The rate of ion leakage from chilling-sensitive tissue does not immediately increase upon exposure to chilling temperatures

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Abstract

Exposure to non-freezing temperatures below ~ 10 °C causes an increase in the subsequent rate of ion leakage from chilling sensitive tissue (e.g. tomato fruit). The conditions of tissue preparation and conductivity measurements necessary to accurately calculate the rate of ion leakage from excised discs of tomato pericarp tissue were determined. Under the proper conditions, the rate of leakage expressed as a percent of the total conductivity per hour was linear for the period of 30–240 min following immersion of the discs in aqueous solutions of 0.2 M mannitol. A kinetic analysis of the efflux data showed that a combination of two exponential equations of the form $y = C_0(1 - e^{-K \cdot t})$, one for a 'fast' extra-cellular reservoir of ions ($C_0 f$, K_f; $y = C_0 f \, 1 - e^{-K_f \cdot t}$), and one for a 'slow' cellular reservoir ($C_0 s$, K_s; $y = C_0 s(1 - e^{-K_s \cdot t})$), fit the original data with an $R^2 > 0.95$. When measured at 25 °C after chilling, chilling at 2.5 °C for 3.5 days increased the rates of ion leakage and the values of $C_0 f$ and K_s, but had no effect on $C_0 s$ or K_f. This implies that chilling increased the permeability (K_s) of the cellular reservoir that allowed ions to leak out during the exposure to chilling and increase the content of $C_0 f$. However, when ion leakage was measured at temperatures from 2 to 20 °C, $C_0 f$ remained unchanged and the changes in K_s paralleled those in K_f. These changes in the rate constants reflect the effects of temperature on diffusion and did not show the abrupt increase predicted by the membrane phase-transition model of chilling injury. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chilling injury occurs in many agriculturally important plants that originated in the tropics and subtropics (e.g. banana, cotton, maize, rice and tomato) when they are exposed to nonfreezing temperatures below ~ 12 °C (Lyons, 1973; Saltveit and Morris, 1990). This physiological disorder is characterized by reduced growth and vigor, altered respiration and development, abnormal fruit ripening, accelerated water loss and senescence, surface pitting and increased disease susceptibility. Symptoms often develop after chilling and are exacerbated by stressful pre- and

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post-chilling environments. Chilling injury has been studied for over a hundred years and an extensive literature exists on conditions that cause chilling injury, on methods to alleviate its deleterious effects, and on the physiological and molecular bases of chilling (Saltveit, 2000).

Almost all of the models that describe how exposure to chilling temperatures can be transduced from a physical into a physiological change involve cellular membranes (Saltveit, 2000). A phase transition or lateral phase separation in some critical portion of the cell's membranes has long been thought to be of primary importance in chilling injury (Raison et al., 1971; Lyons, 1973; Parkin et al., 1989; Raison and Orr, 1990; Marangoni et al., 1996). One of the consequences of a temperature-induced phase transition in cellular membranes would be an alteration in their biophysical properties and consequently their functionality (Marangoni et al., 1996; Saltveit, 2000). Increased membrane permeability and increased rates of ion leakage are associated with chilling of sensitive tissue (Saltveit, 2000), yet the observed increase in permeability usually occurs after prolonged chilling.

To ensure that the rate of ion leakage is an accurate representation of membrane permeability the following three conditions should be observed. First, the tissue should be bathed in an aqueous isotonic solution. When pure water is used as the bathing solution, the osmotic shock that it imparts to the tissue could enhance leakage that would obscure real changes in permeability (Vickery and Bruinsma, 1973; Simon, 1977). Second, the rate of ion leakage must be linear during the sampling period. Ions in the cell wall and extra cellular spaces within the tissue rapidly diffuse from the tissue when it is immersed in an aqueous solution and cause a transient elevated rate of ion leakage. Depending on a number of parameters, this aberration lasts for about 30 min, after which the rate of ion leakage is relatively constant and closely approximates the permeability of the cell membrane (Murata, 1990). Washing the excised tissue to remove cellular contents released by wounding would lessen this transient increase in leakage and reduce the background level of conductivity. Third, if the tissue is freshly wounded, it should be washed and an isotonic solution should be used to reduce the additional stress a hypotonic bathing solution places on the already perturbed membranes. Wounding enhances the rate of ion leakage by exposing physically damaged cells to the bathing solution, and by altering the respiratory and metabolic activity of adjacent physically undamaged cells (Saltveit, 1996). The cellular alterations accompanying fruit ripening may also affect membrane permeability and the rate of ion leakage (Murata, 1990).

