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Effect of Chilling on Respiration and Volatiles of California Lemon Fruit

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Abstract. **The respiration, ethylene production and ethylene, ethyl alcohol, and acetaldehyde content of the internal atmosphere of citrus fruit increased at 20°C following exposures to chilling temperatures (0° and 5°) compared with fruit placed directly at 20°C. The increases were greater the longer the exposure and greater following exposure to 0° than following exposure to 5°. Exposure to 12.8°, a nonchilling temperature, did not elicit a stimulation of these attributes when transferred to 20°. Ethylene, ethyl alcohol, and acetaldehyde in the internal atmosphere of fruit remained at the same levels during the chilling exposures. During storage at 12.8° the acetaldehyde content in the internal atmosphere increased, but the ethylene and ethyl alcohol content did not. The chilling injury sustained by citrus fruit during storage could be evaluated by transferring samples to 20° and determining the respiratory rate, ethylene production or the volatile content in the internal atmosphere 24 hours after transfer to 20°.**

Physiological disorders, referred to as chilling injury, occur in most fruits of tropical and subtropical origin when held at temperatures below 10°C. The literature on chilling injury is summarized in the excellent review by Lyons (6). Empirical observations on temperature tolerance, time-temperature responses and symptomology have been dilineated. Although the membrane lipid physical-phase change appears to be central to the physiological and biochemical disruption of metabolic activity (7, 11) the mechanism of chilling injury is not completely understood. As a result of this disruption, metabolites such as acetaldehyde and ethanol would be expected and have been reported to accumulate in fruit during chilling (2, 3, 6, 10). Stress such as holding in nitrogen also caused increases in the acetaldehyde and ethanol content of citrus fruit (8, 9).

The anomalous respiratory patterns of chilling-sensitive fruits at chilling temperature and the accelerated respiratory rates when transferred from chilling to a nonchilling temperatures have been reviewed (6). In addition, field chilling (1) and freezing field temperatures have increased the ethylene content of the internal atmosphere of citrus fruits (12).

Reported here are the respiratory rates and ethylene production at 20°C following various chilling exposures and the volatile content of the internal atmosphere during holding at chilling temperatures and after transferred to 20° for lemon fruits.

Materials and Methods

Light green lemon fruits (*Citrus limon* [L.] Burm. f. cv. Eureka) were harvested from Experiment Station trees and held at 10°C until the next day when the experiment was established. Fruits of uniform size and color were selected and randomly placed in the different treatments. Treatments consisted of samples placed directly at 20° and storage for 4, 8 and 12 weeks at 0°, 5° and 12.8° and then transferred to 20°. The recommended storage temperature for California lemons is 12.8°. Each treatment was replicated 6 times using individual fruit (6 fruit for respiration and ethylene production and 6 fruit for volatile determinations of the internal atmosphere). Each fruit was marked and weighed initially and at the time of transfer to 20°. Also, similar experiments were conducted with navel and 'Valencia' oranges.

Fruits for respiratory rate and ethylene production determinations were placed in respiratory chambers at 20°C. Humidified air, with the ethylene removed by passing through a column of Purafil and the CO_2 removed by bubbling through 2 N NaOH was metered through the respiratory chambers at 5 liters/hr by calibrated capillaries. CO_2 production of each fruit was determined by a calibrated Beckman infrared CO_2 analyzer equipped with a switching system to sequence the outlet gas from each fruit chamber through the analyzer. Data were taken from the chart every 6 hours for calculation of respiration as ml CO_2/kg-hr. Ethylene production was determined twice daily (8 AM and 4 PM) on 1 ml samples taken from the outlet of each respiratory chamber by a Varian Aerograph flame ionization gas chromatograph equipped with a 2 m × 3.2 mm column packed with 60-80 mesh activated alumina. At each sampling, the gas chromatograph was calibrated with 1 ml samples of a standard ethylene-nitrogen mixture.

The internal atmospheres were sampled by removing the button, inserting the needle of a 1 ml gas-tight syringe into the pithy central core of the fruit and withdrawing the plunger past the 1 ml mark. A rubber septum was placed on the needle to provide a seal between the fruit and the syringe. After the sample was taken the puncture was sealed with silicone stopcock grease. The effect of repeated sampling the same fruit vs. sampling different fruit after various storage treatments was evaluated in a preliminary experiment. The internal carbon dioxide, oxygen, and volatile concentrations were similar for both conditions. The area penetrated by the needle has a low metabolic activity thus, has essentially no effect on the physiology of the fruit. Therefore, the internal atmospheres of the 6 individual fruit designated for this purpose for each treatment were sampled just prior to transfer to 20°C, 7 hr after transfer and daily for 7 days.

