PHYSIOLOGIA PLANTARUM 82: 529–536. Copenhagen 1991

Prior temperature exposure affects subsequent chilling sensitivity

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Saltveit, M. E., Jr. 1991. Prior temperature exposure affects subsequent chilling sensitivity. – Physiol. Plant. 82: 529–536.

The chilling sensitivity of small discs or segments of tissue excised from chilling-sensitive species was significantly altered by prior temperature exposure subsequent to holding the tissue at chilling temperatures as measured by a number of physiological processes sensitive to chilling. This temperature conditioning was reversible by an additional temperature exposure before chilling, and mature-green and red-ripe tomato tissue exhibit similar chilling sensitivities.

Exposing pericarp discs excised from tomato fruit (*Lycopersicon esculentum* Mill. cv. Castelmart), a chilling-sensitive species, to temperatures from 0 to 37°C for 6 h before chilling the discs at 2.5°C for 4 days significantly altered the rate of ion leakage from the discs, but had no effect on the rate of ion leakage before chilling and only a minimal effect on discs held at a non-chilling temperature of 12°C. Exposing chilling-sensitive tissue to temperatures below that required to induce heat-shock proteins but above 20°C significantly increased chilling sensitivity as compared to tissue exposed to temperatures between 10 and 20°C. Rates of ion leakage after 4 days of chilling at 2.5°C were higher from fruit and vegetative tissue of chilling-sensitive species (*Cucumis sativus* L. cv. Poinsett 76, and *Cucurbita pepo* L. cv. Young Beauty) that were previously exposed for 6 h to 32°C than from similar tissue exposed to 12°C. Exposure to 32 and 12°C had no effect on the rate of ion leakage from fruit tissue of chilling tolerant species (*Malus domestica* Borkh. cv. Golden Delicious, *Pyrus communis* L. cv. Bartlett). Ethylene and CO_2 production were higher and lycopene synthesis was lower in chilled tomato pericarp discs that were previously exposed for 6 h to 32°C than the values from tissue exposed to 12°C for 6 h before chilling. Increased chilling sensitivity induced by a 6 h exposure to 32°C could be reversed by subsequent exposure to 12°C for 6 h.

Key words – Apple, carbon dioxide, cucumber, *Cucumis sativus, Cucurbita pepo,* ethylene, ion leakage, lycopene, *Lycopersicon esculentum, Malus domestica,* pear, *Pyrus communis,* squash, tomato.

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Introduction

Many plants of tropical and sub-tropical origin, and some tissues from plants of temperate origin are injured by exposure to non-freezing temperatures below 12°C (Bramlage 1982, Couey 1982, Lyons 1973, Saltveit and Morris 1990). Chilling-sensitive plants include avocados, bananas, cucumbers, peppers and tomatoes, while chilling-resistant plants include apples and pears. Tomato fruit are particularly susceptible to chilling injury at the mature-green stage when they are normally harvested and shipped. The response of tomato fruit to chilling inury is similar to that of most chilling-sensitive plants and is characterized by increased susceptibility to *Alternaria* rot, slow and abnormal ripening, increased rates of CO_2 and C_2H_4 production and increased rates of ion leakage (Autio and Bramlage 1986, King and Ludford 1983, Morris 1982). Increased rates of solute and electrolyte leakage occur in a varity of chilled tissue (Creencia and Bramlage 1971, Murata and Tatsumi 1979), and have been used as measures of the increased permeability of the plasmalemma following chilling (Guinn 1971, Wright and Simon 1973).

Symptoms of chilling injury that are induced by expo-

Received 19 November, 1990; revised 30 March, 1991

sure to chilling temperatures can be reduced in some chilling-sensitive tissues by modifying the pattern of temperature exposure, e.g. periodically warming the chilled tissue above the chilling temperature (Anderson 1982, Cabrera and Saltveit 1990, Davis and Hofman 1973), or by holding the tissue at temperatures near the chilling temperature or above 35°C before chilling (Graham and Patterson 1982, Lafuente et al. 1991). For example, exposure to cool, non-chilling temperatures before chilling reduces chilling injury in fruit of bell pepper (McColloch and Worthington 1952) and grapefruit (Chalutz et al. 1985, Hatton and Cubbedge 1983), tomato seedlings (King et al. 1982, Wheaton and Morris 1967) and ornamental plants (Smith and McWilliams 1979). We recently showed that exposing excised 1-cm discs of cucumber cotyledons to 37°C for 6 h induced heat-shock proteins and reduced chilling sensitivity as measured by reduced ion leakage (Lafuente et al. 1991).