A number of papers have examined the effect of chilling on membrane permeability (e.g. Murata and Tatsumi, 1979; Paull, 1981; Kuo and Parkin, 1989), and have presented data indicating that an increase in ion leakage occurs from sensitive tissue held at a chilling temperature. However, most of these studies did not adhere to the three conditions necessary to accurately calculate the rate of ion leakage. Systematic errors caused by any of these three conditions (i.e. solution concentration, non-linearity, wounded tissue) could have significantly affected the calculated rate of ion leakage at the chilling temperature. For example, Kuo and Parkin (1989) immersed 2 g of freshly excised mesocarp disks from cucumber fruit in 25 ml of 0.4 M mannitol and measured conductivity after 5 h at 22 °C. The disks were not washed, the wound response was not allowed to dissipate, and linearity of leakage was not confirmed. Paull (1981) used chopped tomato leaves within a few hours of preparation and bathed them in a hypotonic (~ 10 mM) medium containing sucrose, $CaCl_2$ and a buffer. The efflux from these wounded leaf pieces pre-loaded with ^3H-leucine or $^{86}Rb^{2+}$ was significantly higher at 1 °C than at 20 °C, but the slope of the increase (i.e. the rate of leakage) at both temperatures was parallel. Although the amount of ^3H-leucine or $^{86}Rb^{2+}$ in the bathing solution was higher at the chilling temperature than at the non-chilling temperature, the rate of leakage during the linear portion was not different. In those experiments in which leakage was not linear over time, the curves at the two temperatures also appear parallel. In another report, Murata and Tatsumi (1979) im-

mersed freshly excised, non-washed tissue discs in 0.0 or 0.4 M mannitol at 0–30 °C for 2 h. Leakage of potassium ions showed a deviation from linearity around the chilling temperature of 5 °C in discs from a number of chilling sensitive cucurbit fruit (e.g. cucumber, melon and pumpkin). However, using the same procedure they also observed a deviation from linearity at 5 °C for chilling insensitive carrot and onion tissue.

Chilling-induced ion leakage is highly correlated with the appearance of chilling injury symptoms in many sensitive tissues and is frequently used to objectively quantify the severity of chilling injury (Saltveit and Morris, 1990). An increase in ion leakage has been used to study chilling injury in a number of crops, including tomato fruit (King and Ludford, 1983; Autio and Bramlage, 1986; Saltveit, 1991). Kinetic studies of ion leakage from tomato pericarp discs showed that enhanced permeability developed slowly during chilling and that several days of chilling were required for leakage rates to become significantly greater than for the non-chilled controls (Saltveit, 1989).

Research reported in this paper was undertaken to determine the conditions necessary to accurately measure the immediate effect of chilling on the rate of ion leakage from chilling sensitive tomato pericarp tissue. Data are presented to show that although prolonged chilling does result in a subsequent increase in the rate of ion leakage, exposing sensitive tissue to chilling temperatures did not immediately increase the rate of ion leakage.

2. Materials and methods

2.1. Plant material

Uniform mature-green tomato (*Lycopersicon esculentum* Mill. 'Castelmart') fruit were picked from the Department of Vegetable Crops field in the morning and carefully transported to the Mann Laboratory where they were washed in dilute sodium hypochlorite (5% aqueous solution of commercial bleach) and air-dried in a laminar-flow transfer hood. Pericarp discs (10-mm diameter) were excised with a stainless steel cork borer and trimmed of locular and epidermal tissue to produce 4-mm thick discs. The discs were washed three times for about 1 min each time in deionized water, blotted dry and 20 randomly selected discs were placed in tared 25×100-mm diameter plastic Petri dishes. The dishes were put into plastic tubs lined with wet paper towels and held overnight (ca. 18 h) at 25 °C to produce aged discs. All procedures were performed under aseptic conditions.

2.2. Determining the isotonic concentration

Weight gain or loss by the discs bathed in the mannitol solutions was measured by pipetting 20 ml of 0.0–0.4 M mannitol solution into each plastic petri dish and gently shaking it on a rotary shaker at 60 cycles/min. The solution was vacuum-aspirated away from the discs after 20, 60, 120 and 240 min, and the tared dishes weighed and fresh solution added.

In another series of experiments, three pericarp discs were put together into 50-ml plastic conical bottom centrifuge tubes containing 20 ml of 0.0, 0.1, 0.2 or 0.3 M mannitol. The conductivity of the solution was measured periodically for 4 h. At the end of the experiment, the absorbance of 1-ml aliquots of the solution was measured at 595 nm and compared to readings from standard aqueous solutions of BSA to approximate the protein content of the mannitol solutions. The tubes with the pericarp discs were then capped and subjected to three cycles of freezing (-20 °C) and thawing (25 °C) before the final conductivity of the solution was measured after 1 h of shaking; this was called 'total conductivity.' There were large differences among the total conductivity readings within and between treatments, but this variability within treatments was significantly reduced when individual readings were converted to percent of total conductivity. Individual conductivity readings were divided by the total conductivity for that tissue and the product multiplied by 100 to convert the readings to percent of total conductivity.

