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Acquisition of Low-temperature Tolerance in Tomatoes by Exposure to High-temperature Stress

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Abstract. **Mature-green tomato *(Lycopersicon esculentum* Mill.) fruit, when kept for 3 days at 36, 38, or 40C before being kept at 2C for 3 weeks, did not develop chilling injury, while unheated fruit placed at 2C immediately after harvest did. When removed from 2 to 20C, the heated tomatoes had lower levels of K^+ leakage and a higher phospholipid content than unheated fruit. Sterol levels were similar in heated and unheated fruit while malonaldehyde concentration was higher in heated fruit at transfer to 20C. The unheated tomatoes remained green, and brown areas developed under the peel; their rate of CO_2 evolution was high and decreased sharply, while ethylene evolution was low and increased at 20C. In contrast, the heat-treated tomatoes ripened normally although more slowly than freshly harvested tomatoes: color developed normally, chlorophyll disappeared, and lycopene content increased, CO_2, and ethylene evolution increased to a climacteric peak and K^+ leakage increased with time. During prestorage heating, heat-stress ethylene production was inhibited, protein synthesis was depressed, and heat-shock proteins accumulated. There appears to be a relationship between the "heat shock response" and the protection of tomato fruit from low-temperature injury.**

Higher plants, being immobile, have a greater need than do most organisms to protect themselves against transient stresses such as high or low temperature, pH changes, salinity, or water deficits. One of the most studied of these stresses has been high temperature, which causes the rapid synthesis of a group of proteins known collectively as heat-shock proteins .(HSP), concomitant with a reduction in the rate of protein synthesis (Czarnecka et al., 1984; Never and Scharf, '1984). The production of HSP confers thermotolerance on the tissue in which they were formed, so that subsequent exposure to a higher temperature, which would normally be lethal, does not cause damage (Key et al., 1985; Krishman et al., 1989).

Observations throughout the past decade have shown that exposure of plants to one stress can elicit responses similar to exposure to other stresses and sometimes protect the plant against another stress. Heat stress was found to protect wheat ***(Triticum aestivum*** Mill.) leaves against metal toxicity (Orzech and Burke, 1988). Water stress conferred cold hardiness on a variety of winter cereals (Cloutier and Siminovitch, 1982). These stresses all changed the pattern of protein synthesis, with some, though not all, changes being common for the different stresses (Czarnecka et al., 1984; Heikkila et al., 1984).

Sudden temperature drops are at least as likely to occur to plants in their natural environment as sudden rises in temperature. Studies with spinach ***(Spinacia oleracea*** L.) leaf disks (Guy et al., 1985) and barley ***(Hordeum vulgar-e*** L.) seedlings (Marmiroli et al., 1986) showed that a temperature drop resulted in an alteration within 4 of the pattern of protein synthesis, although there was no apparent homology with the heat-shock response (Ougham, 1987). However, in other model systems, stresses such as water deficiency or exogenous application of abscisic acid, which cause responses similar to heat shock, conferred resistance to subsequent periods of low temperature (Chen and Gusta, 1983; Cloutier and Siminovitch, 1982).

Subtropical fruits, such as tomato, develop chilling injury when kept below 10C. We have shown previously that a postharvest period of heat stress reversibly inhibited many ripening processes in apples ***(Malus domestics*** Borkh.) (Lurie and Klein, 1990), and that this inhibition was maintained when the fruit was placed at low temperature (Klein and Lurie, 1990). In addition, storage disorders were alleviated by prestorage heat stress (Lurie et al., 1990). Therefore, it was of interest to see if exposing harvested mature-green tomatoes to heat stress could protect them from chilling injury (CI). This paper examines the effect on tomatoes of 3 days of heat stress at 36, 38, or 40C, followed by chilling at 2C.