Changes in the chilling sensitivity of tomato fruit occur naturally during diurnal fluctuations in temperatures in the field (Saltveit and Cabrera 1987). Mature-green tomato fruit harvested at sunrise (i.e. 0630 h), with a pulp temperature of 19°C, were more resistant to chilling at 7°C for 7 days than were fruit harvested at 1300 h or at sunset (i.e. 2030 h) with pulp temperatures of 32 or 29°C, respectively (Saltveit and Cabrera 1987). Fruit harvested on cool, cloudy days during which the fruit were roughly the same temperature throughout the entire day, did not show significant differences in chilling sensitivity with time of harvest. Exposure to air temperatures of 12.5 or 37°C (producing flesh temperatures of 12 or 32°C, respectively) in the laboratory for 7 h induced differences in sensitivity to 4 days of chilling at 2.5°C that were similar to differences in fruit harvested from the field at different temperatures. Short term exposures to 12 or 32°C in the laboratory had no significant effect on the ripening of fruit exposed to non-chilling temperatures.

The physiological responses of chilling-sensitive and resistant tissue to prior temperature exposures that alter chilling sensitivity have not been thoroughly studied. The object of this study was to characterize the effects of prior temperature exposure on the subsequent expression of chilling injury symptoms in a number of chilling-sensitive and resistant species, to examine the reversibility of this conditioning treatment and to determine if changes in chilling sensitivity occur as tomato fruit tissue progresses from the mature green to the red-ripe stage of ripeness.

Materials and methods

Tissue preparation

Tomato fruit (*Lycopersicon esculentum* Mill. cv. Castlemart) were hand-harvested at the mature-green or red-ripe stage from plants grown at the Vegetable Crops field station in Davis, CA, under standard cultural practices. Only uniform, healthy fruit with no external defects were used. Fruit were washed in dilute commercial bleach (1:20 dilution of 5% sodium hypochlorite), air dried under a transfer hood and 1-cm diam. pericarp discs were excised with a sterile stainless steel cork borer. Adhering locular material was trimmed away to produce 3-mm-thick discs. Discs were washed 3 times in autoclaved deionized water, blotted dry and from 4 to 8 discs were placed epidermis down in 15 × 100-mm diam. plastic Petri dishes. Replicate discs were from one fruit and were blocked for intensity of green color across treatments, since light-colored discs produce less lycopene than dark green discs (Saltveit 1989b). Care was also exercised to make sure that the discs remained oriented with their epidermis surface down, since lycopene synthesis was affected by the orientation of the discs. Depending on the size of the experiment (i.e. the number of treatments and replicates), from 1 to 4 uniform furit were used. An 80-mm diam. disc of moist filter paper was stuck to the top lid of the Petri dish to raise the humidity. Dishes were kept overnight at 20°C in a flow of humidified, C_2H_4-free air to allow dissipation of wound responses (Mencarelli and Saltveit 1988).

Pericarp discs were used because they offer many advantages over slices and whole tomato fruit. One-cm diam. epidermal pericarp discs are small and easily handled, yet they exhibit all the physiological changes found in ripening whole fruit: a C_2H_4 and respiratory climacteric, chlorophyll degradation, lycopene synthesis, development of characteristic aroma and softening (Edwards et al. 1983, Gross and Saltveit 1982). From 30 to 60 discs of nearly identical tissue can be prepared from a medium to large fruit. Solutions are easily applied and absorbed through the cut surfaces. Aseptically prepared discs ripen normally without significant invasion by pathogens.

Fruit of apple (*Malus domestica* Borkh. cv. Golden Delicious), pear (*Pyrus communis* L. cv. Bartlett), cucumber (*Cucumis sativus* L. cv. Poinsett 76) and squash (*Cucurbita pepo* L. cv. Young Beauty) were obtained from local markets. Representative fruit from each lot were held at 20°C and experimental results from that lot of fruit were discarded if any of the representative fruit developed chilling injury symptoms. One-cm diameter cylinders of tissue were excised with a sterile stainless steel cork borer from the cortex of apple and pear fruit, and from the mesocarp of cucumber and squash fruit. The cylinders were cut into 3-mm thick discs which were washed 3 times in sterile deionized water, blotted dry, and placed in 15 × 100-mm diam. plastic Petri dishes. One-cm apical root segments were excised from 5-day-old squash (*Cucurbita pepo* L. cv. Young Beauty) seedlings grown in vermiculite at 25°C under 18 h cool-white fluorescent lights giving a photon fluence rate of 70 μmol m^{-2} s^{-1} at plant level. The root segments were placed on moist filter paper in 15 × 100 plastic Petri

dishes. Dishes were kept overnight at 20°C in a flow of humidified, C_2H_4-free air to allow dissipation of wound responses.

