Pitting of Grapefruit that Resembles Chilling Injury

Peter D. Petracek

Florida Department of Citrus, 700 Experiment Station Road, Citrus Research and Education Center, Lake Alfred, FL 33850

Wilfred F. Wardowski²

University of Florida, 700 Experiment Station Road, Citrus Research and Education Center, Lake Alfred, FL 33850

G. Eldon Brown

Florida Department of Citrus, 700 Experiment Station Road, Citrus Research and Education Center, Lake Alfred, FL 33850

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Abstract. A postharvest peel disorder, morphologically similar to chilling injury (CI), was detected on nonchilled 'Marsh' white grapefruit (*Citrus paradisi* Macf.). Like CI, the disorder was characterized by pitting of the peel caused by the collapse of oil gland clusters. This disorder is distinguished from CI in that pitting developed within the first 10 days of postharvest storage on fruit held at high (21.0C), but not low (4.5C), temperatures and on waxed fruit, but not unwaxed fruit. Pathogens isolated from pitted fruit were similar to those of nonpitted fruit. No preharvest pitting or visual clues of fruit susceptibility were observed.

Chilling injury (CI) of grapefruit is a postharvest peel disorder caused by low-temperature (<10C) storage. CI is characterized by pitting of the peel resulting from the sporadic or widespread collapse of oil glands near the peel surface and the eventual bronzing or necrosis of the affected areas (Grierson, 1986; Whiteside et al., 1988). CI does not necessarily affect internal fruit quality, but may result in increased susceptibility to pathogen invasion and reduced marketability. Although the physiology of CI is not fully understood, the development of CI is controlled by preharvest factors, such as time of harvest (Kawada et al., 1978), mineral composition (Slutzky et al., 1981), and postharvest treatments, such as high humidity (Purvis, 1984) and application of the fungicide 2-(4-thiazolyl)-1H-benzimidazole [thiabendazole (TBZ)] (Kokkalos, 1974; McDonald et al., 1991; Wardowski et al., 1975). In practice, the incidence of grapefruit CI is reduced primarily by controlling water loss and storing the fruit above chilling temperatures (>12C) or by conditioning the fruit to progressively lower temperatures during early storage (Chalutz et al., 1985; Eaks, 1960; Hatton and Cubbedge, 1983).

In mid-Feb. 1994, we received inquiries about the cause of pitting of exported white grapefruit. Although the fruit reportedly were not subjected to chilling temperatures, the

pitting initially appeared to be morphologically similar to that caused by CI. Preliminary studies showed that pitting occurred only on fruit stored at ambient temperature and thus confirmed that this disorder was not CI. Our objectives were to morphologically characterize this disorder and to determine how this disorder is affected by selected postharvest handling techniques commonly used in the packing of exported white grapefruit.

Materials and Methods

Plant material. 'Marsh' white grapefruit were harvested from groves in the Martin, St. Lucie, or Indian River counties in Florida. Fruit were packed at the Citrus Research and Education Center (CREC) in Lake Alfred, Fla., or in commercial packinghouses in Lake Hamilton, Haines City, Winter Garden, or Fort Pierce, Fla. All fruit used in these studies were size 36 (10.0 to 11.4 cm in diameter). Fruit were washed with commercially available citrus washing solutions, comprised of ionic and nonionic surfactants, and waxed with commercially available shellac-based, water-emulsion wax within 1 day of harvest, unless otherwise noted.

Morphology. The time course for the development of pitting was determined by visual inspection of 64 fruit [packed on 18 Apr. 1994 in Lake Hamilton and stored for 15 days at 21.0 ± 1.0C at 95% ± 2% relative humidity (RH)] every 2 or 3 days over 35 days. The numbers of sunken glands and clusters of sunken glands were counted, and newly pitted areas were circled with a marking pen. A similar visual assessment of the pit distribution over the surface of the fruit was made by dividing the fruit into six regions (stylar, stem, and four midsection regions) of about equal

area and counting the number of pitted clusters and pitted glands per cluster after 40 days of storage.