2.3. Effect of wounding and washing

To study the effect of wounding, three pericarp discs were put into 50-ml centrifuge tubes with 20 ml of 0.0 or 0.2 M mannitol immediately after excision or after holding at 25 °C for 18 h. To study the effect of washing, three discs were either used as excised (i.e. not washed), or washed three times in deionized water for about 1 min each time. In both series of experiments, conductivity of the solution was measured periodically for 130 min.

2.4. Effect of chilling on ion leakage

Trays of washed discs were transferred from the overnight holding temperature of 25 °C to a chilling temperature of 2.5 °C. After chilling for 1–7 days at 2.5 °C, three discs were put into 50-ml centrifuge tubes with 20 ml of 0.2 M mannitol and the conductivity of the solution measured every 30 min for 150 min. The linear component of leakage was calculated and expressed as a percent of total conductivity per hour.

Kinetics of recovery from chilling-induced ion leakage were studied by periodically measuring the conductivity of 20 ml of 0.2 M mannitol solution containing three aged pericarp discs that had been chilled for 3.5 days at 2.5 °C. The discs were removed from chilling and held at 25 °C for 0, 2, 4 or 6 h before beginning the conductivity measurements. The conductivity was measured every 2 min for 1 h and then less frequently for 240 min.

To study the immediate effect of chilling on the kinetics of ion leakage, three aged pericarp discs were put into each of ten 50-ml centrifuge tubes with 20 ml of 0.2 M mannitol at 2.0 ± 0.2, 5.0 ± 0.3, 10 ± 0.4 and 20 ± 0.5 °C. The temperature of the solution was maintained in a constant temperature water bath and the temperature and conductivity of the solution measured every 2 min for 1 h and then less frequently for 240 min. The data from all kinetic studies were converted to percent of total conductivity and subjected to the kinetic analysis described below.

2.5. Statistical design and analysis

All experiments were repeated at least once with similar results. Each experimental treatment had at least four replicates of three discs each. Appropriate data were combined and means and standard deviations calculated.

A kinetic analysis of the ion efflux data was done using a series of iterative calculations. The data were first expressed as a percent of the total conductivity of the solution, and then zeroed by subtracting the zero time value from all subsequent readings to give the original data set ($D1$). Previous analyses showed that the rate of leakage was linear (i.e. $R^2 > 0.99$) from ~ 30 to 240 min. A linear equation was therefore fitted to the data by starting with data from 90 to 240 min and sequentially including earlier data points until the R^2 value of the equation fell below 0.99. The y-intercept of the linear equation gave the value C_0f, i.e. the percentage of the total conductivity in the 'fast' compartment. The values C_0f and C_0s reflect the percentage of the total conductivity in the 'fast', and 'slow' compartments of the tissue, respectively. I am assuming that the 'fast' compartment represents the extra-cellular portion of the tissue from which ions can readily diffuse, while the 'slow' compartment represents the membrane-bound cellular portion of the tissue. Since the data are expressed as percentages of the total, the percent of total conductivity in the 'slow' compartment was taken as $100 - C_0f$ and is designated C_0s.

Values were computed using only the slope of the linear equation for each sample time and then subtracted from the corresponding original data point to produce the new data set $D2$ (i.e. $D2x = D1x -$ linear value for x). An exponential equation (Eq$_f$) of the form $y = C_0f \ (1 - e^{-K_f t})$ was then fitted to the $D2$ data set by changing K_f in a series of iterative steps to minimize the difference between the calculated values and the data set $D2$. Values from Eq$_f$ were then computed for each sample time and subtracted from the original data set ($D1$) to give the data set $D3$ (i.e. $D3x = D1x - $ Eq$_f x$). A second exponential equation (Eq$_s$) was then fitted to $D3$ by changing K_s in a series of iterative steps to minimize the difference

between the values of $D3$ and those calculated from Es for each sample time (x). Combining the results from equations Ef and Es for each sample time produced a data set that was very close ($R^2 > 0.95$) to the original data set ($D1$). The values K_f and K_s are time constants (units of h^{-1}) in the exponential equation for the rate at which the ions leak from the fast and slow compartments, respectively.