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The percentage carbon dioxide and oxygen in the samples from the internal atmosphere of the fruits were determined by a Varian Aerograph thermo-conductivity gas chromatograph using a 2 column system and a 4-port rotary switch. The carbon dioxide was separated on a 76 cm × 6.35 mm column packed with 50 to 80 mesh Parapak T and the oxygen and nitrogen were separated on a 2 m × 6.35 mm column packed with 60 to 80 mesh molecular sieve 5A. Oven and detector temperatures were 54° and 140°C respectively. The gas chromatograph was calibrated at each sampling with standardized gas mixtures from pressurized gas cylinders.

Volatiles were determined by a Varian Aerograph gas chromatograph equipped with a 2 m × 3.2 mm column packed with 60 to 80 mesh Chromosorb-102. Oven, detector and injection port temperatures were 120°, 170°, and 150° respectively. The gas chromatograph was connected to a recorder and a Varian Aerograph model CDS-111 chromatography data system which printed the peak number, retention time and peak area. Because of difficulties in preparing reliable standard mixtures of the components in the internal atmosphere of citrus, the data were calculated relative to ethylene. The peak area for the standard ethylene mixture used to calibrate the other gas chromatograph for ethylene was converted to peak area units per 1 ppm ethylene and this value was divided into the respective peak areas values for the volatile components to obtain a relative concentration for each volatile.

The fruits were rated for surface pitting, a symptom of chilling injury, at the time of transfer to 20°C and after 1 week at 20°C. Categories of surface pitting were: no pitting = 0, slight pitting = 1, moderate pitting = 2 and severe pitting = 3. After 1 week at 20° the fruits were cut and rated for membranous stain using the same category designations indicated for surface pitting. The data for each chilling symptom were converted to an index for each sample by multiplying the number of fruit in each category by the respective value, summing the products and dividing by the total number of fruit.

Results and Discussion

The rates of weight loss for fruit stored at 0°, 5° and 12.8°C were 0.47, 0.65 and 0.90%/week respectively. Surface pitting was not observed on fruit held at 12.8° at the end of storage or after 1 week at 20° or on fruit held 4 or 8 weeks at 0° or 5° (Table 1). After 1 week at 20° a few fruit held for 8 weeks at 0° and 5° and fruit held for 12 weeks at 5° showed slight pitting. Twelve weeks at 0° resulted in 80% of the fruit showing slight pitting and after 1 week at 20° all such fruit showed slight or moderate pitting (Table 1). Membranous stain was observed only in fruit after 1 week at 20° following 12 weeks storage at 0° (Membranous stain index 0.8).

Table 1. Pitting index of lemon fruit at time of transfer from 0°, 5° and 12.8°C to 20° and after 1 week at 20° for fruit held at 0°, 5° and 12.8° for 4, 8 and 12 weeks (no pitting = 0, slight pitting = 1, moderate pitting = 2, and severe pitting = 3).

Storage temperature (0°C)	Pitting index					
	4 wk storage	After 1 wk at 20°	8 wk storage	After 1 wk at 20°	12 wk storage	After 1 wk at 20°
0	0.0	0.0	0.0	0.5	0.8	1.4
5	0.0	0.0	0.0	0.2	0.4	0.9
12.8	0.0	0.0	0.0	0.0	0.0	0.0

The carbon dioxide and oxygen concentrations in the internal atmosphere of the fruit is given in Table 2. Significant increases in the carbon dioxide concentration and decreases in O_2 concentrations were observed 7 hr after transfer to 20°C from chilling exposures (0° and 5°). The carbon dioxide and oxygen concentration in the internal atmosphere in fruit stored 4 weeks at 0° and 5° return to levels equal to those in fruit placed directly at 20° after 24 hr at 20°C. Fruit held for 8 weeks at 0° and 12 weeks at 0° and 5° took longer for the internal carbon dioxide and oxygen concentrations to return to levels in fruit placed directly at 20°, indicating more severe physiological disturbance due to chilling. Similar increases in the internal concentrations of carbon dioxide and decreases in oxygen concentration have been reported (4). Storage at 12.8°, a nonchilling temperature, had no significant influence on the internal carbon dioxide and oxygen concentrations after transfer to 20°.