Scanning electron micrographs (SEM) of pitted and nonpitted regions from six representative fruit were prepared by freeze-drying the pitted tissue, freeze-fracturing the tissue in liquid N₂, and mounting the tissue on aluminum stubs. Samples were gold-coated, examined with a SEM (model S530; Hitachi, Danbury, Conn.) at 10 kV accelerating voltage, and photographed with Polaroid (Cambridge, Mass.) P/N 55 film.

Pathology. The association of pathogens with pitted and nonpitted areas was evaluated by submerging fruit (packed 18 Apr. 1994 in Lake Hamilton and stored for 15 days at 21.0 ± 1.0C at 95% ± 2% RH) in 70% ethyl alcohol for 2 min. Fruit then were dried thoroughly with paper towels, and 10 pieces (each about 8 mm²) of pitted and nonpitted surface tissue were removed from each of 10 fruit, plated on minimal medium (Brown, 1975), and incubated at 25C for microbial development and identification.

Effect of temperature. For preliminary studies on the effect of storage temperature on pitting, fruit were drenched with water containing chlorine (50 µg·ml⁻¹) and TBZ (700 µg·ml⁻¹) (Merck and Co., Rakway, N.J.), degreened with ethylene (5 to 7 µg·ml⁻¹ at 29.5 ± 2C and 93% ± 3% RH) for 48 or 72 h, washed, and waxed with water-emulsion wax containing TBZ (2000 µg·ml⁻¹) for fruit packed on 25 Mar. and 18 Apr. 1994 in Lake Hamilton and 13 Jan. 1995 in Winter Garden. Fruit packed on 2 Feb. 1995 at the CREC were not drenched or degreened and no TBZ was used in the wax. Fruit were transferred within 6 h of packing to storage rooms, adjusted to 4.5 or 21.0 ± 1.0C and 95% ± 2% RH, at the CREC. Fruit (two boxes of 36 fruit per box for each of two treatments) were rated visually (0 = no pits; 1 = 1 to 3 pits, 2 = 4 to 10 pits, 3 = 11 to 30 pits, 4 = 31 to 100 pits, and 5 = more than 100 pits) 15 and 40 days after packing. The frequency of pitting was expressed as the percentage of fruit with a rating ≥ 2.

For a more detailed study on temperature effects on pitting, fruit were drenched with 1,[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole (imazalil) (250 µg·ml⁻¹, Janssen Pharmaceutica, Beerse, Belgium), washed with surfactant solution, and waxed with water-emulsion wax. Fruit (three boxes of 36 fruit per box for each treatment) were packed on 15 Feb. 1995 in Haines City; transported to the CREC within 1 h of packing; and stored at 4.5, 10.0, 15.5, or 21.0 ± 1.0C and 95% ± 2% RH. Fruit stored at 21.0 ± 1.0C were transferred to 4.5 ± 1.0C at 2, 4, 8, or 16 days after packing or to 10.0 ± 1.0C at 2, 4, or 8 days after packing. The time required to equilibrate the fruit to storage room temperature was <6 h, as determined by thermocouple measurement of core temperatures of similarly packed fruit. Fruit were visually rated for pitting 1, 7, 13, 21, 28, and 35 days after packing.

Effects of degreening, washing, and waxing. For the study on ethylene effects on pitting, fruit (three boxes of 36 fruit per box for

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each treatment) were harvested on 10 Jan. 1995 in Indian River County and were transported to the CREC for packing. The fruit were exposed to ethylene ($9 \mu\text{g}\cdot\text{ml}^{-1}$ at $29.5 \pm 2.0^\circ\text{C}$ and $93\% \pm 3\%$ RH) for 0, 4, 24, 48, or 68 h and then held 72, 68, 48, 24, or 4 h, respectively, at $21.0 \pm 1.0^\circ\text{C}$ and $93\% \pm 3\%$ RH with no ethylene. Fruit were washed with surfactant solution, waxed with water-emulsion wax, stored at $21.0 \pm 1.0^\circ\text{C}$ and $95\% \pm 2\%$ RH, and visually rated for pitting 28 days after packing.

