Phytochemistry, Vol. 28, No. 9, pp. 2293–2301, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

PHASE TRANSITIONS IN MICROSOMAL MEMBRANES FROM CHILLING SENSITIVE AND CHILLING RESISTANT TOMATO PLANTS AND FRUIT

ALEJANDRO G. MARANGONI and DAVID W. STANLEY

University of Guelph, Department of Food Science, Guelph, Ontario N1G 2W1, Canada

(*Received in revised form* 6 *February* 1989)

Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; chilling resistance; tonoplast membranes; phase transitions.

Abstract—The thermal response of tonoplast enriched microsomal membranes from the leaves and mature green fruit pericarp of two tomato varieties was determined by electron paramagnetic resonance (EPR), differential scanning calorimetry (DSC) and fluorescence polarization spectroscopy. Chilling sensitive *Lycopersicon esculentum* cv. Heinz-722 and a chilling resistant hybrid of cv. H-722 and *L. hirsutum* (F-4 generation, strain 58-1), were used for comparative studies. Lipid phase transitions in the microsomal membranes were detected. Transition temperatures were determined by EPR at 12° for the chilling sensitive leaf and fruit and at 4 and 8° for the chilling resistant leaf and fruit, respectively, using 12-doxystearic acid as a spin label. Phase transitions were not detected in 1,6-diphenylhexatriene labelled membranes by fluorescence depolarization. Broad transitions (50° range) were observed by DSC in the sensitive fruit and leaves while none were evident in the resistant counterparts. *In vivo* chlorophyll fluroscence of the leaves showed a greater quenching of fluorescence in the sensitive leaves while ultrastructural studies of the fruit indicated a loss of cytoplasmic structure in the sensitive fruit, but not in the resistant fruit. These results indicate that membrane phase transition temperatures can be used as an indicator of resistance towards chilling.

INTRODUCTION

Chilling injury is a descriptive term for the physiological damage that occurs in many tropical and subtropical plants when exposed to low but non-freezing temperatures in the range 0–15° [1, 2]. The primary cause for chilling injury was first proposed by Lyons [3] to be a thermally induced disruption in the structure of membrane lipids. Membrane lipids undergo co-operative thermotropic phase transitions between a fluid (random) phase and a gel or solid (ordered) phase [4–7].

Direct evidence for this type of membrane phase transitions comes from wide angle X-ray diffraction studies [8, 9]. Other physical techniques which have been used to study lipid phase transitions are EPR (electron paramagnetic resonance) spectroscopy of nitroxyl-stearate labelled membrane lipids [10–17], fluorescence polarization using DPH (1,6-diphenylhexatriene) and *trans*-parinaric acid fluorescent labels [18–21], freeze fracture electron microscopy [22] and differential scanning calorimetry (DSC) [10, 11, 23, 24]. Recent work by Raison and Orr [10] showed that DSC, EPR and fluorescence polarization techniques are valid methods of assessing phase transitions in membrane lipids.

Susceptibility of plants to chilling is related to changes in the molecular ordering of membrane lipids below transition temperatures [15, 23, 25]. Membrane lipids from chilling resistant plants also show phase transitions but at temperatures close to or below 0°. Because plant membranes contain high proportions of *cis*-unsaturated lipids, this would suggest that the overall bulk fluid to solid transition would occur below 0° and would be quite broad. However, there may be discontinuities in the random distribution of bilayer lipid allowing some microenvironments to separate above 0° [12, 26].

Phase transitions in plant mitochondria have been the main focus of membrane structural studies. Membrane rigidification in mitochondria affects membrane associated enzymatic activity and disrupts cellular metabolism [27–29]. However, little work has been performed on the membranes from other subcellular organelles or the plasma membrane. Plasma membrane, vacuoles and endoplasmic reticulum are key regulators of cytosolic ion concentrations, in particular intracellular calcium levels. High cytosolic calcium concentrations induce symptoms similar to those of chilling injury [30]. In addition, vacuoles contain many autolytic enzymes, which if decompartmentalized would degrade cellular organelles and lead to cell autolysis and subsequent death.

Most of the work done on phase transitions has been performed on isolated phospholipids extracted from membranes. Given the importance of specific protein–lipid, sterol–lipid and lipid–lipid interactions on membrane phase behaviour [5–7], it would seem beneficial to perform studies on whole membranes.

The objective of the present work was to observe phase transitions in microsomal membranes derived from two varieties of tomato plants and to correlate them with susceptibility to chilling injury. The varieties used included a chilling-sensitive tomato plant and a hybrid of this sensitive cultivar crossed with a wild, resistant tomato variety that was shown to be more resistant to cold temperatures than the sensitive parent. In addition, we have attempted to determine whether a relationship exists between the resistance to cold temperature in leaf tissue and fruit.

RESULTS

Chlorophyll fluorescence data indicated that the ratio F_0/F_p is higher (closer to unity) for the sensitive cultivar than that for the resistant cultivar after the chilling treatment (Table 1). Both cultivars showed increases in the F_0/F_p ratio upon chilling.

In transmission electron micrographs (TEM) for the tomato pericarp cells chilled for 14 days (Fig. 1), mitochondria appear swollen in the chilled, sensitive fruit (B, C), endoplasmic reticulum is absent (B, C) and mitochon-

Table 1. Chlorophyll fluorescence data for chilling sensitive (H-722) and chilling resistant (58-1) tomato seedlings chilled at 2° for 3 days

Cultivar	Control	Chilled
58-1	0.66 (0.052)	0.80 (0.061)
H-722	0.72 (0.070)	0.88 (0.025)

Values in parentheses are standard errors for eight measurements.