The respiratory rates of lemons at 20°C following chilling (0° and 5°) increased rapidly to a peak rate about 12 hr after transfer and then declined slightly to a fairly constant rate 24 hr after transfer as illustrated by fruit held 8 weeks at the respective storage temperatures before transfer to 20°C (Fig. 1). Similar respiratory rate stimulation for chilled citrus fruit have been reported (4, 5). To illustrate the stimulatory effect of chilling on the respiratory rates the average respiratory rates 24 hr after transfer to 20° are presented in Fig. 2. Fruit stored at 12.8° has a slightly higher respiratory rate than fruit placed directly at 20°, but the duration of storage had no significant effect. Storage at 0° and 5° had a pronounced stimulatory effect on subsequent respiratory rates at 20°

Table 2. Internal carbon dioxide and oxygen percentage of lemon fruit at storage temperature just before transfer (0) and 7, 24 and 48 hr after transfer to 20°C.

Storage		Hours at 20°C							
		0		7		24		48	
Duration (wk)	Temp (0°C)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)
–	–	1.2±.2	16.7±.8	–	–	1.6±.2	15.9±.8	1.2±.1	16.3±.7
4	0	0.7±.1	19.8±.2	4.8±.4	5.1±.9	1.4±.3	16.3±.9	1.2±.2	15.0±.9
	5	1.1±.1	18.2±.8	4.7±.2	5.6±.9	1.3±.3	16.3±.8	1.3±.2	15.1±.9
	12.8	0.9±.1	17.2±.8	2.3±.6	11.2±.6	1.2±.3	15.7±.9	1.4±.2	15.2±.8
8	0	0.8±.1	19.5±.2	5.2±.5	5.7±.9	2.6±.3	13.6±.9	2.2±.3	13.1±.8
	5	1.0±.1	19.2±.3	4.2±.2	9.8±.9	1.7±.2	15.6±.9	1.1±.1	16.2±.7
	12.8	0.9±.1	17.4±.4	1.1±.2	14.2±.8	1.9±.2	13.3±.8	2.1±.1	12.2±.7
12	0	0.8±.1	19.4±.2	6.6±.4	5.1±.5	3.7±.3	10.0±.6	2.0±.2	14.8±.6
	5	1.5±.2	17.4±.6	5.4±.5	9.7±.8	3.8±.3	11.9±.5	2.2±.5	13.4±.8
	12.8	1.0±.1	16.8±.7	2.0±.2	14.0±.9	2.1±.2	13.0±.8	2.0±.2	12.5±.9

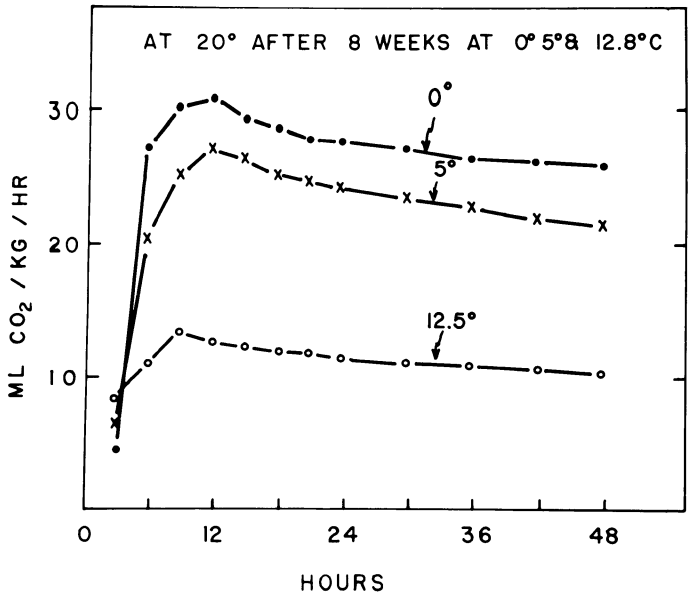

Fig. 1. Respiratory rate of lemon fruit at 20°C after 8 weeks at 0°, 5° and 12.8°.

and there was a significant increase in the respiratory rate as the duration of storage increased.