The effects of commercial washing and waxing techniques on pitting were assessed by three factorial studies. Unless otherwise noted, fruit were drenched with water containing chlorine ($50 \mu\text{g}\cdot\text{ml}^{-1}$) and TBZ ($700 \mu\text{g}\cdot\text{ml}^{-1}$), degreened with ethylene (5 to $7 \mu\text{g}\cdot\text{ml}^{-1}$ at $\approx 30^\circ\text{C}$ and 90% to 95% RH) for 48 h, and then were either 1) washed with surfactant and waxed, 2) washed with surfactant and not waxed, 3) washed without surfactant and waxed, or 4) washed without surfactant and not waxed. Two boxes of 36 fruit per box were used for each treatment. Fruit were transferred to storage rooms at $21.0 \pm 1.0^\circ\text{C}$ and $95\% \pm 2\%$ RH at the CREC within 6 h of packing, and pitting was evaluated 15 and 40 days after packing.

In the first study, a commercially available water-emulsion wax formulation was evaluated on fruit packed on 13 Apr. 1994 in Lake Hamilton. In the second study, another commonly used wax, produced by a second company, was evaluated for fruit packed on 26 May 1994 in Fort Pierce. In addition to the four treatments listed, two additional treatments were added: drenched and degreened fruit were 5) not washed but were waxed or 6) not washed and not waxed. In the final study, a third commercially available wax formulation was evaluated on fruit packed on 10 Jan. 1995 at the CREC.

Internal O_2 and CO_2 levels. A time course on the effect of temperature and waxing on internal gas level was prepared. Fruit were harvested in Indian River County, transported to the CREC, and held overnight at $21.0 \pm 1.0^\circ\text{C}$ and $95\% \pm 2\%$ RH. The fruit (six fruit for each treatment) were washed with surfactant, waxed, and held at 1.0, 4.5, 10, 15.5, 21.0, or $29.5 \pm 1.0^\circ\text{C}$ and $95\% \pm 2\%$ RH. Unwaxed fruit were held at 1.0, 4.5, or $21.0 \pm 1.0^\circ\text{C}$ and $95\% \pm 2\%$ RH. Septa were created by applying dabs (≈ 2 cm in diameter) of silicone (3140 RTV; DOW, Midland, Mich.) to the styler end of the fruit. Gas samples (0.5 ml) were taken by syringe through the septa from the inner core of the fruit 1.5, 3, 6, 12, 24, 48, 144, 336, 624, and 792 h after waxing. Samples were analyzed by a flow-through system, consisting of a paramagnetic O_2 analyzer (model 570A; Servomex, Norwood, Mass.) and an infrared CO_2 analyzer (model AR-400; Anarad, Santa Barbara, Calif.) connected in series with N_2 used as the carrier gas.

Results and Discussion

Pitting of nonchilled fruit was characterized by clusters of collapsed oil glands scattered over the surface of the fruit (Fig. 1A).

Fig. 1. (A) Pitting of nonchilled 'Marsh' white grapefruit. Scanning electron micrographs of freeze-fractured segments of pitted peel with (B) slight and (C) severe collapse of oil glands.

The disorder first was expressed as a slight depression of the peel in regions directly above oil glands. These regions often became bronze in color within 3 days after the initial depressions were noted. When two or more adjacent oil glands collapsed, the peel between the oil glands often became indented. In contrast, damage due to CI tends to be more general rather than focused on oil glands, and discoloration caused by CI tends to be darker brown and more uniformly distributed between the oil glands.

The severity of the disorder was recorded by SEM and marked by the extent of oil gland collapse (Fig. 1 B and C). The layers of small epidermal cells between the surface of the fruit and the oil gland, as well as the large albedo cells underlying the oil gland, appeared to be collapsed or disoriented. Portions of the oil gland near the albedo cells were flattened and glands thus appeared deflated. Depression of the outer surface of the fruit was typically 0.1 to 0.3 mm deep (moderate collapse, Fig. 1B) to >0.7 mm deep (complete collapse, Fig. 1C). Complete collapse did not necessarily affect the morphology of adjacent oil glands.