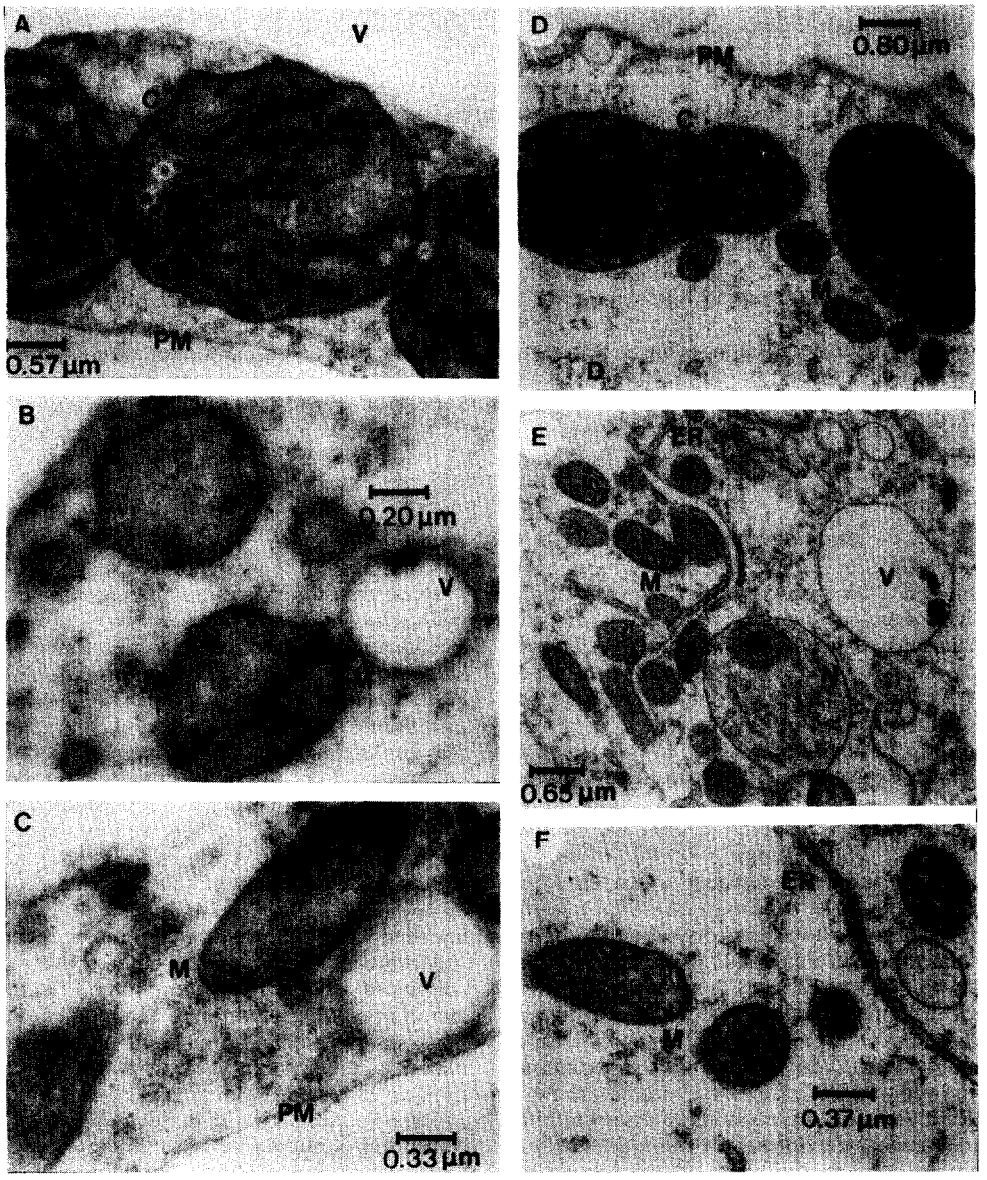

Fig. 1. Electron micrographs of chilling sensitive (A,B,C) and chilling resistant (D,E,F) tomato fruit pericarp tissue chilled for 14 days at 5° (95% RH). (A) Chloroplasts (C) show less staining contrast and plastoglobuli (dark inclusions) are faded (× 17 500). (B) Mitochondria (M) appear swollen and the cytoplasm has lost its integrity. Mitochondrial cristae are not evident (× 48 900). (C) As for (B); plasma membrane (PM) is still discernible (× 30 000). (D) Notice the well defined chloroplast (C) plastoglobuli. Mitochondria (M) show cristae, a dichtyosome (D) is present, plasma membrane (PM) is intact (× 12 600). (E) General subcellular features are evident. Notice the sharp tonoplast around the vacuole (V); the nucleus (N) is evident (× 15 300). (F) Mitochondria show cristae; endoplasmic reticulum (ER) is apparent (× 27 000).

drial cristae are not evident (B, C). Chloroplast plastoglobuli (dark, osmiophilic inclusions) appear faded (A). Sensitive, chilled cells appeared highly vacuolated relative to the control (not shown), nuclei were not present, cytoplasmic integrity was lost and the characteristic features of most subcellular organelles were lost. Plasma membranes, however, still showed high contrast. These effects were not evident in the chilled, resistant fruit cells (D–F) relative to the control (not shown). Figure 2 shows TEM of PACA (phosphotungstic acid-chromic acid) stained membranes from sensitive leaf (A) and fruit (B) and uranyl acetate–lead citrate stained leaf (D) and fruit (D) membranes. The intensity of the stain relative to phosphotungstic acid treated (unstained) grids suggests that the membrane preparation is not particularly enriched in plasma membrane. Marker enzyme studies on the isolated membranes (Table 2) showed an enrichment in tonoplast relative to other membranes (*ca* 66% of total ATPase activity for leaf and *ca* 63% for fruit). Low levels of mitochondrial contamination were evident as determined by oligomycin inhibition of ATPase activity and by cytochrome oxidase activity.

Arrhenius plots of the spin label motion parameter τ_c vs $1/T$ for the chilling sensitive and resistant tomato fruit and leaf tissue (Fig. 3) showed breaks at 12° for the sensitive leaf and fruit and at 8° and 4° for the resistant leaf and fruit, respectively. When resistant pink rather than mature green tomatoes were analysed a transition was observed at 14° (Fig. 4), a temperature similar to that of chilling sensitive mature green tomato fruit (Fig. 3C).