3. Results and discussion

3.1. Determining the isotonic concentration

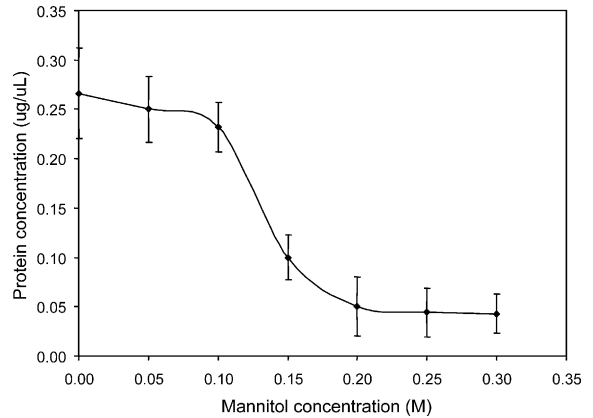Excised tomato fruit pericarp discs initially gained weight when immersed in 0.0–0.4 M

Fig. 2. Protein concentration in the bathing solution of excised pericarp discs of mature-green tomato fruit held in different aqueous concentrations of mannitol for 240 min. Vertical bars represent ± S.D. ($n = 4$).

mannitol solutions (Fig. 1A). After 60 min, discs in 0.0, 0.05 and 0.25–0.4 M solutions showed weight loss, while those in 0.15 and 0.20 M maintained their weight gain. Except for discs in 0.0–0.10 M solutions, weights showed only slight changes from 120 to 240 min, with discs in 0.30–0.40 M solutions showing increasing weight loss with increasing mannitol concentration. When expressed in relation to solution concentration (Fig. 1B), the concentration where there was no net gain or loss of pericarp disc weight after the initial weight gain was around 0.2 M. This concentration was assumed to be isotonic with the tissue. There was a slight increase in this concentration with ripening of the tomato fruit (data not shown), so care was taken to use only mature-green fruit in all experiments.

The protein content of the solution was measured after three discs were immersed in 20 ml of solution for 240 min. When expressed in relation to solution concentration, the greater loss of protein from discs in 0.0–0.1 M solutions than from discs in 0.15–0.30 M solutions can clearly be seen (Fig. 2). The less concentrated solutions probably caused an osmotic shock which lead to the disruption of some cellular membranes and leakage of cytoplasmic proteins into the bathing solution.

Fig. 1. Percent change in weight of excised pericarp discs of mature-green tomato fruit in 20 ml of aqueous solutions of different mannitol concentrations. The percent change in weight is related to (A) time in solution, or (B) solution concentration. Vertical bars represent ± S.D. ($n = 8$).

3.2. Leakage from pericarp discs in solutions of various concentrations

The rate of ion leakage was influenced by the mannitol concentration. The rate of ion leakage was linear ($R^2 > 0.95$) from 30 to 240 min for pericarp discs in 0.1–0.3 M mannitol solutions, while leakage from discs in the 0.0 M solution was quadratic ($R^2 > 0.99$) (Fig. 3). The slopes of the linear equations were 7.2, 2.5 and 3.3% of total conductivity per hour, for 0.1, 0.2 and 0.3 M mannitol solutions, respectively, while the y-intercepts were 6.3, 8.6 and 7.4% of total conductivity, respectively.

Washing the discs after excision is important to reduce the background conductivity readings. Discs were washed 0 or 3 times in deionized water for 1 min before being put into 20 ml of 0.0 or 0.2 M mannitol solutions. The linear regression equations from 30 to 130 min for all treatments had R^2 values > 0.995, but the y-intercepts for the washed and non-washed discs differed greatly (Fig. 4). The intercepts for non-washed discs in the 0.0 and 0.2 M solutions were 13.8 ± 1.6 and $13.6 \pm 1.2\%$ of total conductivity, respectively, while they were 4.4 ± 0.8 and 4.1 ± 0.6 for the

Fig. 4. Effect of washing the excised mature-green tomato pericarp discs in three changes of deionized water on the subsequent cumulative increase in ion leakage as a percent of total conductivity in 0.0 or 0.2 M mannitol. The dashed line represents the linear regression line for each treatment. Vertical bars represent $\pm$ S.D. ($n = 4$).

washed discs. In contrast, the rate of leakage (i.e. slope of the linear regression line) was similar between the washed and non-washed discs for each of the solutions. The slope for 30–130 min was 11.5 ± 2.3 and $11.1 \pm 2.1\%$ of the total conductivity per hour for the non-washed and washed discs, respectively, in the 0.0 M solution, while it was 3.2 ± 0.4 and 3.0 ± 0.6 for the non-washed and washed discs, respectively, in the 0.2 M solution.