Temperature treatments

Pericarp discs of mature-green tomato fruit were exposed to temperatures of 0, 5, 10, 15, 20, 25, 30, 32, 34 and 37°C for 6 h followed by 4 days of exposure at either 12 or 2.5°C. Temperature treatments were applied in controlled temperature rooms under dim light by putting the Petri dishes between 30 × 30-cm plastic bags containing 1-l of water that had equilibrated over 24 h to the temperature of the room. The water bags helped to stabilize the exposure temperature and rapidly establish the desired tissue temperature. Preliminary experiments with a YSI model 47 Scanning Tele-Thermometer equipped with a small #421 temperature probe (Yellow Springs Instrument Co., Yellow Springs, OH, USA) showed that evaporative cooling from the moist filter paper and tissue reduced tissue temperatures below the air temperature. Exposure temperatures were therefore raised an appropriate amount to compensate for this reduction and to produce the desired tissue temperatures that were confirmed by periodic measurements. Weight loss by the tissue was less than 1.5% and not significantly different among any of the temperature treatments. Results from these preliminary experiments indicated that optimal difference in chilling sensitivity was produced by exposing tissue to 12 and 32°C. Control discs were held at 12°C, the lowest non-chilling temperature, to minimize physiological changes caused by ripening during the experiments.

Tissue was subjected to the following four temperature treatments: 1) exposed to 12°C for 6 h and held at 12°C for 4 days, 2) exposed to 32°C for 6 h and held at 12°C for 4 days, 3) exposed to 12°C for 6 h and held at 2.5°C for 4 days, and 4) exposed to 32°C for 6 h and held at 2.5°C for 4 days. Later experiments contained the following two additional temperature treatments: 5) exposed to 32°C for 6 h and then to 12°C for an additional 6 h, and held at 2.5°C for 4 days, and 6) exposed to 12°C for 6 h and then to 32°C for an additional 6 h, and 2.5°C for 4 days.

Measuring chilling injury

Chilling injury was measured as increased ion leakage for all tissues, and also as increased C_2H_4 and CO_2 production and decreased lycopene synthesis for tomato tissue. Ion leakage was measured as increased electrical conductivity in a 0.3 *M* mannitol solution that was used instead of water to reduce osmotic stress on the tissue (Autio and Bramlage 1986, Saltveit 1989a, Vickery and Bruinsma 1973). Tissue was removed from the holding temperature and warmed to 20°C for 0.5 h before being placed into a tared 100-ml beaker. Beakers with tissue were weighed, 30 ml of 0.3 *M* mannitol were added, and the beakers were weighed. The beakers were shaken at 2 cycles s^{-1} between conductivity readings with an Ex-Tech Model 480 digital conductivity meter (Extech Inc., Waltham, MA USA). The beakers were then heated to boiling for 5 min, cooled overnight at 0°C while being shaken, warmed to room temperature and made to their initial weight with deionized water. After the beakers were shaken for 1 h at room temperature, the total

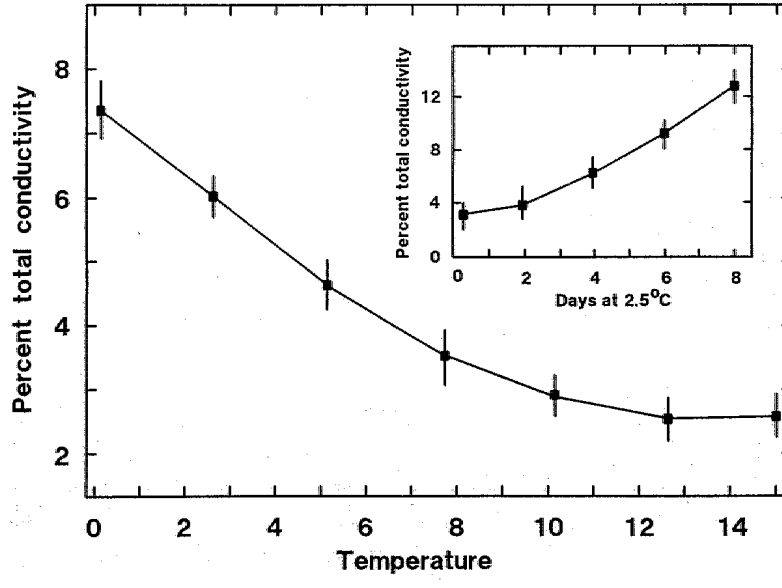

Fig. 1. Effects of temperature and duration of exposure on ion leakage from pericarp discs excised from mature-green tomato fruit. Discs were exposed to temperatures from 0 to 15°C for 4 days (large graph) and to 2.5°C for 0 to 8 days (insert). Data represent triplicate sampling. The vertical error bars represent the SE of the mean.

