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# Chilling-Induced Lipid Degradation in Cucumber (*Cucumis sativa* L. cv Hybrid C) Fruit[1]

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## ABSTRACT

**Chilling at 4°C in the dark induced lipid degradation in cucumber (*Cucumis sativa* L.) fruit upon rewarming at 14°C. Rates of ethane evolution by fruits rewarmed after 3 days of chilling were up to four-fold higher than those evolved by unchilled (14°C) fruits (0.02–0.05 picomoles gram fresh weight$^{-1}$ hour$^{-1}$). This potentiation of lipid peroxidation occurred prior to irreversible injury (requiring 3 to 7 days of chilling) as indicated by increases in ethylene evolution and visual observations. Decreases in unsaturation of peel tissue glycolipids were observed in fruits rewarmed after 3 days of chilling, indicating the plastids to be the site of the early phases of chilling-induced peroxidation. Losses in unsaturation of tissue phospholipids were first observed only after chilling for 7 days. Phospholipase D activity appeared to be potentiated in fruits rewarmed after 7 days of chilling as indicated by a decrease in phosphatidylcholine (and secondarily phosphatidylethanolamine) with a corresponding increase in phosphatidic acid. These results indicate that lipid peroxidation may have a role in conferring chilling injury.**

Several theories have been advanced to account for the nature of CI.[2] The primary lesion has been proposed to involve bulk membrane lipid phase transitions (12), unfavorable and direct low temperature effects on proteins and enzymes (7), the presence of high-melting membrane lipid species (15), and a redistribution of cellular calcium (13). Although data are available to support each of these theories, none appears to be universally accepted as the primary determinant of CI. The existence of a universal mechanism of CI is questionable and alternatively, several of the aforementioned factors may be required to confer low temperature sensitivity. There is also the possibility that some other unidentified factor(s) may be a determinant of CI in susceptible plant tissues.

A common response of plant tissues to environmental stress is lipid peroxidation. This has been noted for plant tissues subjected to salinity (3), wounding (4), freezing (4), and drought (21) stress. Although lipid peroxidation has not been examined as a primary response to chilling stress (in the absence of illumination), there is indirect evidence in the literature that suggests it may have a role in the development of chilling injury. The application of antioxidants to cucumber and pepper fruits delayed or reduced the severity of low temperature injury (26). Chilling also evokes a decrease in catalase activity in cucumber seedlings (18). These studies indicate that antioxidant defenses may be compromised in some CS plants exposed to low temperatures. In the special case where chilling is accompanied by illumination, photooxidation of cellular membranes is believed to play a role in the development of CI (22, 28, 29).

We propose that lipid peroxidation may have a role in the development of CI, and undertook a study to provide an initial evaluation of this new hypothesis. Cucumber fruits were used as a model since the visual manifestations of chilling injury in this tissue are well-defined (25, 26). There is also a lack of studies on CI in fruit tissues compared to whole plants, leaves, and other organs. Fruits were chilled in the absence of light to eliminate the contribution of photooxidative processes in the development of injury.

## MATERIALS AND METHODS

### Cucumber Fruit

Mature cucumber fruits (*Cucumis sativa* L.) cv Hybrid C were obtained from a commercial grower in west central Florida within a day after harvest and air-freighted to Madison, WI, in insulated containers such that the ambient temperature remained above 10°C throughout transit. Problems encountered in coordinating shipment were responsible for our inability to provide zero-time data for all analyses.

### Storage Treatments

Fruits selected for uniformity and absence of damage and defects were randomly divided into two lots. One group was stored at 14°C in containers purged with air at 95% RH and served as controls (unchilled). Another group (chilled) was treated similarly except storage was at 4°C. At selected intervals, some fruit were removed from 4°C storage and transferred (rewarmed) to 14°C storage under the same conditions used for the unchilled fruits. All cucumbers were stored in the dark. At selected intervals, two or three individual fruits from each treatment group were analyzed as described below.

### Analysis for Headspace Gases

Individual fruits were placed in closed glass containers for up to 8 h at the temperature representative of their treatment

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[2] Abbreviations: CI, chilling injury; CS, chilling-sensitive; BHA, butylated hydroxyanisole; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PA, phosphatidic acid; gfw, gram fresh weight; ACC, 1-aminocyclopropane-1-carboxylic acid.

group, after which gases accumulating in the headspace (1.0–5.0 mL sample) were analyzed. Analysis for ethylene and ethane was achieved by gas chromatography (Shimadzu GC-9AM with Chromatopac C-R3A) using a Poropak N, 80/100 mesh column (Supelco). The carrier gas was nitrogen at a flow rate of 30 mL $min^{-1}$, and injector, column, and flame ionization detector temperatures were 150, 36, and 150°C, respectively. Quantification of each gas was made relative to external standard curves prepared with 1000 ppm ethylene and 100 ppm ethane (both balance of nitrogen) calibration gases (Alltech).

### Electrolyte Leakage

Freshly excised discs (2.0 mm thick, 9.5 mm diameter, 10–11 discs, about 2 g total) of mesocarp tissue from each fruit were placed in 25 mL 0.4 M mannitol and incubated at ambient temperature. Conductivity of the suspending solution was measured after 5 h and again after autoclaving at 121°C for 30 min (taken as 100%) with a YSI model 32 conductivity meter (Yellow Springs).