The rate of ethylene production at 20°C followed a pattern similar to that of the respiratory rates. Therefore, the rate of ethylene production 24 hr after transfer to 20° illustrates the rate response to chilling (Fig. 2). The rate of ethylene production at 20° after storage at 12.8° was very low with a slight increase as the storage period increased. Fruit stored at 0° or 5° produced ethylene at a significantly higher rate at 20° as the storage period increased with fruit stored at 0° producing more ethylene than fruit stored at 5°.

It is tempting to postulate that the high respiratory rates are a response to the ethylene synthesized by the fruit. However, the treatment of citrus fruit with exogenous ethylene does not induce a respiratory response until 6 to 8 hr after beginning the treatment. Therefore, the rapid respiratory rise at 20° following chilling (Fig. 1) is probably caused by metabolic disturbances; possibly the accumulation of metabolic intermediates during the chilling exposure which are available for rapid metabolism.

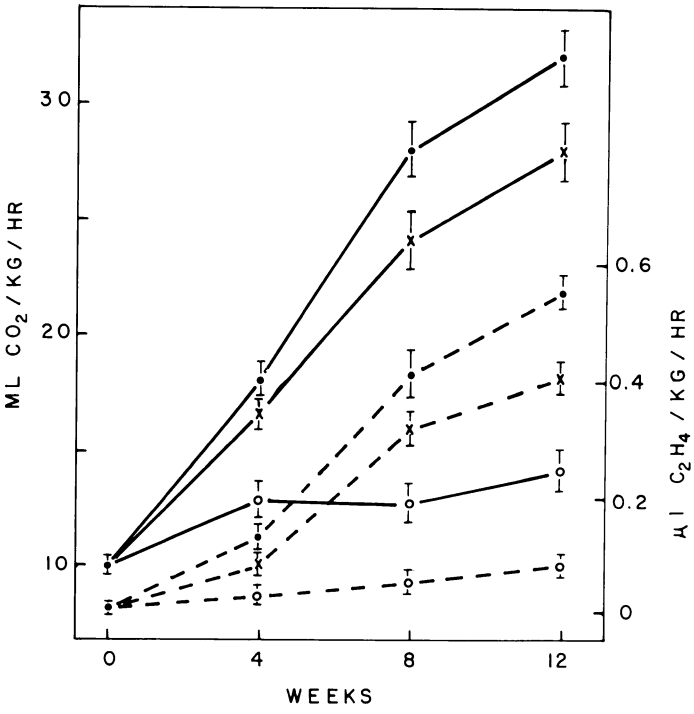

Fig. 2. Respiratory rate (solid line) and ethylene production dotted line) of lemon fruit 24 hr after transfer to 20°C after 0, 4, 8 and 12 weeks at 0° (•), 5° (x) and 12.8° (○). Vertical bars indicate SE.