Materials and Methods

Mature-green tomatoes of uniform size and color were picked directly from the greenhouse and divided into three lots. Three replicates of 10 fruits each were used to follow postharvest ripening during 10 days at 20C. The second lot of fruit was placed immediately at 2C, while the third lot was heated for 3 days and then transferred to 2C. Heating was in a temperature-controlled chamber with the fruit in plastic trays inside non-sealed plastic bags to retard water loss. The fruit weight was monitored and did not exceed a loss of l% over the 3 days of heating. After 3 weeks at 2C, fruits were removed to 20C and kept there 7 days, during which time they were examined at 2-day intervals for color development, chilling injury symptoms and rot development. Color development was measured objectively as the 'a' value on a Gardner calorimeter (Gardener Laboratory, Bethesda, Md.) and subjectively as percent of red fruit.

Three replicates, consisting of 10 g of pericarp tissue from three fruits in each treatment, were frozen and then lyophilized for subsequent determination of malonaldehyde, phospholipid, sterol, chlorophyll, and Iycopene contents. For each of the chemical determinations, 100 mg of lyophilized tissue was used. Malonaldehyde was extracted and assayed according to Kosugi and Kikugawa (1985). Phospholipids and sterols were extracted and assayed as described by Lurie et al. (1987). Chlorophyll and Iycopene were extracted by grinding in chloroform, filtering, and reading absorption at 665 nm for chlorophyll and 455

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nm for lycopene. Extinction coefficients were as reported in Goodwin (1976).

From the same fruit that was sampled for Iyophilization, two pericarp disks weighing ≈1 g were removed from each fruit with a cork borer, washed twice in distilled water, and incubated in 4 ml of 0.3 M sorbitol, 10 mM Tris·Mes pH 7. After 4 h of incubation, the disks were removed from the incubation medium, frozen in 4 ml of fresh incubation medium, and then thawed for determination of K^+ remaining in the tissue. Potassium content was measured with a flame photometer. Leakage was expressed as the ratio of the 4 h leachate's K^+ content to total tissue" K^+ content.

Ethylene and CO_2 evolution were measured by placing two fruits in l-liter jars and closing them for 1 h each day. Four replicate pairs of fruit from each treatment were assayed. Gas samples were withdrawn with a syringe through a septum in the jar lid. Ethylene was measured on a gas chromatography using a flame ionization detector with an alumina column kept at 80C with N as the carrier gas. Carbon dioxide was measured with a thermal conductivity detector with a Poropak N column kept at 25C and helium as the carrier gas. The jars were left open and ventilated between measurements.

Protein synthesis was determined using tomato disks incubated under sterile conditions with (^{35}S)methionine. Two replicates of pericarp disks from three tomatoes were placed in 12-well multiwell plates in 1 ml of incubation medium (0.3 M sorbitol, 10 mM potassium phosphate, pH 6). One group of replicates was placed at 20C and the other at 38C. After 15 min for temperature equilibrium, 560 kBq of (^{35}S)methionine (42 PBq·mol $^{-1}$, were added. Disks were removed after 4 and 12 h, rinsed with fresh unlabeled medium, and frozen in liquid nitrogen. The protein complement was extracted, assayed for radioactivity, and run on SDS-PAGE as described by Lurie and Klein (1990).