### Isolation of Tissue Lipids

Peel tissue (about 5 g) was excised as thin strips (1–2 mm thick) and homogenized in 36 mL isopropanol containing 400 μg methyl heptadecanoate as an internal standard. The resulting suspension was combined with 54 mL hexane and rehomogenized. After filtering over Whatman No. 1 paper, the homogenization vessel and filter cake were washed with 10 mL isopropanol: hexane (3:2) containing 0.05% BHA and the pooled extracts were placed in a separatory funnel to which 46 mL 6.0% sodium sulfate were added. The total mixture was vigorously agitated and the hexane-rich organic phase was dried over crystalline sodium sulfate, decanted, and evaporated under nitrogen at 45°C. The residue was resuspended in 1.5 mL chloroform:acetic acid:BHA (100:1:0.005, v/v/w) and stored under nitrogen at −40°C until analyzed.

### Fractionation of Lipids

A 0.5 mL sample of the lipid extract was applied to a 500 mg silica Sep-Pak cartridge (Waters) and sequentially eluted (under 3 in vacuum) by 12 mL chloroform:acetic acid (100:1), 6 mL acetone followed by 6 mL acetone:acetic acid (100:1), and 6 mL chloroform:methanol (2:1) followed by 6 mL methanol, to obtain neutral lipid, glycolipid, and phospholipid fractions, respectively (11). The efficacy in separating these lipid fractions was verified by TLC (see below). For each fraction, solvent was evaporated under nitrogen and the residue resuspended in 0.5 mL chloroform containing 0.005% BHA. The suspension was stored under nitrogen at −40°C until analyzed.

### Fatty Acid Composition

Esterified fatty acids in each fraction (0.15 mL) were converted into their methyl ester derivatives by reaction at 55°C for 10 min 0.3 mL tetrahydrofuran and 1 mL dry methanolic KOH (0.5 M). Methylpentadecanoate (100 μg) was added as an internal standard. After addition of 0.1 mL acetic acid and 4 mL saturated NaCl, the methyl esters were partitioned into 8 mL hexane, dried under nitrogen, and resuspended in 0.1 mL hexane. Analysis of fatty acid methyl esters was achieved by gas chromatography (Shimadzu) fitted with a glass column packed with 10% SP-2330 (100/200 mesh) Chromosorb WAW (Supelco) with nitrogen as carrier gas at a flow rate of 50 mL $min^{-1}$. Injector and flame ionization detector temperatures were 270 and 300°C, respectively, and column temperature was programmed from 190 to 220°C at 6° $min^{-1}$.

### Separation of Glycolipid and Phospholipid Fractions

Between 100 to 150 μL of each fraction was separated on boric acid-impregnated LK-5 silica gel thin layer plates (Whatman) as described by Leray and Pelletier (15). Identification of MGDG, DGDG, PC, PE, PI, PG, and PA was made by cochromatography with standards (Sigma) and specific spray reagents. Detection of lipid bands was achieved with brief exposure to iodine vapor or 0.02% rhodamine 6G in ethanol. Bands corresponding to identified lipids were scraped from the plate and analyzed for lipid-phosphorous by wet-ashing with $Mg(NO_3)_2$ and subsequent reaction with ascorbic acid and sodium molybdate (1) or galactose after digestion by 2 *N*-sulfuric acid and reaction with phenol in 29 *N*-sulfuric acid (20). Quantification was made relative to inorganic phosphate and galactose external standards, respectively.

### Treatment of Data

Due to the wide fruit-to-fruit variation, data are presented for each fruit analyzed, and lipid analyses are reported after normalization. Actual values for peel tissue total glycolipid and phospholipid contents were at 1477 ± 434 (SD) and 598 ± 151 nmol $gfw^{-1}$, respectively, which is similar to a previous determination of peel tissue polar lipid content (26). The ratio of MGDG:DGDG observed for all samples analyzed was 1.92 ± 0.69. The data were plotted using polynomial curve-fitting software.

## RESULTS

### Development of Injury in Chilled Cucumber Fruits

Changes in electrolyte leakage of mesocarp discs and visual observations of intact fruit were used to assess the development of chilling injury. There were only modest increases (from 20–25%, to 35–40%) in electrolyte leakage of tissue discs excised from fruits continuously held at 4 and 14°C (control) over 22 d of storage (Fig. 1A). There was also little difference between chilled and control fruits. A marked increase in electrolyte leakage for fruits transferred from 4 to 14°C occurred only after 18 d of chilling (Fig. 1B). The visual assessment of chilling injury in stored fruits is reported in Figure 2. Controls began to senesce by 10 d as indicated by a fading and yellowing of the peel tissue. Continuously chilled fruits developed the first signs of injury after 18 d and injury became pronounced by 22 d. Fruits transferred after 3 d of chilling showed no signs of injury and started to senesce by the end of the 8 d rewarming period. Fruits chilled for at least