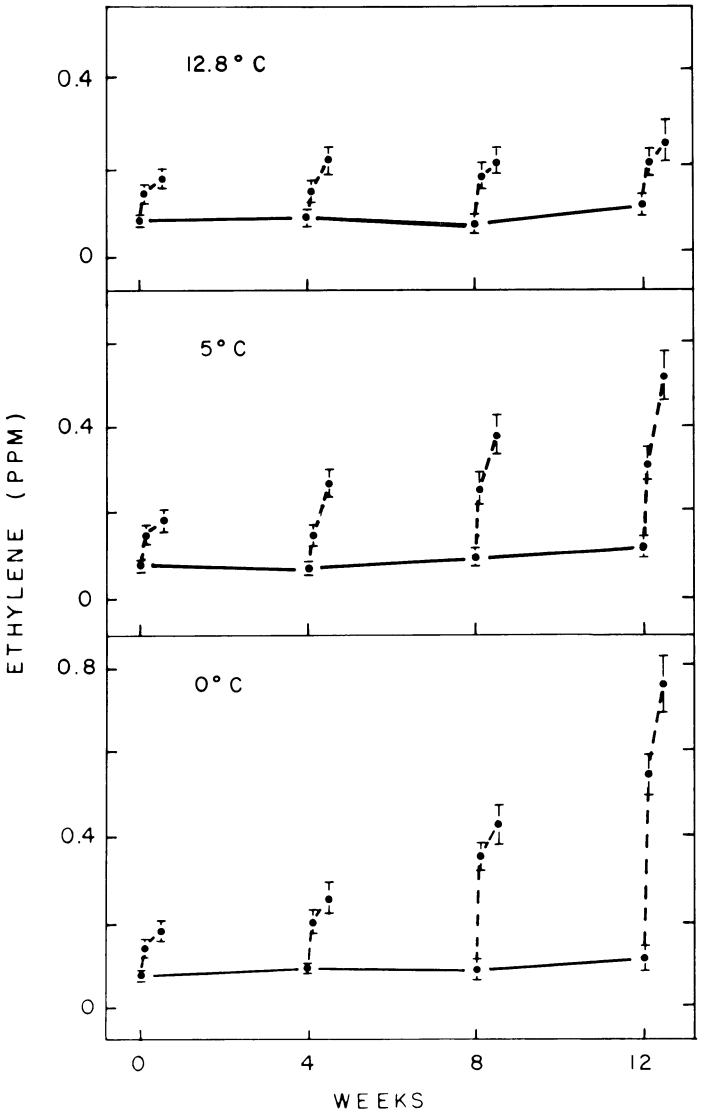

Fig. 3. Ethylene in the internal atmosphere of lemon fruit during storage for 0, 4, 8 and 12 weeks at indicated temperature (solid line) and 1 and 5 days after transfer to 20°C (dotted line). Vertical bars indicate SE.

The internal ethylene concentrations of lemons did not change significantly during storage at 0°, 5°, and 12.8°C (Fig. 3). When transferred to 20° after 4, 8 and 12 weeks storage at 12.8°, a nonchilling temperature, the internal ethylene concentrations were slightly higher than in the initial samples, but no differences existed among storage periods. Internal ethylene concentrations of fruit at 20° after storage at 0° and 5° showed a significant effect of storage duration, the increases being greater after each storage period for fruit held at 0° than for those held at 5°.

The ethyl alcohol content in the internal atmosphere of lemons (Fig. 4), in general, followed patterns similar to those observed for ethylene (Fig. 3). However, the ethyl alcohol

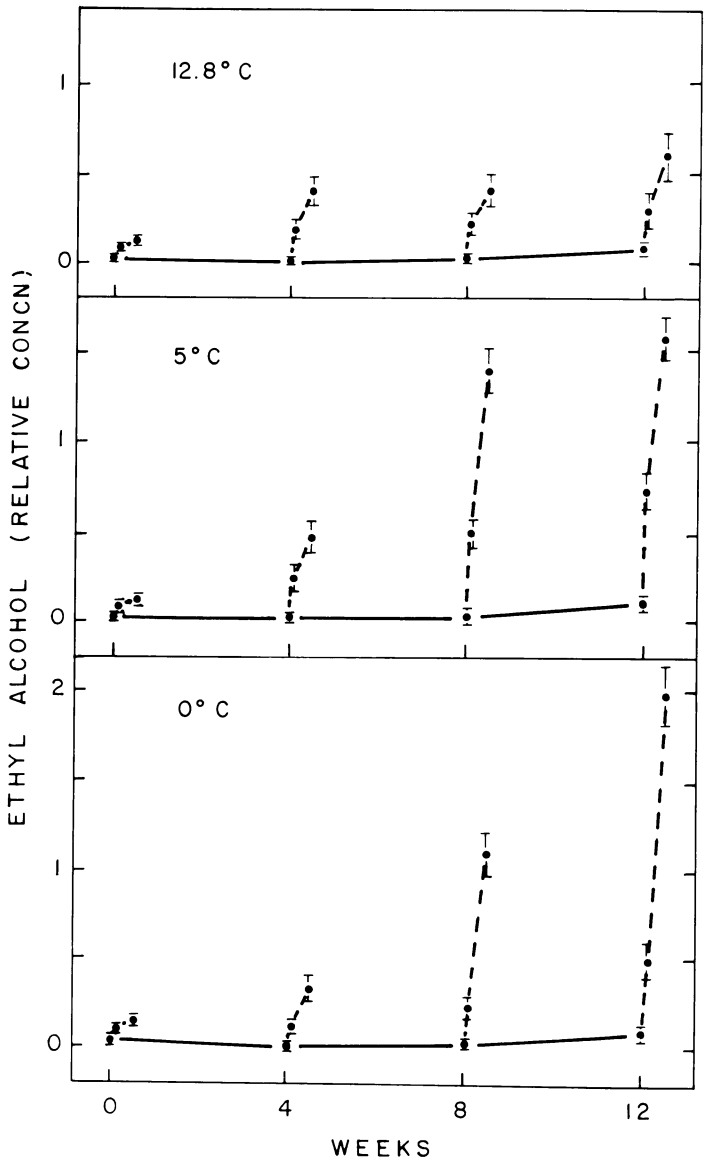

Fig. 4. Ethyl alcohol in the internal atmosphere of lemon fruit during storage for 0, 4, 8 and 12 weeks at indicated temperature (solid line) and 1 and 5 days after transfer to 20°C (dotted line). Vertical bars indicate SE.

