIG. 1. SEM view of fruit surface of untreated grapefruit showing at × 1600 a stoma, wax platelets, and other irregular bodies of natural wax deposited over the cuticular surface.

and lenticels (12), the air-filled stem scar (11, 12, 15, 16), and air-filled spaces between the epidermal cells (19), all have been suggested as transport routes. Other workers claim that the stomata and lenticels are nonfunctional, closed, or occluded, so that gases and water move mainly through the cuticle (2, 3, 15, 30), constrained by the properties of the hydrated cuticular polymer (28, 29) or, in addition, by the resistance of the air-filled spaces that separate the epicuticular wax platelets (17). Alternatively, it has been suggested that CO<sub>2</sub> moves through the skin and O<sub>2</sub> through the lenticels of fruits (23), and more recently Burg and Kosson (14) proposed that water moves preferentially through a liquid water phase, whereas gases move through air-filled pores.

The present study uses a SEM and gas exchange measurements to investigate the mechanisms of exchange of gases and liquids with seal-packaging and waxing of citrus fruits. The results are

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<sup>4</sup> Abbreviations: HDPE, high density polyethylene; SEM, scanning electron microscope.

discussed in relation to the hypothesis that water and gas move by different pathways in harvested fruits.

### MATERIALS AND METHODS

For SEM observations, grapefruit (*Citrus paradisi* Macf., cvs Marsh and Duncan) and oranges (*C. sinensis* L. Osbeck, cvs Shamouti, Valencia, and Hamlin) were either left untreated or were treated by washing, disinfecting with  $1,000 \mu\text{L}^{-1}$  aqueous thiabendazole, drying, and waxing with FMC<sup>®5</sup> solvent wax. This coating is a coumarone indene resin (a polymerization product of crude heavy coal tar of naphtha) dissolved in petroleum base solvent. Samples of both waxed and untreated fruit were sealed in HDPE film, 10  $\mu\text{m}$  in thickness, using a sealer manufactured by Weldotron<sup>®</sup> (6). For SEM observations, fruit was stored at 21°C, 90% RH. Samples were prepared for SEM observations using the method of Albrigo *et al.* (2, 3). Thin, tangential peel strips for observation with the SEM were removed from the fruit and dried in closed Petri dishes. The peel oil caused the slices to adhere to the disposable plastic Petri dishes. Smaller sections (2 × 2 mm) were usually cut from the dried sections for mounting and coating with Gold-Palladium (60% Au) on a rotating stage. The orange peel surfaces were observed with a Cambridge Stereoscan SEM by directing the electron beam toward the sample