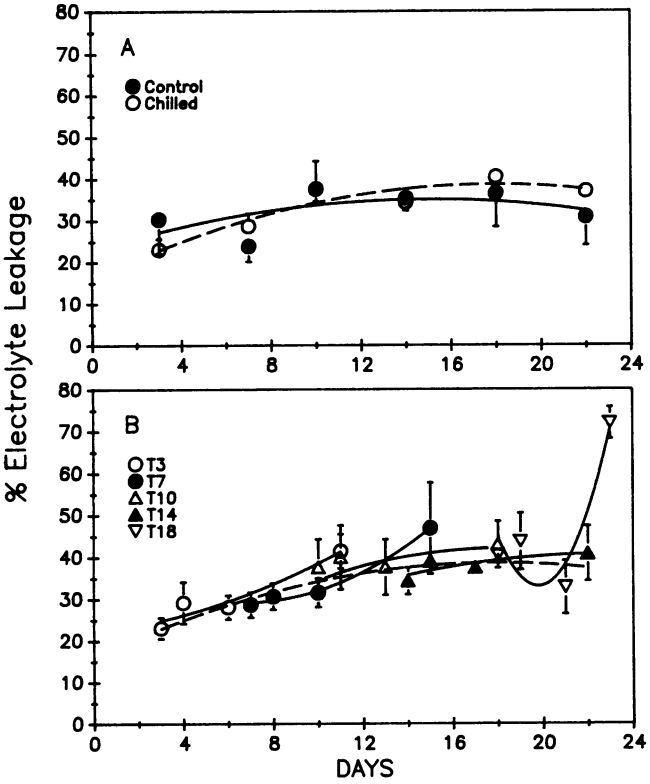


**Figure 1.** Electrolyte leakage in tissue discs of stored cucumbers. A, Fruits stored continuously at 4 or 14°C; B, fruits rewarmed at 14°C after chilling for 3 (T3), 7 (T7), 10 (T10), 14 (T14), and 18 (T18) d where the dashed line represents the response of the continuously chilled fruits. Bars = SD.

7 d showed visible signs of injury after rewarming. From these observations, we conclude that for this cultivar, irreversible injury due to chilling requires between 3 and 7 d of continuous low temperature exposure. Our results concur with previous observations on the chilling sensitivity of cucumbers and that chilling injury is initially observed after rewarming (25, 26).

### Ethylene and Ethane Evolution by Stored Fruits

Ethylene production, often used as an indicator of stress, in stored fruits is described in Figure 3. Continually chilled fruits evolved more ethylene than did controls during storage for the first few days and after about 2 weeks (Fig. 3A). Upon transfer of chilled fruits to 14°C, a surge in ethylene production was consistently observed (Fig. 3B). More pronounced ethylene formation during rewarming was associated with a longer duration of chilling for up to 10 d. The attenuation of the surge in ethylene evolution when rewarming follows extended durations of chilling has been previously noted for cucumber fruit (25).

Ethane evolution was used to follow the course of lipid peroxidation in stored fruits (Fig. 4). Low levels of ethane were evolved in continuously chilled and control fruits (Fig.4A). Ethane evolution was enhanced in continuously chilled fruits after 20 d, when visible signs of injury were evident, compared to the controls. Low levels of ethane evolution for the controls is consistent with the development of senescence which is believed to involve lipid peroxidative processes (24). Rewarming of chilled fruits was consistently accompanied by an increase in ethane evolution, with this increase being more pronounced when rewarming followed protracted chilling (Fig. 4B).

### Changes in Fatty Acid Unsaturation

Glycolipid and phospholipid fractions were analyzed for changes in fatty acid composition (16:0,[3] 18:0, 18:1, 18:2, 18:3). Changes in the ratio of unsaturated:saturated fatty acids [(18:1 + 18:2 + 18:3)/(16:0 + 18:0)] for the glycolipid fraction are shown in Figure 5. There was little change in this ratio with time and little difference in this ratio between control and continuously chilled fruits (Fig. 5A). Others have noted little change in this ratio for a polar lipid fraction isolated from peel tissue of cucumber fruit chilled for short durations (26). Rewarmed fruits showed a decrease in unsaturation of the glycolipid fraction (Fig. 5B). This decrease in unsaturation appeared to be reversible only for fruits rewarmed after 3 d of chilling, where the levels of unsaturation recovered to those observed for continuously chilled and unchilled fruits.

For the phospholipid fraction no differences were noted in the degree of unsaturation between the control and continuously chilled fruits, nor were there any changes in unsaturation during storage (Fig. 6A). Upon rewarming a decline in phospholipid unsaturation was noted for fruits previously chilled for at least 7 d (Fig. 6B). This trend became more pronounced as the fruits were chilled for longer periods up to 14 d prior to transfer. Fruits rewarmed after chilling for only 3 d showed no decline in phospholipid unsaturation relative to continuously chilled and control fruits.

### Changes in Glycolipids and Phospholipids

There were no consistent or discernible changes in the MGDG and DGDG contents, the MGDG:DGDG ratio or the relative levels of PE, PI, and PG in lipid fractions isolated from peel tissue of chilled, control, and rewarmed fruits (data not shown). Changes in percent PC content during storage of control and continuously chilled fruits yielded no obvious patterns or differences (Fig. 7A). However, during rewarming, the percent PC decreased, especially after 7 and 14 d of chilling (Fig. 7B). After 10 d of chilling there was little change in PC during rewarming; however, PE declined from about 30 to 20% (data not shown).

The changes in percent PA in control and continuously chilled fruit are described in Figure 8A. The relatively high baseline percent PA in all of the phospholipid preparations was not due to the potentiation of phospholipase D activity during homogenization or extraction. Lipid extractions prepared with an initial step of homogenization with hot isopropanol gave similar results. We believe the higher than normal levels of PA are a result of initially slicing the thin strips of peel tissue from the fruit, yielding a large degree of cellular damage due to the large surface area of excised tissue. Phospholipase activities are known to be rapidly activated upon

[3] Fatty acids are designated by X:Y, where X is the carbon number and Y is the number of double bonds.