EPR spectra of the 12-doxystearic acid labelled chilling sensitive leaf microsomes at −22° (frozen), 0° and

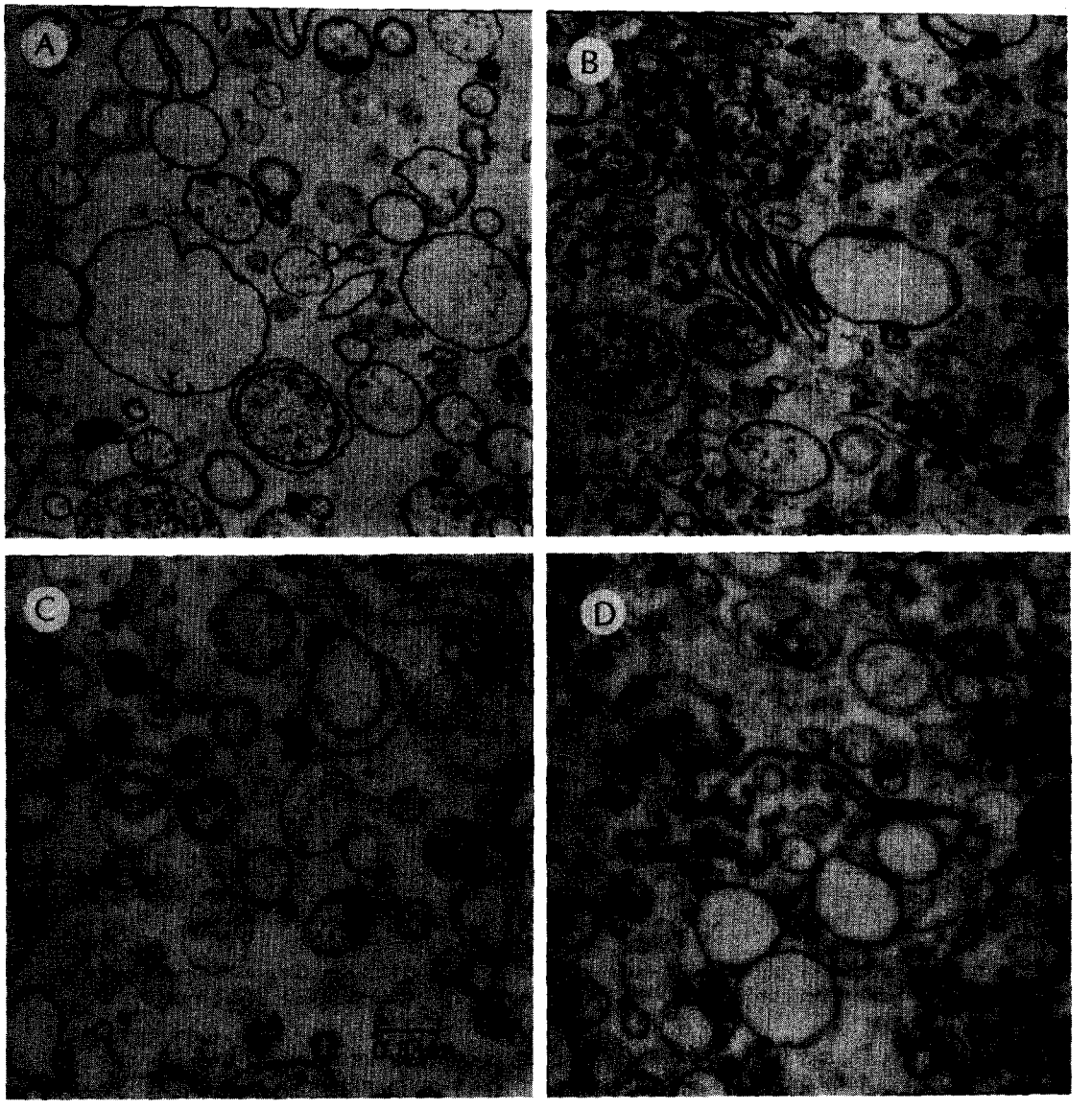

Fig. 2. Electron micrographs of the microsomal membrane vesicles derived from chilling sensitive leaf and fruit tissue (× 35 000). Phosphotungstic acid–chromic acid stained leaf (A) and fruit (B) membranes and uranyl acetate–lead citrate stained leaf (C) and fruit (D) membranes.

Table 2. Marker enzymes activities associated with the microsomal membranes from chilling sensitive tomato leaf and fruit tissue

Marker enzyme	Activity of leaf (%)	Activity of fruit (%)
K^+ stimulated Mg-ATPase (nmol/μg protein/hr)	17.37 (100%)	15.30 (100%)
+ Oligomycin	13.34 (23.2%)	10.53 (31.2%)
+ KNO_3	5.88 (66.1%)	5.58 (63.5%)
+ NH_4VO_3	14.84 (14.6%)	12.22 (20.1%)
+ Triton X-100	16.33 (94.0%)	14.98 (97.9%)
Cytochrome *c* oxidase (μmol/mg prot./min)	1.16	0.76
Antimycin A insensitive NADH-cytochrome *c* reductase (μmol/mg prot./min)	0.18	0.13
Triton-stimulated UDPase (nmol/μg prot./hr)	43.2	52.1

Values are averages of two determinations.