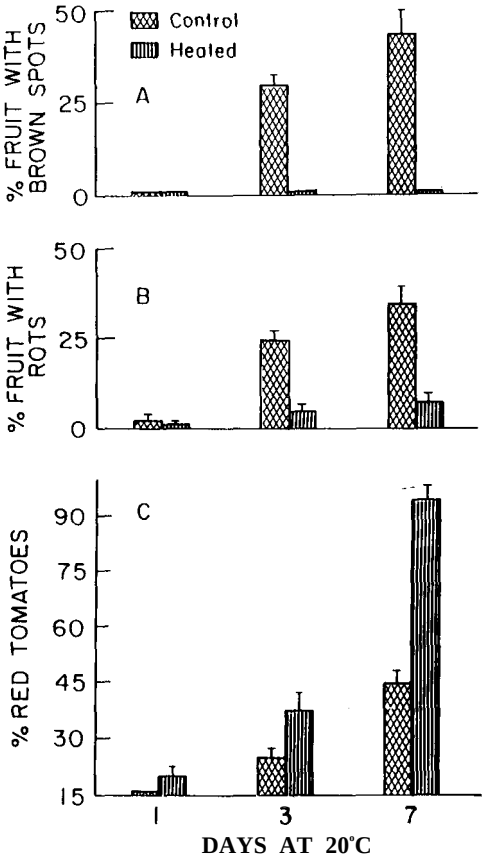

Fig. 1. Incidence of brown peel areas (A), occurrence of pathogen infection **(B),** and percentage red tomatoes (C) for fruit kept at 20C for 7 days following 3 weeks at 2C. Heated tomatoes were kept at 38C for 3 days before being placed at 2C. SD is indicated by bars.

Results

Tomatoes that had been heated 3 days at 38C before being kept 3 weeks at 2C developed no signs of Cl when transferred to 20C (Fig. 1). This lack of· CI symptoms was found also in fruit heated at 36 or 40C (data not shown). After 3 days at 20C, browning was apparent in untreated fruit, while even after 7 days the heat-treated fruits were free from signs of CI. Fungal infection was also much higher on the untreated tomatoes, the main pathogen having been *Altermaria altemata* (Fr.) Keissler. After 7 days at 20C, > 90% of the heat-stressed fruit had turned red, while >50% of the control tomatoes were still green. Chlorophyll disappearance and lycopene accumulation commenced in the heat-stressed tomatoes after transfer from 2 to 20C (Fig. 2). In the unheated tomatoes, lycopene did not increase during 5 days at 20C, but chlorophyll decreased, although its concentration remained higher than in heat-stressed tomatoes. One day after removal to 20C, the chlorophyll content of the heat-stressed fruit was significantly lower than that of the untreated fruit, and this difference was retained during subsequent days. Lycopene content, although similar in all three treatments after 1 day at 20C, had almost doubled after 5 days at 20C in tomatoes heat-treated at 40C (Fig. 2). Heat stress at 40C caused greater changes in pigment concentration than did stress at 38C.

Potassium leakage in untreated tomatoes remained static at ≈50% during most of the period at 20C, while heat-stressed tomatoes showed low initial K^+ leakage, which increased as the tomatoes ripened (Fig. 3). The leakage of heated and unheated tomatoes was higher than the 31% for a 4-h incubation found in freshly harvested tomatoes. Changes in lipid membrane components, which may be a cause of enhanced leakage, were measured by determining sterol, phospholipid, and malonaldehyde contents. Freshly harvested green tomatoes contained 1500 µg phospholipid, 188 µg sterol, and 505 nM malonaldehyde per 100 mg lyophilized tissue. Following 3 weeks at 2C, plus 1 or 7 days at 20C, the major difference between heat-stressed and unheated tomatoes was a 50% decrease in phospholipid content in the nonheated fruit (Fig. 4A). The phospholipid content of heat-stressed tomatoes was similar after 7 days at 20C as at harvest. In contrast, sterol content of heat-stressed tomatoes was lower than that of unheated tomatoes by 7 days after transfer from 2 to 20C (Fig. 4B). Malonaldehyde levels were higher in heat-stressed tomatoes than in the control and decreased during holding at 20C (Fig. 4C).

Ethylene and $C0_2$ production were monitored during the heat treatment and after removal from low temperature. Ethylene production was inhibited in mature-green tomatoes while being kept at 36C (Fig. 5A). The low levels of ethylene produced by mature-green tomatoes at harvest began to rise within 1 day when the fruits were placed at 20C, reached a climacteric peak within 4 to 7 days, and then declined. The highest rate of ethylene production occurred as the fruit became pink. During the 3 days of heat stress, ethylene production by tomatoes remained very low, but upon transfer to 20C recovery was rapid, and within a day ethylene production surpassed that of fruits kept continuously at 20C. The heat-stressed fruit reddened with a slight delay, but reached full color development (Table 1). The