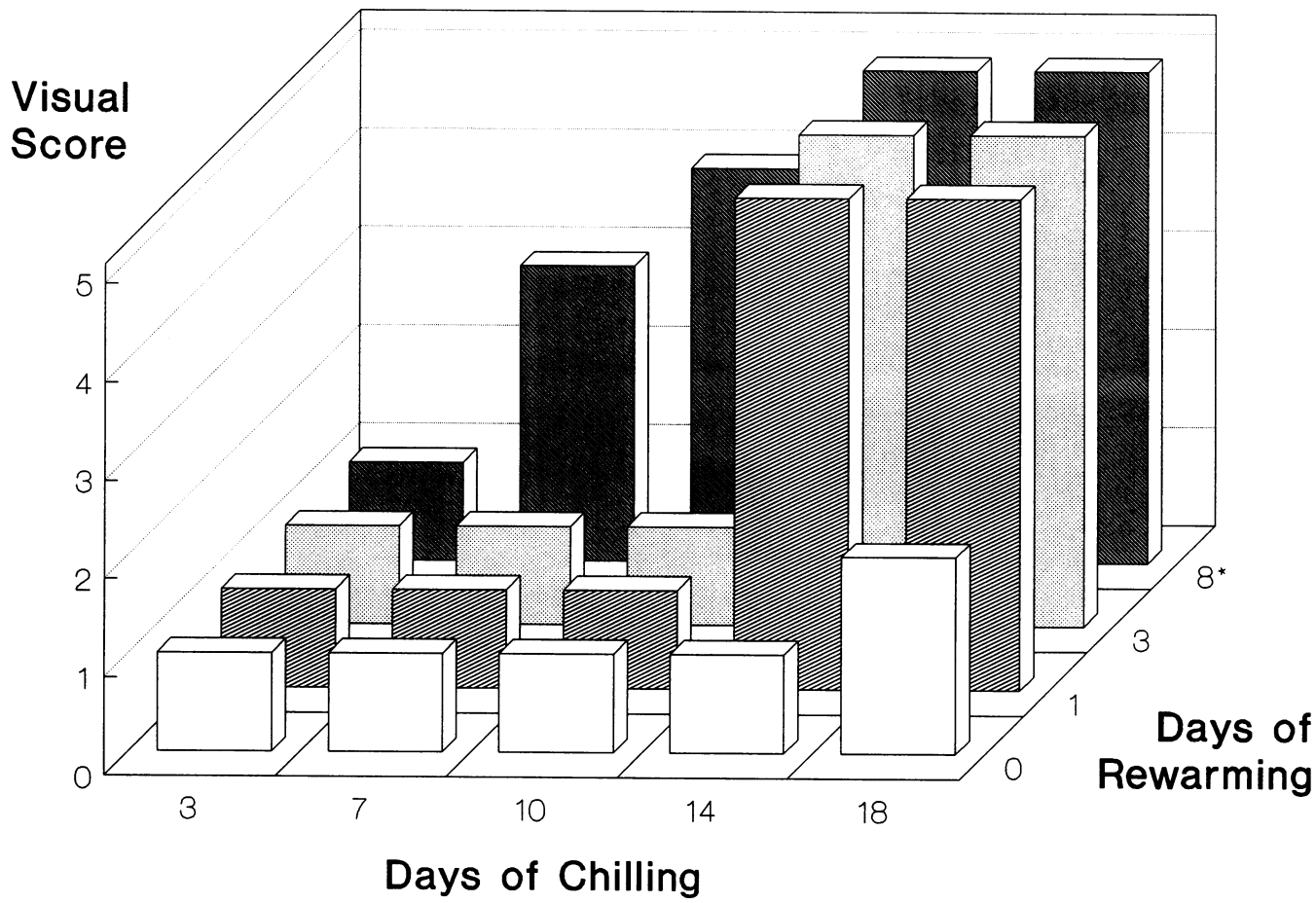


**Figure 2.** Visual assessment of chilling injury in stored fruit. 1, No injury; 2, trace injury as indicated by shriveling/pitting in some fruits; 3, slight injury as indicated by shriveling/pitting in all fruits; 4, moderate injury as indicated by pitting, depressions in peel, and internal browning; and 5, severe injury as indicated by tissue collapse and incipient decay. (*), Evaluated after 5 d of rewarming for fruit chilled 18 d.

tissue disruption, especially in leaf and fruit tissues of curcurbits (14). A phospholipid fraction extracted with boiling isopropanol from excised discs of cucumber leaf tissue also had a percent PA content of about 4% (8). Therefore, for the present study, levels of PA above 4 to 5% are considered as increases taking place in the tissue during storage. There were only minor changes in percent PA in continuously chilled fruits for the duration of storage and for the control fruits until about 18 d of storage (Fig. 8A). Between 18 and 22 d, percent PA increased in the controls and this was the time period associated with advanced senescence. Rewarming of fruits previously chilled for at least 7 d was associated with marked increases in percent PA content (Fig. 8B). These increases were quantitatively similar to the corresponding decreases in percent PC (Fig. 7B) and PE (when rewarmed after 10 d of chilling).