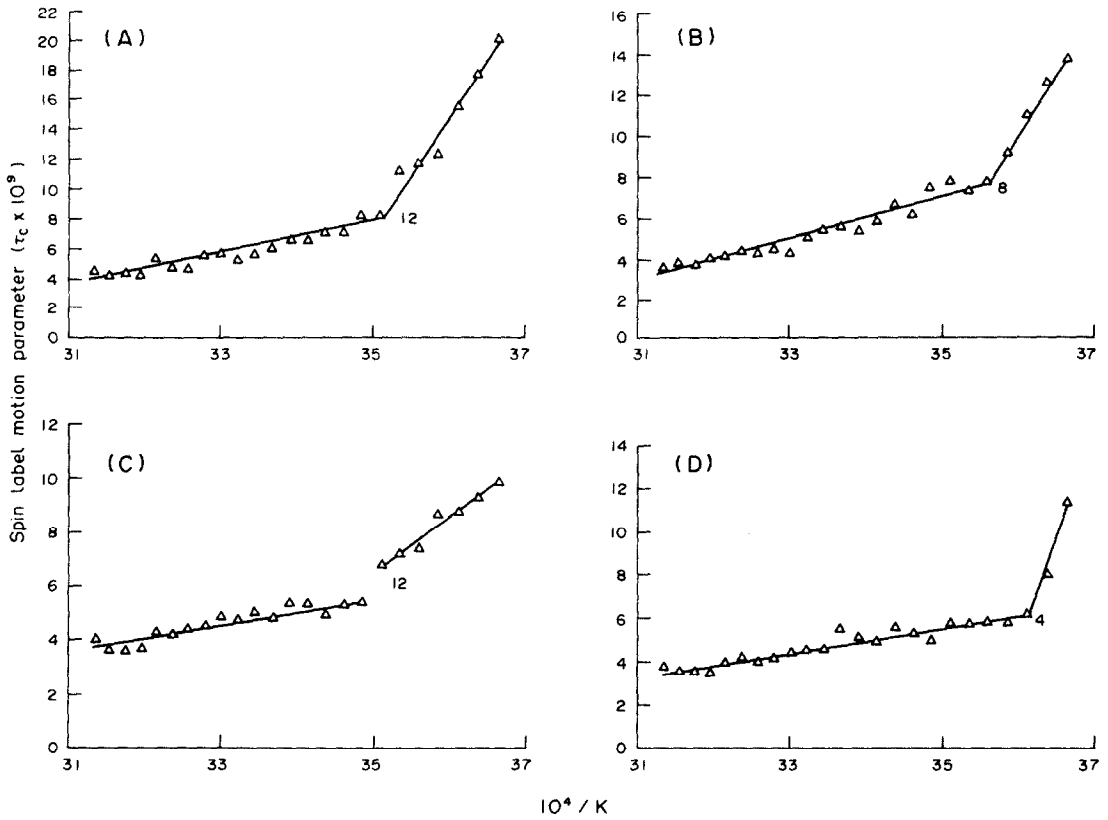

Fig. 3. Arrhenius plots of spin label motion parameter (τ_c) vs $1/T$ for 12 DS labelled microsomal membranes derived from chilling sensitive leaf (A) and fruit (C) tissue and chilling resistant leaf (B) and fruit (D) tissue.

38° are shown in Fig. 5. The signal at 0° is a composite of two different spectra: a broad spectrum arising from a more immobilized probe in a completely solid membrane (−22°) and a sharp spectrum arising from a fluid membrane (38°). The probe seems to partition into both the fluid and gel phases and gives rise to the two signals, depending on its degree of immobilization. Dynamic changes in the membrane structure and partitioning of the probe in the two phases could be studied in this way.

Changes in the lipid structural order parameter (S_{DPH}) value for DPH in microsomal membranes of the sensitive and resistant leaf and fruit tissue are shown in Fig. 6. No

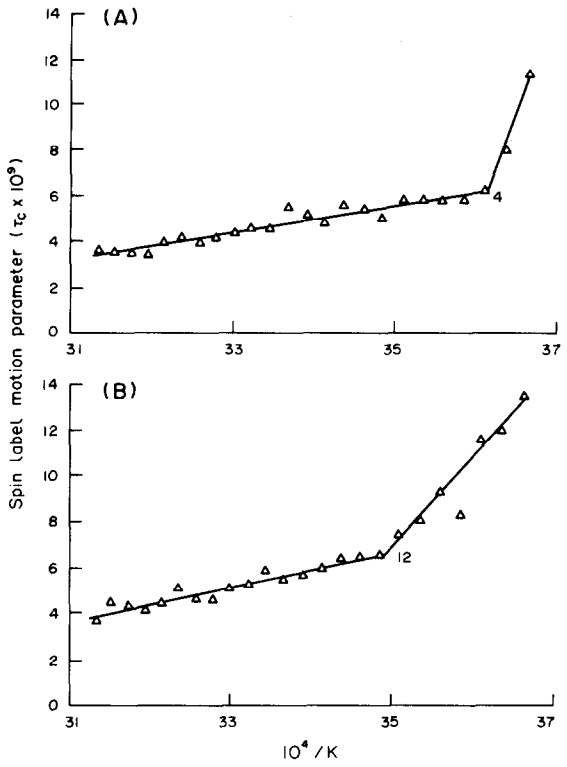

Fig. 4. Arrhenius plots of spin label motion parameter (τ_c) vs $1/T$ for 12-hydroxystearic acid labelled microsomal membranes derived from chilling resistant fruit in mature green stage (A) and in the pink stage (B).

transitions were detected. Deviations from linearity in the S_{DPH} vs T plots below 10° have been reported before [20] and should not be interpreted as breaks in the curves.

DSC results from the isolated membranes are shown in Fig. 7. Only broad and small thermal transitions were determined for the sensitive leaf (A) and fruit (B) membranes while none were evident in the resistant varieties (C, D). Ranges of transitions were 30–50°. Transitions were only evident after heat denaturing the membrane, which agrees closely with former work [24].

DISCUSSION

Chlorophyll fluorescence has been previously used to assess chilling injury in plants [31]. Our experiments showed that Photosystem II-associated chlorophyll fluorescence was quenched more after chilling in the sensitive tomato cultivar than in the resistant one. Chlorophyll fluorescence was also quenched in the resistant seedlings, but to a lesser extent than in the sensitive seedlings. These data were taken as an indication of increases in chilling tolerance in the resistant hybrid.

Ultrastructural changes in the tomato fruit pericarp cells are consistent with those found by other workers [32–35]. Drastic ultrastructural changes in the chilling sensitive fruit cells and their absence in the resistant fruit were considered to be indicative of a greater chilling resistance in the more tolerant hybrid. Tonoplast and mitochondrial membranes were not evident after chilling and chloroplast plastoglobuli had degraded. These effects may all be due to chill induced membrane degradation and were not evident in the resistant fruit. Plasma membrane, however, could still be recognized in the chilled sensitive varieties. It would seem that this membrane is able to withstand the effects of low temperature better than the tonoplast.