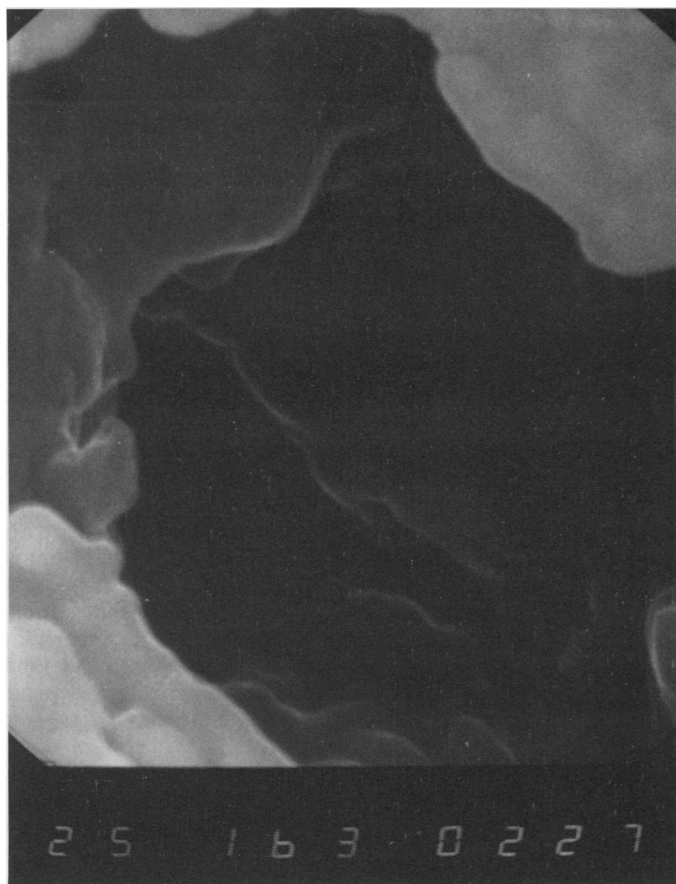


FIG. 2. Stomatal pore. A greater magnification ( $\times 10,000$ ) probes deeply into the pore, showing the partial opening of the stoma between the two guard cells, the various protrusions into the pore, as well as the cuticular walls at the surface opening of the pore.

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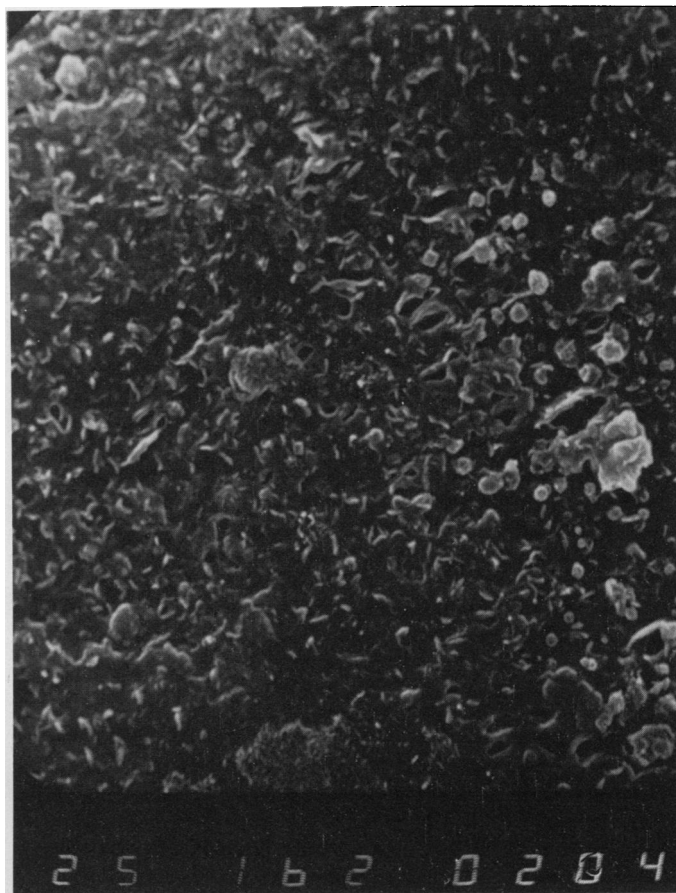


FIG. 3. The wax surface formed after waxing with FMC solvent wax.

surface at a 45° angle. Distribution of the wax was observed using sections 3 mm wide and approximately 1 cm long, measured from the button. Four sections from each of five fruits were dried and six sections were selected at random and mounted for observation with the SEM. Other sections were fixed at 4°C in 5% aeration for 24 h, followed by 1% osmium tetroxide for 24 h and then rinsed in H<sub>2</sub>O for 24 h. The flavedo was then removed, air-dried, and coated.

Ethylene and CO<sub>2</sub> production were measured by GC after enclosing both sealed and nonsealed fruits in closed jars for a limited time. It was previously established that such a brief exposure does not affect the rate of CO<sub>2</sub> or ethylene production (5, 9). The internal atmosphere was sampled by inserting the needle of a 5-ml syringe into the central space of the orange; both were immersed in water, the sample withdrawn and O<sub>2</sub>, CO<sub>2</sub>, and ethylene assayed by GC (1, 8, 9, 10, 13).

To analyze acetaldehyde + ethanol, vials containing 10 ml of juice obtained from five fruits were sealed with rubber serum caps and rotated at 35°C for 1 h in a water bath. Acetaldehyde + ethanol were detected in the head space by GC using a flame ionization detector at 180°C with a 20% Carbo wax 20 M column, carrier N<sub>2</sub>, injection temperature 110°C, and oven temperature 80°C (18).