conductivity was measured. Rates of ion leakage are expressed as changes in the percent of the total conductivity h^{-1}.

Production of CO_2 and C_2H_4 was measured by enclosing tissue in glass jars at 20°C for an appropriate length of time for C_2H_4 and CO_2 to accumulate to measurable levels (about 2 h). One-ml gas samples of the headspace were taken periodically and analyzed for C_2H_4 by flame ionization gas chromatography and for CO_2 by infrared analysis (Saltveit 1982).

Tomato discs were ripened at 20°C for 5 days in 20-l glass jars that were flushed with humidified air containing 10 ul l^{-1} C_2H_4. Dishes were stored at –20°C until the discs were assayed for lycopene. Two discs from each dish were weighed, homogenized with 10 ml of acetone for 10 s in a Sorvall Omnimixer (Ivan Sorvall Inc., Newtown, CT, USA) at maximum speed, centrifuged at maximum speed in an International clinical Model CL (International Equipment Co., Needham, MA, USA) table-top centrifuge and the absorbance of the clear supernatant was measured at 503 nm. Lycopene concentration is expressed as absorbance per gram fresh weight.

Each experiment had at least 3 replicates per treatment and was repeated twice. Data within each experiment were subjected to an analyses of variance with 5% LSD values calculated to separate significantly different treatment means.

Results

Holding discs of tomato pericarp tissue at temperatures from 0 to 12°C for 4 days resulted in a progressive increase in ion leakage with decreasing temperature (Fig. 1). The response declined linearly from 0 to 5°C with a significant reduction in the slope occurring after 7.5°C. The transition between chilling and non-chilling temperatures occurs between 7.5 and 12°C for mature-green tomato pericarp discs. A temperature of 12°C was chosen for the non-chilling exposure and holding temperature because it was the lowest consistently non-chilling temperature. The chilling temperature of 2.5°C caused a steady increase in the rate of ion leakage with time after a delay of 2 days (insert in Fig. 1). A 4-day exposure to 2.5°C was chosen as the chilling treatment because it was the shortest period that consistently caused significant increases in ion leakage, and a short holding period would reduce ripening differences between the two holding temperatures.

Exposing tomato discs to temperatures from 0 to 37°C for 6 h had no significant effect on subsequent ion leakage immediately after exposure (Fig. 2). Leakage from discs held at 12°C for 4.5 days after the 6 h exposure was roughly constant across exposure temperatures from 15 to 37°C, and increased slightly from those discs exposed to 0 to 10°C for 6 h. In contrast, there were dramatic differences in the rates of ion leakage from discs held at 2.5°C for 4.5 days after conditioning. Leakage was higher from tissue previously exposed to the chilling temperatures of 0 and 5°C for 6 h than from tissue exposed to 10 and 15°C. The higher rate of leakage may have been the result of the additional 6 h of chilling at 0 or 5°C. It is difficult to imagine, however, how an additional 0.25 days of chilling could have such a pronounced effect over that of 4.5 days. The highest rate of leakage was from tissue previously exposed to 25, 30 and 32°C, while the lowest rate of leakage was from tissue exposed to 34 and 37°C. We have previously