Our membrane preparation was enriched in tonoplast (Table 2). Niki *et al.* [33, 34] have shown that the integrity of the tonoplast may be the key factor determining the ability of a plant cell to recover from chilling stress. These authors suggested that when the tonoplast degraded, autolytic enzymes would be released from the vacuole into the cytosol, leading to the degradation of subcellular organelles and membranes. Breaks in the EPR Arrhenius plots were observed in the microsomal membrane preparation from the resistant and sensitive leaf and fruit pericarp tissue. This suggests that changes in the physical

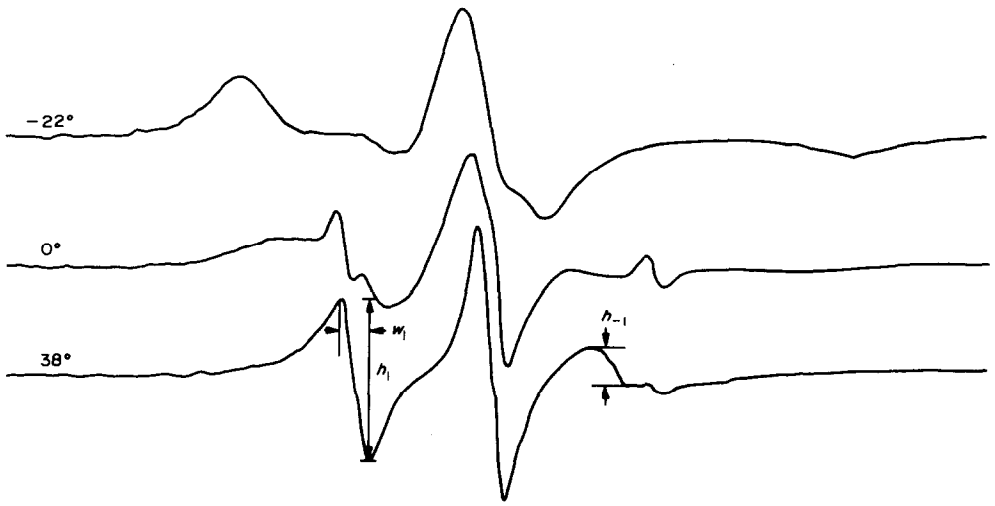

Fig. 5. Electron paramagnetic resonance spectra of 12-doxystearic acid labelled microsomal membranes from chilling sensitive leaf tissue at −22° (frozen), 0° and 38°.

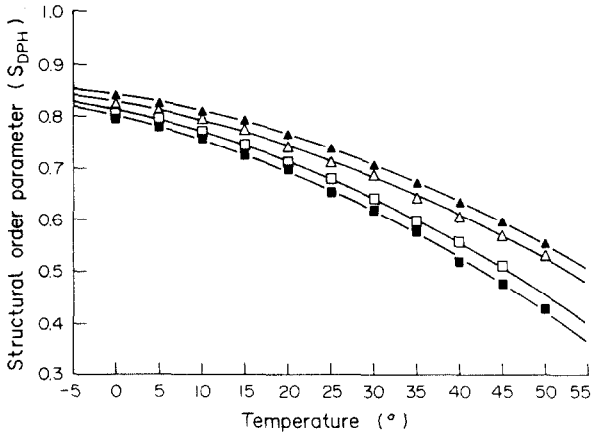

Fig. 6. Fluorescence depolarization structural order parameter of DPH labelled microsomal membranes as a function of temperature for chilling sensitive (△——△) and chilling resistant (▲——▲) tomato leaves and for chilling sensitive (□——□) and chilling resistant (■——■) tomato fruit.

structure of microsomal membrane lipids do occur and correlate with the usual temperature below which injury develops [15], a phenomenon previously observed in mitochondria. Phase transitions in the tonoplast or plasma membrane could be responsible for ion and solute leakage that is generally associated with chilling injury [28] and could affect membrane bound enzymes such as Ca-ATPases which regulate cytosolic calcium concentrations [36]. Increases in cytosolic calcium levels have been found to lead to a cessation of cytoplasmic streaming, inactivation of mitochondrial oxidase activity [30] and membrane degradation through phospholiphase D activation [37]. On the other hand, a breakdown of the tonoplast would release degradative enzymes into the cytosol, leading to cellular degradation. From the EPR data it may be observed that chilling sensitive leaf and fruit membranes show breaks at higher temperatures than the chilling resistant species. The onset of injury would then occur at higher temperatures, making the plant more prone to chilling injury.

Breaks were not detected in the structural order parameter vs temperature plots for the DPH labelled microsomal membranes. Other groups [20] have failed to detect phase transitions in several biomembranes using DPH as a probe. Lynch *et al.* [18] found breaks in DPH labelled bean microsomes and microsomal lipids. Yoshida *et al.* [38] also found subtle breaks in DPH labelled *Vigna radiata* plasma membranes. When using other fluorescent probes such as *trans*-parinaric acid on membrane lipids [19, 21], however, clear breaks are observed in polarization vs temperature plots, perhaps as it is possible that fatty acid based probes such as parinaric acid for fluorescence polarization and nitroxyl stearate probes for EPR associate with membrane microenvironments that undergo phase transitions in physiological temperature ranges. This may explain the lack of breaks in the DPH labelled membranes as the measurement in unfractionated DPH labelled membranes yields only time-averaged order parameters, i.e. they do not give resolution into particular lipid domains which may exist in a biological membrane. These observations, where phase transitions are probe specific, imply that only certain regions of the membranes undergo phase transitions. This agrees with findings that just 5% of the membrane lipids undergo phase transitions as determined by the enthalpies of transition [10, 12, 39].