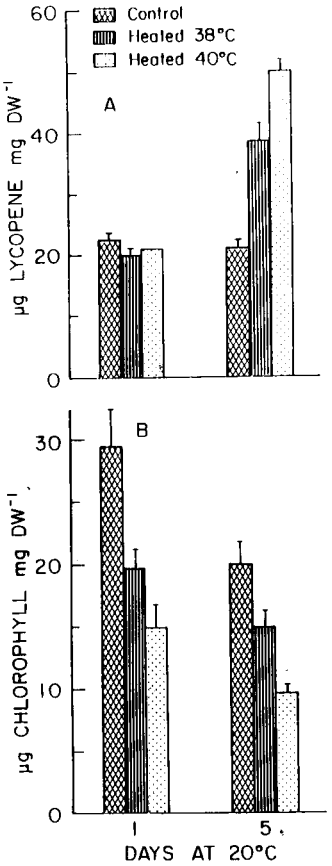

Fig. 2. Lycopene (A) 'and chlorophyll (B) concentrations in extracts from control tomatoes and those heated for 3 days at 38 or 40C. Tomatoes then were kept 3 weeks at 2C and before their transfer to 20C. SD is indicated by bars.

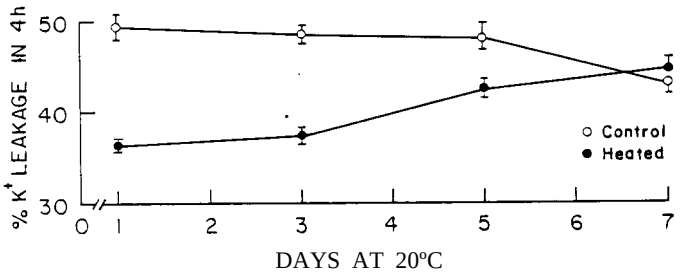

Fig. 3. K+ leakage from disks of control or heat-stressed (38C) tomatoes during 7 days at 20C following 3 weeks at 2C. Leakage during 4 h from disks of freshly harvested green tomatoes was 31% + 0.5%. SD is indicated by bars.

inhibition of ethylene occurred whether the fruit was held at 36, 38, or 40C (Table 2), although the full recovery of ethylene production took 2 or 3 days when tomatoes were held at 38 or 40C (data not shown).

In contrast to the inhibition of ethylene production by heating, CO_2 evolution was much higher during the 3 days tomatoes were kept at 36C than in the controls (Fig. 6A, Table 2). Upon transferring tomatoes to 20C, the respiratory rate plunged to a level below that of fruit kept continuously at 20C and then recovered to levels similar to, but slightly lower than, those of unheated fruit. Fruit kept continuously at 20C showed a normal climacteric rise of CO_2 evolution, paralleling that of ethylene production.

After 3 weeks at 2C and transfer to 20C, normal ripening-

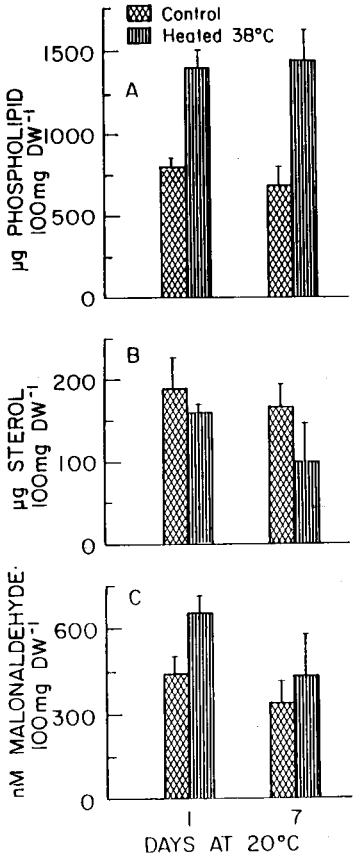

Fig. 4. Amounts of phospholipid (A), sterol (B), and malonaldehyde (C) in lyophilized tissue from control and from heat-stressed (38C) tomatoes 1 day and 7 days after transfer from 2 to 20C. SD is indicated by bars. Concentrations in freshly harvested fruit were: 1500 µg phospholipid; 188 µg sterol; 505 nM malonaldehyde; all per 100 mg lyophilized tissue.