## DISCUSSION

The objective of this study was to determine if lipid peroxidation was associated with the development of CI. We attempted to correlate the development of injury in cucumber fruit to increases in electrolyte leakage, a parameter that has often been used to indicate physical damage to the plasmalemma resulting from low-temperature stress (16). The cultivar of cucumber (Hybrid C) used in this study did not display the rapid and marked increases in electrolyte leakage during chilling stress that other cucumbers do (16), including those (Marketmore and Carolina) used in other studies in our laboratory (SJ Kuo, KL Parkin, unpublished data). Furthermore, we found substantial increases in electrolyte leakage took place only after fruit displayed visible signs of CI. Therefore, it can be questioned from our results whether electrolyte leakage is always an adequate assessment of CI in cucumber fruit. While an increase in electrolyte leakage is an early response to chilling in several fruits it does not appear to be a symptom shared by all CS plants (16). This forced us to rely primarily on a visual assessment of CI, which is commonly done for studies of CI in cucumber fruit (25, 26). It is acknowledged that this type of assessment is not adequate for evaluating the early stages of injury, but it did allow a determination (after rewarming) of whether irreversible injury due to chilling had occurred in stored fruit. Based on our observations, irreversible injury due to chilling required between 3 to 7 d of continuous chilling before CI could be detected following an additional 8 d of rewarming. In continuously chilled fruit, signs of injury were first noticed after 18 d. The

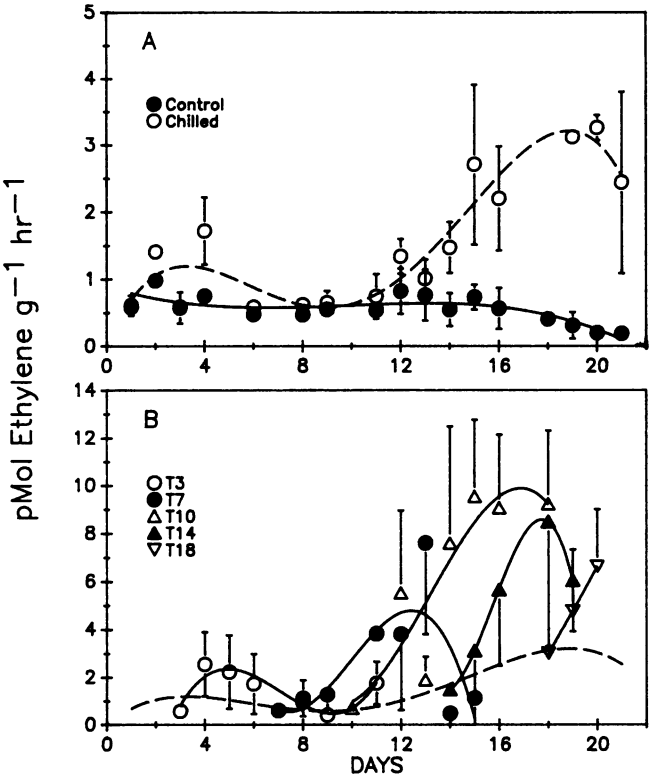


**Figure 3.** Ethylene evolution in stored fruits. A, Fruits stored continuously at 4 or 14°C; B, fruits rewarmed at 14°C after chilling for 3 (T3), 7 (T7), 10 (T10), 14 (T14), and 18 (T18) d where the dashed line represents the response of the continuously chilled fruits. Bars = SD.

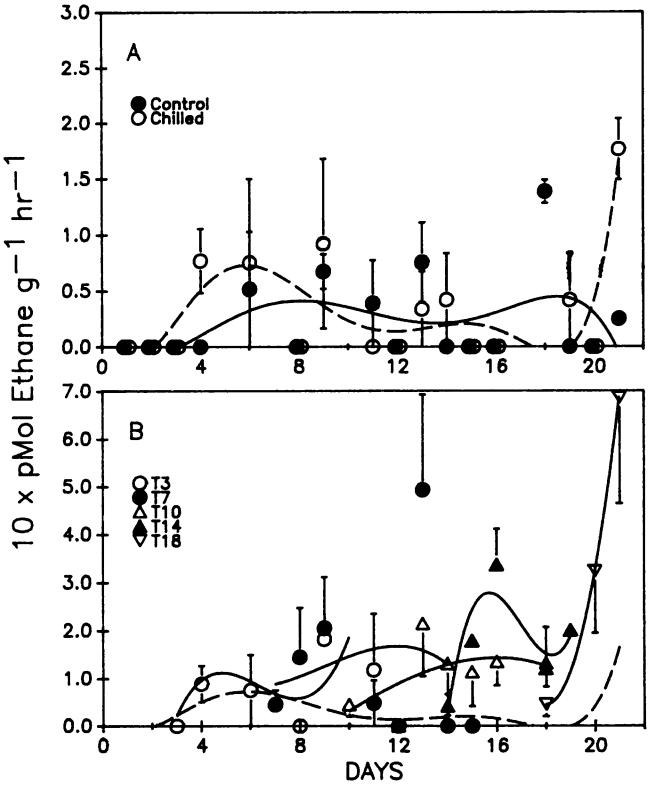


**Figure 4.** Ethane evolution by stored fruits. A, Fruits stored continuously at 4 or 14°C; B, fruits rewarmed at 14°C after chilling for 3 (T3), 7 (T7), 10 (T10), 14 (T14), and 18 (T18) d where the dashed line represents the response of the continuously chilled fruits. Bars = SD.