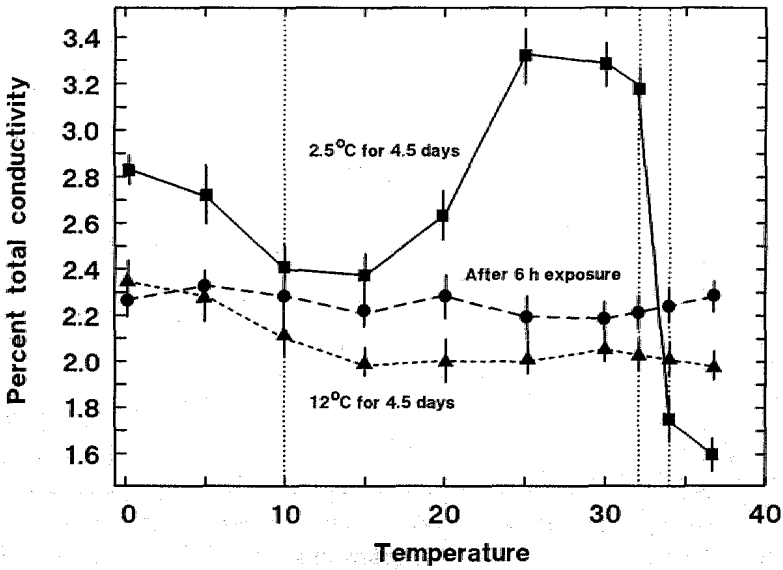

Fig. 2. Effects of prior temperature exposure for 6 h of mature-green tomato pericarp discs on their subsequent rates of ion leakage immediately after conditioning (–●–●–) and after holding for 4.5 days at 12°C (–▲–▲–) or at 2.5°C (–■–■–). Data represent triplicate sampling. The vertical error bars represent the SE of the mean.

reported that the higher temperatures induce heat-shock proteins (Lafuenta et al. 1991). To accentuate differences, exposure temperatures of 12 and 32°C were selected for subsequent experiments.

Exposing mature-green epidermal tomato pericarp discs to 2.5°C for 4 days significantly increased the rates of ion leakage and C_2H_4 and CO_2 production during the 2-h period immediately after they were warmed to 20°C, and decreased the lycopene concentration in discs held for an additional 4 days at 20°C in an atmosphere containing 10 ul l^{-1} C_2H_4 (Fig. 3). Chilling caused these changes in tissue previously exposed to both 12 and 32°C for 6 h, but the changes were significantly greater for discs exposed to 32 than to 12°C. In contrast, exposing discs to 12 or 32°C for 6 h had no significant effect on their response when they were held at the non-chilling temperature of 12°C for 4 days.

While there were significant differences in all 4 measurements of chilling injury between discs exposed to 12 and 32°C, the most pronounced effect of conditioning at 12°C was on the rate of ion leakage (Fig. 3). Ion leakage from chilled discs conditioned at 32°C was almost 2-fold higher than from discs conditioned at 12°C. Production of C_2H_4 and CO_2 was increased by about 1.3- and 1.2-fold, respectively, while lycopene concentration was reduced by around 20% by the 32°C conditioning treatment in comparison to the 12°C conditioning treatment.

The rates of ion leakage remained significantly higher from discs exposed to 32°C than from discs exposed to 12°C prior to chilling for the four 1.5 h sampling periods that extended for 6 h from immediately after removal of the discs from the chilling temperature. Although there was a decline in the rates of leakage from discs in all treatments over this 6 h period, the statistically significant differences among the temperature conditioning and holding treatments remained the same.

Increases in conductivity during the 0.0 to 0.5 h period immediately following warming to 20°C exhibited greater variability within and among experiments than did measurements made during other sampling periods. Because of the greater response of ion leakage than of C_2H_4 and CO_2 production or lycopene concentration to temperature conditioning (Fig. 3), changes in electrical conductivity during a 0.5 to 2.5 h period immediately after warming to 20°C were used as a measure of the extent of chilling injury in all subsequent experiments.

The effect of temperature conditioning on chilling sensitivity was not confined to tomato pericarp tissue, but was also observed in tissue excised from other chilling-sensitive species (Tab. 1). In each of the chilling-sensitive tissues (i.e. cucumber, squash and tomato), exposure to 32°C for 6 h significantly increased the rate of ion leakage after 4 days of chilling at 2.5°C over that of tissue exposed to 12°C. Neither conditioning temperature had a significant effect on electrolyte leakage from tissue held at the non-chilling temperature of 12°C. In contrast to the pronounced effect of temperature conditioning on ion leakage from chilling-sensitive tissue, chilling-resistant tissue (i.e. apple and pear) showed no significant changes in ion leakage as the result of either the exposure or holding temperature. Within the experimental conditions, temperature conditioning uniquely affect the rate of ion leakage from chilling-sensitive tissue, and not from chilling-resistant tissue.