The pits initially appeared as early as 2 days after packing, and appearance concluded about 28 days thereafter. The rate of appearance was highest in the first 2 weeks after packing (e.g., Fig. 2). Susceptibility of the fruit or specific regions on the fruit was not predictable by visual inspection of the fruit before pitting. Industry reports that pitting occurred in the field or appeared within hours after packing were not verified. Pitted areas consisted of an average of three oil glands per cluster (range, 1 to 27). The number of oil glands for a given cluster did not increase with time. The average number of clusters per fruit was 22. Clusters were evenly distributed over the fruit surface with two exceptions: 1) the stem end had $\approx 60\%$ fewer pits than other regions of the peel, and 2) about a quarter of the midsection region of the peel had few or no pits.

Pitting was not associated with the presence of an isolated pathogen. As many as 70% and 59% of the tissue pieces were sterile for pitted and nonpitted regions, respectively. *Colletotrichum*, the most commonly isolated pathogen, was recovered from 16% and 25% of the tissue pieces from pitted and nonpitted regions, respectively. Occasionally, other organisms were isolated (<10% occurrence), including *Mycosphaerella*, *Chaetomium*, *Penicillium*, *Candida*, and *Fusarium*, with similar occurrences for pitted and nonpitted regions.

For all studies, postharvest pitting was absent on fruit that were stored at 4.5°C within several hours after packing but was observed on fruit held at 21.0°C (Table 1). Detailed evaluation of temperature effects revealed that the rate of pit appearance (Fig. 2) and the extent of pitting (Table 2) were most effectively controlled when fruit were stored at 4.5°C within 1 h after packing. Storage at 10.0°C or transferring fruit from 21.0 to 4.5 or 10.0°C after 2 days also suppressed pitting, but to a lesser extent. Pitting for fruit stored at low temperature (4.5 or 10.0°C) within 1 hour after

packing was primarily due to CI. Pitting was not inhibited by transferring fruit to 4.5 or 10.0°C after 4 or more days of storage at 21.0°C or by storage at 15.5°C (Table 2).

Exposure of the fruit to ethylene up to 68 h before washing and waxing did not affect

pitting (data not shown). Pitting was not significantly affected by washing either with or without surfactant solution (Table 3). However, pitting was present only on waxed fruit. Wax applications usually improve storage of citrus because they reduce water loss. The

Fig. 2. Effect of storage temperature on the number of grapefruit pits following packing. (A) Fruit stored at constant temperature: (●) 4.5, (○) 10.0, (■) 15.5, or (□) 21.0 $\pm$ 1.0°C. (B) Fruit stored at variable temperatures: 21.0 $\pm$ 1.0°C for (◆) 2, (▲) 4, (▼) 8, or (×) 16 days; then at 4.5 $\pm$ 1.0°C or at 21.0 $\pm$ 1.0°C for (◇) 2, (△) 4, or (▽) 8 days; and then at 10.0 $\pm$ 1.0°C. Symbols represent the mean for three boxes of 36 fruit per box ($\pm$ SE).

Table 1. Effect of storing grapefruit 40 days at 4.5 or 21.0°C following packing on severity of pitting.

Storage temp (°C)	Pitting (rating) ^a			
	Packing dates			
	25 Mar. 1994	18 Apr. 1994	10 Jan. 1995	2 Feb. 1995
4.5	0.0 $\pm$ 0.0 (0)	0.0 $\pm$ 0.0 (0)	0.0 $\pm$ 0.0 (0)	0.0 $\pm$ 0.0 (0)
21.0	1.0 $\pm$ 0.2 (28)	1.1 $\pm$ 0.2 (24)	0.7 $\pm$ 0.1 (15)	1.2 $\pm$ 0.3 (25)

^aMean score ($\pm$ SE) for 72 fruit. Pitting was rated from 0 (none) to 5 (most severe). Percentage of fruit with four or more collapsed oil glands is in parentheses.

Table 2. Effect of storing grapefruit 35 days at 4.5, 10.0, 15.5, or 21.0°C following packing on severity of pitting.