Hybrid C cultivar appears to be of moderate sensitivity to chilling compared to others (25, 26).

During the first week of storage average rates of ethane evolution (lipid peroxidation) were similar for continuously chilled and unchilled fruits (about 0.02–0.05 pmol $gfw^{-1}$ $h^{-1}$). However, fruits rewarmed after only 3 d of chilling evolved ethane at rates up to four-fold higher (up to 0.20 pmol $gfw^{-1}$ $h^{-1}$) than continuously chilled or unchilled fruits. Fruits chilled 3 d and rewarmed 8 d showed no visible signs of CI and appeared to enter into a normal senescent pattern upon rewarming. This indicated that the effects of chilling were reversible after at least 3 d of low temperature exposure and that an increase in lipid peroxidation was induced prior to the onset of irreversible injury. The surge of ethane evolution during rewarming of fruit chilled 3 d corresponded to a transient fall and rise in the degree of unsaturation in the tissue glycolipids, whereas little change in this parameter was observed for the phospholipid fraction. This implicates the plastid as being the primary target of chilling-induced lipid peroxidation. Although the levels of ethane evolved by chilled/rewarmed fruit were low, these levels approach those that are evoked by wounding upon the excision of tissue discs from beet leaf (about 1 pmol $gfw^{-1}$ $h^{-1}$, [(4]). Since cucumber peel is the tissue most sensitive to chilling (26) and comprises only a few percent of the total weight of the fruit, our calculations for ethane evolution based on the entire fruit may represent large underestimations of that taking place in the peel. By comparison, the more stressful conditions of chilling-enhanced photooxidation in cucumber leaves evokes about 125 pmol ethane $gfw^{-1}$ $h^{-1}$ (28, 29).

When chilling was extended to 7 d or more, rewarmed fruit began to display visible signs of CI. The increased rates of ethane evolution became more pronounced when rewarming followed protracted chilling. Losses in fatty acid unsaturation in peel tissue glycolipids and phospholipids were associated with the chilling-potentiated rise in peroxidative activity. The high rates of ethane evolution observed in chilled and rewarmed fruit during the latter stages of this study are probably a reflection of cumulative damage suffered from chilling stress.

Our results showed that chilling stimulated lipid peroxidation in cucumber fruit prior to the development of irreversible injury. Increases in rates of lipid peroxidation (and losses in glycolipid unsaturation) preceded increases in rates of ethylene evolution, indicating that lipid peroxidation may be responsible for creating stress conditions in chilled fruit. Although lipid peroxidation yields ethylene under certain conditions it is uncertain to what extent this pathway is operative in plants *in vivo* (27). Under conditions of combined chilling and photooxidative stress lipid peroxidation is not responsible for ethylene production (29). The enhanced levels of ethylene evolution in rewarmed cucumber fruit and other CS plant tissues is due to the accumulation of ACC and elevation of ACC synthase during and/or rewarming (25).

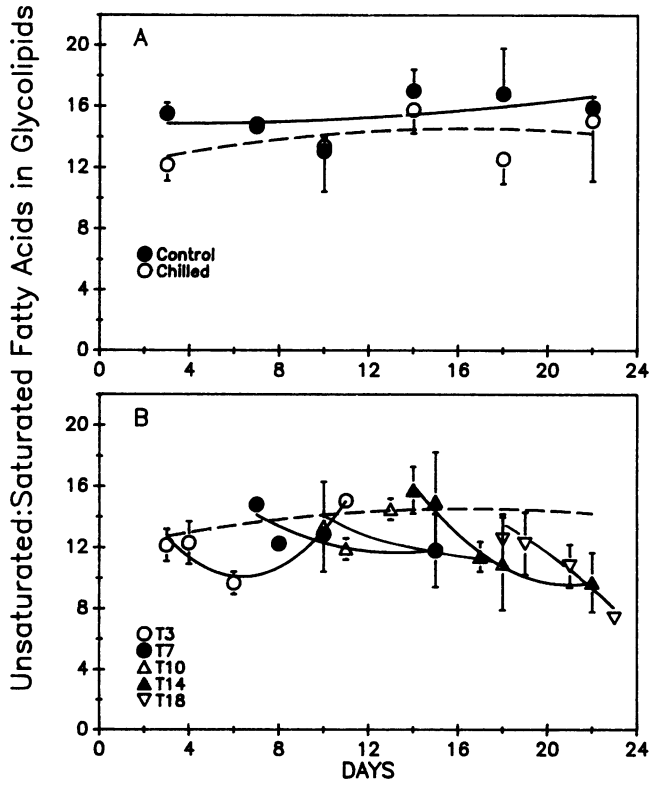


**Figure 5.** Changes in degree of unsaturation of peel tissue glycolipids. Degree of unsaturation is expressed as [(18:1 + 18:2 + 18:3)/(16:0 + 18:0)]. A, Fruits stored continuously at 4 or 14 °C; B, fruits rewarmed at 14°C after chilling for 3 (T3), 7 (T7), 10 (T10), 14 (T14), and 18 (T18) d where the dashed line represents the response of the continuously chilled fruits. Bars = SD.