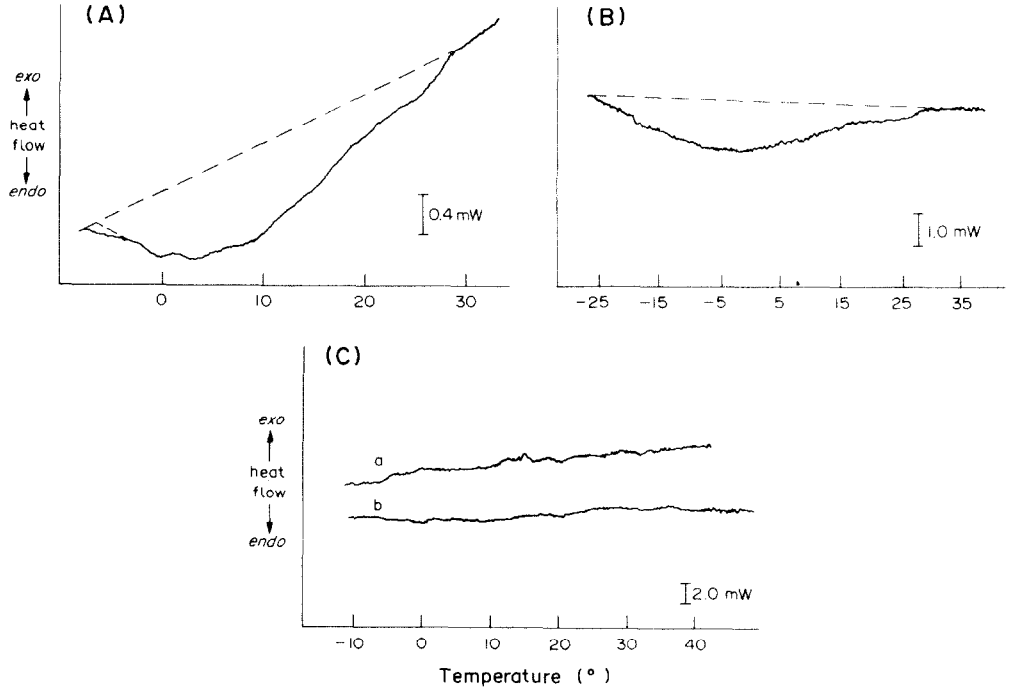

Fig. 7. Differential scanning calorimetric thermograms of microsomal membranes derived from chilling sensitive leaf (A), fruit (B) and chilling resistant (C) leaves (a) and fruit (b).

DSC results agreed closely to that of other workers and similar effects were evident [24]. Phase transitions were found to be broad and small for the sensitive fruit and leaf membranes while no transitions were detected in the resistant leaves or fruit. These transitions, though, were only evident after heating the membranes at 95° for 5 min. A more gentle heat treatment (60° for 3 min) did not have a marked effect on the phase properties of the membranes; this phenomenon has been reported before [24]. McMurchie [24] regarded this effect as a 'lipid–lipid' interaction as it was also observed in the extracted lipids. This effect seems to be specific for tomatoes. There is the possibility of specific proteins modulating the phase properties of the membranes as well. Broad transitions are probably due to the high sterol content of biological membranes which would decrease phase transition enthalpies. A high structural order parameter was found for both the fruit and leaf membranes, suggesting a rigid structure in these membranes. The lipid structural order parameter is related to the degree of molecular packing and this suggests an inverse relationship with the lateral diffusion of membrane lipids and proteins. The use of this parameter as opposed to the widely used 'microviscocity' is theoretically more sound [20].

Transition temperatures, as determined by EPR, were shown to shift from 4° to 14° in chilling resistant fruit when developing from a mature green to a pink stage. As plant cells senesce, cellular membranes are known to become less fluid [8, 9, 18]. This does not imply that pink tomatoes are more prone to chill-injury than mature green ones. After the senescent process has been initiated and the fruit has reached physiological maturity, cold temperature will only slow down the effects of senescence.

It has been demonstrated that phase transitions occur in microsomal membranes and that chilling resistant tomato fruit and leaf membranes show lower phase transition temperatures than chilling sensitive ones. This suggests that one of the characteristics bred into the chilling resistant hybrid is a membrane structure less prone to undergo phase transitions at lower temperatures, leading to a greater resistance towards chilling.

A greater chilling resistance was also observed in the fruit of the resistant hybrid. This could have important implications in the field of postharvest cold storage of horticultural crops as chilling sensitivity is a major deterrent to the long term storage of many crops [3]. Perhaps it will be possible to use the detection of chilling resistance in leaf tissue as a prediction of fruit response, a procedure that could speed breeding experiments.

EXPERIMENTAL

Plants. Two varieties of tomato differing in their chilling sensitivity, but which were derived from the same genetic pool, were chosen for the study. Chilling sensitive (*L. esculentum* cv. Heinz-722) and chilling resistant [F-4 generation *L. esculentum* cv. Heinz-722 crossed and back-crossed with *L. hirsutum* (wild South American species), strain 58-1] plants were grown in University of Guelph greenhouses during the months of May, June and July, 1987.

Plants were grown from seed in 1:1:1 soil base–peat moss–perlite and fertilized twice weekly with 20-20-20 NPK and $Ca(NO_3)_2$. Leaf tissue from a variety of maturities was collected randomly from the plants, but senescent leaves were avoided. Mature green fruit (stages 2, 3 and 4) were used as judged by established criteria [40].

Determination of chilling resistance. Seedlings (4–5 weeks old) were subjected to a chilling treatment (2° for 3 days) in the light (14 hr light cycle, 300 μE light intensity), dark adapted for 15 min and chlorophyll fluorescence of the leaf [31] was measured upon return to ambient temp. as a function of time (excitation wavelenth: 670 nm, emission wavelength recorded: > 710 nm, light intensity: 4000 ergs/cm^2/sec). Four fluorescence measurements were made on each of the 3rd and 4th fully expanded leaves of the plant. The ratio F_0/F_p was used to assess chilling resistance quantitatively. F_0 is the initial level of fluorescence before the induced rise and F_p is the peak level of induced fluorescence. A decrease in the ratio was interpreted as an indication of chilling damage [31].

Electron microscopy of tomato fruit. Chilling sensitive and chilling resistant mature green fruit, stages 3 and 4 was chilled for 14 days at 5° (95% RH) and pericarp samples examined by TEM for ultrastructural changes associated with chilling.

Chilling resistant and chilling sensitive fruit pericarp, control and chilled, was fixed in 70 mM Pi buffer, pH 6.8, 2% in glutaraldehyde, 1% in paraformaldehyde for 16 hr at 20°. Samples were postfixed in aq. OsO_4, dehydrated through a graded EtOH series and embedded in Epon resin. Thin sections were stained with satd uranyl acetate in 50% aq. EtOH for 20 min, and with Reynold's Pb citrate, 0.25% aq. Pb citrate for 10 min. TEM was performed on a Phillips 300 electron microscope at an operating voltage of 60 kV.