Storage temp (°C)	Storage pitting (rating) ^a	Incidence of collapsed oil glands (no.) ^b
4.5	0.1 $\pm$ 0.1 a	0.7 $\pm$ 0.7 a
10.0	0.2 $\pm$ 0.1 ab	3.0 $\pm$ 2.0 ab
15.5	0.9 $\pm$ 0.3 cd	10.3 $\pm$ 3.8 bc
21.0	1.1 $\pm$ 0.2 d	13.0 $\pm$ 3.5 c
21.0 for 2 days, then 4.5	0.4 $\pm$ 0.1 a-c	5.0 $\pm$ 1.0 a-c
21.0 for 4 days, then 4.5	0.9 $\pm$ 0.2 b-d	10.0 $\pm$ 2.5 bc
21.0 for 8 days, then 4.5	0.8 $\pm$ 0.2 b-d	8.3 $\pm$ 2.9 a-c
21.0 for 16 days, then 4.5	1.1 $\pm$ 0.2 d	12.7 $\pm$ 1.8 c
21.0 for 2 days, then 10.0	0.5 $\pm$ 0.2 a-c	4.0 $\pm$ 2.6 ab
21.0 for 4 days, then 10.0	0.9 $\pm$ 0.3 b-d	9.3 $\pm$ 3.0 bc
21.0 for 8 days, then 10.0	0.6 $\pm$ 0.2 a-d	6.3 $\pm$ 3.8 a-c

^aMean score ($\pm$ SE) for three boxes of 36 fruit per box. Pitting was rated from 0 (none) to 5 (most severe).

^bNumber of fruit per box of 36 with four or more collapsed oil glands. Mean separation within columns by Duncan's new multiple range test at $\alpha = 0.05$.

detrimental effects of applied wax often are related to stimulation of pathogen development (Brown, 1992; Waks et al., 1985) and production of anaerobic off-flavors (Davis and Hofmann, 1973; Hagenmaier and Baker, 1994). Wild (1991) reported peteca rind pitting of lemons [*Citrus limon* (L.) Burm. f.] was stimulated by waxing, although the cause was not determined. It is unlikely that the pitting observed in our studies was due to a phytotoxic response or "burning" of the peel. Phytotoxic response of citrus peels to wax application is uncommon and reportedly is characterized by localized collapse of the entire peel within several days after waxing. In contrast, the pitting we observed was characterized by scattered collapse of patches of oil glands, which sometimes appeared several weeks after application. We also note that waxes used in our studies came from two companies and consisted of three formulations. Moreover, reports from packinghouses indicated that fruit treated with water-emulsion waxes from other companies also pitted, which indicates that the problem is not associated strictly with one specific wax formulation.

To our knowledge, the effects of wax formulation on pitting have not been evaluated yet. Furthermore, a detailed study on the effects of fungicides that are incorporated in the wax may be beneficial. However, because pitting developed on fruit not treated with fungicide (Table 3, 10 Jan. 1995 packed date), we assume that fungicide treatment is not essential for pitting.

The interaction between temperature and waxing on pitting gas exchange and internal gas compositions may be intermediary factors. Studies on internal gas levels of waxed and nonwaxed grapefruit showed that steady-state O₂ and CO₂ levels were attained in <48 h, and the time to attain steady-state conditions decreased with increasing temperature (e.g., ≈48 h at 1.0C and 6 h at 29.4C, detailed data not shown). Internal gas levels varied little with temperature for nonwaxed fruit (18.5% and 19.5% for O₂ and 0.9% and 0.7% for CO₂ for 21.0 and 4.5C, respectively) but changed substantially with temperature for waxed fruit (2.5% and 13.4% for O₂ and 6.0% and 1.9%

for CO₂ for 21.0 and 4.5C, respectively). Steady-state internal gas levels were nearly linear functions of temperature: O₂ (percent) = $-0.32 \times \text{temperature (degrees Celsius)} + 14.04$, $r^2 = 0.84$; CO₂ (percent) = $0.25 \times \text{temperature (degrees Celsius)} + 4.04$, $r^2 = 0.98$.

Although high-temperature storage and waxing stimulated pitting and produced low O₂ and high CO₂ levels, the relationship between internal gas composition and pitting was not clear. Correlations between the extent of pitting and internal O₂ and CO₂ levels were low ($r^2 = 0.06$ and 0.04 , respectively). Also, sampling of internal gases of nearly 200 pitted fruit revealed that pitted fruit had internal levels of O₂ as high as 10% and CO₂ as low as 2%. Controlled-atmosphere studies may help define the role of gases and wax in pitting.