Surprisingly, pericarp discs from both mature-green and red-ripe tomato fruit showed similar significant increases in the rate of ion leakage upon exposure to 32°C over that at 12°C (Tab. 1). Conditioning at 32°C resulted in a 23% increase in ion leakage from chilled mature-

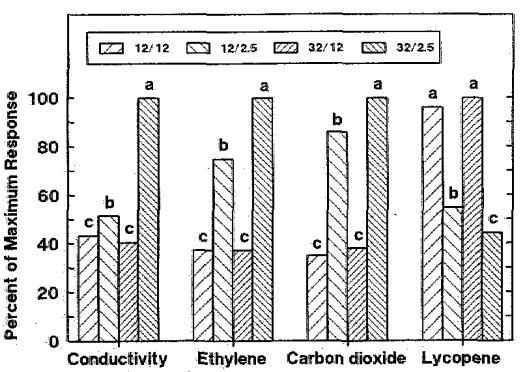

Fig. 3. Effects of holding mature-green tomato epidermal pericarp discs at 12 or 2.5°C for 4 days after conditioning at 12 or 32°C for 6 h on the rates of ion leakage and the production of C_2H_4 and CO_2 during the 2 h period immediately after warming to 20°C, and on lycopene concentration after ripening for 4 days at 20°C in an atmosphere containing 10 ul l^{-1} C_2H_4. Data represent triplicate sampling from two experiments. Bars within each type of measurement that are associated with the same letter are not statistically different at the 5 % level.

Tab. 1. Effects of holding various tissues at 12 or 2.5°C for 4 days after conditioning at 12 or 32°C for 6 h on the percent ion leakage during a 0.5 to 2.5 h period immediately after warming to 20°C. Data represent triplicate sampling from two experiments. Means for each tissue were separated by LSD at the 5 % level, and means followed by the same letter are not statistically different at the 5 % level.

	Conditioning temperatures			
	12°C		32°C	
	Holding temperatures		Holding temperatures	
Tissue	12°C	2.5°C	12°C	2.5°C
Apple cortex discs	5.7a	5.8a	5.8a	6.0a
Pear cortex discs	5.8a	5.6a	5.9a	6.0a
Squash root segments	2.0c	3.2b	1.9c	4.9a
Squash mesocarp discs	1.7c	3.1b	1.6c	5.6a
Cucumber mesocarp discs	3.5c	4.4b	3.6c	4.9a
Tomato pericarp discs				
Mature-green	3.9c	4.3b	3.7c	5.3a
Red-ripe	1.8c	2.0b	1.7c	2.7a

green discs over that of discs conditioned at 12°C, while a 35% increase was noted for discs from red-ripe fruit.

All 4 measurements of chilling injury were significantly reduced when the mature-green tomato pericarp discs that had been exposed to 32°C for 6 h were subsequently exposed to 12°C for 6 h before chilling. Ion leakage was reduced to that of chilled discs conditioned only at 12°C. For C_2H_4 and CO_2 production the reduction was even greater, i.e. to the level of the non-chilled discs. Lycopene concentration was significantly increased to an amount halfway between the chilled and non-chilled treatments. The increase in chilling sensitivity brought on by exposure to 32°C for 6 h was apparently reversed by a 6 h period at 12°C. In contrast, additional exposure to 32°C for 6 h of discs first conditioned at 12°C for 6 h increased all 4 measurements of chilling to that of discs initially exposed to 32°C.

The results of additional conditioning on ion leakage from mature-green and red-ripe pericarp discs are shown in Fig. 4. Mature-green and red-ripe tomato pericarp discs exhibited the previously described alterations in the rate of ion leakage when exposed to 32 or 12°C before chilling at 2.5°C for 4 days. Holding discs that had been exposed to 12 or 32°C for an additional 6 h at 32 or 12°C, respectively, resulted in rates of ion leakage comparable to that from discs originally exposed to 32 or 12°C, respectively. The initial rate of electrolyte leakage during 0.0 to 0.5 h from all treatments was around 2-fold greater from red-ripe discs than from mature-green discs. In contrast, leakage from red-ripe discs during the normal 0.5 to 2.5 h sampling period was about 50% of that from mature-green discs.