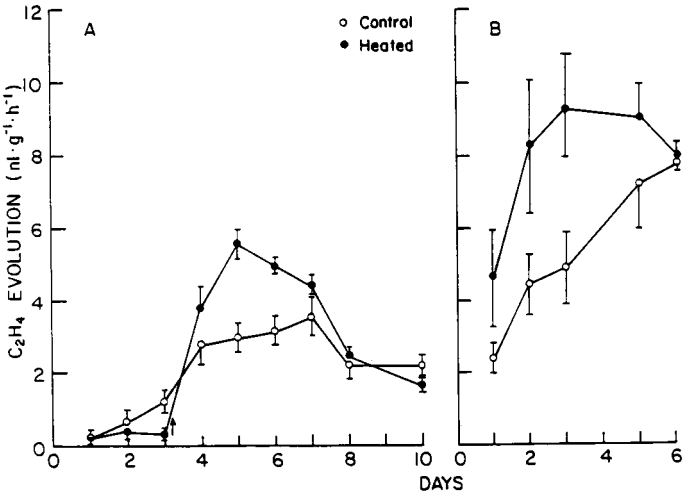

Fig. 5. Ethylene production of tomatoes. (A) Tomatoes were kept for 3 days at 36C (heated) and then, where indicated by the arrow, transferred to 20C; control tomatoes were kept continuously at 20C. (B) Tomatoes kept at 20C following 3 weeks at 2C. SD is indicated by bars.

associated increases were apparent in the ethylene and $C0_2$ evolution of heat-stressed tomatoes. Ethylene production of control fruit began at a lower level than that of heated fruit and increased continuously during the time the fruits were kept at

Table 1. Color development at 20C of control and heat-stressed (3 days at 36C) mature-green tomatoes just after harvest and after an additional 3 weeks at 2C before keeping at 20C.

	Hunter 'a' value				
	Days from harvest			Days at 20C following 3 weeks at 2C	
Heat stress	1	4	10	1	5
No	−4.8	3.1	14.0	−4.5	0.5
Yes	−4.5	0.1	15.0	−4.0	8.0

Table 2. Ethylene and CO_2 production by tomatoes heat-stressed at various temperatures, as percentage of that of tomatoes kept continuouslv at 20C.

	Ethylene		CO_2	
	Days of heat stress			
Temp (°C)	1	3	1	3
	% of control			
36	61	31	256	160
38	50	20	270	175
40	33	3	312	193

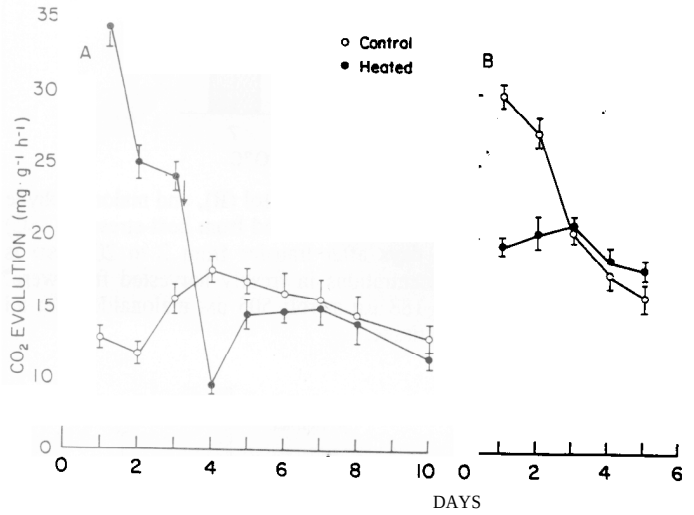

Fig. 6. Carbon dioxide evolution of tomatoes. (A) Tomatoes were kept 3 days at 36C (heated) and then, where indicated by the arrow, transferred to 20C, control tomatoes were kept continuously at 20C. (B) Tomatoes kept at 20C following 3 weeks at 2C. SD is indicated by bars.