Rate of weight loss was measured by weighing 10 different fruits during the second day after harvest. Water loss was calculated by subtracting the carbon loss in the respiratory activity.

Gas transport resistance was computed from the expression for mass transfer:

$$J = (P_i - P_a) A_i / R_D T r$$

where  $P_i$  and  $P_a$  are the steady state gas partial pressures (atm) in the intercellular spaces and in the ambient atmosphere a

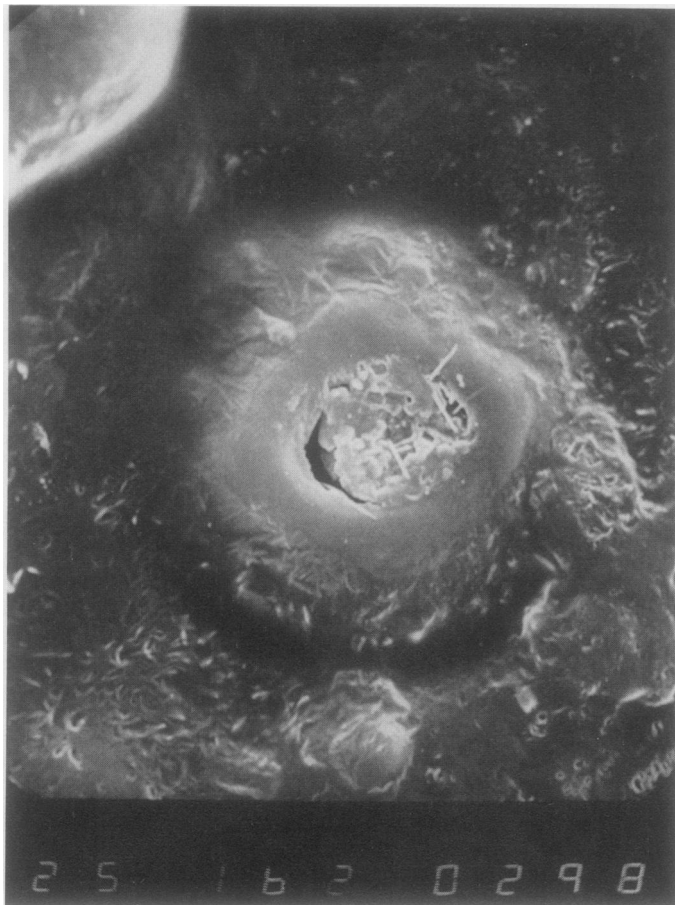


FIG. 4. Stomatal pore partially clogged by the applied wax,  $\times 1600$ .

substantial distance from the cuticular surface, respectively;  $A_t$  is the fruit's surface area ( $\text{cm}^2$ );  $R_D$  is the gas constant per gram in  $\text{atm} \cdot \text{cm}^3 \cdot \text{K}^{-1}$ ;  $T$  is the absolute temperature;  $r$  is the resistance ( $\text{s cm}^{-1}$ ); and  $J$  is the flux ( $\text{g s}^{-1}$ ) per  $\text{cm}^2$  of surface. Citrus meet the prerequisite for such a computation, that the intercellular resistance must be insignificant compared with the surface resistance (5, 12).

## RESULTS AND DISCUSSION

**SEM Observations.** The SEM micrographs of untreated fruits confirm reports (2) that the cuticular surface is pebbled and covered with wax platelets and other irregular shapes. A notable external feature of the stomata is the raised cuticular ridges which surround the opening of the outer vestibular chamber (Fig. 1). Probing into the pore of this chamber with a  $\times 10,000$  magnification, the stomatal aperture is often seen to be closed, but occasionally (Fig. 2) an open split is observed between two guard cells. The pore of the upper vestibular chamber often is partially clogged by foreign objects or by natural wax protrusions (2, 3, 32). Commercial waxing results in a new surface layer with a different structure compared with the natural extracuticular wax platelets (Figs. 3–5). The new layer has many pits and cracks (Fig. 3). The commercial wax, presumably because it flows into the pore as a liquid, is far more effective than the natural extracuticular wax and other occlusions in clogging the opening both of the upper vestibular chamber and the stoma itself. Figures 4 and 5 show the clogging of the outer stomatal (vestibular) pore by the newly applied wax layer, recognizable by small, characteristic pits.

