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Electrolyte Leakage, Respiration, and Ethylene Production as Indices of Chilling Injury in Grapefruit

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Abstract. **Storage of 'Marsh' white seedless grapefruit (*Citrus paradisi* Macf.) for 2 weeks at 5C resulted in the development of chilling injury (CI). Electrolyte leakage from chilled fruit did not increase significantly until CI had become severe, and was therefore considered to be of limited value as an early indicator of CI. In contrast to electrolyte leakage, respiration and ethylene evolution were significantly higher in chilled than in nonchilled fruit, even before the onset of visual symptoms of CI. Respiration rates ranged from ≈8 to 11 and 5 to 7 ml CO_2/kg per hour in chilled and nonchilled fruit, respectively. Ethylene evolution was not detected from nonchilled fruit, whereas chilled fruit produced from 45 to 250 nl ethylene/kg per hour. Results of this study indicate that electrolyte leakage does not increase until visible pitting of the flavedo has occurred, whereas stimulation of respiration and ethylene evolution occur early in the development of CI.**

Grapefruit develop chilling injury (CI) when stored below 10 to 12C. Injury symptoms include surface pitting, scald, and increased susceptibility to decay when fruit are returned to ambient conditions (Schiffman-Nadel et al., 1971). Although numerous studies have been conducted to determine the influence of various factors on the development and prevention of CI in grapefruit (for a recent review see Grierson, 1986), few (Forney and Peterson, 1990; Pantastico et al., 1968) have reported on underlying physiological changes related to the development of CI in grapefruit.

Pantastico et al. (1968) reported that electrical conductivity of the leachate from whole fruits of oranges, limes, and grapefruit increased in response to chilling. Forney and Peterson (1990) reported that enhanced leakage of K ions was an effective indicator of CI in grapefruit albedo callus. Chilling stimulated respiration in oranges and lemons, the magnitude of which was increased by increased duration of chilling and lower chilling temperatures (Eaks, 1960, 1965, 1980). Exposure of grapefruit attached to the tree to diurnal cycles of chilling was found to stimulate ethylene evolution (Cooper et al., 1969). Eaks (1980) reported that chilling temperatures stimulated ethylene evolution in California lemon fruit and suggested that the extent of CI sustained during storage could be evaluated by transferring the fruit to 20C, and after 24 h at 20C determining the respiration rate and ethylene evolution. Physiological measures of CI would be useful for evaluating effects prior to the development of visual symptoms, and may also provide information on the physiological changes involved in the development of CI. In this paper we report on the effects of storage at chilling temperature on electrolyte leakage, respiration, and ethylene evolution from grapefruit.

'Marsh' white seedless grapefruit were harvested by hand on 13 Oct. and 16 Nov. 1989 from a grove near Merritt Island, Fla. The fruit were transported to the laboratory where they were degreened in a room with ≈20 µl ethylene/liter [30C, 90% ± 2% relative humidity (RH)] for 48 h. Following degreening, the fruit were washed, waxed (Sta-Fresh 320), and placed into standard fiberboard citrus cartons. The fruit were stored at 5 or 15C (±0.1C) at 86% ± 5% RH. Samples of 30 fruit were removed from storage at 1-week intervals and visually rated for pitting, a symptom of CI. Data presented represent the percentage of fruit that were pitted. Five fruit were selected at random from each sample and used for the determination of tissue electrolyte leakage. The rind was removed from the fruit, and disks (13 mm in diameter) were cut from the rind using a 13-mm-diameter cork borer. The albedo and flavedo of each disk were separated using a razor blade. In an initial experiment we determined that conductivity of the bathing solution did not increase appreciably with incubation times >4 h. Therefore, initial electrolyte leakage was determined following incubation of five disks of albedo or flavedo in 25 ml of 0.4 M mannitol at 23 ± 2C for 4 h with constant shaking. Electrical conductivity of the bathing solution was measured using a conductivity meter (Markson, Del Mar, Calif.). The tissue was then autoclaved for 20 min, held overnight, and total conductivity was measured. Electrolyte leakage was calculated as (initial/total) × 100.

A second sample of five fruit selected at random was used for determination of whole-fruit respiration and ethylene evolution. These fruit were transferred to a room at 20C and allowed to equilibrate to ambient temperature. For gas analysis, the fruit were placed

Table 1. Electrolyte leakage from 'Marsh' grapefruit flavedo tissue following storage for 8 weeks at nonchilling (15C) or chilling (5C) temperatures.

Condition	Electrolyte leakage (% of total electrolyte leakage)
Nonchilled	9.3 ± 3.5[z]
Chilled nonpitted	16.9 ± 2.6
Chilled pitted	52.0 ± 5.8

[z]Values represent the means of five samples ± SD.