These data show that serious errors could be encountered in comparing treatments if the discs are not washed after excision and if the rate of leakage is not calculated for the linear portion of the leakage curve. Not washing the discs increased the background level of conductivity 3.3-fold from 4.2 ± 0.6 for the washed discs to $13.7 \pm 1.6\%$ of total conductivity (Fig. 4). Given a small difference between two treatments, this higher background level would make it more difficult to detect this small difference as significant. The kinetics of leakage from discs in the 0.0 M solution was quadratic, however, it was quite accurately approximated by the linear regression. A simple second degree polynomial gave a very precise fit to the washed, 0.0 M data, but the initial
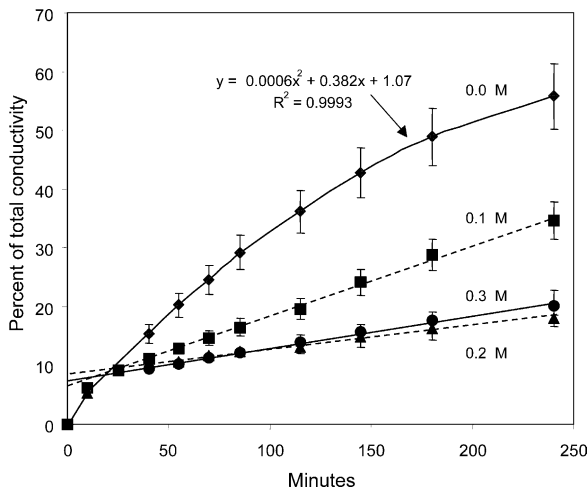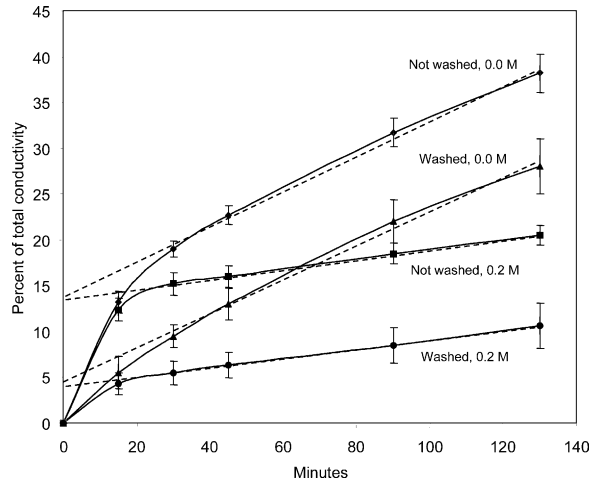

Fig. 3. Cumulative increase in ion leakage as a percent of total from washed and aged pericarp discs of mature-green tomato fruit held in different aqueous concentrations of mannitol. The lines represent the quadratic regression for the 0.0 M solution, and the linear regressions for the 0.1, 0.2 and 0.3 M mannitol solutions. Vertical bars represent $\pm$ S.D. ($n = 4$).

large efflux from the not washed discs in the 0.0 M treatment required the elimination of readings before 30 min from the data set to get as good a fit. The slopes of the linear portion of each treatment were relatively independent of whether the tissue had been washed or not, indicating that it is a robust measurement of ion leakage.

The rate of leakage from discs immediately after excision was influenced by the concentration of the mannitol solution (Fig. 5). Leakage from discs in 0.0 M solution declined about 25% from 18.1 ± 1.4 immediately after excision to 13.7 ± 1.2 percent of total conductivity per hour for discs held in moist air for 18 h at 25 °C before being immersed in the mannitol solution for the conductivity readings. In contrast, the rate of leakage was almost identical at 2.9 ± 0.5 from both freshly excised and aged discs when bathed in 0.2 M mannitol. Again, the importance of using a concentration close to the isotonic value to minimize extraneous influences, such as the effects of excision on ion leakage is apparent.

3.3. Ion leakage from previously chilled tissue

After a lag of 1 day, the rate of ion leakage from washed and aged pericarp discs increased with the time of chilling at 2.5 °C (Fig. 6). Four

Fig. 6. Effect of chilling at 2.5 °C on the subsequent rate of ion leakage (percent of total conductivity per h) from pericarp discs of mature-green tomato fruit held in a 0.2 M mannitol solution. Tissue was immersed in the solution within 20 min of removal from chilling. Vertical bars represent ± S.D. ($n = 4$).