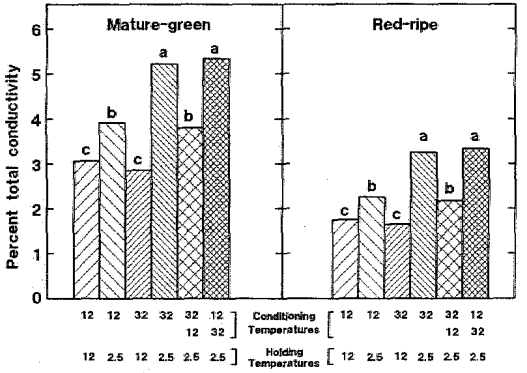

Fig. 4. Effects of holding mature-green and red-ripe tomato epidermal pericarp discs at 12 or 2.5°C for 4 days following 1 of the 6 temperature conditioning treatments: 1) 12/12 = conditioned at 12°C for 6 h and held at 12°C for 4 days, 2) 12/2.5 = conditioned at 12°C for 6 h and held at 2.5°C for 4 days, 3) 32/12 = conditioned at 32°C for 6 h and held at 12°C for 4 days, 4) 32/2.5 = conditioned at 32°C for 6 h and then at 12°C for an additional 6 h and held at 2.5°C for 4 days, and 6) 12/32/2.5 = conditioned at 12°C for 6 h and then at 32°C for 6 h and held at 2.5°C for 4 days. Data represent triplicate sampling from two experiments. Bars within each maturity class that are associated with the same letter are not statistically different at the 5 % level.

Discussion

Both field and laboratory data have shown that the chilling sensitivity of tomato fruit tissue is significantly affected by the temperature of the tissue prior to chilling (Saltveit and Cabrera 1987). In addition, the present study has shown that exposure of several chilling-sensitive plant tissues to 32°C for 6 h increases their chilling sensitivity over tissue exposed to 12°C for 6 h as measured by all 4 quantitative indicators of chilling injury. Chilling-resistant species were unaffected by any of the temperature exposures or chilling treatments. Increased rates of ion leakage were shown to be a sensitive measure of the changes in chilling sensitivity induced by prior temperature exposure.

The lower rates of ion leakage from discs of red-ripe fruit than from discs of mature-green fruit were unexpected, since electrolyte leakage has been reported to be around twice as great from red-ripe as from mature-green tomato tissue (King and Ludford 1983). This inconsistency perhaps resulted from my measuring ion leakage after a total of 5 days from excision (i.e. an 18 h incubation after excision, a 6-h conditioning treatment and a 4-day holding period), rather than measuring leakage from freshly excised tissue as was done in the other study. I also observed that the rate of ion leakage from discs held at 12 or 20°C declined as the length of time after excision increased.

The 32°C conditioning treatment by itself did not cause stress to the tissue sufficient to affect the rate of ripening or any of the measurements used to quantify chilling injury. While prolonged exposure to temperatures around 32°C is known to inhibit lycopene synthesis during tomato fruit ripening, our previous results (Saltveit and Cabrera 1987) agreed with those of Hall (1964) that short periods of exposure to high temperatures do not significantly affect ripening or color development. Hall (1964) reported that holding whole tomato fruit for 6 h at 32 to 43°C did not significantly affect either softening or color development during 7 days of ripening at 20°C. It has been reported recently that mature-green tomato fruit held for 1 day at 40°C produced a normal respiratory and C_2H_4 climacteric and ripened normally during subsequent storage at 25°C (Inaba and Chachin 1988). In addition, I have shown that exposure to 32°C did not result in significantly increased CO_2 or C_2H_4 production, increased ion leakage or reduced lycopene content from treated tissue in comparison to tissue exposed to 12°C (Tab. 1 and Fig. 3). Furthermore, the effect of the 32°C conditioning treatment was not permanent. A 6-h exposure to 12°C after the 32°C exposure was sufficient to completely reverse the 32°C effect (Fig. 4). The reversibility of the 32°C conditioning treatment in chilled chilling-sensitive tissue, the failure of the 32°C conditioning treatment to affect any non-chilled tissue, and the normal ripening of tissue exposed to higher temperatures for longer periods of time all indicate that, by itself, the 6 h exposure

to 32°C did not cause a signficant or persistent stress to the tissues.

Since many symptoms of chilling injury involve alterations of normal ripening, it is commonly thought that ripe fruit are more resistant to chilling injury than unripe fruit (Bramlage 1982, Couey 1982, King and Ludford 1983, Morris 1982). The fact is that ripe fruit may appear more chilling-resistant simply because they can not exhibit alterations in their already accomplished processes of ripening. The ion leakage data presented above suggest that the physiological basis for chilling injury in tomato fruit is equally present in both ripe and unripe pericarp tissue.

The chilling sensitivity of mature-green and red-ripe tomato pericarp discs was dependent on the last temperature to which they were exposed. A 6-h period of temperature conditioning prior to 4 days of chilling resulted in a reversible change in a number of measurements of chilling injury in tissue from chilling-sensitive species. The reversibility of these temperature-induced changes in chilling sensitivity implies involvement of an active physiological process rather than passive changes.