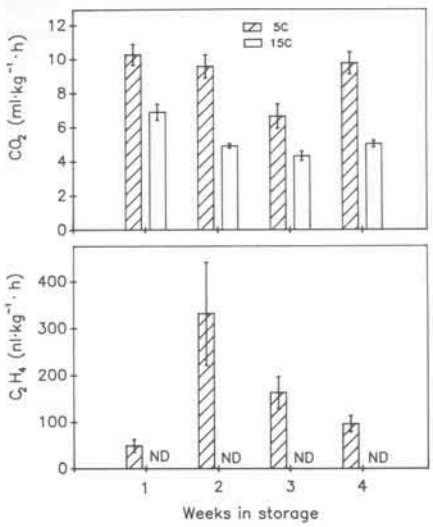

Fig. 1. Rates of CO_2 evolution (**top**) and ethylene evolution (**bottom**) from grapefruit following storage at chilling (5C) or nonchilling (15C) temperatures. ND = none detected. Values are means of five samples, vertical lines above bars represent SE.

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into 3.8-liter glass jars with lids fitted with rubber serum stoppers. In a preliminary experiment, we determined that CO_2 and ethylene accumulation in the headspace atmosphere was linear over a 24-h period. Sampling for CO_2 was conducted after an incubation time of 1 to 3 h, and for ethylene after an incubation time of 20-24 h. Before sampling, the headspace atmosphere was agitated 10 times with a 30-cc syringe. Samples of headspace atmosphere were removed using gas-tight syringes, and the analyses were conducted using standard gas chromatography methods.

The experiment was a completely randomized design with 30 single-fruit replicates for determining the presence of CI and five single-fruit replicates for electrolyte leakage and gas analysis. The data from the two harvests were pooled, and an analysis of variance was conducted for electrolyte leakage and gas analysis data.

Chilling incidence was 0%, 50%, 55%, and 59% after 1, 2, 3, and 4 weeks at 5C, respectively. Fruit stored at 15C showed no symptoms of CI.

In an initial experiment, we determined that electrolyte leakage was greatest from pitted areas of chilled fruit, intermediate in nonpitted areas of chilled fruit, and least in nonchilled fruit (Table 1). Therefore, we reasoned that electrolyte leakage would be a useful index of CI in grapefruit. We measured electrolyte leakage in flavedo and albedo tissues separately because the flavedo is the tissue to first show signs of CI and Forney and Peterson (1990) reported that leakage from calli grown from grapefruit albedo was a useful measure of CI.

Electrolyte leakage was consistently between 13% and 18% of total from albedo and between 10% and 14% of total from flavedo throughout the entire storage duration (data not shown). The slightly higher values for albedo tissue may have been the result of albedo disks having had two cut surfaces, whereas the flavedo tissue had only a single cut surface. Electrolyte leakage was not influenced by storage temperature in either tissue. The duration of chilling in our studies may not have been long enough to induce an increase in electrolyte leakage; however, visual symptoms of CI were apparent within the durations employed. The higher amount of electrolyte leakage in pitted areas of chilled fruit is most likely a secondary symptom of CI.

Respiration rates of chilled grapefruit were significantly ($P < 0.001$) higher than rates of nonchilled grapefruit (Fig. 1, top). Respiratory rates of both chilled and nonchilled grapefruit tended to decrease during the first 3 weeks of storage. This finding contrasts with the pattern observed for chilled oranges (Eaks, 1960, 1965) and lemons (Eaks, 1965, 1980), in which increasing the duration of chilling resulted in a greater stimulation of respiration.

Ethylene evolution from nonchilled grapefruit was below the limits of detection, whereas chilled fruit produced measurable quantities of ethylene (Fig. 1, bottom). An enhanced rate of ethylene evolution was apparent following 1 week of storage at 5C and preceded the appearance of visual CI symptoms. Changes in ethylene evolution were not consistent with time of chilling; ethylene evolution increased during the first 2 weeks at 5C, then decreased. The nondetectable level of ethylene in nonchilled fruit is in agreement with the results of Vines et al. (1968) who were able to detect ethylene only in grapefruit that had suffered freezing stress.

We found electrolyte leakage to be an ineffective index of CI in grapefruit. Electrolyte leakage was higher in severely pitted areas of chilled fruit than in those unaffected, but did not precede visual symptoms of CI. Rates of CO_2 and ethylene evolution were stimulated by storage at chilling temperature, and the effect was detectable before the development of visual symptoms; however, respiration and ethylene evolution cannot be used as quantitative measures of CI in grapefruit. Carbon dioxide evolution rates of chilled grapefruit were consistently higher than those of nonchilled fruit, but these rates decreased during storage in both chilled and nonchilled fruit. Although ethylene was detected only from chilled fruit, ethylene evolution did not change with increased duration of chilling. Additional research is needed to identify useful early indicators of CI in grapefruit.

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