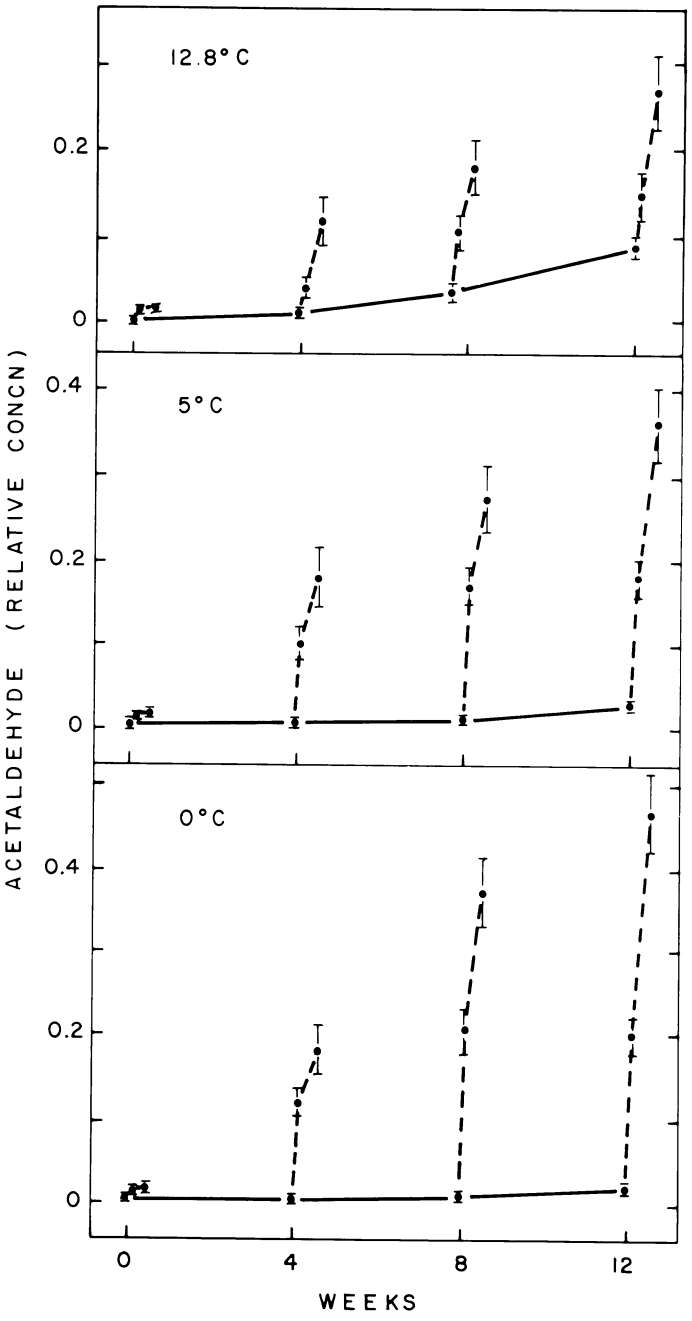

Fig. 5. Acetaldehyde in the internal atmosphere of lemon fruit during storage for 0, 4, 8 and 12 weeks at indicated temperature (solid line) and 1 and 5 days after transfer to 20°C (dotted line). Vertical bars indicate SE.

content of 20° after 8 and 12 weeks at 0° and 5° increased in a more linear fashion than did ethylene which showed a greater increase after 1 day at 20° than from the 1st to the 5th day.

Acetaldehyde in the internal atmosphere of lemons increased significantly during storage at 12.8°C, but not at 0° or 5° (Fig. 5). After transfer to 20° all treatments increased in acetaldehyde content. The increases at 20° were greater as the storage period increased at each temperature. The increases were greatest following 8 and 12 weeks storage at 0° and 12 weeks at 5°.

The internal atmosphere of lemons during storage and after transfer to 20° also contained methyl alcohol, ethyl acetate and other unidentified compounds. Volatile emanations from 'Valencia' oranges (8, 9) contained the same compounds as reported here for lemons. Also the stress of storage in a nitrogen atmosphere increased the amount of ethyl alcohol and acetaldehyde in the volatile emanations (8, 9).