days of chilling doubled the rate of leakage from 2.4 ± 0.3 to 4.9 ± 0.4% of total conductivity per hour. A further 40% increase in the chilling-induced rate of ion leakage occurred after 6 days of chilling when the rate of leakage reached 5.7 ± 0.5% of total conductivity per hour.
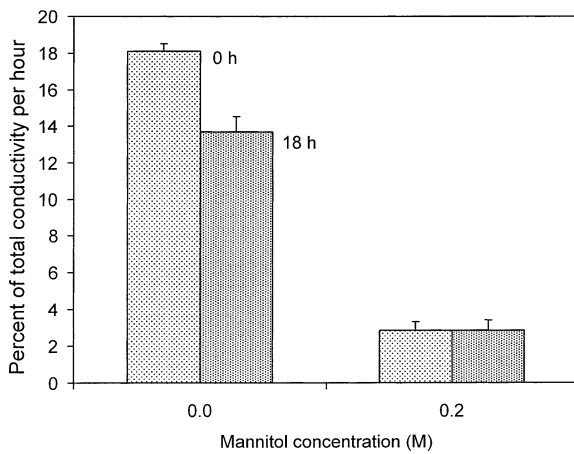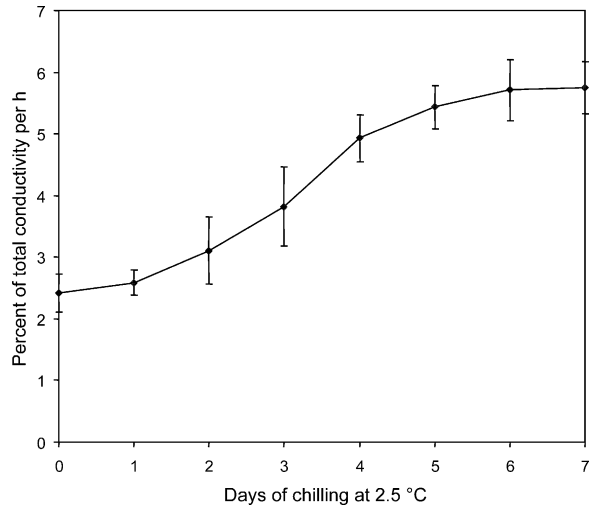

Fig. 5. Effect of time after excision on the rate of ion leakage from pericarp discs of mature-green tomato fruit held in a 0.0 or 0.2 M mannitol solution. Vertical bars represent ± S.D. ($n = 4$).
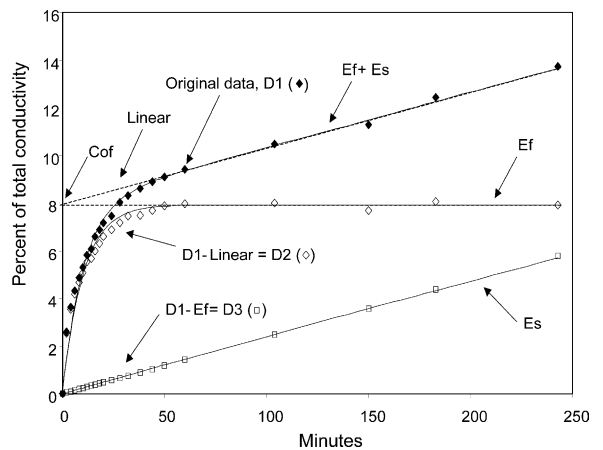

Fig. 7. Representative analysis of ion efflux data from excised tomato pericarp discs chilled for 3 days at 2.5 °C and then put into a 0.2 M mannitol solution using the procedure described in Section 2 to find C_0f, $D2$, F_f, $D3$, E_s and thereby K_f and K_s.

A graphic depiction of the kinetic analysis described in Section 2 (Fig. 7) shows how an original data set ($D1$) is resolved into the various components of C_0f, $D2$, Ef, $D3$, and Es. As long as the tomato pericarp discs were washed, aged and bathed in a 0.2 M mannitol solution, this analysis produced a combined set of equations (Ef + Es) that fit the original data set with an $R^2 > 0.95$. This method was therefore used to analyze ion efflux data from chilled pericarp discs.

As the length of chilling increased from 0 to 7 days, the time constant for the fast compartment (K_f) remained relatively constant at 0.24 ± 0.05 h^{-1}, while C_0f showed a 5-fold increase from 0.6 ± 0.2 to $3.1 \pm 0.3\%$ of total conductivity. In comparison, the value for C_0s decreased from 99.4 ± 0.2 to $96.9 \pm 0.3\%$ of total conductivity, while K_s increased 8-fold from $1.3 \pm 0.2 \times 10^{-4}$ to $10.4 \pm 1.4 \times 10^{-4}$ h^{-1}. These changes are similar to those suggested during a preliminary study (Saltveit, 1989). Although these changes occurred over the 7 days of chilling, they are large enough so that even if a small portion of the change occurred immediately upon chilling, it should be easily detected using the precautions and procedures described in this paper.