Table 3. Protein synthesis, expressed in counts per minute (CPM), in tomato pericarp disks held at 20 or 38C as measured by incorporation of (^{35}S)methionine into TCA precipitable compounds.

	Protein synthesis (CPM)	
	Duration of incubation (h)	
Temp (“C)	4	12
20	6,560	48,300
38	3,300	20,100

20C (Fig. 5B). In contrast, the untreated tomatoes showed high C 0_2 evolution upon transfer from 2 to 20C, but this rate declined during the time at 20C (Fig 6B).

The fruit heated at 38C had a lower level of protein synthesis than control fruit as measured by incorporation of (^{35}S)methionine into TCA-precipitable material (Table 3). F1uorographs of proteins synthesized in disks from tomatoes kept at 38 or 20C showed differences in the polypeptide pattern (Fig. 7), depending on the temperature of incubation of the disks. Similar bands were seen after incubation for 4 or 12 h at either temperature. Several bands that were Iabelled after 4 h at 38C and became . more prominent after 12h at 38C were either absent or very faint in tomato disks incubated at 20C. Most of these bands were either of high (> 69 kDa) or low (< 30 kl)a) molecular weight.

Discussion

Several theories have been advanced to account for the nature of CI. The main one, which is restated periodically with modifications, is that the primary lesion is at;t the membrane level (Lyons.1973). In many plant tissues, correlations between low-temperature injury and increased electrolyte leakage have been found (Inaba and Crandall, 1988), as were observed in this study. This increase is an indication of an alteration in membrane properties.

A common response of plant tissues to environmental stress is lipid peroxidation (Parkin and Kuo, 1989), as has been noted for plant tissues subjected to wounding, freezing, and drought stress (Elstine and Konze, 1976). Cucumbers ***(Cucumis sativus*** L.) subjected to low temperatures for 3 days evolved ethane upon rewarming before visual signs of CI. appeared (Parkin and Kuo, 1989). Ethane, one product of lipid peroxidation, appeared before losses in phospholipids were noted (Parkin and Kuo, 1989). We did not observe any ethane production after removal of the fruit from low temperature in the experiments reported here. The concentration of malonaldehyde, another indicator of lipid peroxidation (Kosugi and Kikugawa, 1985), was higher in heat-stressed than in control tomatoes, indicating that this stress may cause lipid peroxidation. However, malonaldehyde content

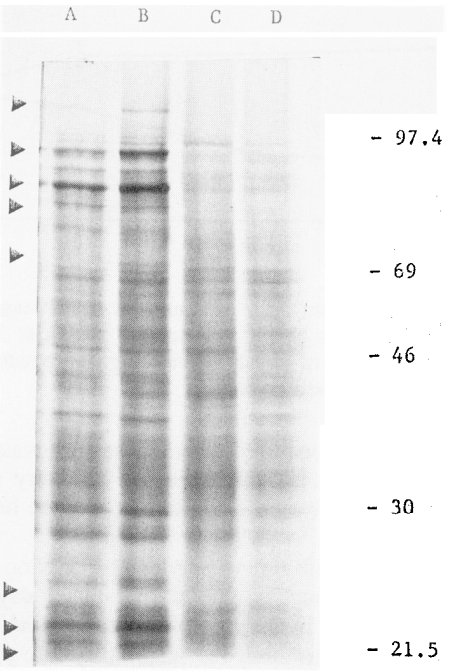

Fig. 7. Audioradiogram of one-dimensional SDS-PAGE 10% gel of proteins synthesized in tomato disks incubated at 20 or 38C for 4 or 12 h. 50,000 cpm were loaded on each lane. The arrows indicate proteins synthesized more prominently at 38C than at 20C. Lane A, 4 h at 38C; lane B, 12 h at 38C; lane C, 12 h at 20C; lane D, 4 h at 20C.

showed no correlation with chilling injury and did not increase in the unheated fruits that developed CI. Therefore, it appears that lipid peroxidation is not involved in the development of CI in tomatoes.