Microsomal isolation. Microsomes were isolated from whole leaf tissue and from fruit pericarp tissue. A 20 g sample of leaf tissue or 50 g of fruit pericarp tissue was diced and homogenized with 100 ml cold buffer consisting of 100 mM MOPS (Sigma), 250 mM sucrose (Sigma), 28 mM ascorbate (Fisher), 10 mM EGTA (Sigma), 2.5 mM sodium metabisulphite (Fisher), 10 mM 2-mercaptoethanol (Bio-Rad), 0.5% (w/v) insol PVP (Sigma) and adjusted to pH 7.2 with 0.1 M NaOH for fluorescence polarization and DSC studies and TEM of the membranes. For EPR studies the homogenizing buffer consisted of 100 mM MOPS, 0.4 M mannitol (Sigma), 0.005 M EDTA (Fisher), 10 mM 2-mercaptoethanol, 1% insol. PVP, adjusted to pH 7.0 with 0.1 M NaOH.

Tissue was homogenized at room temp. in a Waring-type blender by 3-5 sec bursts with a 10 sec intervening cooling period. The homogenate was filtered through 4 layers of cheesecloth and the filtrate centrifuged at 13000 g for 15 min at 4° in a Beckman L8-70 ultracentrifuge using a fixed angle 45 Ti rotor. The resulting supernatant was filtered through 4 layers of cheesecloth and centrifuged at 135000 g for 30 min at 4° using a 70 Ti rotor. Pellets were resuspended and washed in 10 ml of fresh homogenizing buffer (without mercaptoethanol or PVP for EPR studies) using a loosely fitting Potter–Elvehjem homogenizer. The suspension was recentrifuged at 135000 g for 30 min at 4°, yielding a pellet of microsomal membranes. These were freshly prepd prior to each expt.

Characterization of microsomal membranes. Enzyme markers for the characterization of the microsomal membranes were chosen and performed as described in ref. [41]. Triton X-100 stimulation of ATPase activity was performed as described in ref. [38]. Oligomycin inhibition of ATPase activity (mitochondrial marker) was performed as described in ref. [24]. For inorganic phosphate estimation, a method was utilized that eliminates the high background problem inherrent in this determination [42].

Electron paramagnetic resonance (EPR). Prior to spin labelling, microsomal pellets were resuspended in homogenizing buffer (1:2 w/v membrane–buffer) without mercaptoethanol or PVP. The spin label used was 12-doxystearic acid (Sigma). A 10 μl sample of a 2 mM soln of spin label in MeOH was evapd

onto the sides of a small test tube with dry N_2 and a 75 μl portion of the membrane suspension added. The tube was shaken for 10 sec and the sample transferred to a capillary tube for EPR studies. These labelling conditions are similar to those described in ref. [43] and yielded a membrane phospholipid to spin label ratio of 100:1. No spin–spin interactions were observed at this concn. EPR spectra were recorded on a Varian E-109 EPR spectrometer equipped with a variable temp accessory. Microwave power was set at 20 mW. A time constant of 0.128 sec combined with a modulation amplitude of 2 Gauss and a scanning time of 2 min gave the best results. Measurements were performed every 2° with a 2 min equilibration period at each temp. Values for rotational correlation time (τ_c) were calculated from spectra recorded from the labelled microsomes as described in ref. [43]:

$$\tau_c = 6.9 \times 10^{-10} w_1 [(h_1/h_{-1})^{+1/2} - 1] \text{ sec}$$

where w_1 and h_1 are the width and height of the low field line and h_{-1} the height of the high field line (Fig. 5). Three determinations on three different samples were performed. Arrhenius plots of τ_c vs $1/T$ were constructed and the most representative plots of three identical runs presented.

Fluorescence depolarization. *Ca* 80 mg of wet microsomal pellets were resuspended in 5 ml of homogenizing buffer (*ca* 50 μg protein/ml). To this, a 5 ml aliquot of 2 μM DPH (1,6-diphenyl hexatriene, Sigma) in 100 mM MOPS, pH 7.2, was added and the mixt. incubated for 2 hr at 4°. Measurements were then performed on a SLM 8000 fluorimeter from 0 to 50° every 5°. The heating rate was 1°/min and a 5 min equilibration time was allowed at each temp. Temp. was controlled with a refrigerated H_2O bath. Excitation and emission wavelengths were 360 and 435 nm, respectively; integration time was 10 sec. Chlorophyll florescence was removed with a bandpass filter (400–450 nm), a cut-off filter (425 nm) and a monochromator. Samples were not corrected for light scattering. All samples were kept in the dark at 4° prior to the first measurement. The polarization value was expressed as:

$$P = \frac{I_{||} - I_{||}}{I_{||} + I_{\perp}}$$

where $I_{||}$ and $I_{\perp}$ are the emission intensities through an analyser oriented parallel ($I_{||}$) and perpendicular ($I_{\perp}$) to the direction of polarization of light [20].

The lipid structural order parameter of the DPH labelled membranes was calculated as described previously [20]:

$$S_{DPH} = [r_\infty/r_0]^{1/2} \qquad 0 \le S \le 1$$

where r_∞ is the limiting fluorescence anisotropy (static factor) and r_0 is the maximal fluorescence anisotropy. r_∞ can be calculated from r_s, the steady-state fluorescence anisotropy,

$$r_\infty = 4/3 r_s - 0.1$$

or from the polarization value by

$$r_s = (2P)/(3 - P)$$

For these conditions the value of r_0 has been determined empirically to be 0.4 [20]. One determination on each of two different samples was performed.

Differential scanning calorimetry (DSC). A 40 mg sample of the wet microsomal pellet (1–1.5 mg lipid) was resuspended in an equivalent wt of ethylene glycol. A 10 μl aliquot of the suspension was pipetted into an aluminum pan and sealed. A 3–4 mg quantity of Sephadex G-25 in 10 μl of 50% ethylene glycol–MOPS, pH 7.2, was used as ref. providing an equivalent thermal mass. Thermograms were obtained using a DuPont 1090 thermal analyser. Samples were scanned twice; the first was performed at 10°/min from −15° to 95° with a 5 min isothermal hold at 95°. The second scan was performed at 5°/min from −30 to 50°. At least 5 determinations on 3 different samples were performed.

Acknowledgements—The authors thank Dr B. D. McKersie and Dr M. Walker for supplying plant material and the chlorophyll fluorescence data, and Dr Y. Kotake for assistance with EPR studies. This project was supported in part by a Natural Sciences and Engineering Research Council of Canada (NSERC) Strategic Grant and the Ontario Ministry of Agriculture and Food.