Personnel in production, packing, and shipping industries were surveyed informally to determine whether additional factors contributed to the disorder. Although the reports were sometimes contradictory, some observations seemed consistent. 'Marsh' white grapefruit was the cultivar most affected, although similar pitting was identified in pink and red grapefruit. The pitted fruit came from groves in three counties of eastern Florida. Pitting was seen primarily on fruit shipped to Japan. Some pitting was reported on red grapefruit shipped to Europe but was not reported for domestic fruit. However, we note that these observations are confounded by market preference, season, shipping mode, and time. The factors must be interpreted accordingly. Pitting was reported initially in mid-Feb. 1994, was extensive throughout March and April, decreased in early May, and continued to the end of the season (early June). Pitting was reported again in the following season in late Dec. 1994 and continued until mid-Feb. 1995.

No single preharvest treatment, such as spray application, apparently caused the disorder. Fruit from some groves reportedly had excessive pitting. Rootstock, tree age, irrigation method, and fertilization strategies were suspected as playing roles, although no consistent trend accounted for differences in the incidence of the disorder. Several industry reports stated that fruit from adjacent rows or

groves were, alternately, pitted extensively or virtually nonpitted, depending on the day harvested, the hour packed, or the mode by which they were shipped. While these reports have been unsubstantiated by experiment, they suggest that fruit susceptibility and postharvest stress may be involved.

We suspect that this disorder has been observed in previous seasons at lower incidence rates and may have been cursorily diagnosed as CI or physical damage. Pitting characterized in this study was distinguishable from CI: 1) CI requires low temperatures (<10C) to develop, whereas the pitting we observed developed at high temperatures (21.0C); 2) CI is reduced by waxing (Grierson, 1971), whereas pitting required waxing; and 3) CI requires several weeks of storage to develop, whereas pitting developed within the first week of storage.

Pitting is distinguishable from physical damage caused by sand abrasion, puncturing, or insect damage. Physical damage of the peel often is characterized by the presence of a wound periderm and of individual pits or isolated wound areas. Pitting was characterized by scattered clusters of pitted areas with no associated wound periderm. Moreover, physical damage was not always focused on oil glands and does not increase in frequency during storage as is characteristic of pitting.

Other sporadically occurring citrus peel disorders that involve the collapse of oil glands or surrounding areas include rumpole or peteca of lemon, "nuxan" blemish of 'Shamouti' orange [*Citrus sinensis* (L.) Osb.], pitting of 'Pineapple' and 'Valencia' orange, aging, and stem end rind breakdown (Pantastico et al., 1975; Smoot et al., 1971). Further research is required to determine common causal factors between these disorders and the pitting observed for grapefruit. More extensive studies on the influence of storage temperature, wax composition, and changes in peel physiology may explain this newly defined disorder better.

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Table 3. Effect of surfactant washing and waxing on pitting of grapefruit held for 40 days at 21.0C after packing.

Treatment		Pitting ^z	
Washing	Surfactant ^y	Wax ^x	No wax
13 Apr. 1994			
+	+	1.5 ± 0.3 (38)	0.0 ± 0.0 (0)
+	-	1.1 ± 0.2 (21)	0.0 ± 0.0 (0)
6 May 1994			
+	+	1.0 ± 0.2 (16)	0.0 ± 0.0 (0)
+	-	0.9 ± 0.1 (14)	0.0 ± 0.0 (0)
-	-	0.9 ± 0.1 (12)	0.0 ± 0.0 (0)
10 Jan. 1995			
+	+	0.9 ± 0.2 (20)	0.0 ± 0.0 (0)
+	-	1.1 ± 0.2 (23)	0.0 ± 0.0 (0)

^zMean score (±SE) for 72 fruit. Pitting was rated from 0 (none) to 5 (most severe). Percentage of fruit with four or more collapsed oil glands is in parentheses. The main treatment of wax application was significant at $\alpha = 0.05$ for each packing date.

^ySurfactants were commercially available solutions comprised of ionic and nonionic surfactants.

^xWaxes were commercially available, shellac-based, water-emulsion wax formulations.

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