One such passive change could be water loss. Water loss is known to increase the content of sugar, proline and ABA in many herbaceous plants, and concomitantly increase chilling reistance (Lyons 1973, Wang 1982). This possibility was eliminated by designing the treatment containers to minimize water loss from the pericarp discs. In actuality, water loss did not differ significantly among any of the temperature treatments.

Resistance of tomato plants to low temperature injury has been positively associated with the endogenous carbohydrate content (King et al. 1988). The 32°C conditioning treatment possibly could have stimulated respiration or altered metabolism to deplete some important carbohydrate component to levels that increased chilling sensitivity. However, the reversibility of the conditioning treatments rules out the possibility that stimulated respiration was the primary cause. The possibility exists that a conversion of carbohydrates during the conditioning treatments (e.g. the inter-conversion of starch to sugars) was responsible for the changes in chilling sensitivity. For example, increasing the endogenous level of sugars by the application of 14 m*M* sugar solutions to excised tomato seedlings reduced subsequent chilling injury (King et al. 1988). The protective effect was most pronounced with sucrose, less with glucose and fructose and mannitol actually increased chilling injury. A positive relationship also exists between levels of proline and chilling resistance, but it is not clear whether proline accumulation was a result of chilling or a protective adaptation. These possibilities warrant additional study.

Chilling resistance has also been related to increased ABA content (Markhart 1986). Increased ABA levels induced by a variety of stresses, including high temperature stress and exogenous application, have conferred resistance to subsequent chilling treatments (Rikin et al. 1976). ABA broadens the phase temperature range by directly affecting the fluid properties of the lipid bilayer (Markhart 1986). If the 32°C conditioning treatment was a sufficient temperature stress to induce synthesis of ABA, then the added ABA should have conferred protection from chilling. The subsequent 6-h period of conditioning at 12°C that reversed the previous conditioning effect could have allowed catabolism of ABA to normal levels with increased chilling sensitivity. However, the chilling responses of tissue conditioned at 32°C and of tissue reconditioned at 12 after 32°C are in direct opposition to those predicated on ABA changes. The 32°C conditioned tissue which should have had the highest ABA level and should, therefore, have had the highest level of chilling resistance, was the most chilling-sensitive.

Many organisms, including bacteria, yeast, plants and poikilothermic animals maintain optimal membrane viscosity and function as the environmental temperature varies by altering the proportion of unsaturated and saturated fatty acids in their phospholipids, a response termed "homeoviscous adaptation" (Sinensky 1974). For example, tomato fruit grown in cooler climates or during cooler seasons are more resistant to chilling injury than fruit grown under hotter conditions (Abdel-Maksoud et al. 1974). Such adaptation may be necessary to maintain the spatial orientation of membrane-bound enzymes and the permeability characteristics of the cellular membranes, since there is evidence that changes in the physical properties of cellular membranes at chilling temperatures can be a source of injury (Lyons et al. 1979).

Observed diurnal changes in chilling sensitivity may result from modification of the composition of plant membranes to maintain a constant viscosity at different temperatures (Graham and Patterson 1982, Lyons et al. 1979). One consequence of changes in membrane composition to maintain constant viscosity at different temperatures would be that phospholipids synthesized or incorporated into the membranes at higher temperatures would exhibit progressively higher solid-to-liquid-crystalline phase transition temperatures (Chapman et al. 1967). Therefore, membranes synthesized by cells to produce the desired viscosity at 12°C would be much less likely to undergo phase transition upon a 10°C drop to a chilling temperature of 2.5°C than would membranes synthesized to produce a desired viscosity at 32°C. These "high temperature" membranes would more likely experience phase transition upon cooling 30°C to a chilling temperature of 2.5°C. Additional studies on the physiological basis of temperature conditioning are in progress to differentiate among these possibilities.

Previous studies of the physiology and biochemistry of chilling injury have compared plants with differing levels of chilling sensitivity. These types of comparison studies are hampered by the high degree of variability

among the cultivars or species being compared. This source of variability has been greatly reduced by inducing statistically significant differences in chilling sensitivity in excised tissue from the same fruit. Tissue, nearly identical except for a 6-h temperature exposure that induced differences in chilling sensitivity, could be compared to detect differences too small to be observed in more heterogeneous tissue.

Acknowledgments – The author expresses his appreciation to Mr Roberto M. Cabrera for assistance with preliminary experiments.

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Edited by T. C. Vogelmann