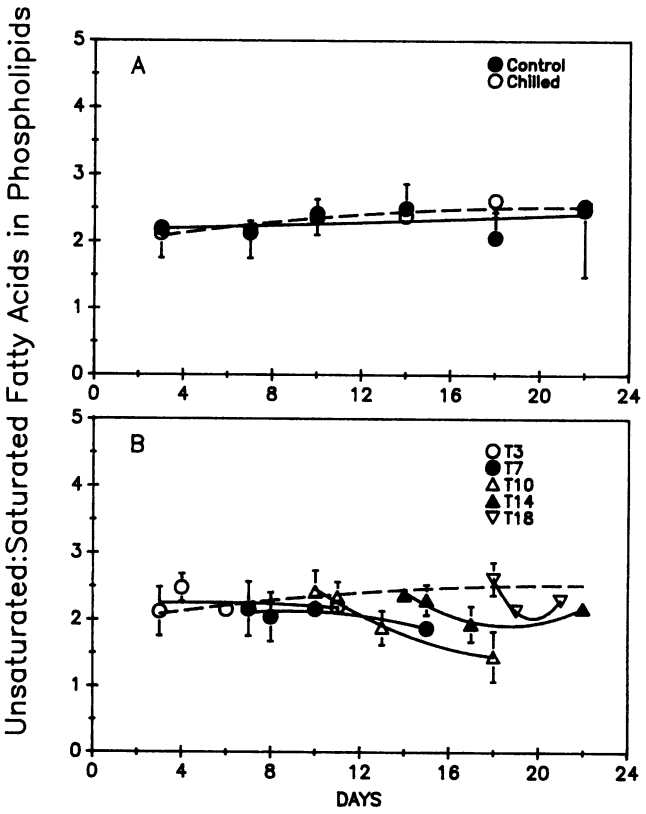


**Figure 6.** Changes in degree of unsaturation of peel tissue phospholipids. Degree of unsaturation is expressed as [(18:1 + 18:2 + 18:3)/(16:0 + 18:0)]. A, Fruits stored continuously at 4 or 14°C, B, fruits rewarmed at 14°C after chilling for 3 (T3), 7 (T7), 10 (T10), 14 (T14), and 18 (T18) d where the dashed line represents the response of the continuously chilled fruits. Bars = SD.

Although enzymic lipid peroxidation is facilitated by deesterification of unsaturated fatty acids, lipoxygenase can oxidize fatty acids in intact glycolipids (30). In addition, nonenzymic lipid peroxidation can proceed in membranes without a requirement for fatty acid deesterification (19). Therefore, the release of fatty acids from intact membrane lipids may not be a prerequisite for the elevated rates of lipid peroxidation observed for chilled and rewarmed fruit. Although we did not analyze for free fatty acids, our results indicated that phospholipase D activity is potentiated in fruit rewarmed after at least 7 d of chilling. Therefore, it is possible that other lipolytic activities may also be potentiated by chilling. An increase in free fatty acid levels of chloroplasts isolated from cucumber leaves chilled for 3 to 4 d in the dark has been reported (6). Free fatty acids also accumulate in the microsomal fraction of leaves of tomato plants chilled in the presence of light (22) and it is unlikely that this effect is induced primarily by illumination.

The preferential breakdown of PC (and secondarily PE) to PA in chilled and rewarmed cucumber fruit appeared to temporally follow the elevation in the rates of lipid peroxidation. Therefore, the potentiation of phospholipase D appeared to be a secondary response to chilling. PC also appears to be the phospholipid preferentially degraded during low temperature stress in leaves of cucumber plants (5). Due to its membrane fluidizing properties (8) the loss of PC may further increase tissue sensitivity to chilling stress. The activation of phospholipase D in chilled/rewarmed cucumber fruit may be an indirect effect of prior lipid peroxidation and/or phospholipase action on cellular membranes. These latter two processes can yield products which are calcium ionophores and lead to an increase in cellular calcium which is an activator of phospholipase D (10).

From our experiments it cannot be concluded if the increase in lipid peroxidative activity in chilled/rewarmed fruit is a partial cause or solely an effect of CI. While the latter view is likely more popular, it can be argued that lipid peroxidation may have a role in conferring CI. Although the potentiation of lipid peroxidation did not occur immediately upon chilling, this response did precede the development of irreversible injury in chilled and rewarmed cucumber fruit. Moreover, rapid responses to chilling such as membrane lipid phase transitions, loss of cytoplasmic streaming or a redistribution of cellular calcium alone cannot account for the requirement of many plants and their tissues to be exposed to several days of chilling prior to being injured. In such cases, some secondary dysfunction must be required to confer CI. If one takes the view that CI results from the irreversible lesion(s) evoked by low temperature stress, the features of lipid peroxidation in plants can fulfill this requirement. Lipid peroxidation leads to the proliferation of free radicals capable of indiscriminately