Adaptation to low temperature often involves increases in phospholipid content (Raison et al., '1980). It increased during low-temperature storage of apples (Lurie et al., 1987): Conversely, phospholipase D activity increased in chilled cucumbers, and an increase was observed in phosphatidic acid (Parkin and Kuo, 1989). During the development of low-temperature injury, y general increase has been observed in phospholipases. This increase could account for the large difference in phospholipid content of heat-stressed and nonheated tomatoes upon removal to 20C and the continuing loss of phospholipids observed in unheated tomatoes during the time they were held at 20C. Accelerated loss of phospholipids in the unheated tomatoes might lead to the large increase in K^+ leakage observed and the development of the brown tissue areas symptomatic of CI. The heat stress inhibited this loss of phospholipids, leading to a lower rate of K^+ leakage and protection against development of CI.

The objective of this study was to determine whether heat stress could confer tolerance to low-temperature stress. The data presented here clearly demonstrate that a treatment in the range of 36 to 40C before placing tomato fruit at a low temperature prevented the development of CI symptoms when the fruits were removed from the low temperature. The heat-stressed fruit showed normal $C0_2$ and ethylene climacteric patterns and color development after removal from low temperature, while unheated tomatoes had abnormal CO_2 and ethylene evolution, a high rate of K^+ leakage, and CI symptoms.

High temperatures inhibit ripening in tomatoes and other fruits. Biggs et al. (1988) found that ethylene production in tomatoes was reversibly inhibited above 34C, and correlated this inhibition with a rapid decline in l-aminocyclopropane-l-carbo@ic acid synthase activity. The enzyme activity recovered upon cooling, but recovery was inhibited in the presence of cycloheximide. We have found a similar inhibition and recovery in this study and observed a similar response in apples (Klein, 1989; Lurie and Klein, 1990).

High temperature was found to delay ripening of tomatoes even after the temperature stress was removed (Cheng et al., 1988; Inaba and Chachin, 1988; Saltveit and Cabrera, 1987). They, as we, found that CO_2 production was high in the stressed fruit and decreased when the stress was removed. Depending on the study, mitochondria isolated from fruit held at elevated temperatures showed either a higher or lower rate of respiration and respiratory control rate (Cheng et al., 1988; Inaba and Chachin, 1989). The disparity in results may be due to the methods of sampling and preparation of the mitochondria.

Saltveit and Cabrera (1987) heated tomatoes to 37C for 7 h before chilling them for 4 days at 2.5C. These tomatoes ripened more slowly than nonheated fruit, which the authors interpreted as symptomatic of CI. Based on the results of our work, another interpretation might be that the heat stress caused inhibition of ripening, that this inhibition was maintained at 2.5C, and was removed only after transfer to 20C.

The inhibition of ripening originates at least partially at the level of gene expression. Some mRNAs that normally increase with ripening were inhibited in tomato kept at high temperature (Picton and Grierson, 1988). In parallel with the decrease in normal mRNA and protein synthesis, plants respond to high-temperature stress by synthesizing HSP. The kinetics of HSP synthesis, their electrophoretic profiles, and intracellular localization have been studied in tomato (Never and Scharf, 1984; Neumann et al., 1987), but the role of these stress proteins in thermal tolerance is not known. The same proteins synthesized in leaves at high temperature were not apparent in leaves when transferred to low temperature (Ougham, 1987). However, chilling of mung bean *Vigna* ~ L.) hypocotyls initiated the production of three proteins that were also initiated by heat shock (Kawata and Yoshida, 1988). In our study, inhibition of normal protein synthesis and the production of HSP were found at high temperature. However, a causal relationship between the presence of HSP and protection against CI was not demonstrated.

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