Significant changes in the coefficients describing ion leakage were observable upon recovery of the pericarp tissue from moderate chilling. Holding tomato pericarp discs at 25 °C for up to 6 h after chilling for 3.5 days reduced the amount of leachable ions in the fast compartment (C_0f) (Fig. 8). The percent of total conductivity in the fast compartment declined in a linear fashion [$C_0f = 2.48 - (0.35 \times \text{hours})$; $R^2 = 0.996$] with hours at 25 °C after chilling. The fast time constant remained fairly stable ($K_f = 0.264 \pm 0.023$) over the 0–6 h holding period. In contrast, the slow constant (K_s) steadily increased from $11.7 \pm 2.2 \times 10^{-4}$ to $23.4 \pm 1.2 \times 10^{-4}$ h^{-1} according to the equation $K_s = 11.27 \times 10^{-4} + (2.04 \times 10^{-4} h^{-1} \times \text{hours})$ with an R^2 of 0.986.

Repair over time after chilling of the cellular membranes damaged during chilling could decrease the chilling-induced increase in membrane permeability (K_s), and possibly allow reabsorption of the ions that had leaked out of the cell into the intercellular spaces (and contributed to C_0f) dur-
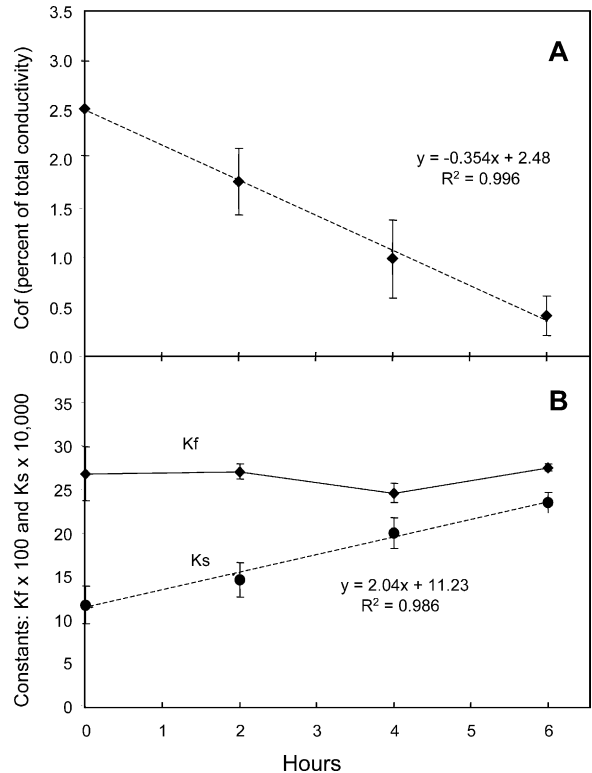

Fig. 8. Components of the kinetic analysis of ion leakage from excised pericarp discs of mature-green tomato fruit held in a 0.2 M mannitol solution 0, 2, 4 and 6 h after removal from 3.5 days of chilling at 2.5 °C. Panel A and B show the change in C_0f, and K_f and K_s, respectively, after removal from chilling. The dashed line represents the linear regression line. Vertical bars represent $\pm$ S.D. ($n = 5$).

ing chilling. Reabsorption appears to have occurred since C_0f showed a steady decline at 25 °C after chilling (Fig. 8). Instead of decreasing, however, K_s actually increased after chilling. This may possibly show that the ability of a chilling injured cell to repair cellular membranes was insufficient to counter the detrimental effects of chilling. Presumably, such repair capacity does ultimately arise since chilling sensitive tissue can recover from these moderate levels of chilling-induced injury. Since efflux from the fast, presumably extra-cellular portion of the tissue, is not restricted by an intervening membrane barrier, its rate constant (K_f) should, and did remain constant during the post-chilling period of holding. These descriptive values for the rate of leakage

from the proposed membrane-bound region show both the detrimental effect of chilling (increases in K_s) and the ability of the tissue to recovery from chilling when removed to warm, non-chilling temperatures (decreases in C_0f).