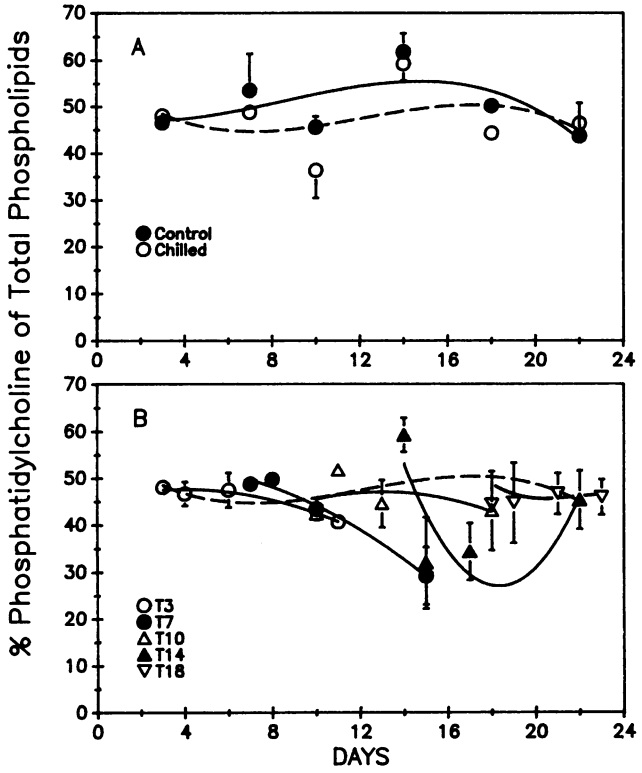


**Figure 7.** Changes in relative levels of PC in peel tissue phospholipids. A, Fruits stored continuously at 4 or 14°C; B, fruits rewarmed at 14°C after chilling for 3 (T3), 7 (T7), 10 (T10), 14 (T14), and 18 (T18) d where the dashed line represents the response of the continuously chilled fruits. Bars = SD.

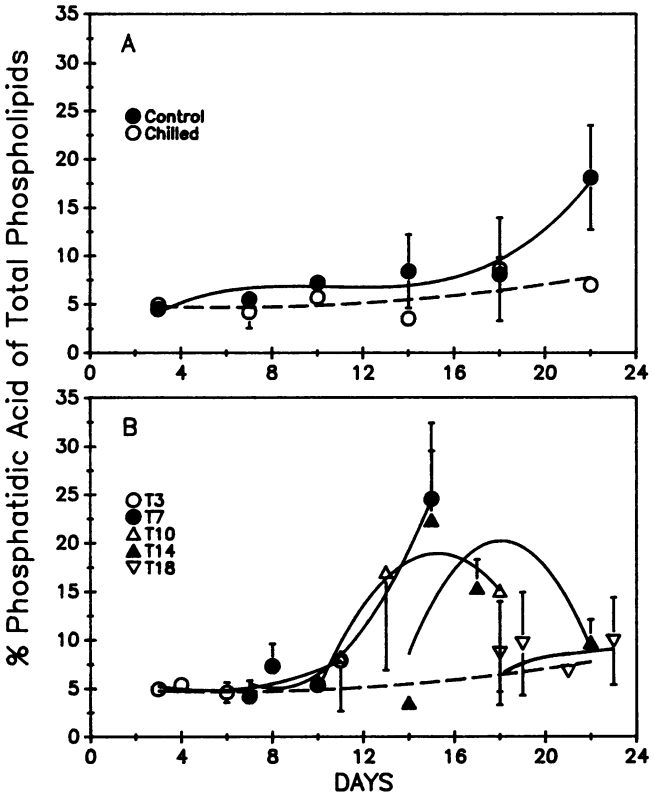


**Figure 8.** Changes in relative levels of PA in peel tissue phospholipids. A, Fruits stored continuously at 4 or 14°C; B, fruits rewarmed at 14°C after chilling for 3 (T3), 7 (T7), 10 (T10), 14 (T14), and 18 (T18) d where the dashed line represents the response of the continuously chilled fruits. Bars = SD.

damaging membranes and proteins (24), and deesterifying membrane fatty acids (17). Lipid peroxidation also induces the molecular ordering of membranes with a concomitant loss in membrane semipermeability (24). This could result in an irreversible collapse of gradients for key metabolic affectors, including calcium. Even low level lipid peroxidation is capable of causing pronounced changes in membrane structure (2). Membrane lipid peroxidation also induces phospholipase A activity, a response presumed to reflect cellular repair mechanisms (23). While plant tissues have a variety of antioxidant defenses, prolonged peroxidative stress will likely deplete the ability to withstand such stress. This is one rationale offered for the irreversible injury to cucumber leaves caused by chilling-induced photooxidation (28).

We are not proposing that lipid peroxidation is the primary or sole determinant of CI. Rather, our view on its potential role is predicated on the previous observations and theories of others. Rapid responses of plants to chilling, including membrane lipid phase changes/separations and a rise in cytosolic calcium may be required for lipolytic and lipid peroxidative activity to be initiated, especially if peroxidation is pursuant to deesterification of fatty acids. Lipolytic attack on cellular membranes is activated by calcium (10) and potentiated by the presence of blemishes or defects on the membrane surface (23). If the high-melting PG species promote such blemishes by undergoing a gel phase separation, this would implicate the plastids as being particularly sensitive to lipolytic attack. Others have noted an increase in the level of free fatty acids in chloroplasts during chilling (6) and we observed a decrease in the degree of unsaturation of the glycolipid fraction to be an early response to chilling stress in cucumber fruit. The observation that the potentiation of lipid peroxidation was an early response to chilling in cucumber fruit, and the participation of free-radical processes in senescence make it tempting to speculate that senescence and CI may share some common feature(s). In any event, the findings reported here warrant further investigation into the potential role lipid peroxidation may have in conferring CI.

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