REFERENCES

1. Jackman, R. L., Yada, R. Y., Marangoni, A. G., Parkin, K. L. and Stanley, D. W. (1988) *J. Food Quality* **11**, 253.
2. Raison, J. K. and Lyons, J. M. (1986) *Plant Cell Environ.* **9**, 685.
3. Lyons, J. M. (1973) *Ann. Rev. Plant Physiol.* **24**, 445.
4. Parkin, K. L., Marangoni, A. G., Jackman, R. L., Yada, R. Y. and Stanley, D. W. (1989) *J. Food Biochem.* (in press).
5. Benga, G. and Holmes, R. P. (1984) *Prog. Biophys. Molec. Biol.* **43**, 195.
6. Quinn, P. J. (1981) *Prog. Biophys. Molec. Biol.* **38**, 1.
7. Quinn, P. J. and Williams, W. P. (1978) *Prog. Biophys. Molec. Biol.* **34**, 109.
8. McKersie, B. D. and Thompson, J. E. (1977) *Plant Physiol.* **59**, 803.
9. McKersie, B. D., Thompson, J. E. and Brandon, J. K. (1976) *Can J. Botany* **54**, 1074.
10. Raison, J. K. and Orr, G. R. (1986) *Plant Physiol.* **80**, 638.
11. Raison, J. K. and Orr, G. R. (1986) *Plant Physiol.* **81**, 807.
12. Raison, J. K. (1985) in *Frontiers of Membrane Research in Agriculture* (Berlin, E. and Jackson, P. C., eds), p. 383. Rowman & Allanheld, Totowa.
13. Raison, J. K., Pike, C. S. and Berry, J. A. (1982) *Plant Physiol.* **70**, 215.
14. Patterson, B. D., Kenkrick, J. R. and Raison, J. K. (1978) *Phytochemistry* **17**, 1089.
15. Raison, J. K., Chapman, E. A., Wright, L. C. and Jacobs, S. W. L. (1979) in *Low Temperature Stress in Crop Plants: the Role of the Membrane* (Lyons, J. M., Graham, D. and Raison, J. K., eds), p. 177. Academic Press, New York.
16. Sinensky, M. (1974) *Proc. Natl Acad. Sci. U.S.A.* **71**, 522.
17. Miller, R. W., de la Roche, I. and Pomeroy, M. K. (1974) *Plant Physiol.* **53**, 426.
18. Lynch, D. V., Lepock, J. R. and Thompson, J. E. (1987) *Plant Cell Physiol.* **28**, 787.
19. Murata, N. and Yamaya, J. (1984) *Plant Physiol.* **74**, 1016.
20. Van Blitterswijk, W. J., Van Hoeven, R. P. and Van der Meer, B. W. (1981) *Biochim. Biophys. Acta* **644**, 323.
21. Pike, C. S., Berry, J. A. and Raison, J. K. (1979) in *Low Temperature Stress in Crop Plants: The Role of the Membrane* (Lyons, J. M., Graham, D. and Raison, J. K., eds), p. 305. Academic Press, New York.
22. Platt-Aloia, K. A. and Thomson, W. W. (1987) *Protoplasma* **136**, 71.
23. Raison, J. K. and Wright, L. C. (1983) *Biochim. Biophys. Acta* **731**, 69.
24. McMurchie, E. J. (1979) in *Low Temperature Stress in Crop Plants: the Role of the Membrane* (Lyons, J. M., Graham, D. and Raison, J. K., eds), p. 163. Academic Press, New York.
25. Lyons, J. M., Raison, J. K. and Steponkus, P. L. (1971) in *Low Temperature Stress in Crop Plants: The Role of the Membrane* (Lyons, J. M., Graham, D. and Raison, J. K., eds), p. 1. Academic Press, New York.

26. Marangoni, A. G. and Stanley, D. W. (1988) *Bioch. Bioph. Res. Comm.* **153**, 104.
27. Graham, D. and Patterson, B. D. (1982) *Ann. Rev. Plant Physiol.* **33**, 347.
28. Levitt, J. (1980) *Physiological Ecology, Responses of Plants to Environmental Stresses* 2nd Edn, Vol. 1, p. 23. Academic Press, New York.
29. Wolfe, J. (1978) *Plant Cell Environ.* **1**, 241.
30. Minorsky, C. (1985) *Plant Cell Environ.* **8**, 75.
31. Smillie, R. M., Nott, R., Hetherington, S. E. and Oquist, G. (1987) *Physiol. Plantarum* **69**, 419.
32. Ilker, R., Breidenbach, R. W. and Lyons, J. M. (1979) in *Low Temperature Stress in Crop Plants: The Role of the Membrane* (Lyons, J. M., Graham, D. and Raison, J. K., eds), p. 97. Academic Press, New York.
33. Niki, T., Yoshida, S. and Sakai, A. (1978) *Plant Cell Physiol.* **19**, 139.
34. Niki, T., Yoshida, S. and Sakai, A. (1979) *Plant Cell Physiol.* **20**, 899.
35. Moline, H. E. (1976) *Phytopathology* **66**, 617.
36. Poovaiah, B. W., Reddy, A. S. N. and McFadden, J. J. (1987) *Physiol. Plantarum* **68**, 569.
37. Paliyath, G. and Thompson, J. E. (1987) *Plant Physiol.* **83**, 63.
38. Yoshida, S., Kawata, T., Uemura, M. and Niki, T. (1986) *Plant Physiol.* **80**, 152.
39. Moeller, C. H., Mudd, J. B. and Thomson, W. W. (1981) *Biochim. Biophys. Acta* **643**, 376.
40. Kader, A. and Morris, L. L. (1976) *Proceedings 2nd Tomato Quality Workshop*. University of California, Davis.
41. Briskin, D. P., Leonard, R. T. and Hodges, T. K. (1987) in *Methods in Enzymology* (Packer, L. and Douce, R., eds) Vol. 148, p. 542. Academic Press, New York.
42. Chifflet, S., Torriglia, A., Chiesa, R. and Tolosa, S. (1988) *Anal. Biochem.* **168**, 1.
43. Legge, R., Thompson, J. E., Baker, J. E. and Lieberman, M. (1982) *Plant Cell Physiol.* **23**, 161.