3.4. Ion leakage from tissue during chilling

In contrast to the effects of prolonged chilling on increasing the subsequent rate of ion leakage at warm, non-chilling temperatures, tomato pericarp discs did not show an immediate increase in the rates of ion leakage when chilled (Fig. 9). The content of C_0f (i.e. the extra-cellular fast compartment) was consistent across all temperatures ($1.1 \pm 0.2\%$ of total conductivity), and therefore, since C_0s is defined as $100 - C_0f$, C_0s was also
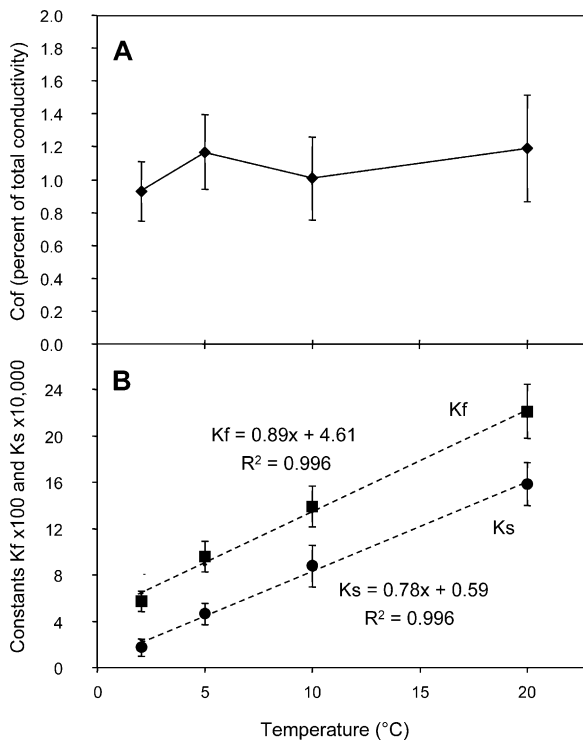

Fig. 9. Components of the kinetic analysis of ion leakage from excised pericarp discs of mature-green tomato fruit held in a 0.2 M mannitol solution at chilling and non-chilling temperatures. Panel A and B show the values of C_0f, and K_f and K_s, respectively, at chilling and non-chilling temperatures. The dashed line represents the linear regression line. Vertical bars represent $\pm$ S.D. ($n = 8$).

consistent across all temperatures used in these experiments. The two rate constants (K_f and K_s) showed a similar linear decline with reductions in temperature. This linear decline continued from non-chilling through to chilling temperatures with no significant deviation from linearity at the chilling temperature. The fast constant (K_f) changed according to the linear equation $K_f = 0.0461 + (0.0089 \times T \, °C)$ with an R^2 of > 0.99, while the slow constant (K_s) changed according to the linear equation $K_s = 0.59 \times 10^{-4} + (0.78 \times 10^{-4} \times T \, °C)$ with an R^2 of > 0.99.

Since K_f reflects the rate of leakage from the extra-cellular compartment (e.g. cell walls and intra-cellular spaces within the pericarp tissue), changes in its rate with temperature are expected because of the effect of temperature on the rate of diffusion. In contrast, since K_s reflects the rate of leakage from the membrane bound portion of cells its value should change as the permeability of the cell changes at different temperatures. However, although offset by a 100-fold difference in magnitude, the changes in K_f and K_s over temperature are strikingly similar. If the theory is correct that a phase change occurs at the chilling temperature in chilling sensitive tissue, then the values of K_s at 2 and 5 °C should have deviated from the linear regression line for the 10 and 20 °C values and been larger than those observed. That they lie on the linear regression line implies that the change in the rate of ion leakage from the cells was more influenced by the effect of temperature on rates of diffusion through the membrane than by its effect on changing membrane permeability by inducing a substantial phase transition.

4. Conclusion

Storage at chilling temperatures for a few days did induce an increase in the subsequent rate of ion leakage from sensitive tissue when measured after chilling, but chilling did not immediately increase the rate of ion leakage from cells. Rather, there was a slow increase in both the amount of leachable ions in the extra-cellular component of the tissue and an increase in the permeability of the membrane bound region as the length of

chilling increased. Chilling does not appear to immediately cause a change in the permeability of the cellular membranes, but it does seem to cause a progressive increase in permeability over a few days of chilling. It is difficult to imagine a temperature-induced phase transition in cellular membranes that would be of sufficient magnitude to produce the significant changes in the activity of associated enzymes described by Raison (1973) and Lyons (1973) without causing a measurable change in permeability.

Although membrane lipid phase separation is associated with chilling of tomato fruit, the phase separation has been ascribed to an indirect effect of low temperature (Sharom et al., 1994). They observed that the phase transition was not reversible when the tissue was warmed above the chilling temperature. In fact, there was an increase in gel phase lipids upon returning the chilled tissue to 25 °C that coincided with the development of visual chilling symptoms. The authors concluded that active phospholipid catabolism and accumulation of free fatty acids in the membranes followed chilling and preceded formation of gel phase lipids.

Data presented in this paper do not support the chilling model in which a membrane phase transition immediately occurs upon chilling and alters membrane permeability, but they do show that changes in membrane permeability accumulate gradually during exposure to chilling. An abrupt temperature-induced phase transition in membranes of chilling sensitive plant tissue may not be the physical event that transduces an exposure to chilling temperatures into the physiological event that causes chilling injury.

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