Similar experiments were conducted with 'Valencia' and navel oranges and the patterns of respiration, ethylene production and internal volatile content for these cultivars stored for 4, 8 and 12 weeks at 0°, 5° and 10°C were similar to those presented for lemons.

Although the physiological, biochemical and physical changes associated with chilling injury in various plant materials differs, the temperature-induced phase transition in cellular membranes is common to all chilling sensitive tissues (6). As a consequence of this phenomenon it has been suggested that this leads to metabolic aberrations which result in the accumulation of metabolites, some, such as acetaldehyde and ethyl alcohol may be toxic. Increases in acetaldehyde and ethyl alcohol have been reported during storage at chilling temperatures in the juice of citrus (3) and in other fruits (6). However, the internal

atmosphere of citrus reported here did not accumulate ethyl alcohol or acetaldehyde during exposure to chilling temperatures. Acetaldehyde did increase during storage at 12.8°C, a nonchilling temperature, and appears to be associated with senescence. The previous report of accumulation of ethyl alcohol and acetaldehyde in citrus during chilling (3) analyzed head space gases over juice and these compounds could have been formed as a result of exposure to nonchilling temperatures during juicing operations and holding time in the can. However, the response of citrus reported here indicate that the respiration, ethylene production and internal ethylene, ethyl alcohol and acetaldehyde content increased at 20° after chilling characteristic of metabolic disturbances caused by chilling, i.e., the longer the exposure or the lower the storage temperature the greater the increase. Any one of these responses has the potential for evaluating the damage caused by chilling injury during storage of citrus fruit.

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Effects of Irrigation Regimes on Yield and Water Use of Snap Bean (*Phaseolus vulgaris* L.)[1,2]

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Additional index words. soil water tension, evapotranspiration, pan evaporation, irrigation scheduling

Abstract: **Two snap bean cultivars, 'Galagreen' and 'Eagle', were grown in rainfall sheltered irrigation plots as spring and fall crops. Pod yield of snap beans irrigated when the soil water tension reached 25 kPa (0.25 bar) averaged 11.9 MT/ha. Application of irrigation at soil water tensions of 50 kPa (0.5 bar) and 75 kPa (.75 bar) reduced yield by 41% and 48%, respectively. The reduction in water use was proportionately less than yield decreases, resulting in water use efficiencies of 0.62, 0.45 and 0.40 MT of pods/cm of water for the 25, 50 and 75 kPa irrigation treatments. Water use by the cultivars was similar, but pod yield and water use efficiency of 'Eagle' was greater than 'Galagreen'. Pod yields were reduced when plants were subjected to a 75 kPa soil water stress during pre-blossom, blossom or pod development growth stages. The relationships of snap bean water use (ET) to evaporation from an open pan (PA) were established throughout growth. The crop factor value (ET/PA) varied with plant age and irrigation regime.**

Irrigation is increasing rapidly throughout the United States especially in humid areas where need for irrigation has previously been considered marginal. In Georgia, which has an average annual rainfall near 1250 mm/yr, irrigation has increased from 78,500 ha in 1973 to an estimated 400,000 ha in 1979, with about 20,000 ha devoted to production of horticultural crops (9).

Snap bean is an important crop in the intensive, diversified, multi-cropped production programs being developed under year-round irrigation in the Southeast. Normally it is a high value crop, is fully mechanized and has a short growing season (≃ 60 days) when once over harvested.

Snap bean is susceptible to soil water stress, although the most critical developmental stage of the plant to soil water stress susceptibly has not been definitely established. Dubetz and Mahalle (1) reported soil water stress (800 kPa) during flowering was most damaging to yields, while Kattan and Fleming (6) and Maurer et al. (7) found postbloom stress to be most detrimental. Gableman and Williams (3) reported that pre-bloom irrigation was not necessary if beans were planted in moist soil, but adequate soil moisture during bloom and pod development was required. Other researchers (1, 2, 6, 7, 8, 10, 11) found that adequate, full season soil moisture contributed to highest yields.

Janes (4) reported water use by irrigated beans of 4.4 mm/day (June 30 – July 22) and 4.9 mm/day (July 18 – August 15) at two stages of growth. These evapotranspiration rates were equal to 90 and 94% of potential evaporation as determined by atmometers, and 65 and 71% of measured pan evaporation.

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