**Resistance to Gas and Water Transport.** The parameters needed to compute the resistance of fruit to water,  $\text{CO}_2$ ,  $\text{O}_2$ , and

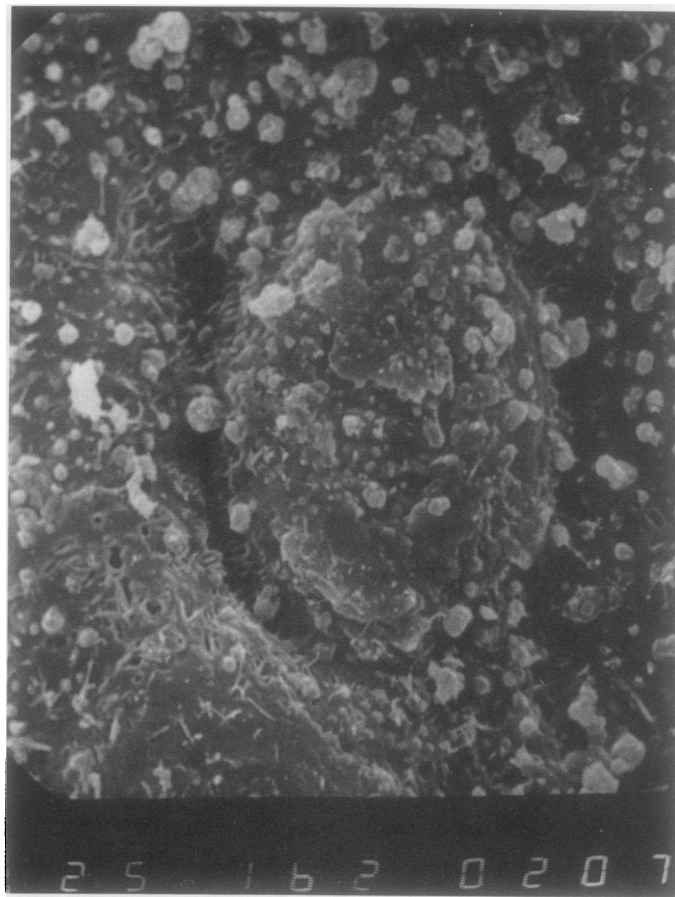


FIG. 5. Stomatal pore completely covered by the new wax layer,  $\times 1600$ .

ethylene transport were measured for Valencia oranges (Table I; 4–9).

The data in Table II, computed using values from Table I, indicate that the surface resistance of untreated Valencia oranges is similar for ethylene,  $\text{CO}_2$ ,  $\text{O}_2$ , and very high, whereas the resistance to water is 60-fold lower. This large difference, recently reported for numerous fruits (14, 15), apparently was overlooked previously because of the convention of expressing transpirational conductance and gaseous resistance in units which are not easily compared. For the system, air/membrane/air, the transport resistance is  $r = \mu \Delta x / DKS$ , where  $\mu$  is the tortuosity of the diffusion path,  $\Delta x$  is the barrier thickness (cm),  $D$  is the vapor's diffusion coefficient in the membrane ( $\text{cm}^2 \text{s}^{-1}$ ),  $K$  is the partition coefficient between air and the membrane, and  $S$  is the fractional membrane area available for diffusion (25). Diffusivity depends on the square root of the mol wt, and therefore for  $\text{O}_2$ ,  $\text{CO}_2$ , and ethylene it cannot differ by more than  $\pm 12\%$  in any medium, but these same gases have widely differing air/water and air/oil partition coefficients. Consequently, it is difficult to conceive of a mechanism other than an air-phase which could transfer these gases through the fruit's surface with equal ease (Table II). Although 12 to 60% of the stomata in oranges have been occluded by natural wax prior to harvest (2, 3, 32), they still open and close in response to light and are highly effective in conducting water and  $\text{CO}_2$  (24, 27). Their resistance to water ranges from  $13 \text{ s cm}^{-1}$  when they are open to  $>50 \text{ s cm}^{-1}$  when they are closed (24). Based on diffusivity, the resistances to  $\text{O}_2$ ,  $\text{CO}_2$ , and ethylene must be  $<21 \text{ s cm}^{-1}$  when the stomata are open, compared with  $6000 \text{ s cm}^{-1}$  after harvest (Table II). Clearly, the stomata are shut after harvest, for only  $<0.4\%$  ( $100 \text{ 21 s cm}^{-1} / 6000 \text{ s cm}^{-1}$ ) of the initial fully open pore area is needed to



Table III. Effect of Waxing with Broshar Wax and Seal-Packaging with HDPE Film and Cryovac D-950 on Levels of Acetaldehyde and Ethanol in Juice of Valencia and Shamouti Oranges and Marsh Grapefruit

	Treatment	Acetaldehyde		Ethanol	
		Waxed	Unwaxed	Waxed	Unwaxed
$\mu\text{l}\cdot\text{L}^{-1}$					
Valencia oranges, 13 weeks at 15°C	Not sealed	23.5a*	18.5b	556a	338b
	Sealed with HDPE	23.4a	17.4b	542a	338b
	Sealed with Cryovac-D-950	26.9a	21.1ab	595a	359b
	$\bar{X}$	24.6a	19.0b	564a	345b
	%	100.0	77.0	100	61
Marsh grapefruit, 14 weeks at 17°C	Not sealed	6.9a	2.3b	147a	71b
	Sealed with Cryovac D-950	5.1a	3.5b	145a	64b
	$\bar{X}$	6.0a	3.4b	146a	68b
	%	100.0	57.0	100	47
	Sealed with Cryovac D-950	40.0a	29.0b	1513a	541b
Shamouti oranges, 4 weeks at 6°C and 1 week at 17°C	%	100.0	73.0	100	36

\* Mean separation by Duncan's test, 1% level. Values followed by a different letter are significantly different at 1% level.

sufficiently to induce partial anaerobiosis. In three cultivars of citrus, Valencia and Shamouti oranges, and Marsh grapefruit, the levels of acetaldehyde and, to an even greater extent the level of ethanol, is significantly higher in juice of waxed fruit than it is in the juice of nonwaxed fruits. Sealing fruit with two different plastic films, 0.01 mm thick HDPE or 0.015 mm Cryovac D-950, did not affect the ethanol and acetaldehyde contents. A similar response to waxing and seal-packaging was reported by Hale *et al.* (22) using a different film, 15  $\mu\text{m}$ -thick Clysar ECH-50, predominantly a polypropylene copolymer. They found that waxing affected ethanol and acetaldehyde contents much more than sealing did.

In summary, the following observations suggest that the mass transport of water and fixed gases occur by different mechanisms in fruits: (a) the resistances to  $\text{CO}_2$ ,  $\text{O}_2$ , and ethylene mass transport are similar, but the apparent resistance to water is 60- to 100-fold smaller (14, 15), suggesting that water moves preferentially in a liquid aqueous phase in the cuticle (28, 29); and (b) waxing inhibits the transport of  $\text{CO}_2$ ,  $\text{O}_2$ , and ethylene, but not of water, whereas the 10- to 15- $\mu$ -thick film restricts mainly the transport of water (28, 29). This conclusion is in agreement with previous morphological observations which showed lack of a direct relationship between the number of stomata and the rate of transpiration in oranges. Water loss from detached fruit kept in darkness is greater from the calyx half than from the stylar half of Valencia oranges even though stomatal density is greater in the stylar half than in the calyx half (21). The rates of transpiration per unit surface area of young Shamouti orange fruit and leaves are similar in the sun in the bright hours of the day (27), even though the stomatal density of young fruit is many times lower than that of leaves. All these observations could now be better explained by considering the greater proportion that cuticular transpiration takes place in fruits as compared with the stomatal transpiration.

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